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DESCRIPTIONS OF SOME OF THE GLOCHIDIA OF THE UNIONIDAE
(MOLLUSCA:BIVALVIA)

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ABSTRACT

The primary objective of this study was to describe glochidia of the family Unionidae. Glochidia from 82 nominal taxa representing 30 genera in four subfamilies of the Unionidae were examined. The glochidium of the European *Unio elongatulus glaucinus* was found to share many characters with those of the Anodontinae. Both have triangular valves and styliform hooks. Furthermore, the glochidium of *Simpsonaias ambigua* and *U. e. glaucinus* share exterior valve sculpture and styliform hook fine structure.

The glochidia of some members of the Alasmidontini (subfamily Anodontinae), including some species in the genera *Lasmigona* and *Alasmidonta*, and that of *Pegias fabula* are depressed-pyriform, with large adductor muscles, looped exterior valve sculpture, and a double row of microstylets on the hook. These glochidia are very similar to those of *Strophitus*. The glochidia of *Arcidens* and the remaining members of *Alasmidonta* and *Lasmigona* are high-pyriform, with small adductor muscles, beaded to rosette exterior valve sculpture, and complex hooks with at least four rows of microstylets.

The glochidia of the Ambleminae demonstrate structures also found in the primitive lampsiline genera. The Lampsilinae are divided into four groups with the main lineage including *Ptychobranthus*, *Actinonaias*, *Obovaria*, *Ligumia*, *Venustaconcha*, *Villosa* and *Lampsilis*. Branches from this lineage include: (1) *Obliquaria*, *Cyrogenia* and *Dromus*; (2) *Ellipsaria*, *Leptodea* and *Potamilus*; and (3) *Epioblasma*.

Key words: Unionidae, glochidia, glochidial morphology, electron microscopy.

INTRODUCTION

Leeuwenhoek made the first substantial contribution to the study of glochidia (Leeuwenhoek, 1695). He correctly interpreted these tiny bivalves as young molluscs. He also observed limited development of the larvae and saw the characteristic snapping behavior they display when mature. It does not appear that he ever doubted that these tiny molluscs, which had developed within the gills of a female, were her young. However, like so many others during the century and a half to follow, he was unable to provide an environment outside of the female where development could continue. This, combined with the observations that these tiny molluscs were almost identical in both *Anodonta* and *Unio*, numbered within the gills of the larger mollusc by the thousands, and had structures, either real or imagined, that were quite different from those of the larger mollusc, led some to believe that the small shelled animals were not larvae but parasites. The tiny bivalve para-

sites were given the name *Glochidium parasiticum* by Rathke (1797).

The next major advance in the understanding of the life history of the Unionacea came with the observations of Carus and Leydig. Carus (1832) watched the brightly colored ova of *Potamida littoralis* (Cuvier, 1797) pass from the oviduct to the outer gills of a female mollusc. Continued observation demonstrated that the animal known as *G. parasiticum* was not a species separate from the larger mollusc but its larval stage. Leydig's (1866) discovery of glochidia embedded in the fins of a fish solved the primary developmental mystery and led the way for life history investigation. One of the primary objectives of these early life history investigations was to document the changes that occur during parasitism, when the glochidium transforms into a juvenile (Braun, 1878; Schmidt, 1885; Schierholz, 1878, 1888; Harms, 1907a, b, c, 1908, 1909). These studies also demonstrated that artificial infection could be used to indicate the susceptibility of fish to glochidia. Fueled by the com-

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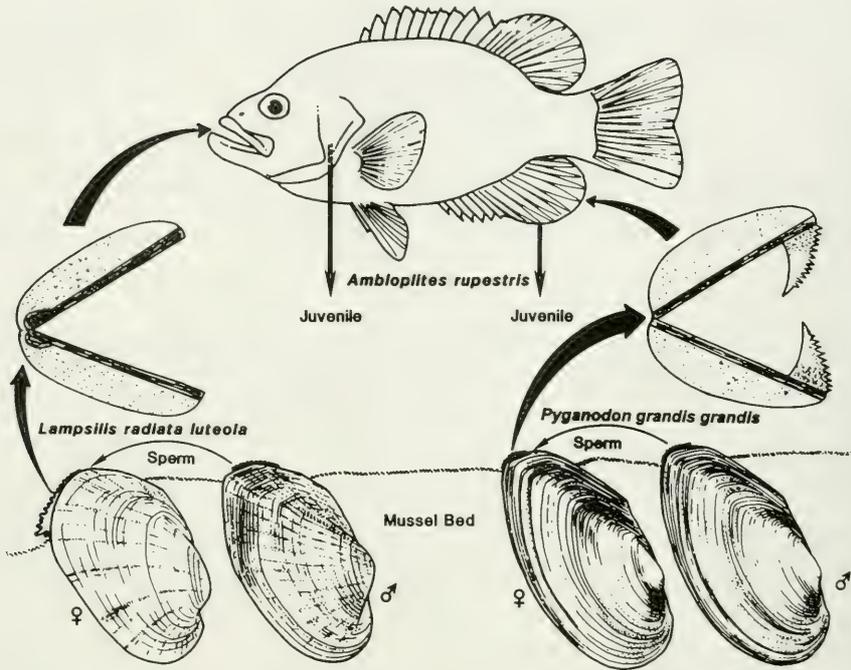


FIG. 1. Typical life histories of two species of Unionidae. (Drawing of *A. rupestris* after Trautman, 1981, and drawings of adult shells of *P. g. grandis* and *L. r. luteola* after Burch, 1975.)

mercial importance of North American unionid shells in the button industry, and the dwindling supply of those shells, the staff of the U. S. Bureau of Fisheries began to use artificial infection to determine the hosts for commercially important species (Lefevre & Curtis, 1910, 1912; Coker & Surber, 1911; Surber, 1912, 1913, 1915; Howard, 1912, 1914a, b, c). Diagrams of the unionid life cycle, were soon to follow.

Figure 1 demonstrates that there are essentially two very different life histories. Not only are the larvae themselves morphologically different (triangulate, ligulate, etc.), but so are their sites of eventual parasitism and the way in which they gain access to their host. The relatively large, subtriangular glochidium of the Anodontinae bears a hook on the ventral margin of each valve. If this glochidium clamps down on the fin of a passing fish and pierces the fin epithelium, it may become encapsulated by host tissue. The smaller, subelliptical glochidium of the Lampsilinae lacks hooks. If this glochidium is taken in through the mouth of its host, travels to the gills and clamps down on a gill filament, irritating the gill epithelium, it too may become

encapsulated by host tissue. Attraction devices, such as the mantle flaps of *Lampsilis*, facilitate the exchange of glochidium from unionid to fish (Morrison, 1973).

The dissimilarities in these life histories reveal three periods when selection might be acting upon the glochidium: (1) during release from the female, (2) during initial contact with the host, and (3) during encapsulation. The selective advantage of glochidial structures might be understood, therefore as they facilitate attraction of the host, attachment to the host, or induction of encapsulation (Hoggarth & Gaunt, 1988). Encapsulation provides a stable environment where transformation can occur, a ready source of nutrients (whether used or not), and protection from predators and from being washed or tumbled downstream (Coker & Surber, 1911; Howard & Anson, 1922; Arey, 1932a, b; Kat, 1984). Furthermore, the host functions as a dispersal mechanism, and releases the newly transformed juvenile into suitable habitats. With the use of scanning electron microscopy, minute glochidial structures can be examined and used to interpret relationships among unionid species. It is the objective of this paper to de-

scribe the glochidia of a large portion of the North American fauna to begin this endeavor.

MATERIALS AND METHODS

Specimens Examined

Glochidia were removed from the marsupia of 150 female unionids representing 82 nominal taxa from 30 genera. Forty-three lots of material were processed from specimens collected or received during this study. These specimens were deposited in the collection of The Ohio State University Museum of Zoology (OSUM), but glochidia samples from each specimen were retained by the author and have MAH catalog numbers. The remaining specimens were located in collections of unionids at The Ohio State University Museum of Zoology, University of Michigan Museum of Zoology (UMMZ), University of Wisconsin Zoological Museum (UWZYM), and The Illinois Natural History Survey (INHS).

Glochidia removed from the marsupia of female unionids were preserved (for freshly collected specimens) and stored in a solution of 80% alcohol, 5% glycerin, and 15% water. Each vial of glochidia was labeled with the catalog number of the female from which the sample came. Subsamples of glochidia taken from a vial for dehydration in acetone, and subsamples of these that were placed on a stub for viewing with scanning electron microscopy (SEM) were also labeled with the catalog number of the female mollusc. Therefore, each glochidium examined can be traced back to its maternal parent.

Procedures for Scanning Electron Microscopy

Preserved and freshly collected glochidia were cleaned by the removal of the glochidial soft parts. Only glochidia that had valves gaping apart (in the case of the preserved material) or that were actively snapping their valves (in the case of freshly collected glochidia) were processed for the SEM.

The initial step in cleaning was to wash the glochidia in three changes of distilled water. Each sample was suspended in distilled water and then allowed to settle, after which the supernatant was removed by using a Pasteur pipette. Generally, the glochidia settled to the bottom of the vial within 10–15 sec, whereas

small pieces of the marsupium and the matrix, within which the glochidia may be found within the marsupium, were still suspended. These impurities were removed with the supernatant.

Freshly collected glochidia were cleaned according to the method of Calloway & Turner (1979). These glochidia initially were washed as above, with two drops of 1N NaOH added to the final wash (10 ml). The glochidia were allowed to stand in this slightly basic solution for ten minutes and then washed in three changes of distilled water.

Previous studies using SEM to examine glochidia employed only freshly collected material from which the soft parts were removed as described above, or preserved material from which the soft parts had not been removed (Giusti, 1973; Giusti et al., 1975; Clarke, 1981a, 1985; Rand & Wiles, 1982). No practical method for the removal of the preserved soft parts had been developed. However, the procedure outlined below presents a method that was found to produce adequate specimens for SEM examination. This procedure is sufficiently flexible to allow for differences in preservation, initial valve gape, and amount of extraneous material in the sample.

Following the final rinse in distilled water, specimens were placed in 10 ml of 1% aqueous trypsin solution (Sorensen's Phosphate Buffer, pH 7.00) and thoroughly mixed. This was accomplished by drawing and expelling the liquid in the vial into a pipette 10–15 times. The action of the rapidly moving liquid often dislodged the soft parts from the valves, thereby reducing the time required in the trypsin solution. The sample was placed in an incubator at 37°C, visually inspected every 15 min, and removed when the valves began to gape at an angle approaching 180°. The digestion process was discontinued as soon as the first valves began to open, rather than after all valves had opened. The supernatant was carefully removed from the sample and the sample was washed in three changes of distilled water. Any remaining soft parts were mechanically dislodged by drawing a large number of glochidia into a pipette and expelling them 20–30 times per wash.

Cleaned glochidia, whether freshly collected or preserved, then were dehydrated in an ascending gradation of acetone (10%, 30%, 50%, 70%, 90%, 95%, 100%, 100%) and stored in Borosilicate Glass Scintillation Vials (VWR Scientific) in the final acetone

wash. Subsamples of these glochidia were processed for the SEM. Glochidia were air dried on a clean glass slide and mounted on double stick tape (3M) on 13 mm aluminum stubs. The tape was rimmed with silver paint and then the entire stub was placed in a vacuum desiccator for 24 h. Critical point drying was found to be unnecessary since the soft parts, which would tend to resist desiccation, were removed. Following desiccation, the specimens were coated with 30 nm of gold-palladium in a Hummer VI Sputter Coater, and then viewed in a Cambridge Stereoscan S4-10 SEM or a Hitachi S-500 SEM at an acceleration voltage of 20kV.

Orientation of Specimens on Figures

The orientation of the glochidial valve, demonstrated by the electron micrographs on each plate, follows Hoggarth (1987). The dorsal aspect of the valve has been oriented toward the top of the page, ventral is down. The anterior margin is toward the right hand margin of the page and posterior is to the left.

Characters Examined

Each character examined was defined in terms of two or more states or expressions of that character. An index to glochidial valve characters is found in Table 1. Measurements were made directly from SEM micrographs with care taken when glochidia were placed on the stub to ensure that some were flat and not tilted in respect to the stub surface. Micrographs of these glochidia were taken at 0° tilt.

Length – Glochidial valve length was measured as the greatest distance from anterior to posterior margins. This measurement was made parallel to the hinge (Fig. 2). Table 2 contains the morphometric data for each specimen of glochidium examined.

Height – Glochidial valve height was measured as the greatest distance from dorsal to ventral margins. This measurement was made perpendicular to length (Fig. 2).

Hinge length – The hinge was measured in a straight line from the points where the dorsal margins intersect the anterior and posterior margins, regardless of whether the hinge was curved or straight (Fig. 2).

Hinge ligament length – In the glochidium the hinge ligament extends the entire length of the hinge. A portion of the ligament can be

seen when viewing the valve externally and another portion can be viewed internally. Herein, central ligament refers to the expanded central portion of the hinge ligament or that portion that can be viewed internally. Posterior ligament refers to the posterior portion of the hinge ligament, measured from the posterior margin of the hinge to the posterior margin of the central ligament. Anterior ligament is the portion of the hinge ligament from the anterior margin of the hinge to the anterior margin of the central ligament.

Central ligament position – Central ligament position was found by adding one half the central ligament length to the length of the posterior ligament and then dividing by the length of the hinge. The mid-point of the central ligament was expressed as % length of the hinge from posterior to anterior.

Valve shape – Valve shape refers to the outline of the shell when viewed sagittally (the plane bisecting anterior and posterior). The terms used to describe valve shape have been loosely defined in the past. Valve shape descriptions and representative electron micrographs are indexed in Table 1.

Lateral valve gape – With the valves fully adducted some species of glochidia were found to possess anterior and posterior valve gape. This character was expressed as either absent or present (Table 1 provides an index to these and all subsequent glochidial valve characters).

Dorsal alae – This structure was found at the dorsal-anterior and dorsal-posterior margins of most lampsiline glochidia. They can best be described as arch-like extensions of the glochidial valve. This character was expressed as absent, short or long.

Microstylets – Clarke (1981a) proposed the term microstylet for the larger (> 1.0 µm long) points on the ventral margin of the glochidial valve. Microstylets were, (1) absent, (2) many and unorganized, (3) arranged in one distal row on the hook, (4) arranged in two distal rows on the hook, or (5) arranged in many distal rows on the hook.

Micropoints – Clarke (1981a) used the term micropoints for the small (< 1.0 µm long) points on the ventral margin of the glochidial valve. Micropoints were, (1) lanceolate, arising as single attenuate points from the ventral margin of the valve, (2) lamellate, arising as single plate-like points, or (3) coronal, with three to seven attenuate points arising from a common base.

TABLE 1. Glochidial shell characters used in analysis of relationships between the species of Unionidae. Following each character state is a reference to a figure illustrating the character state.

1. Length	8. Dorsal alae
a. short (46A)	a. absent (36A)
b. moderate (57A)	b. short (47A)
c. long (17A)	c. long (62A)
2. Height	9. Microstylets
a. short (32A)	a. absent (61C)
b. moderate (58A)	b. many unorganized (15D)
c. high (24A)	c. one distal row (13D)
3. Hinge length	d. two distal rows (16D)
a. short (48C)	e. many distal rows (28D)
b. moderate (64G)	10. Micropoints
c. long (4F)	a. lanceolate (29E)
4. Central ligament length	b. lamellate (49E)
a. short (47E)	c. coronal (33E)
b. long (9C)	11. Micropoint organization
5. Central ligament position	a. unorganized (36H)
a. posterior (7F)	b. horizontal rows (71C)
b. central (50F)	c. broken vertical rows (59E)
6. Valve shape	d. complete vertical rows (45D)
a. subelliptical (36B)	12. Hook
b. depressed subelliptical (71B)	a. absent (55D)
c. subrotund (38B)	b. styliform (25E)
d. subspatulate (63B)	c. supernumerary (70C)
e. fabelliform (40B)	d. lanceolate (50D)
f. subligulate (45B)	13. Exterior surface sculpture
g. ligulate (51B)	a. rough (68E)
h. subtriangulate (5B)	b. beaded (15E)
i. lachrimiform (11B)	c. rosette (26E)
j. pyriform (27B)	d. loose looped (9D)
k. depressed pyriform (20B)	e. ribbed loose looped (5C)
l. quadrate (23B)	f. tight looped (23E)
7. Lateral valve gape	g. vermiculate (51E)
a. absent (64C)	
b. present (50C)	

Micropoint organization – Micropoints were, (1) unorganized, (2) arranged in horizontal rows, (3) arranged in broken vertical rows, or (4) arranged in complete vertical rows.

Hook – As pointed out by Clarke (1981a), this term has been used indiscriminately to apply to independently derived structures that serve a similar function. He proposed using the term stylet to refer to the complex hook of the anodontine glochidia. In this study, the glochidial hook was either, (1) absent, (2) styliform, a V-shaped extension of the ventral margin of the glochidium, (3) lanceolate, a re-

curved, attenuate extension at each corner of the ventral margin of the glochidia of the genus *Potamilus*, or (4) supernumerary, the straight and sharply pointed hook found in an area along the ventral margin of the genus *Epioblasma*.

Exterior surface sculpturing – This refers to the fine structure of the exterior surface of the glochidial valve (viewed between 10,000 to 20,000 x). Exterior surface sculpture was, (1) rough, (2) beaded, (3) rosette, (4) loose-looped, (5) ribbed, loose-looped, (6) tight-looped, or (7) vermiculate.

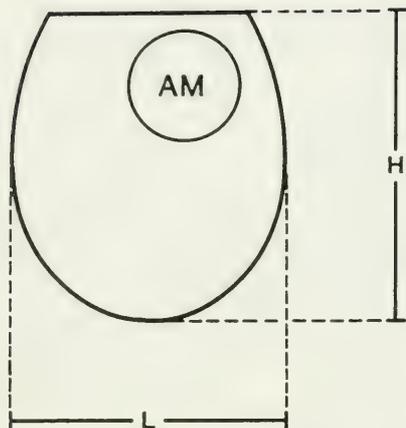


FIG. 2. Subelliptical glochidium of the Lampsiliinae demonstrating length (L) and height (H). The dorsal margin of the valve is toward the top of the page, ventral down, anterior to the right, and posterior to the left. The adductor muscle (AM) is located in the dorsal-anterior quadrant of the valve.

DESCRIPTIONS OF GLOCHIDIA

Subfamily Unioninae

Unio elongatulus glaucinus Porro, 1838 (Fig. 3A–F)

Material Examined

MAH 2055-Rivanazzano, Pavia, Italy, ex Coll. F. Giusti.

Description

Glochidium subtriangular, length 218 to 232 μm , ($227 \pm 6.45 \mu\text{m}$, $n = 4$), height 210 to 218 μm ($216 \pm 4.08 \mu\text{m}$, $n = 4$). Anterior and posterior margins arcuate, meeting at base of hook to form a broadly rounded ventral terminus. Valve outline only slightly asymmetric, with anterior margin slightly more produced than posterior margin. Exterior surface of valve malleated (large dimple-like depressions) and pitted (smaller depressions generally within malleated valve surface), except along smooth valve border. Fine sculpture of exterior valve surface beaded (Fig. 3E). Central ligament (seen in internal views of valve) 55 to 58 μm ($56 \pm 1.73 \mu\text{m}$, $n = 3$) in length, located about 40% from posterior to anterior. Posterior ligament 41 to 44 μm (42 ± 1.53

μm , $n = 3$) in length; anterior ligament 68 to 75 μm ($72 \pm 3.60 \mu\text{m}$, $n = 3$) long. Dorsal margin straight, 164 to 175 μm ($171 \pm 4.36 \mu\text{m}$, $n = 4$) in length. Styliform hook extending from ventral terminus of each valve as a broad triangular plate. Hook gradually and uniformly tapered to a broad point distally, located about 50% from both lateral margins. Surface of hook covered by microstylets ($> 1.0 \mu\text{m}$ long points on hook) and micropoints ($< 1.0 \mu\text{m}$ long points on the hook). Most microstylets (about 35) bluntly pointed, with some of more proximal microstylets multifaceted, sharply pointed. Micropoints extending from lateral and proximal margins of styliform hook covering most of its lateral surface, except within narrow band distally.

Remarks

The glochidium of *Unio* has been described as subtriangular with a ventral hook (Ortmann, 1912). Giusti (1973) stated that other than its smaller size, this glochidium resembles that of *Anodonta*. Ortmann (1918), however, reported the absence of a hook in *Unio caffer*, but Heard & Guckert (1970) suggested that this was the result of examining immature specimens. They noted that Giusti (1973) demonstrated hooks in *U. e. glaucinus* and that McMichael & Hiscock (1958) found hooks on mature glochidia of *Velesunio ambiguus*, even though the species was earlier described as having hookless glochidia (Hiscock, 1951).

Ortmann (1912) and Heard & Guckert (1970) placed this genus near the North American genus *Pleurobema* (Ambleminae) on the basis of similar shell morphology and anatomy. However, Ortmann (1912) recognized the similarity between the glochidia of the Unioninae and the Anodontinae and suggested that the former may have given rise to the latter. Morrison (1955) agreed with Ortmann's conclusion regarding the origin of the Anodontinae. He chose to stress developmental characters (the similarity in glochidia) over adult characters and therefore placed the subfamily Unioninae near the Anodontinae. Harms (1908, 1909) and Haas (1910) gave length and height measurements of 290 μm for the glochidium of *U. pictorum*. Giusti (1973) noted that the glochidium of *U. e. glaucinus* is smaller than that of *Anodonta*, but gave no measurements.

TABLE 2. Morphometric data for glochidia examined during this study. All measurements in μm .

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>U. e. glaucinus</i>	MAH 2055	225	218	172	75	56	41
	MAH 2055	218	216	175			
	MAH 2055			168	68	57	43
	MAH 2055	231	210	164			
	MAH 2055			173	74	55	44
<i>A. cygnea</i>	MAH 2055	232	218	175			
	OSUM 20911.1	358	352	268	112	79	77
	OSUM 20911.1	351	348	273			
	OSUM 20911.1	345	345	268			
	OSUM 20911.1			279	115	85	79
	OSUM 20911.1	347	356	277			
	OSUM 20911.1	351	356	279	115	88	76
	OSUM 20911.1	351	352	279	118	83	78
	OSUM 20911.1	357	344	272			
	OSUM 20911.1	346	346	270			
<i>A. anatina</i>	OSUM 20911.1			268	115	85	68
	OSUM 20911.1	350	357	279			
	OSUM 20912.1	348	361	273			
	OSUM 20912.1	355	354	267			
	OSUM 20912.1	352	352	262			
	OSUM 20912.1	353	361	264	116	91	57
	OSUM 20912.1	353	350	260	114	86	60
	OSUM 20912.2	356	358	274			
<i>A. beringiana</i>	OSUM 20912.2	361	361	280	125	96	59
	OSUM 3711.1	289	289	212			
	OSUM 3711.1	292	293	214			
	OSUM 3711.1	288	288	211			
	OSUM 3711.1	286	290	210	118	58	34
<i>A. kennerlyi</i>	OSUM 3711.1	288	284	209			
	OSUM 52882.2	354	340	264	111	88	65
	OSUM 52882.2			257	110	88	59
	OSUM 52882.2	352	345	260	111	89	60
	OSUM 52882.2			267	114	89	64
<i>A. implicata</i>	OSUM 52882.3			250	106	85	59
	OSUM 52882.3	350	350	253	114	84	55
	OSUM 52463.7	342	345	163			
<i>A. suborbiculata</i>	OSUM 52463.7	343	350	166	52	77	37
	OSUM 52463.7			160	48	74	38
	OSUM 13634	328	328	237	80	101	56
<i>P. g. grandis</i>	OSUM 13634	326	324	228	74	100	54
	OSUM 13634	326	323	233	81	98	54
	OSUM 13634	323	320	232	78	102	52
	OSUM 13634	325	322	231			
	OSUM 38467.10	358	360	250	104	92	54
<i>P. g. corpulenta</i>	OSUM 38467.10	355	355	250			
	OSUM 38467.10	355	360	257	111	94	52
	OSUM 38467.10	365	352	250			
	INHS 2247	353	355	259	107	90	62
	INHS 2247	350	353	250			
<i>P. c. cataracta</i>	INHS 2247	358	350	252			
	OSUM 47890	350	367	260			
	OSUM 47890	350	368	275			
<i>P. c. cataracta</i>	OSUM 53653	343	363	263	100	93	70
	OSUM 52462.27	380	368	288	102	115	71
	OSUM 52462.27	374	366	277			
	OSUM 52462.27	375	370	277			
	OSUM 52462.35	375	358	291			
	OSUM 52462.35	378	351	289	98	116	75

(continued)

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length			
					Ante.	Cent.	Post.	
<i>P. c. marginata</i>	OSUM 38962.6	358	364	276	102	110	64	
	OSUM 38962.6	360	369	277				
<i>P. doliaris</i>	OSUM 26405	317	317	240				
	OSUM 26405	405	368	305				
<i>U. imbecillis</i>	MAH 435	291	297	246	95	90	61	
	MAH 435	310	297	242				
	OSUM 9436.2	310	300	245				
	OSUM 9436.2	306	295	251	98	93	60	
	OSUM 9436.2	299	292	243				
<i>U. imbecillis</i>	OSUM 9436.2	300	289	242				
	OSUM 9436.2	299	303	240				
	OSUM 9436.2	310	304	243				
	OSUM 9436.2	309	303	249				
	OSUM 9436.2	300	303	256	103	93	60	
	UWZY 24971.1	313	306	252				
	UWZY 24971.1			255	103	89	63	
<i>A. ferussacianus</i>	UWZY 24971.1	303	305	250				
	OSUM 18275.2	323	325	235				
	OSUM 18275.2	325	326	234	83	92	59	
	OSUM 18275.2	326	327	238				
	MAH 989.4	322	322	231				
	MAH 989.4	325	325	238	85	91	62	
	MAH 989.4	321	320	231				
	MAH 989.4	319	322	233	89	88	56	
<i>S. ambigua</i>	UMZY 22658	253	256	168				
	UMZY 22658	251	257	168	65	58	45	
	UMZY 22658	255	265	167				
	UMZY 22662	258	264	170	61	63	49	
	UMZY 22662	257	264	170				
	UMZY 22672			166	63	54	49	
<i>S. u. undulatus</i>	OSUM 55995	255	259	164				
	OSUM 49443	369	299	281				
	OSUM 52458.4	360	289	271	101	97	73	
	OSUM 52458.4	360	296	280				
	OSUM 52458.4			272	109	87	76	
	OSUM 52458.4			281	108	103	70	
<i>S. u. tennesseensis</i>	OSUM 52458.4			280	110	92	78	
	OSUM 33381.2			268	96	94	78	
	OSUM 55449	346	298	268				
	<i>S. subvexus</i>	OSUM 36240	354	288	271			
		OSUM 36240	348	292	275			
<i>A. viridis</i>	OSUM 36240			277	123	84	70	
	OSUM 36240	359	289	273				
	OSUM 47518	300	245	245	100	80	65	
	OSUM 47518	313	250	260	108	81	71	
	OSUM 47518	313	246	250				
	OSUM 47518	300	253	250				
	OSUM 47518	319	258	258				
	OSUM 47518	303	250	250	109	73	68	
OSUM 47518	300	250	245					
<i>A. heterodon</i>	OSUM 25106.2	330	267	253				
	OSUM 25106.2	338	258	266				
	OSUM 25106.2	332	268	265	110	80	75	
	OSUM 25106.2	335	258	255				
<i>A. undulata</i>	OSUM 52434.4	358	378	258	105	98	55	
	OSUM 52434.4	356	365	247	103	89	55	
	OSUM 52434.4	355	375	260				
	OSUM 52434.4	354	368	257				
	OSUM 52434.4	343	369	247	105	89	53	

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>A. marginata</i>	MAH 277.1	340	360	233			
	MAH 277.1	341	362	235	107	67	61
	MAH 277.1	335	372	232	100	73	59
	MAH 724.1	341	367	230			
<i>P. fabula</i>	OSUM 41308.3	386	325	210	74	73	63
	OSUM 41309.1	388	319	200	69	68	62
	OSUM 41309.1	385	323	208	79	73	56
<i>A. confragosus</i>	OSUM 52015	357	353	240	100	70	70
	OSUM 52015	352	354	252	108	74	70
	OSUM 52015	363	355	237			
	OSUM 52015	360	355	252	115	78	59
	OSUM 52015			250	115	63	72
<i>L. compressa</i>	OSUM 23179.1	322	285	234			
	OSUM 23179.1	327	287	233	82	84	67
	MAH 702	320	286	230			
	MAH 702	317	283	239			
	MAH 727	327	288	232	81	83	68
<i>L. subviridis</i>	OSUM 27131.66	370	309	253	100	88	65
	OSUM 27131.66	383	318	259			
	OSUM 27131.66	380	311	244			
	OSUM 27131.68	368	310	260	105	87	68
	OSUM 27131.68	381	312	253	107	89	57
<i>L. holstonia</i>	OSUM 55826.6	286	294	234	109	73	52
	OSUM 55826.6	291	276	235			
	OSUM 55826.6	281	275	221			
	OSUM 55826.7	290	275	228	110	63	55
	OSUM 55826.7	284	291	221			
<i>L. costata</i>	MAH 585	343	365	240	97	79	64
	MAH 585	347	375	239			
	MAH 585	343	373	239	104	71	64
	MAH 585	345	364	248	105	82	61
	MAH 882.1	343	368	241	90	83	68
	MAH 882.1	348	377	244	99	84	61
	MAH 882.1	340	363	239			
<i>L. complanata</i>	MAH 278.2			193	75	68	50
	MAH 278.2	290	295	197	79	65	53
	MAH 278.2	296	310	201	88	60	53
	MAH 278.2	289	293	195	82	62	51
	MAH 278.2	294	298	199			
	MAH 278.2	293	297	208			
	MAH 278.2	295	308	206			
<i>M. nervosa</i>	OSUM 13032.66	268	347	155			
	OSUM 54178	254	348	145			
	OSUM 54178			153	60	49	44
	OSUM 54178	262	350	147			
	OSUM 1986:22	260	340	145			
	OSUM 1986:22			155	60	52	43
<i>M. boykiniana</i>	OSUM 1986:22			155	63	49	43
	OSUM 51107.5	245	350	150			
<i>P. dombeyana</i>	OSUM 53273.2	223	238	132	55	46	31
	OSUM 53273.2	223	240	134			
	OSUM 53273.2	228	259	135			
	OSUM 53273.2			133	58	43	32
	OSUM 53273.3	224	252	134			
	OSUM 53273.3	230	251	132	59	42	31
	OSUM 53273.3	225	243	133			
	OSUM 43011	223	250	136			
	OSUM 43011	226	243	132			
	OSUM 43011	231	241	134			
	OSUM 43011	223	243	133	51	47	35

(continued)

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>T. verrucosa</i>	MAH 654.1	92	101	45			
	MAH 654.1			46	9	32	5
	MAH 654.1	88	100	40	6	31	3
	MAH 654.1			43	6	33	4
	MAH 654.1	90	100	46	8	33	5
	MAH 654.1	85	97	44			
	MAH 654.1	94	101	45			
	MAH 654.1			44	9	30	5
<i>Q. infucata</i>	MAH 654.1	93	99	44	6	34	4
	OSUM 48537.1			104	28	54	22
	OSUM 48537.1	234	275	104	26	58	20
	OSUM 48537.2	240	287	105			
	OSUM 48537.2	242	285	102			
<i>E. dilatata</i>	OSUM 48537.2	242	285	102			
	MAH 946.9	217	225	140	56	45	39
	MAH 946.9	210	219	142	56	50	36
	MAH 946.9	219	219	142			
<i>P. fasciolaris</i>	MAH 946.9	219	219	147			
	MAH 641	170	195	89			
	MAH 641	174	184	85	26	40	19
<i>P. occidentalis</i>	MAH 651	175	182	80	22	36	22
	OSUM 45361.14	197	241	101	30	48	23
	OSUM 45361.17	203	234	102			
	OSUM 45361.17			104	34	44	26
<i>P. greeni</i>	OSUM 19025.2	189	227	96	26	49	21
	OSUM 19025.2	190	228	93			
	OSUM 19025.2	183	226	90			
<i>P. subtentum</i>	OSUM 43156.5	195	251	85	25	36	24
	OSUM 43156.5	181	239	90			
	OSUM 43156.5	191	251	85			
	OSUM 43156.5			83	26	36	21
	OSUM 43156.5	190	236	82			
<i>O. reflexa</i>	OSUM 43156.5	194	239	83	25	38	20
	OSUM 54361.1	219	221	119			
	OSUM 54361.1	213	219	127	44	52	31
	OSUM 54361.1	219	206	121	37	54	30
<i>C. stegaria</i>	OSUM 6298.21	204	164	113			
	OSUM 6298.21	206	167	116	46	40	30
	OSUM 6298.21	208	170	120			
	OSUM 6298.21			115	43	39	33
<i>C. aberti</i>	OSUM 6298.21			114	47	35	32
	OSUM 48067	200	145	128	50	29	49
	OSUM 48067	207	155	136			
	OSUM 48067	200	143	125	48	31	46
	OSUM 48067	211	156	128	51	36	41
	OSUM 48067	218	161	132			
	OSUM 48067	211	161	131	60	28	43
	OSUM 48067			136	61	32	43
<i>D. dromas</i>	OSUM 23209			171	76	39	56
	OSUM 20407.1	230	120	182			
	OSUM 20407.1	230	114	182	82	39	61
	OSUM 20407.1	222	118	169	78	40	51
	OSUM 20407.1	221	120	160			
<i>A. pectorosa</i>	OSUM 20407.1	219	120	182			
	OSUM 24337	253	270	139	46	62	31
	OSUM 48748.3	248	270	151	52	62	37
	OSUM 48748.3	244	260	144	47	58	39
<i>A. l. carinata</i>	MAH 842.1	220	243	125	38	57	30
<i>O. retusa</i>	UUMZ UNCAT.	233	278	115			
	UUMZ UNCAT.	218	272	117			
	UUMZ UNCAT.			119	38	45	36

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>O. olivaria</i>	OSUM 51282.2	202	254	107			
	OSUM 51282.2	198	261	106			
	OSUM 51282.2			105	32	44	29
	OSUM 51282.2			101	41	34	26
<i>O. subrotunda</i>	OSUM 51282.2	206	258	110	43	40	27
	MAH 659.2			94	29	40	25
	MAH 659.2	174	197	89			
	MAH 659.2	180	210	95			
	MAH 805.1	178	205	92			
	MAH 805.1	180	210	88	24	43	21
<i>O. jacksoniana</i>	MAH 805.1	174	199	85			
	OSUM 50233.8	175	243	89	26	42	21
	OSUM 50233.8	183	230	95			
	OSUM 50233.8	184	230	100			
<i>O. unicolor</i>	OSUM 50233.8	187	240	97	31	41	25
	OSUM 33158	172	222	85			
	OSUM 33158	174	224	88			
	OSUM 33158	180	229	96	41	32	23
	OSUM 47696.6	168	218	93	32	33	28
	OSUM 47696.6	168	234	89			
<i>E. lineolata</i>	OSUM 47696.6			88	32	32	24
	OSUM:1984:14	240	324	93	23	50	20
	OSUM:1984:14	229	319	88	27	37	24
	OSUM:1984:14	240	325	91			
	OSUM:1984:14	235	310	87	29	35	23
	OSUM:1984:14	245	325	96	23	51	22
<i>L. fragilis</i>	OSUM:1984:14	233	319	91			
	OSUM:1984:14	241	325	89			
	MAH 626.1	73	82	35	14	11	10
	MAH 626.1	72	80	35			
<i>L. ochracea</i>	MAH 626.1	72	83	30	11	11	8
	MAH 896	246	289	109	32	47	30
	MAH 896			110	36	42	32
	MAH 896	246	293	102			
	MAH 896	241	289	105	34	43	28
	MAH 896	241	291	105			
<i>P. ohioensis</i>	MAH 896	241	291	110	37	39	34
	OSUM 54521			42	13	16	13
	OSUM 54521	126	182	45			
	OSUM 54521	122	187	42	15	21	14
	OSUM 54521	120	175	47			
<i>P. amphichaena</i>	OSUM 33163.13	113	171	41	11	20	10
	OSUM 33163.13	111	170	40			
<i>P. alatus</i>	OSUM 55465	206	371	104			
	OSUM 55465	208	371	96	35	42	19
	OSUM 55465	227	386	109	38	42	29
	OSUM 55465	223	386	96			
<i>P. purpuratus</i>	OSUM 55465	217	375	106			
	OSUM 15738.2	200	356	106	37	41	28
	OSUM 15738.2			103	37	37	29
	OSUM 15738.2	195	347	100			
<i>L. recta</i>	OSUM 15738.2	190	348	109			
	OSUM:1984:2	205	257	105			
	OSUM:1984:2	219	263	112			
	OSUM:1984:2			115	41	40	34
	OSUM:1984:2			110	45	35	30
	OSUM:1984:2	207	257	105			
	OSUM:1984:2	211	265	110			

(continued)

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>V. ellipsiformis</i>	MAH 947.2	230	280	110	39	40	31
	MAH 947.2			102	34	40	28
	MAH 947.2	226	287	105			
	MAH 947.2	226	287	103			
	MAH 947.2			104	34	38	32
	MAH 947.2	223	284	102			
<i>V. trabalis</i>	OSUM 9516.49	213	278	94			
	OSUM 9516.49	211	277	95	36	31	28
	OSUM 9516.49			99	36	36	27
	OSUM 9516.49	214	279	96	40	31	25
	OSUM 9516.49	216	280	97			
<i>V. perpurpurea</i>	OSUM 16262	165	241	88			
<i>V. villosa</i>	OSUM 45943	246	300	114			
	OSUM 45943	245	298	105			
	OSUM 45943	246	296	110	35	42	33
	OSUM 45947	250	308	116			
	OSUM 45947	248	304	108			
	OSUM 45947	240	307	110			
	OSUM 45947	241	308	113	43	37	33
	OSUM 45947	241	308	113	43	37	33
<i>V. vibex</i>	OSUM 54631	230	298	117			
	OSUM 24124	231	302	120			
	OSUM 24124	232	304	102	29	44	29
	OSUM 24124	231	302	117			
	OSUM 24124	239	299	105	32	45	28
	OSUM 48628	231	300	109			
	OSUM 48628	225	297	105			
	OSUM 48628	224	299	103			
	<i>V. i. iris</i>	MAH 641.1	230	289	112		
MAH 641.1		217	289	113	38	42	33
MAH 641.1		230	300	112			
MAH 641.1		232	303	115	40	41	34
MAH 641.1		228	305	115			
OSUM 55828.3		220	293	113			
OSUM 55828.3		227	300	114	39	42	33
OSUM 55828.3		217	290	107			
<i>L. t. teres</i>	OSUM 36409	189	256	103			
	OSUM 36409			113	54	27	32
	OSUM 36409	189	255	100			
	OSUM 36409	190	255	107			
	OSUM 36409	191	256	108			
	OSUM 36409			115	49	34	32
	OSUM 36409			106	42	34	30
	OSUM 36531.1	190	258	106			
	OSUM 36531.1			107	42	33	32
	OSUM 51669.2	190	259	115			
	OSUM 51669.2			116	49	37	30
	OSUM 51669.2	192	261	115			
	OSUM 51669.2	193	265	112	41	39	32
	OSUM 51669.2	194	260	112			
	<i>L. t. anodontoides</i>	OSUM 35612	197	255	105		
OSUM 35612		206	249	115	46	36	33
OSUM 35612		207	254	110			
OSUM 35612				107	39	36	32
OSUM 41762.2				115	47	38	30
OSUM 41762.2		187	249	105			
OSUM 41762.2		199	254	110			
OSUM 41762.2		204	249	118	50	37	31
OSUM 41762.2		194	249	118	46	42	30

TABLE 2. (Continued)

Species	Catalog #	Length	Height	Hinge Length	Ligament Length		
					Ante.	Cent.	Post.
<i>L. r. radiata</i>	MAH 897.1	255	311	128	50	46	32
	MAH 897.1	260	295	129			
	MAH 897.1	250	304	122	46	47	29
	MAH 897.1	255		121	49	45	27
<i>L. r. luteola</i>	MAH 321.6	230	288	110	37	40	33
	MAH 321.6	232	295	112			
	MAH 587.1	227	280	109			
	MAH 587.1	235	290	118	39	46	33
	MAH 587.1	230	280	107			
	MAH 587.1			120	40	40	40
	MAH 587.1			119	42	45	32
	MAH 628.1	232	280	113			
	MAH 628.1			115	37	42	36
	MAH 628.1			110			
<i>L. r. luteola</i>	MAH 727.2	232	280	110			
	MAH 727.2			120	40	41	39
	MAH 727.2	230	285	123			
<i>L. abrupta</i>	OSUM 13303	208	251	96			
	OSUM 13303	214	252	112			
	OSUM 13303	207	257	98			
	OSUM 13303			111	46	38	27
	OSUM 13303	210	259	106			
	OSUM 38841	214	252	96			
<i>L. higginsii</i>	OSUM 38841	208	251	97			
	OSUM 49024.1	214	257	108			
	OSUM 49024.1	217	254	111			
	OSUM 49024.1			108	38	39	31
<i>L. ovata</i>	OSUM 49024.1			118	40	47	31
	OSUM 43164.1	232	271	113			
	OSUM 43164.1	232	276	116			
	OSUM 43164.1			116	39	43	34
	OSUM 43164.1			119	40	44	35
<i>L. ornata</i>	OSUM 54661	203	258	98			
	OSUM 54661	204	257	100	38	33	29
	OSUM 54661	198	260	96			
<i>L. satura</i>	OSUM 38973.51			117	42	40	35
	OSUM 38973.51	220	269	115			
	OSUM 38973.51			116	43	40	33
	OSUM 38973.52	223	268	113			
<i>L. ventricosa</i>	MAH 846.1	255	292	111			
	MAH 846.1	253	287	109			
	MAH 846.1			118	45	41	33
	MAH 846.1	254	291	111	38	40	33
	MAH 954.1	245	275	117	45	39	33
	MAH 954.1	250	274	108			
	MAH 954.1	240	274	106			
	MAH 954.2	255	286	105			

Subfamily Anodontinae

Anodonta cygnea (Linnaeus, 1758)

(Fig. 4A–F)

Material Examined

OSUM 20911.1 – “Maubroux (Genval) petit etang pres du lac”, Belgium [etang = pool, pond], 24 January 1949, W. Adam.

Description

Glochidium subtriangular, length 345 to 358 μm ($351 \pm 4.33 \mu\text{m}$, $n = 9$), height 344 to 357 μm ($351 \pm 4.41 \mu\text{m}$, $n = 9$). Anterior margin rounded, slightly more produced than posterior margin, with maximum inflation at approximately 50–60% from dorsal to ventral. Posterior margin gently curved throughout its length,

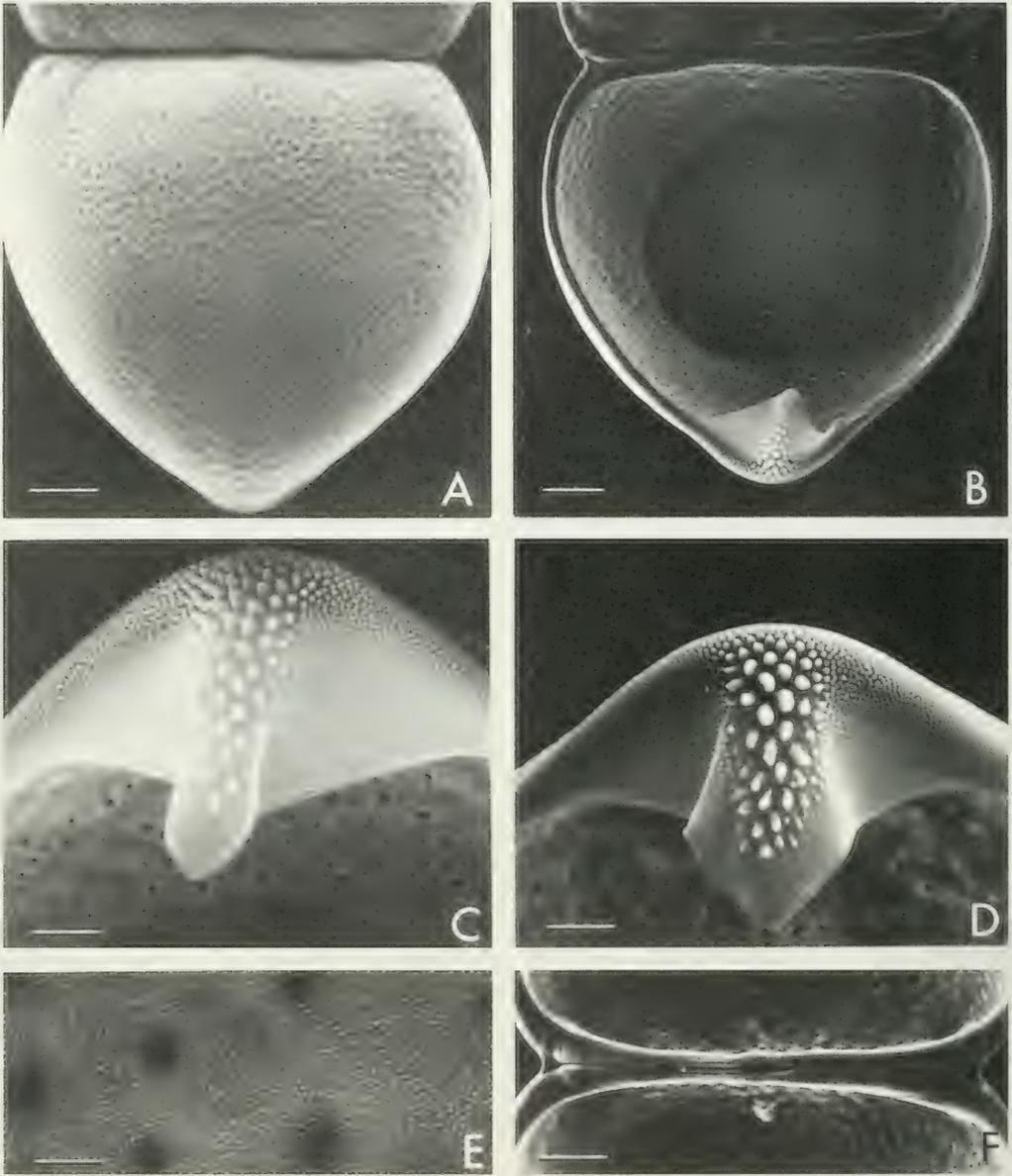


FIG. 3. Glochidium of *Unio elongatulus glaucinus*, MAH 2055; A. exterior valve, bar length = 30 μ m; B. interior valve, bar length = 30 μ m; C. styliform hook, bar length = 10 μ m; D. styliform hook, bar length = 10 μ m; E. exterior valve sculpture, bar length = 1 μ m; F. hinge, bar length = 25 μ m.

producing a moderately asymmetric valve outline. Ventral terminus narrowly pointed, located about 40% from posterior to anterior. Exterior surface of valve malleated and pitted, except in a narrow band along lateral margins. Pit density reduced in the area of adductor muscle scar (Fig. 4B, C). Loose-looped sculp-

turing covering exterior surface of valves (Fig. 4E). Central ligament 79 to 88 μ m ($84 \pm 3.27 \mu$ m, $n = 5$) in length, centered about 43% from posterior to anterior. Posterior ligament 68 to 79 μ m ($73 \pm 5.22 \mu$ m, $n = 5$) in length; anterior ligament 112 to 118 μ m ($115 \pm 2.12 \mu$ m, $n = 5$) long. Hinge straight, 268 to 279 μ m ($274 \pm$

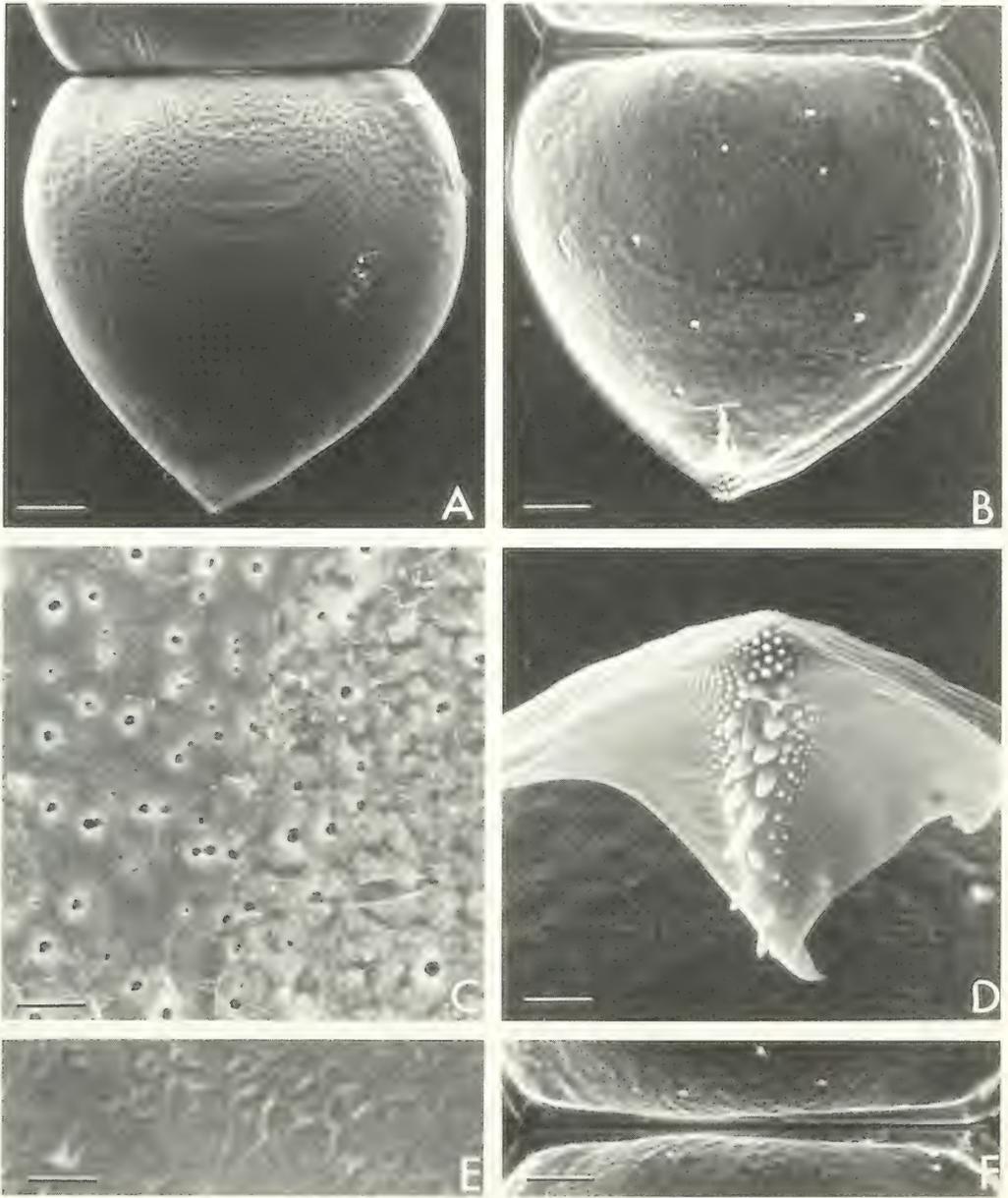


FIG. 4. Glochidium of *Anodonta cygnea*, OSUM 20911.1; A. exterior valve, bar length = 55 μm ; B. interior valve, bar length = 50 μm ; C. interior valve pitting, bar length = 5 μm ; D. styliform hook, bar length = 20 μm ; E. exterior valve sculpture, bar length = 1 μm ; F. hinge, bar length = 40 μm .

3.35 μm , $n = 11$) in length. A styliform hook extends from ventral terminus as a broad triangular plate. Lateral hook margins concave, rapidly narrowed, producing a sharp point. Microstylets lanceolate, sharply pointed, ar-

ranged in four rows near ventral terminus, reduced to single row distally. Micropoints covering ventral terminus and lateral surfaces of the hook, leaving narrow unsculptured band along distal edge of the hook.

Remarks

The glochidium of *A. cygnea* can be distinguished from that of *Unio* by its shape, hook structure, exterior valve sculpturing and size. Length and height measurements of 350 μm have been given for this glochidium by Harms (1909), Haas (1910), and Ortmann (1912). It has been figured by Ortmann (1912: pl. 19, fig. 2) and Wood (1974: figs. 3, 4).

Anodonta anatina (Linnaeus, 1758)
(Fig. 5A-G)

Material Examined

OSUM 20912.1, 20912.2 – "Ixelles, etang", Belgium [etang = pool, pond], 16 March 1950, W. Adam.

Description

Glochidium subtriangular, moderately asymmetric, length 350 to 361 μm ($357 \pm 3.91 \mu\text{m}$, $n = 7$), height 348 to 361 μm ($354 \pm 4.46 \mu\text{m}$, $n = 7$). Anterior margin broadly curved near the dorsal margin, more moderately curved ventrally. Posterior margin gently and evenly curved throughout its length. Malleations and pits covering exterior surface of valve, except along the valve margin. Interior surface uniformly pitted, without a noticeable adductor muscle scar. Exterior valve sculpturing ribbed loose-looped, consisting of raised loops restricted to bands that run parallel to the dorsoventral axis of valve, separated by unsculptured bands (Fig. 5C). Larval thread present (Fig. 5B, E, F). Central ligament 86 to 96 μm ($91 \pm 5.00 \mu\text{m}$, $n = 3$) in length, centered about 43% from posterior to anterior. Posterior ligament 57 to 60 μm ($59 \pm 5.51 \mu\text{m}$, $n = 3$) long; anterior ligament 114 to 125 μm ($118 \pm 5.86 \mu\text{m}$, $n = 3$) long. Hinge straight, with a length of 260 to 280 μm ($269 \pm 6.37 \mu\text{m}$, $n = 7$). Styliform hook with biconcave lateral margins, forming sharp distal point. Microstylets (about 15) lanceolate (Fig. 5G), sharply pointed, reduced to a single row distally. Micropoints numerous, covering ventral terminus and lateral surface of hook. Unsculptured distal hook margin narrow. Hook located about 40% from posterior to anterior.

Remarks

This glochidium is distinguished from that of *A. cygnea* by its unique exterior valve scul-

pturing. Giusti (1973) and Giusti et al. (1975) were the first to demonstrate this unusual sculpturing. They refer to their species as *A. cygnea* (*Anodonta piscinalis*). *Anodonta piscinalis* was placed in the synonymy of *A. cygnea* by Simpson (1900, 1914) and Ortmann (1912). *Anodonta anatina* has also been synonymized with *A. cygnea* by Ortmann (1912), although it was treated as a variant of *A. cygnea* by Simpson (1900, 1914).

There is little question that there are two species of European *Anodonta* based on glochidial characters. These species have many characters in common, but their exterior valve sculpturing is sufficiently different to separate them. Glochidia of *A. anatina* were figured by Flemming (1875: p1. 3, fig. 11), Schierholz (1888: p1. 2, fig. 29), Ortmann (1912: p1. 19, fig. 3, as *A. complanata*), Giusti (1973: figs. 13–25, as *A. cygnea*), and Giusti et al. (1975: figs. 10–21, as *A. cygnea*).

Anodonta beringiana Middendorff, 1851
(Fig. 6A-F)

Material Examined

OSUM 3711.1 – Outlet of Peper Lake, Kenai Peninsula, Alaska, 13 August 1957, R. Rausch.

Description

Glochidium subtriangular, moderately asymmetric, length 286 to 292 μm ($289 \pm 2.28 \mu\text{m}$, $n = 5$), height 284 to 293 μm ($290 \pm 3.42 \mu\text{m}$, $n = 5$). Anterior and posterior margins about equal, more or less gently curved, with anterior margin slightly more produced, especially in the dorsal half of valve. Valve outline only slightly asymmetric. Exterior surface of valve malleated and pitted. Pits uniformly distributed, except along the valve margin, where they are absent. Loose-looped sculpture covering exterior surface of valve. Larval thread present (Fig. 6A, C). Hinge straight, length 209 to 214 μm ($211 \pm 2.35 \mu\text{m}$, $n = 5$). Posterior, central and anterior ligaments of a single specimen, 34 μm , 58 μm and 118 μm , respectively. Central ligament far posterior, at 31% from posterior to anterior. Styliform hook arising as a broad biconcave triangular plate. Microstylets robust, multifaceted few in number (about 15). Micropoints bluntly lanceolate, on ventral rim of valve, along lateral edges of the microstylets and for

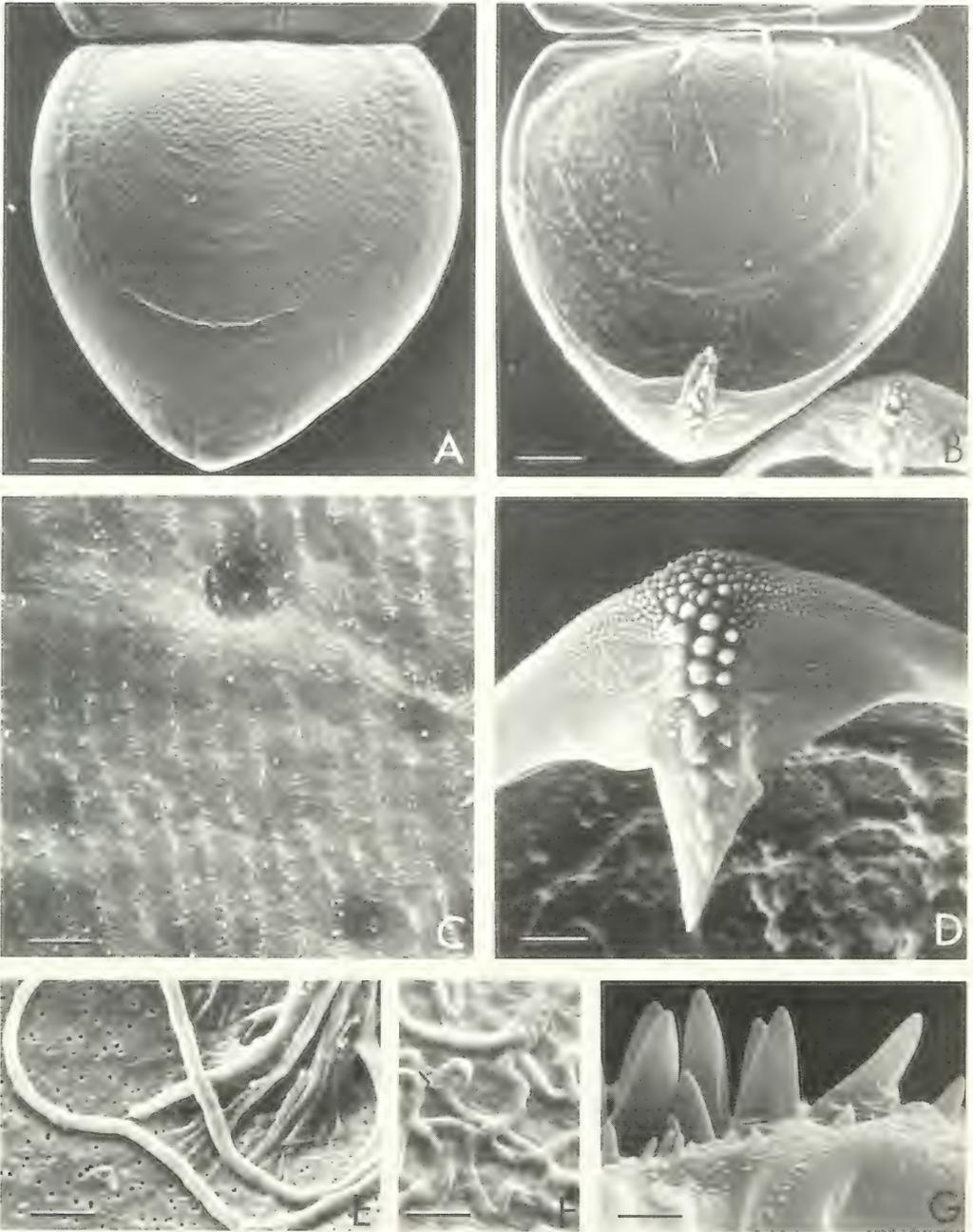


FIG. 5. Glochidium of *Anodonta anatina*: A. exterior valve, OSUM 20912.2, bar length = 55 μ m; B. interior valve, OSUM 20912.2, bar length = 50 μ m; C. exterior valve sculpture, OSUM 20912.1, bar length = 1 μ m; D. styliform hook, OSUM 20912.2, bar length = 20 μ m; E. larval thread, OSUM 20912.2, bar length = 25 μ m; F. larval thread, OSUM 20912.2, bar length = 15 μ m; G. microstylets, OSUM 20912.2, bar length = 5 μ m.

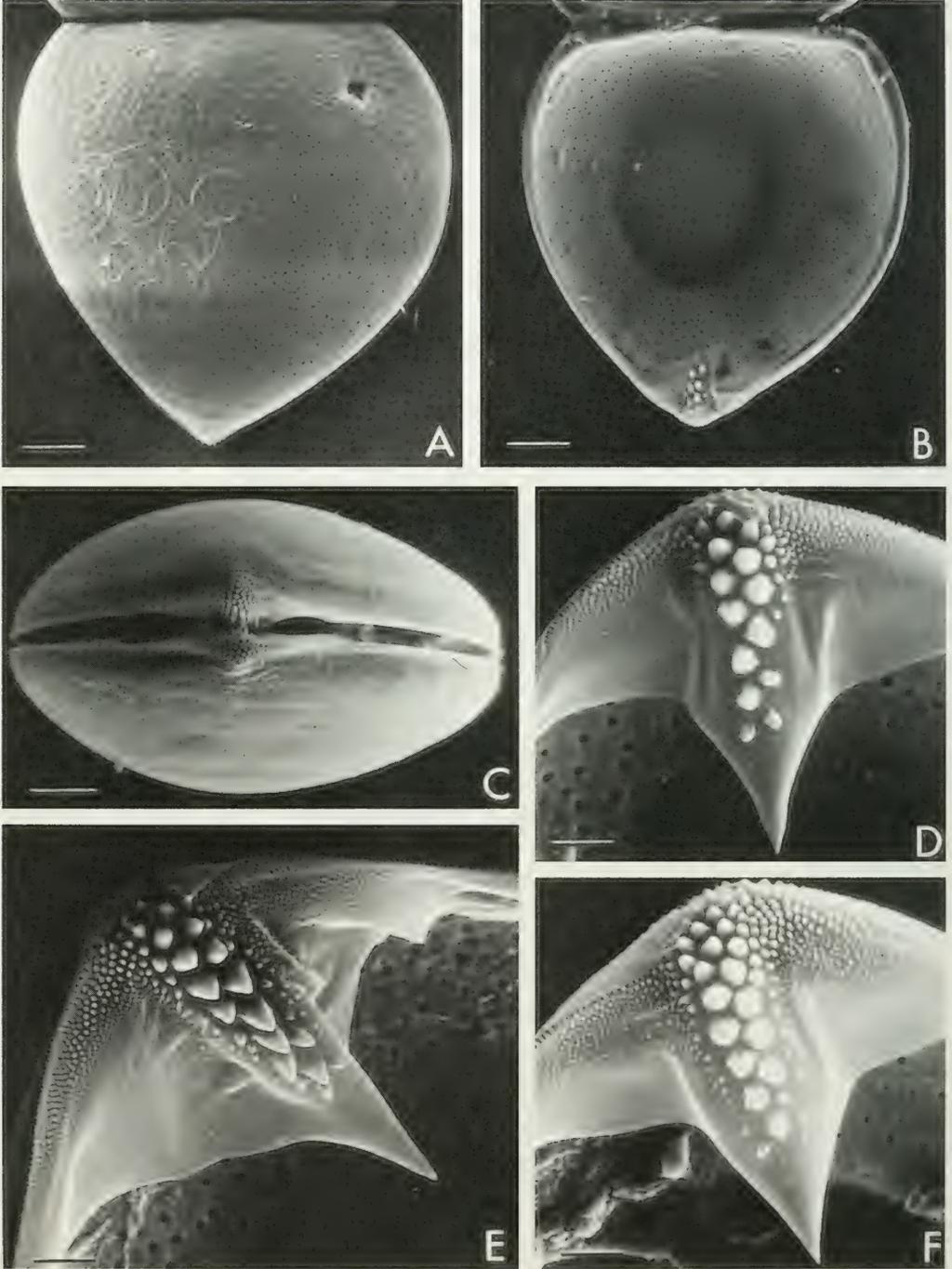


FIG. 6. Glochidium of *Anodonta beringiana*, OSUM 3711.1; A. exterior valve, bar length = 45 μ m; B. interior valve, bar length = 50 μ m; C. ventral valve view, bar length = 40 μ m; D. styliform hook, bar length = 10 μ m; E. styliform hook, bar length = 10 μ m; F. styliform hook, bar length = 10 μ m.

a short distance on lateral, surfaces of hook. Unsculptured distal hook margin, wide. Hook located approximately 41% from posterior to anterior.

Remarks

This species was allied, on the basis of adult shell morphology, to *A. cygnea* by Simpson (1914). The shape of this glochidium also allies *A. beringiana* with *A. cygnea*. The glochidium of *A. beringiana* can be distinguished from that of *A. cygnea* by its smaller overall size and hook structure. Inaba (1941) gave 296 μm for the length and height of this glochidium, and Cope (1959) reported length and height measurement of 275 $\mu\text{m} \times 300 \mu\text{m}$.

Anodonta kennerlyi Lea, 1860
(Fig. 7A–F)

Material Examined

OSUM 52882.2, 52882.3 – “Griffin Creek,” Snohomish Co., Washington, January 1972, L. Gilbertson.

Description

Glochidium subtriangular, moderately asymmetric, length 350 to 354 μm ($352 \pm 2.08 \mu\text{m}$, $n = 3$), height 340 to 350 μm ($344 \pm 5.13 \mu\text{m}$, $n = 3$). Anterior margin rounded, tapering to meet a slightly curved posterior margin at ventral terminus. Ventral terminus narrowly pointed, located about 40% from posterior to anterior. Malleations and pits uniformly distributed on valve, except along valve margin, where dorsally converging longitudinal ridges occur (Fig. 7C). Exterior valve sculpturing intermediate between beaded and loose-looped (Fig. 7E), resembling short lengths of strung beads closely packed on surface of valve. Hinge straight, 250 to 267 μm ($259 \pm 7.31 \mu\text{m}$, $n = 6$) in length. Central ligament 84 to 89 μm ($87 \pm 2.07 \mu\text{m}$, $n = 6$) long, centered about 40% from posterior margin. Posterior ligament 55 to 65 μm ($60 \pm 3.25 \mu\text{m}$, $n = 6$) in length; anterior ligament 106 to 114 μm ($111 \pm 2.94 \mu\text{m}$, $n = 6$) long. Styliform hook sharply pointed, with fewer than 20 microstylets (Fig. 7B, D). Microstylets lanceolate, sharply pointed, arranged in four proximal rows, reduced to a single row distally. Two to three microstylets, and as many as seven micropoints, forming a cluster near tip of

hook. Additional micropoints covering lateral surfaces of hook and along lateral borders of microstylets, leaving narrow unsculptured distal hook edge.

Remarks

This species was also allied to *A. cygnea* by Simpson (1914) due to similarity in adult shell morphology. The shape of this glochidium is also similar to that of *A. cygnea*, although this species can be distinguished by its exterior surface sculpturing and the cluster of microstylets and micropoints near the point of the hook. This aspect of the styliform hook resembles that of *Pyganodon cataracta* and related species east of the Rocky Mountains.

Anodonta implicata Say, 1829
(Fig. 8A–F)

Material Examined

OSUM 52463.7 – Great Herring Pond, S shore by the Herring River, 0.8 mi. N of Bournedale, 1.6 mi. WNW of Sagamore, Barnstable Co., Massachusetts, 4 October 1982, D. H. Stansbery & K. E. Wright.

Description

Glochidium lachrimiform, asymmetric, length 342 to 343 μm ($343 \pm 0.71 \mu\text{m}$, $n = 2$), height 345 to 350 μm ($348 \pm 3.54 \mu\text{m}$, $n = 2$). Posterior margin gently curved, anterior margin subrotund. Maximum inflation of anterior margin at about 50% from dorsal to ventral, broadly rounded ventral terminus occurring about 35% from posterior to anterior. Exterior valve surface finely malleated and pitted, except at valve margin. Pits uniformly distributed in the malleated surface, exterior valve surface with sculpturing intermediate between beaded and loose-looped (Fig. 8F). Hinge straight, 160 to 166 μm ($163 \pm 3.06 \mu\text{m}$, $n = 3$) long. Central ligament 74 to 77 μm ($76 \pm 2.12 \mu\text{m}$, $n = 2$) in length, centered about 46% from posterior border of hinge. Posterior ligament 37 to 38 μm ($38 \pm 0.71 \mu\text{m}$, $n = 2$) long; anterior ligament 48 to 52 μm ($50 \pm 2.36 \mu\text{m}$, $n = 2$) long. Hook styliform, broadly triangular, gradually tapered to blunt point (Fig. 8C). Microstylets lanceolate, gradually increasing in size toward center, arranged in four rows near proximal border of hook. Micropoints numerous, covering lateral surfaces of hook. Unsculptured distal hook margin, narrow.

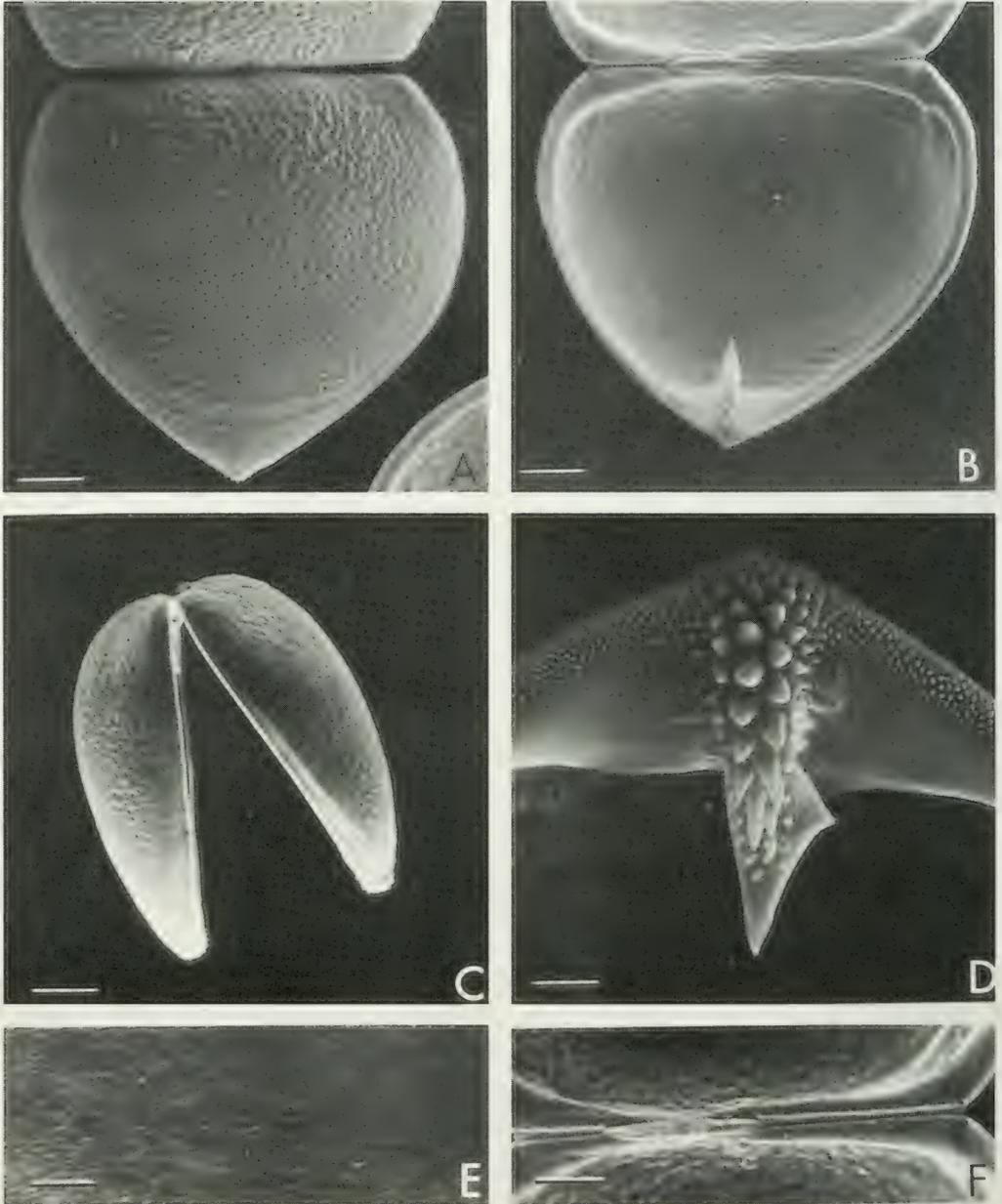


FIG. 7. Glochidium of *Anodonta kennerlyi*; A. exterior valve, OSUM 52882.2, bar length = 50 μ m; B. interior valve, OSUM 52882.2, bar length = 50 μ m; C. lateral view, OSUM 52882.3, bar length = 55 μ m; D. styliform hook, OSUM 52882.2, bar length = 15 μ m; E. exterior valve sculpture, OSUM 52882.3, bar length = 1 μ m; F. hinge, OSUM 52882.3, bar length = 35 μ m.

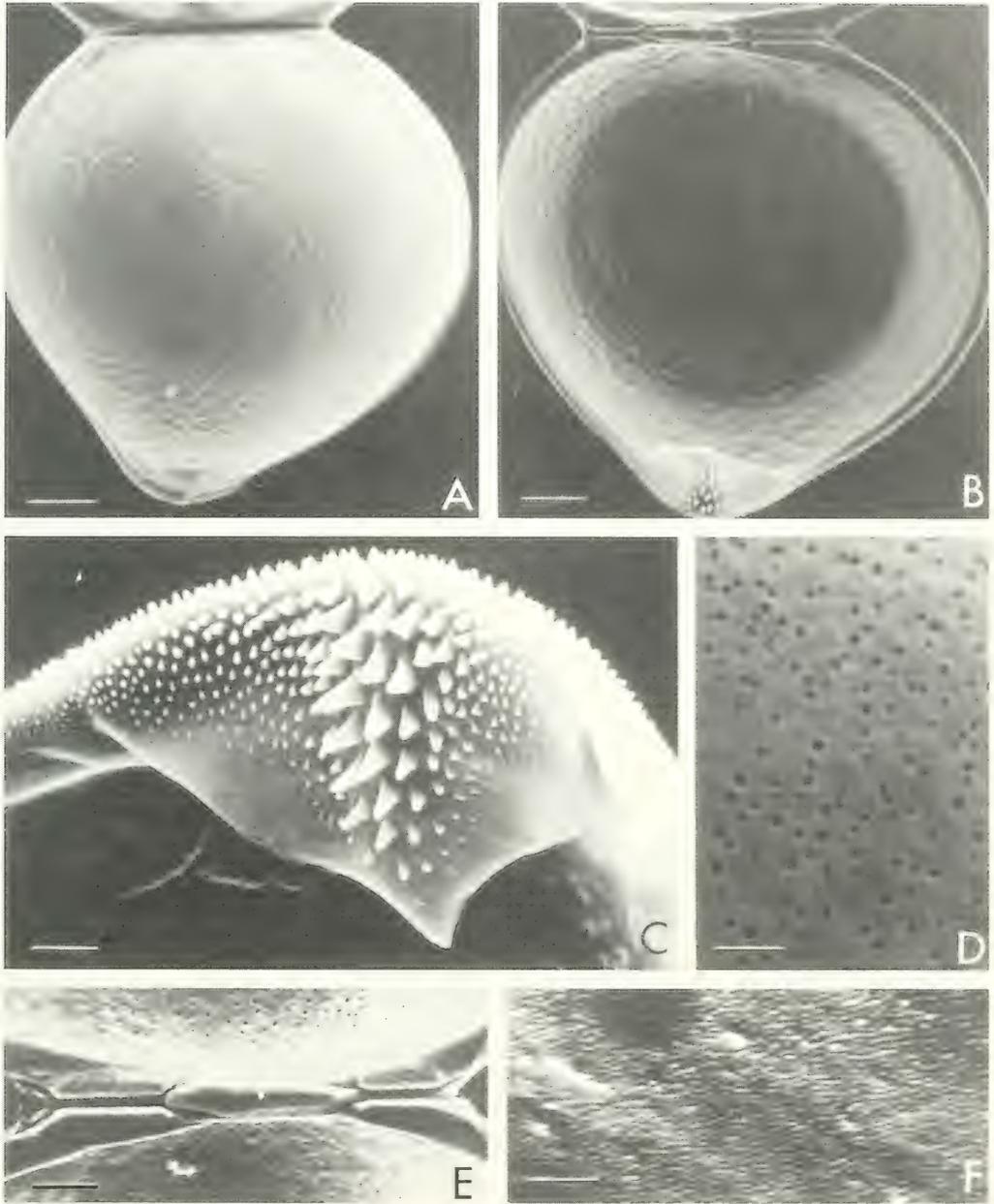


FIG. 8. Glochidium of *Anodonta implicata*. OSUM 52463.7; A. exterior valve, bar length = 50 μ m; B. interior valve, bar length = 45 μ m; C. styliform hook, bar length = 10 μ m; D. valve pitting, bar length = 15 μ m; E. hinge, bar length = 25 μ m; F. exterior valve sculpture, bar length = 2 μ m.

Remarks

Johnson (1946) describes this glochidium as, "typical of the genus *Anodonta*," even though his figure shows the short hinge line and greatly inflated anterior margin, both characters far from typical for this genus. Rand & Wiles (1982) have also failed to recognize that this species can be distinguished from all other glochidia simply by the tear-drop outline of the valve. This glochidium is further distinguished by its hook structure and exterior valve sculpture. The glochidium of this species is figured by Johnson (1946: pl. 16, fig. 3), Wiles (1975: figs. 1, 2, as *A. implicata* and *A. cataracta*), and Rand & Wiles (1982: figs. 5–8). Rand & Wiles gave length and height measurements of $345 \mu\text{m} \times 345 \mu\text{m}$.

Anodonta suborbiculata Say, 1831
(Fig. 9A–F)

Material Examined

OSUM 13634 – Black River at U.S. Rt. 67 bridge W of Hendrickson, Butler Co., Missouri, 14 October 1964, C. B. Stein.

Description

Glochidium subtriangular, length 323 to 328 μm ($325 \pm 2.08 \mu\text{m}$, $n = 5$), height 320 to 328 μm ($323 \pm 3.46 \mu\text{m}$, $n = 5$). Dorsal margin straight, 231 to 237 μm ($232 \pm 2.41 \mu\text{m}$, $n = 5$) long. Posterior margin gently curved; anterior margin, broadly curved. Maximum anterior inflation at about 50% from dorsal to ventral. Ventral terminus, narrowly pointed, located about 42% from posterior to anterior. Exterior surface malleated, pitted (Fig. 9E), except along the edge of valve (Fig. 9C); fine structure of exterior surface consisting of fine non-overlapping lines referred to here as vermiculate sculpturing (Fig. 9F). Central ligament 98 to 102 μm ($100 \pm 1.71 \mu\text{m}$, $n = 4$) in length, centered about 45% from posterior to anterior (Fig. 9B). Posterior ligament 52 to 56 μm ($54 \pm 1.83 \mu\text{m}$, $n = 4$) long; anterior ligament, 74 to 81 μm ($78 \pm 2.99 \mu\text{m}$, $n = 4$) long. Hook styliform, with about 40 lanceolate microstylets and many micropoints. Unsculptured distal margin of hook narrow.

Remarks

This glochidium can be distinguished by its exterior valve sculpturing and hook. It resem-

bles *A. cygnea* in shape but it has no other characters to tie it to that species. Surber (1915) stated that in, "general outline [the glochidium of] *suborbiculata* closely resembles *Anodonta grandis* but may be distinguished by its smaller size". He gave measurements of $325 \mu\text{m} \times 320 \mu\text{m}$ for length and height. However, the glochidia of these species are only superficially similar, and a close relationship between this species and any other member of the genus is not supported by glochidial characters. The glochidium of *A. suborbiculata* is figured by Surber (1915: pl. 1, fig. 1) and Utterback (1915–1916: fig. 7).

Pyganodon grandis grandis (Say, 1829)
(Fig. 10A–F)

Material Examined

P. g. grandis: OSUM 38467.10 – Miami River, R.Mi. 82.4, at I-75 bridge at Dayton, just above mouth of Mad River, Harrison Twp., Montgomery Co., Ohio, 6 February 1976, D. H. Stansbery. MAH 668-Olentangy River below Fifth Ave. bridge near Ohio State University main campus, Columbus, Franklin Co., Ohio, 30 September 1984, K. Wright & K. Gallant. INHS 2247-Kankakee River at Kankakee, below hydroelectric plant, Kankakee Co., Illinois, 11 October 1985, J. M. Kasprowicz. *P. g. corpulenta*: OSUM 47890-Stonelick Creek at Stonelick Reservoir, 1.1 mi. SW of Edenton, 2.8 mi. N of Newtonville, Wayne Twp., Clermont Co., Ohio, 1 October 1978, D. H. Stansbery & K. G. Borrer. OSUM 53653-Ohio River bank, R.Mi. 442.8–443.0, 0.3–0.4 mi. NW of Moscow, 2.2–2.4 mi. S of Point Pleasant, 6.9 mi. SE of New Richmond, Clermont Co., Ohio, 22 October 1984, K. E. Wright et al.

Description

Glochidium subtriangular, asymmetric, length 350 to 365 μm ($356 \pm 5.56 \mu\text{m}$, $n = 7$), height 350 to 360 μm ($355 \pm 3.10 \mu\text{m}$, $n = 7$). Posterior margin gently curved throughout its length. Anterior margin broadly curved to its point of maximum inflation at about 70% from dorsal to ventral. Ventral terminus broadly rounded, located about 40% from posterior to anterior. Valve surface malleated and pitted, except along its margin, where dorsally converging longitudinal ridges are found (Fig. 10C). Adductor muscle scar not evident. Coarse loose-looped exterior valve sculpture covering surface of valve (Fig. 10F). Hinge

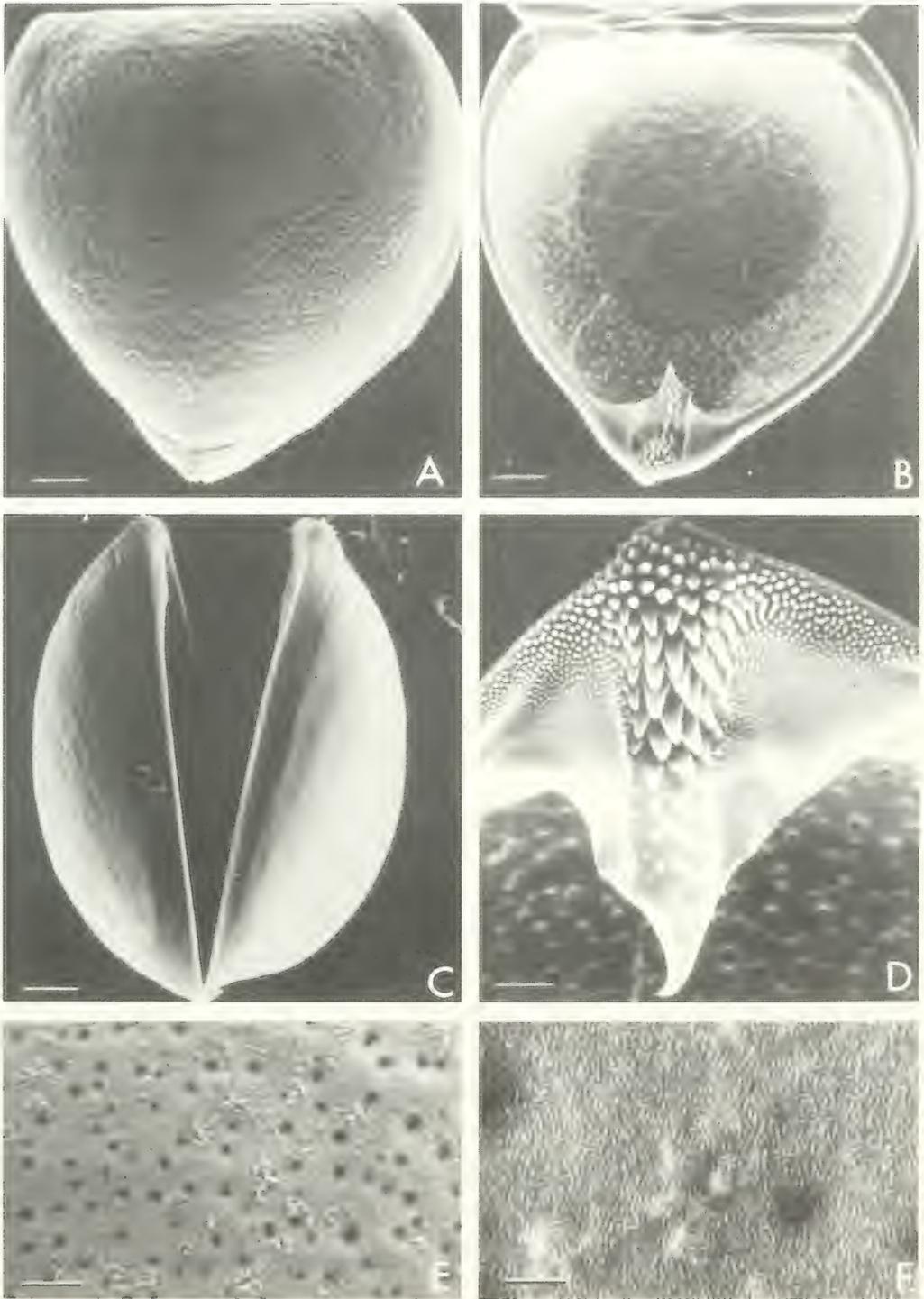


FIG. 9. Glochidium of *Anodonta suborbiculata*. OSUM 13634; A. exterior valve, bar length = 40 μ m; B. interior valve, bar length = 40 μ m; C. lateral view, bar length = 40 μ m; D. styliform hook, bar length = 10 μ m; E. valve pitting, bar length = 10 μ m; F. exterior valve sculpture, bar length = 2 μ m.

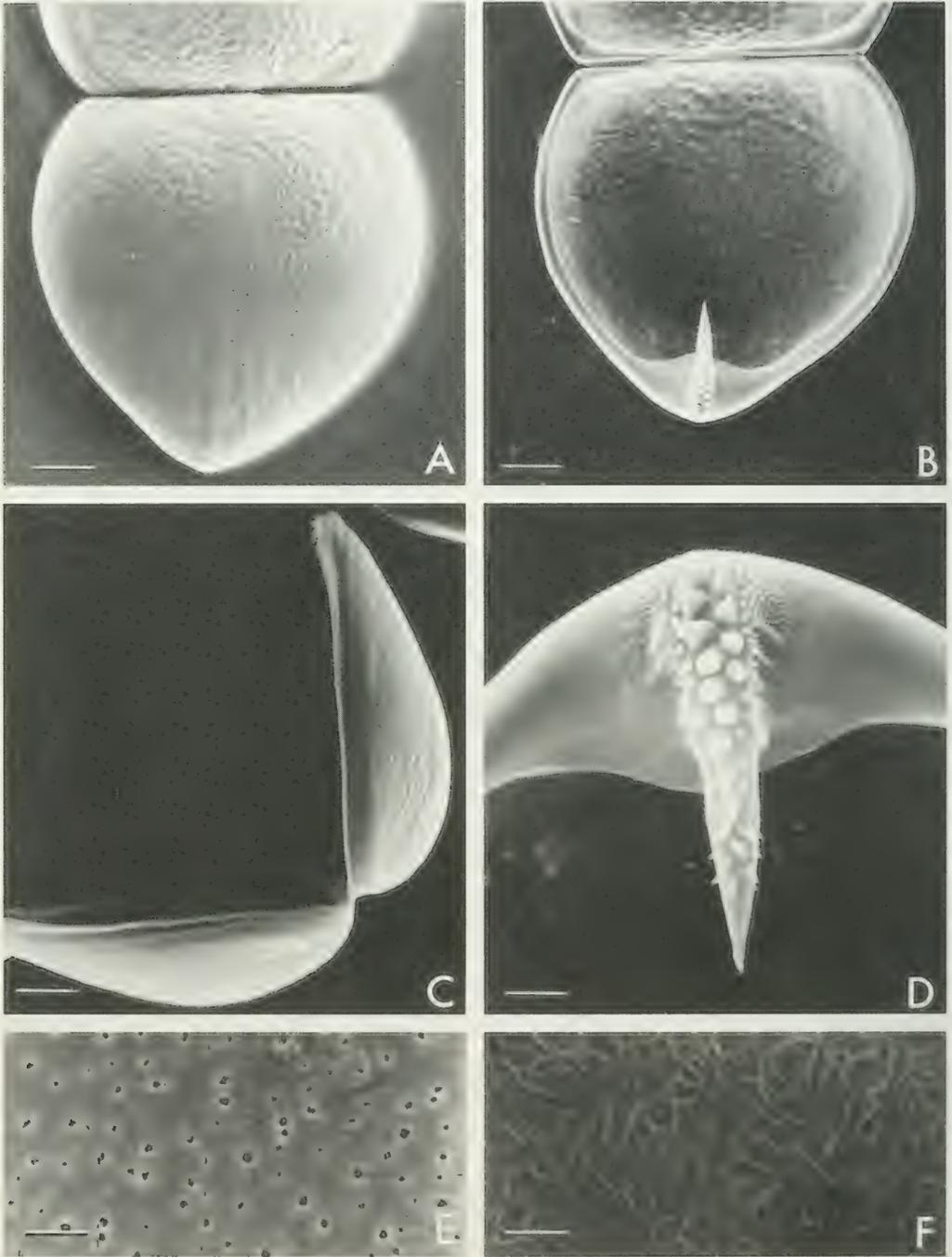


FIG. 10. Glochidium of *Pyganodon g. grandis*, OSUM 38467.10; A. exterior valve, bar length = 55 μ m; B. interior valve, bar length = 55 μ m; C. lateral view, bar length = 55 μ m; D. styli-form hook, bar length = 15 μ m; E. interior valve pitting, bar length = 15 μ m; F. exterior valve sculpture, bar length = 1 μ m.

straight, 250 to 259 μm ($253 \pm 3.20 \mu\text{m}$, $n = 7$) in length. Central ligament 90 to 94 μm ($92 \pm 2.08 \mu\text{m}$, $n = 3$) long, centered between 41–42% from posterior margin. Posterior ligament 54 to 62 μm ($57 \pm 4.04 \mu\text{m}$, $n = 3$) long; anterior ligament 104 to 111 μm ($107 \pm 3.61 \mu\text{m}$, $n = 3$) in length. Hook styliform, with about 20 microstylets. Microstylets arranged in four proximal rows, reduced to a single row distally (Fig. 10B, D). Five to six sharply pointed microstylets forming a cluster near tip of hook. Micropoints few in number, found only along ventral rim of valve and along borders of microstylets. Unsculptured distal hook margin very wide.

The glochidium of *P. g. corpulenta* is nearly identical to that of *P. g. grandis*, with a length of 343 to 350 μm ($348 \pm 5.13 \mu\text{m}$, $n = 3$), a height of 363 to 368 μm ($366 \pm 3.54 \mu\text{m}$, $n = 3$), and a hinge length of 260 to 275 μm ($266 \pm 7.94 \mu\text{m}$, $n = 3$). The posterior, central and anterior ligaments of a single specimen were 70 μm , 93 μm and 100 μm in length, respectively. Surber figured this glochidium (1912: fig. 4; 1913: fig. 1) and gave length and height measurements of 350 μm .

Remarks

Ortmann (1912) gave 360 $\mu\text{m} \times 370 \mu\text{m}$ for length and height of the glochidium of *P. g. grandis*, and Tucker (1928) reported the following ranges: length, 350–398 μm ; height, 343–390 μm . However, Surber gave measurements of 410 $\mu\text{m} \times 420 \mu\text{m}$. This extremely large range in size led Tucker (1928) to suggest that this was the result of examining the glochidia of a far ranging, variable species. Her material, Ortmann's and mine came from small streams, whereas Surber's material probably came from the Mississippi River and probably represents *P. g. gigantia*. The glochidium of *P. g. grandis* was figured by Lea (1858: pl. 5, figs. 32, 33, 34, as *A. lewisii*, *A. ovata* and *A. decora* = *P. g. grandis*, *fide* Ortmann, 1919) and Surber (1912: pl. 3, fig. 45). Lea's figures are slightly different from each other, and none show the correct outline. Surber's figure demonstrates the asymmetrical valves of this glochidium.

Pyganodon cataracta cataracta (Say, 1817)
(Fig. 11A–E)

Material Examined

P. c. cataracta; OSUM 52462.27, 52462.35
– Great Herring Pond, S Shore by the Herring

River, 0.8 mi. N of Bournedale [1.6 mi. WNW of Sagamore], Barnstable Co., Massachusetts, 4 October 1984, D. H. Stansbery & K. Wright. *P. c. marginata* – OSUM 38962.6 “Black Moshannon Lake” [near or part of Black Moshannon Creek?], Snowshoe Twp., Centre Co., Pennsylvania, September 1975, D. S. Gussman.

Description

Glochidium subtriangular, asymmetric, length 374 to 380 μm ($376 \pm 2.61 \mu\text{m}$, $n = 5$), height 351 to 370 μm ($363 \pm 7.40 \mu\text{m}$, $n = 5$). Dorsal margin straight; posterior margin gently, evenly curved; anterior margin broadly curved to its maximum inflation at about 70% from dorsal to ventral. Ventral terminus broadly pointed, about 43% from posterior to anterior. Malleations and pits uniformly distributed on the valve, except along valve margin. Coarse loose-looped sculpture covering exterior surface of valve (Fig. 11D). Hinge straight, 277 to 291 μm ($284 \pm 5.92 \mu\text{m}$, $n = 5$) long. Central ligament 98 to 102 μm ($100 \pm 2.83 \mu\text{m}$, $n = 2$) in length, centered about 42% from posterior to anterior. Posterior ligament 71 to 75 μm ($73 \pm 2.83 \mu\text{m}$, $n = 2$) in length; anterior ligament, 115 to 116 μm ($116 \pm 0.71 \mu\text{m}$, $n = 2$) long. Hook styliform, with about 20 lanceolate microstylets (Fig. 11E). Microstylets arranged in four rows near ventral terminus, reduced to a single row distally, clustered near tip of hook (four to six per hook). Micropoints limited to proximal half of hook, except along lateral margins of microstylets, where they form a single row, leaving wide unsculptured distal hook margin.

The glochidium of *P. c. marginata* had a length of 358 to 360 μm ($359 \pm 1.41 \mu\text{m}$, $n = 2$), a height of 364 to 369 μm ($367 \pm 3.54 \mu\text{m}$, $n = 2$), and a hinge length of 276 to 277 μm ($277 \pm 0.71 \mu\text{m}$, $n = 2$). The posterior, central and anterior ligaments of a single specimen were 64 μm , 110 μm and 102 μm in length, respectively.

Remarks

Ortmann (1912) believed this species was the eastern representative of the *P. grandis* complex. The similarities between the glochidia of these two species support the view of close relationship; however, the glochidium of *P. cataracta* can be distinguished from that of *P. grandis* by its longer central ligament. The glochidia of both *P. c. cataracta* and *P. c. marginata* have longer anterior ligaments

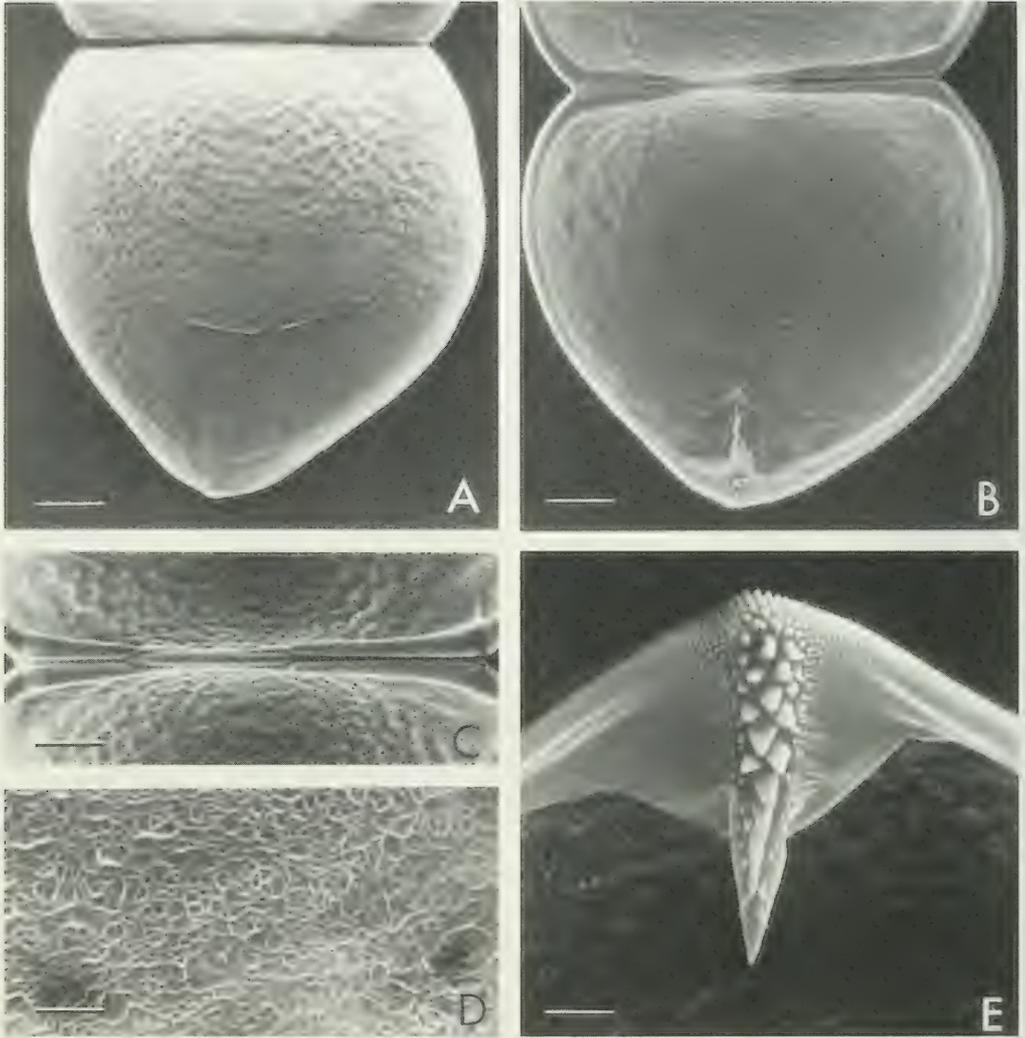


FIG. 11. Glochidium of *Pyganodon c. cataracta*: A. exterior valve, OSUM 52462.27, bar length = 55 μ m; B. interior valve, OSUM 52462.35, bar length = 55 μ m; C. hinge, OSUM 52462.27, bar length = 40 μ m; D. exterior valve sculpture, OSUM 52462.27, bar length = 2 μ m; E. styliform hook, OSUM 52462.27, bar length = 20 μ m.

than any species in the Anodontinae. Rand & Wiles (1982) gave length and height measurement of 382 μ m \times 383 μ m, whereas Ortmann (1912) reported 360 μ m \times 370 μ m (identical to his figures for *P. g. grandis*). This glochidium is figured by Lefevre & Curtis (1910: fig. C; 1912: fig. 1C), Calloway & Turner (1979: pl. 3, figs. 1, 3, 5, 7, 8), Wiles (1975: figs. 3, 7), and Rand & Wiles (1982: figs. 1–4).

Pyganodon doliaris (Lea, 1863)
(Fig. 12A–D)

Material Examined

OSUM 26405 – Potomac River along W shore Theodore Roosevelt Island, W. edge Washington D.C., just E of Arlington, Va., District of Columbia, 22 February 1970, K. Hef-felfinger Olson.

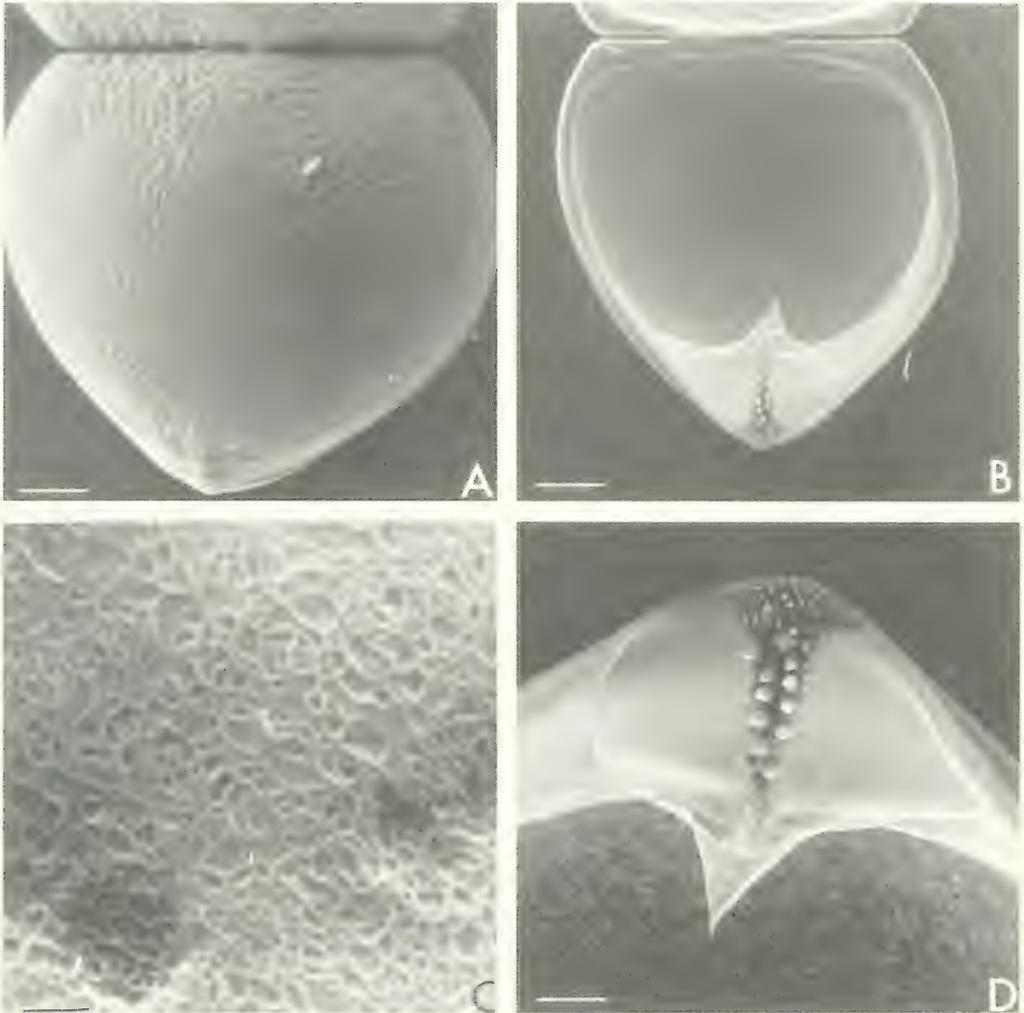


FIG. 12. Glochidium of *Pyganodon doliaris*, OSUM 26405: A. exterior valve, bar length = 55 μm ; B. interior valve, bar length = 55 μm ; C. exterior valve sculpture, bar length = 1 μm ; D. styliform hook, bar length = 20 μm .

Description

The glochidium of this species is essentially identical to that of *P. g. grandis* and *P. c. cataracta*. Its shape and hinge structure, exterior valve sculpture (Fig. 12C) and hook structure (Fig. 12D) place it firmly with these species. One glochidium measured 317 μm \times 317 μm \times 240 μm (length \times height \times hinge length) (Fig. 12B), while another measured 405 μm \times 368 μm \times 305 μm (Fig. 12A). Both

glochidia were removed from the marsupium of the same female.

Remarks

With such a limited amount of material and such great variability in size, it would not be appropriate to suggest limits. However, the larger measurements seem out of range for glochidia in this group, with the exception of *P. g. gigantia*.

Utterbackia imbecillis (Say, 1829)
(Fig. 13A–F)

Material Examined

OSUM 9436.2 – Lake Erie off East Sister Island, Ontario, Canada, 13 July 1960, D. Mount. OSUM 34463 – Clear Fork Mohican River at Clear Fork State Park Campground B, 5 mi. NNW of Jelloway, Hanover Twp., Ashland Co., Ohio, 13 July 1973, E. W. Tittsler. MAH 435 – Hellbranch Run at Bausch Rd. (Co. Rt. 11) bridge, 0.7 mi. S of Galloway, 10.0 mi. SW of Columbus, Prairie Twp., Franklin Co., Ohio, 15 June 1983, M. A. Hoggarth. UWZY 24971.1 – Baraboo River, T11N, R6E, Sec. 1, NW 1/4 of NE 1/4, 25 meters downstream of dam at Baraboo Sauk Co., Wisconsin, 12 October 1984, D. J. Heath.

Description

Glochidium subtriangular, length 291 to 313 μm ($304 \pm 6.41 \mu\text{m}$, $n = 12$), height 289 to 306 μm ($300 \pm 6.11 \mu\text{m}$, $n = 12$). Dorsal margin straight 240 to 256 μm ($246 \pm 5.97 \mu\text{m}$, $n = 13$) long. Anterior and posterior margins slightly and evenly curved. Anterior margin only slightly more produced than posterior margin, producing a slightly asymmetric valve outline. Surface of valve weakly malleated and pitted, except along smooth valve margin. Surface of valve covered with a uniform looped pattern, referred to here as tight-looped sculpture (Fig. 13F). Central ligament 85 to 93 μm ($91 \pm 3.50 \mu\text{m}$, $n = 4$) long, centered about 42% from posterior border of hinge. Anterior ligament 95 to 103 μm ($100 \pm 3.79 \mu\text{m}$, $n = 4$) in length; posterior ligament 60 to 63 μm ($61 \pm 1.50 \mu\text{m}$, $n = 4$) long. Hook styliform, arising as a broad triangular plate from ventral terminus. Microstylets lanceolate near distal margin of hook, multifaceted near proximal border (Fig. 13C, D), arranged in three to four rows near ventral terminus, reduced to a single row distally. A cluster of microstylets and micropoints near point of hook present in some material (Fig. 13C); however, most micropoints cover proximal half of lateral surface of hook, leaving wide unsculptured distal margin. Ventral terminus located about 45% from posterior to anterior.

Remarks

This glochidium can be distinguished from others in the subfamily by its exterior valve

sculpture, its rather small size, and its relatively long central ligament. It resembles *A. suborbiculata* in these last two characters, but differs in valve sculpture and hook structure. The outline of this glochidium is like that of *A. cygnea*, whereas the hook allies this species to *P. grandis*. Ortmann (1912) gave length and height measurements of 300 $\mu\text{m} \times$ 310 μm (for *A. imbecillis*) and 290 $\mu\text{m} \times$ 300 μm (for *A. henryana*, = *U. imbecillis*, *vide* Johnson, 1970). Surber (1912) gave 310 $\mu\text{m} \times$ 290 μm , and Tucker (1927) reported 290 $\mu\text{m} \times$ 300 μm . This glochidium was figured by Lea (1858: pl. 5, fig. 36), Ortmann (1911: pl. 89, fig. 13), Surber (1912: pl. 1, fig. 2), and Tucker (1927: pl. 10, figs. 1, 2).

Anodontoides ferussacianus (Lea, 1834)
(Fig. 14A–E)

Material Examined

OSUM 18275.2 – South Branch Phelps Creek at Rt. 322 bridge, 1.5 mi. E of Huntsburg, 5.5 mi. NE of Middlefield, Huntsburg Twp., Geauga Co., Ohio, 23 March 1966, R. E. Jezerinac. MAH 989.4 – Silver Creek, R.M. 4.6, at St. Rt. 15 bridge, 1.5 mi. N of Pioneer, 8.4 mi. NNE of Montpelier, T9S, R2W, Sec. 8/9, Madison Twp., Williams Co., Ohio, 2 October 1986, M. A. Hoggarth.

Description

Glochidium subtriangular, only slightly asymmetric, length 319 to 326 μm ($323 \pm 3.20 \mu\text{m}$, $n = 7$), height 320 to 327 μm ($324 \pm 2.64 \mu\text{m}$, $n = 7$). Hinge 231 to 238 μm ($234 \pm 2.93 \mu\text{m}$, $n = 7$) in length. Dorsal margin straight. Posterior and anterior margins about equally curved to a bluntly rounded ventral terminus. Exterior surface malleated and pitted, except along lateral margins and within a circular dorsomedial area or umbo (Fig. 14A, C). Exterior surface of valve covered by loose-looped sculpture (Fig. 14E). Central ligament 88 to 92 μm ($91 \pm 2.31 \mu\text{m}$, $n = 3$) long, centered about 42% from posterior to anterior. Anterior ligament 83 to 89 μm ($86 \pm 3.06 \mu\text{m}$, $n = 3$) in length; posterior ligament 56 to 62 μm ($59 \pm 3.06 \mu\text{m}$, $n = 3$) long. Hook styliform, with two rows of microstylets extending distally for at least three quarters of its length, located about 50% from posterior to anterior. A cluster of microstylets and micropoints occurring at distal end of hook. Micropoints located

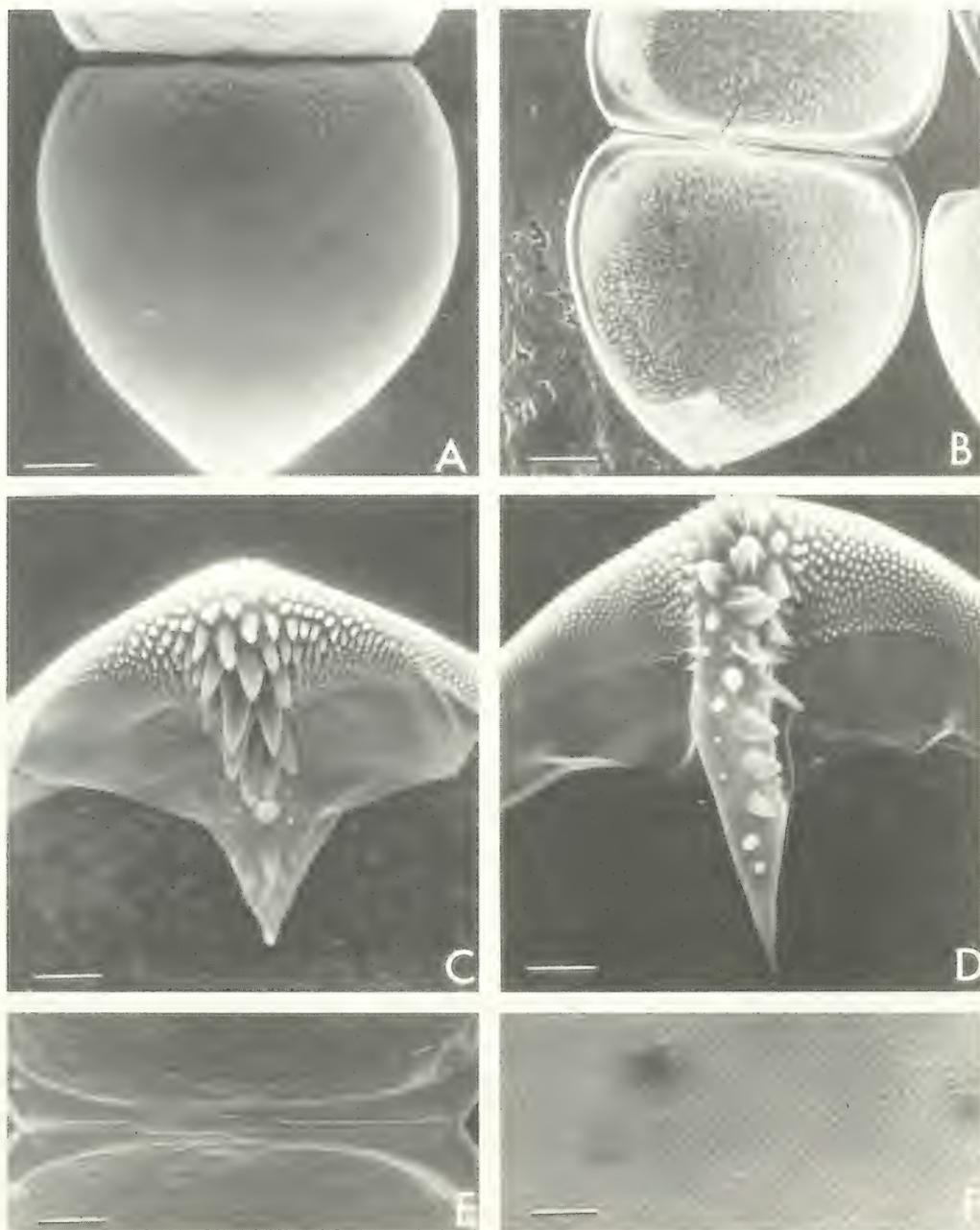


FIG. 13. Glochidium of *Utterbackia imbecillis*; A. exterior valve, UWZY 24971.1, bar length = 50 μ m; B. interior valve, OSUM 9436.2, bar length = 60 μ m; C. styliform hook, UWZY 24971.1, bar length = 15 μ m; D. styliform hook, MAH 435, bar length = 15 μ m; E. hinge, UWZY 24971.1, bar length = 2 μ m; F. exterior valve sculpture, UWZY 24971.1, bar length = 2 μ m.

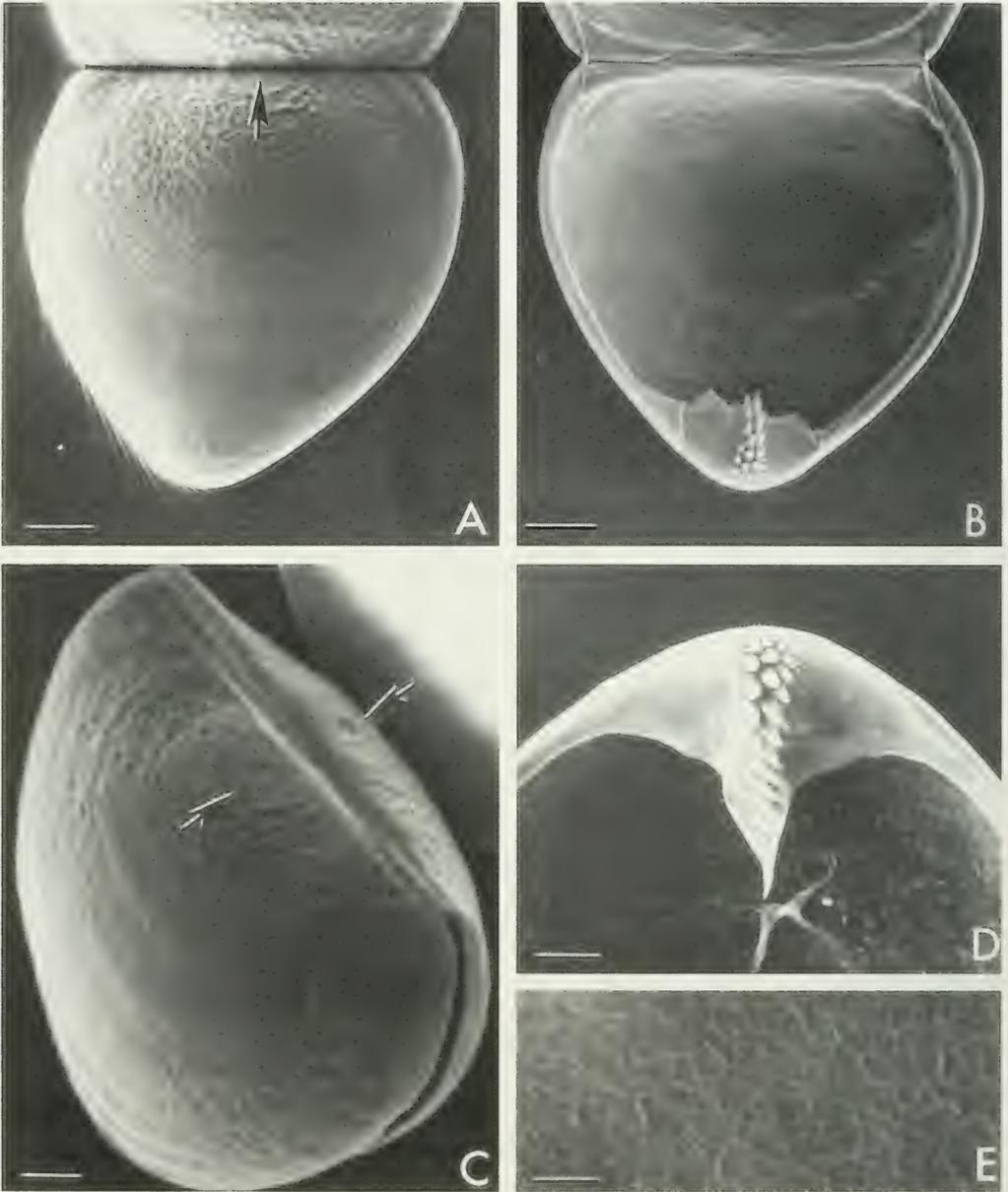


FIG. 14. Glochidium of *Anodontoides ferussacianus*; A. exterior valve, MAH 989.4, bar length = 50 μm ; B. interior valve, MAH 989.4, bar length = 50 μm ; C. hinge ligament, OSUM 18275.2, bar length = 30 μm ; D. styliform hook, MAH 989.4, bar length = 25 μm ; E. exterior valve sculpture, MAH 989.4, bar length = 1 μm .

on ventral rim of valve and on proximal surfaces of hook, leaving wide unsculptured distal hook margin.

Remarks

The glochidium of *Anodontoides ferussacianus* can be distinguished from above-described members of the Anodontinae by its double row of microstylets distally and its symmetry. The hook of this glochidium resembles that of *Strophitus*, but the outline of its valve is essentially like that of *Anodonta*. Ortmann (1912) and Surber (1912) gave the following size range for the glochidium of *A. ferussacianus*; 320–330 μm \times 320–330 μm . This glochidium has been figured by Lea (1858: pl. 5, fig. 35), Ortmann (1911: pl. 89, fig. 12), and Surber (1912: pl. 13, fig. 43). Lea figured this glochidium without hooks, but Ortmann (1919) suggests that Lea's specimens were immature.

Simpsonias ambigua (Say, 1825)
(Fig. 15A–F)

Material Examined

UWZY 22658, 22662, 22672 – Wisconsin River, T8N, R1E, Sec. 5, 9.5 mi. S. of Richland Center, Richland Co., Wisconsin, 16 July 1984, D. J. Heath. OSUM 55995 – Wisconsin River, T8N, R1E, Sec. 5, N side of river, 100 meters upstream of public boat landing, 9.5 mi. S. of Richland Center, Richland Co., Wisconsin, 20 April 1985, D. J. Heath.

Description

Glochidium ovate subtriangular, slightly asymmetric, length 250 to 258 μm ($255 \pm 3.39 \mu\text{m}$, $n = 6$), height 256 to 265 μm ($261 \pm 3.43 \mu\text{m}$, $n = 6$). Dorsal margin straight, 164 to 170 μm ($168 \pm 2.22 \mu\text{m}$, $n = 7$) in length. Posterior margin gently and evenly curved. Anterior margin slightly more rounded than posterior margin. Maximum anterior inflation occurring near 60% from dorsal to ventral. Ventral terminus bluntly rounded, about 45% from posterior to anterior. Exterior surface of valve finely malleated, uniformly pitted, except along smooth valve margin and umbo. Exterior surface sculpture beaded (Fig. 15E). Central ligament 54 to 63 μm ($58 \pm 4.51 \mu\text{m}$, $n = 3$) long, centered about 45% from poste-

rior to anterior. Posterior ligament 45 to 49 μm ($48 \pm 2.31 \mu\text{m}$, $n = 3$) long; anterior ligament 61 to 65 μm ($63 \pm 2.00 \mu\text{m}$, $n = 3$) in length. Hook styliform, arising as a broad biconcave triangular plate from ventral terminus, tapering to a sharp point. Micropoints grading more centrally located microstylets. Proximal microstylets multifaceted, sharply pointed, arranged four to five abreast. Distal microstylets and micropoints lanceolate. Micropoints located on ventral terminus and lateral surfaces of hook, leaving narrow unsculptured distal hook margin.

Remarks

The glochidium of *S. ambigua* has only rarely been available for description. Lea (1858) described the glochidium of *Margaritana hildrethiana* (= *S. ambigua*, *vide* Simpson, 1900) as subrotund, with a straight or slightly incurved dorsal line and lacking hooks. He suggested, however, that hooks might be present in more mature specimens. Howard (1915, 1951) described the glochidium as triangular, with well-developed hooks, and Clarke (1985) added that the glochidium is slightly asymmetric with malleated surfaces. Clarke's description, however, is of Howard's figure rather than of glochidia he examined.

This glochidium can be distinguished from most others by its shape, and from that of the other Anodontinae by its size, hook structure and exterior valve sculpturing. In regards to valve symmetry, size, hook structure, hinge structure and exterior valve sculpture, this glochidium is reminiscent of *Unio*. The glochidium of *S. ambigua* was figured by Lea (1858: pl. 5, fig. 31), and Howard (1951: figs. 4a, b). Lea's figure is much too round and lacks hooks. Howard's figures show the outline of this glochidium correctly, but the drawings lack microstylets on the styliform hook. Howard's figures are reprinted in Clarke (1985: fig. 20d).

Strophitus undulatus undulatus (Say, 1817)
(Fig. 16A–F)

Material Examined

S. u. undulatus – OSUM 49443 Mississippi River, R. Mi. 635.0, East Channel across from Prairie du Chien, N tip of island above U.S. Rt. 18 bridge, Crawford Co., Wisconsin, 21 March

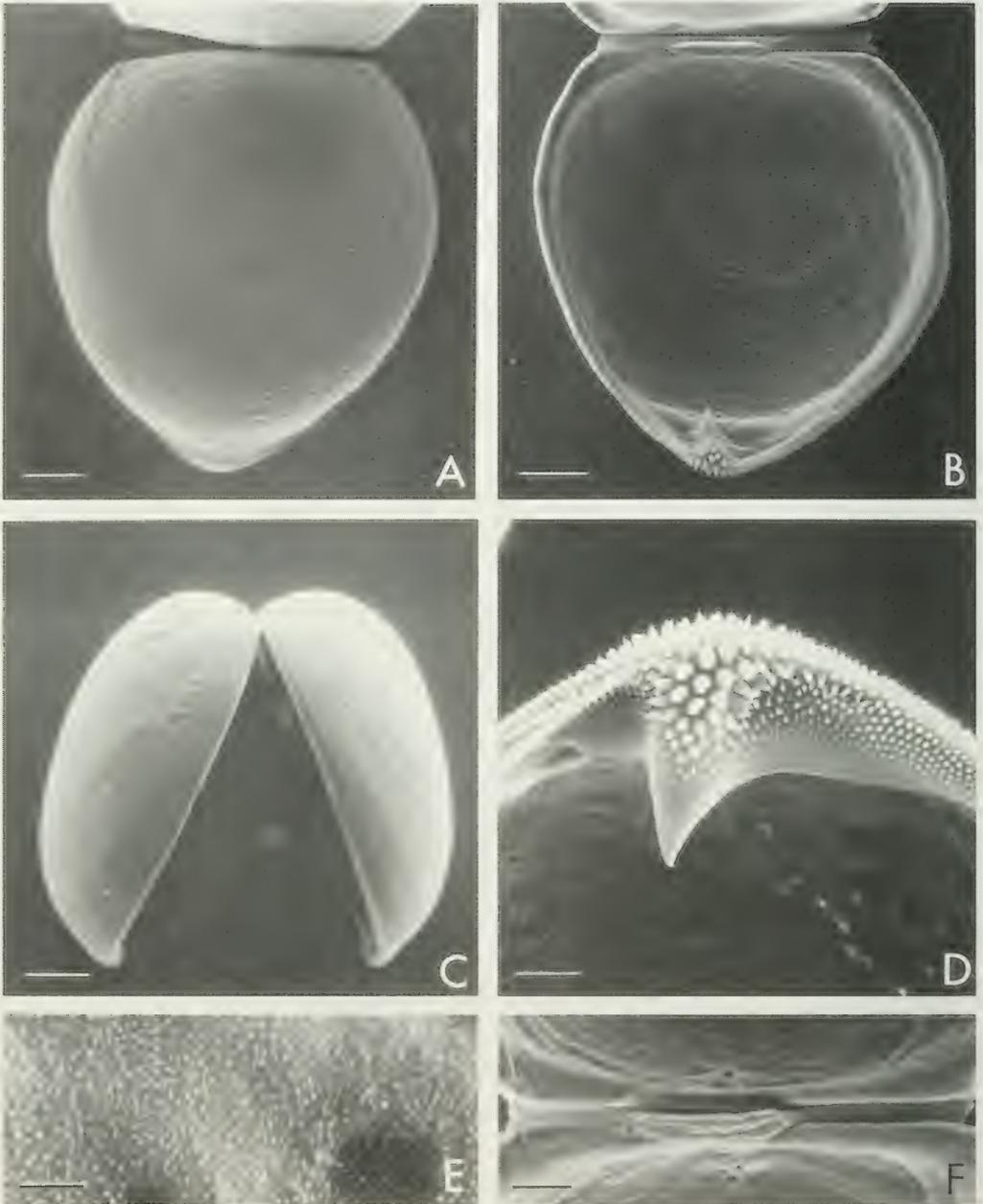


FIG. 15. Glochidium of *Simpsonaias ambigua*; A. exterior valve, UWZY 22658, bar length = 40 μ m; B. interior valve, UWZY 22662, bar length = 40 μ m; C. lateral view, UWZY 22662, bar length = 45 μ m; D. styliform hook, UWZY 22658, bar length = 10 μ m; E. exterior valve sculpture, OSUM 55995, bar length = 2 μ m; F. hinge, UWZY 22672, bar length = 20 μ m.

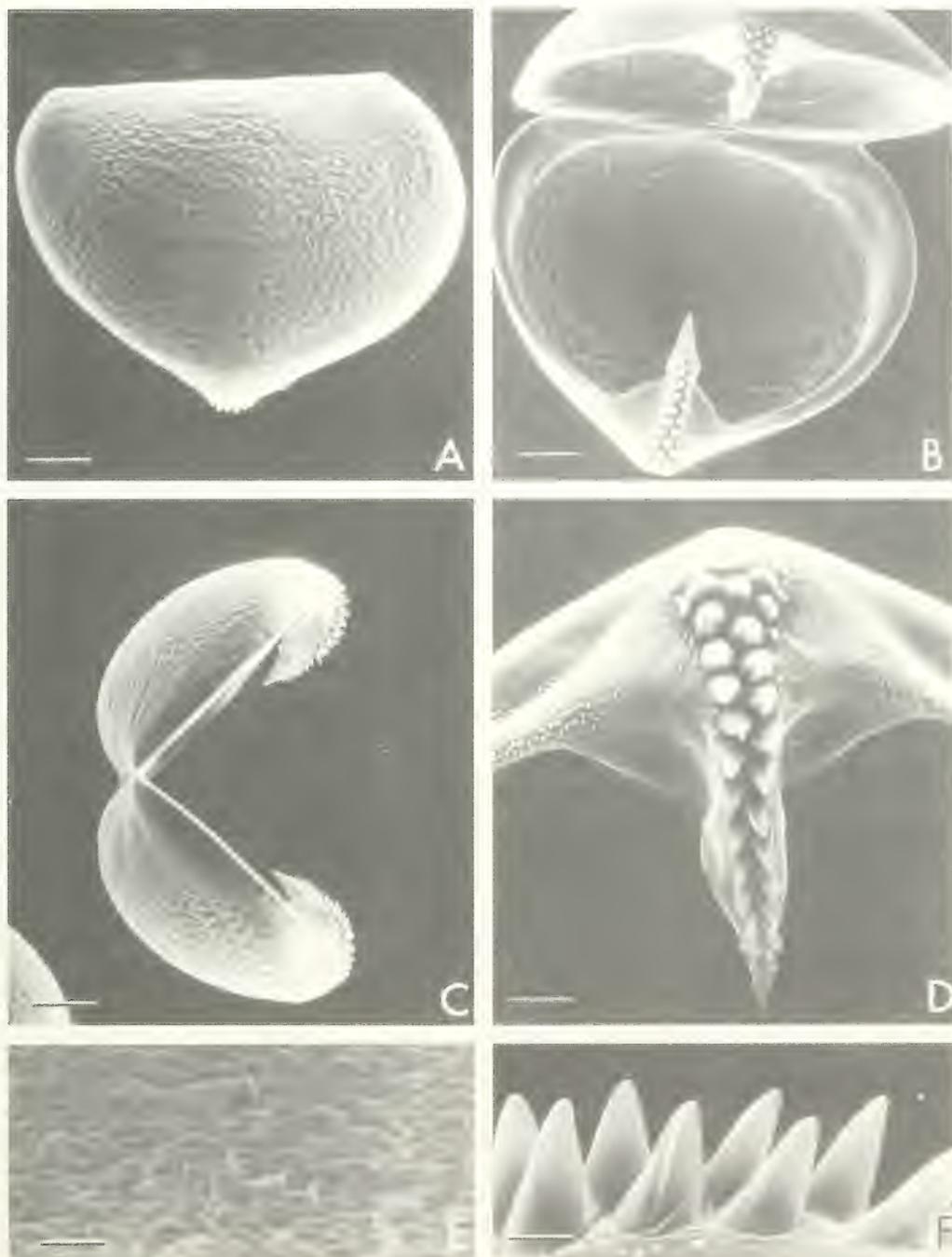


FIG. 16. Glochidium of *Strophitus u. undulatus*; A. exterior valve, OSUM 49443, bar length = 50 μm ; B. interior valve, OSUM 52458.4, bar length = 50 μm ; C. lateral view, OSUM 52458.4, bar length = 70 μm ; D. styli-form hook, OSUM 52458.4, bar length = 20 μm ; E. exterior valve sculpture, OSUM 49443, bar length = 1 μm ; F. microstylets, OSUM 52458.4, bar length = 10 μm .

1981, M. E. Havlik et al. OSUM 52458.4 – Ashuelot River 0.4 mi. S of Surrey Mountain Dam, 4.3 mi. NNW of Keene, Cheshire Co., New Hampshire, 22 August 1982, K. Wright & J. LeBlanc. MAH 792.1 – Fish Creek 0.4 mi. above its mouth at Co. Rt. 49 bridge, 1.1 mi. N of Edgerton, 10.4 mi. W of Bryan, St. Joseph Twp., Williams Co., Ohio, 29 October 1986, D. H. Stansbery et al. *S. u. tennesseensis* – OSUM 33381.2 Laurel Creek 0.4 mi. N of Bradford along Va. Rt. 91, 6.3 mi. NE of Saltville, Rich Valley District, Smyth Co., Virginia, 29 September 1971, D. H. Stansbery & W. J. Clench. OSUM 55449 – Clinch River, R.Mi. 270.6–270.9, 0.7–1.0 mi. SW of Cleveland, 1.5–1.8 mi. NE of Carbo, Russell Co., Virginia, 3 October 1985, G. T. Watters.

Description

Glochidium depressed pyriform, asymmetric, length 360 to 369 μm ($363 \pm 5.20 \mu\text{m}$, $n = 3$), height 289 to 299 μm ($295 \pm 5.29 \mu\text{m}$, $n = 3$). Dorsal margin straight, 271 to 281 μm ($278 \pm 3.78 \mu\text{m}$, $n = 6$) in length. Posterior margin broadly arcuate. Anterior margin almost round, meeting posterior margin at a slightly rounded and ventrally produced, nipple-like, ventral terminus. Ventral terminus located about 44% from posterior to anterior. Exterior valve surface malleated and pitted. Pits uniformly distributed throughout valve, except along valve margin and at umbo. Coarse loose-looped sculpture covering exterior surface of valves (Fig. 16E). Central ligament 87 to 103 μm ($95 \pm 6.55 \mu\text{m}$, $n = 4$) in length, located about 44% from posterior margin. Posterior ligament 70 to 78 μm ($74 \pm 3.30 \mu\text{m}$, $n = 4$) long; anterior ligament 101 to 110 μm ($107 \pm 4.08 \mu\text{m}$, $n = 4$) long. Hook styliform, covered with about 30 microstylets and numerous micropoints. Proximal microstylets bluntly pointed. Distal microstylets, multifaceted, sharply pointed (Fig. 16F). Two rows of microstylets extend distally, and a few microstylets and micropoints forming a cluster near sharply pointed terminus of hook. Unsculptured distal hook margin narrow.

The glochidium of *S. u. tennesseensis* is identical to that described above. One glochidium had the following dimensions: length, 346 μm ; height, 298 μm ; hinge length, 268 μm ; central ligament length, 94 μm ; posterior ligament length, 78 μm ; anterior ligament length, 96 μm .

Remarks

The glochidium of *Strophitus* is depressed-pyriform and possesses a styliform hook at the ventral terminus of each valve. Lea (1858) described the glochidia of *Strophitus edentula* (= *S. undulatus*, *vide* Johnson, 1970) and *S. undulata* as subtriangular, with a long straight dorsal margin, inflated side margins (Lea erroneously viewed all glochidia as symmetrical about the dorsoventral axis), and a large hook with four rows of "granules" proximally, reduced to two rows distally. Other than shape, Lea's description is surprisingly accurate.

The glochidium of *S. u. undulatus* is unlike any so far described. Its shape, hook structure and coarse looped sculpture will distinguish it from other species examined. Glochidia of *S. u. undulatus* have been figured by Lea (1858: pl. 5, fig. 37, as *S. edentula*, pl. 5, fig. 38, as *S. undulatus*), and Surber (1912: pl. 1, fig. 3, as *S. edentula*). Surber gave length and height measurements of 350 $\mu\text{m} \times 285 \mu\text{m}$, and Ortman (1912) gave 360 $\mu\text{m} \times 300 \mu\text{m}$.

Strophitus subvexus (Conrad, 1834)
(Fig. 17A–F)

Material Examined

OSUM 36240 – Buttahatchie River about 0.5 mi. above its mouth, 12 mi. NNW of Columbus, T16S, R19W, Lowndes Co., Mississippi, 4 October 1974, R. Grace et al.

Description

Glochidium depressed pyriform, asymmetric, length 348 to 359 μm ($354 \pm 5.51 \mu\text{m}$, $n = 3$), height 288 to 292 μm ($290 \pm 2.08 \mu\text{m}$, $n = 3$). Dorsal margin straight, 271 to 277 μm ($274 \pm 2.50 \mu\text{m}$, $n = 4$) in length. Posterior margin broadly curved; anterior margin rounded. Lateral margins meeting at a narrowly rounded, nipple-like ventral terminus located about 50% from posterior to anterior. Surface of valve coarsely malleated, uniformly pitted, except along valve margin and at umbo (Fig. 17B, C). Coarse loose-looped sculpture covering the exterior surface of valves (Fig. 17E). Central ligament about 84 μm in length, centered about 40% from posterior to anterior. Posterior ligament about 70 μm long; anterior ligament about 123 μm long. Hook styliform, with proximal microstylets bluntly pointed dis-

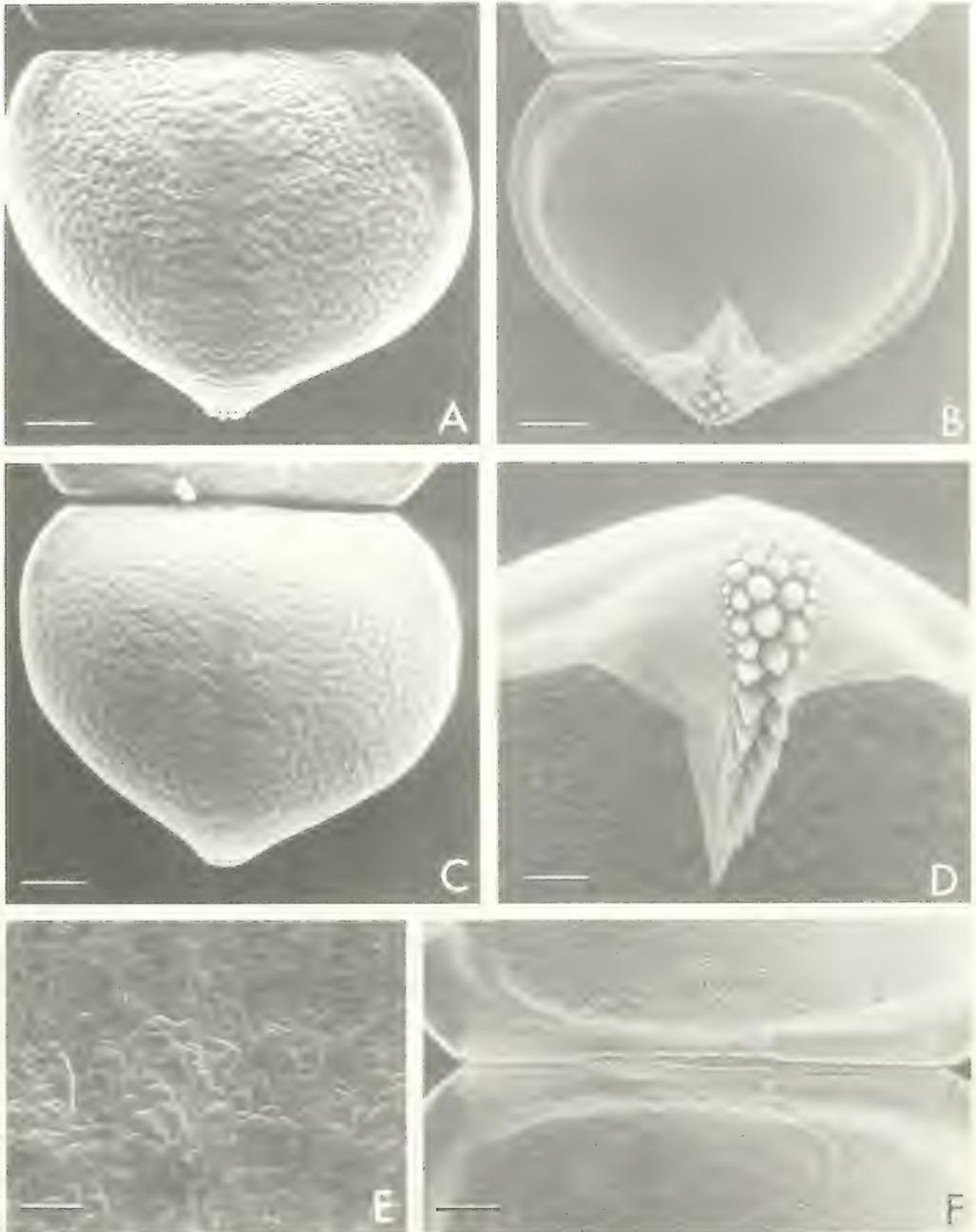


FIG. 17. Glochidium of *Strophitus subvexus*. OSUM 36240; A. exterior valve, bar length = 50 μ m; B. interior valve, bar length = 50 μ m; C. exterior valve, bar length = 50 μ m; D. styliform hook, bar length = 20 μ m; E. exterior valve sculpture, bar length = 2 μ m; F. hinge, bar length = 35 μ m.

tal microstylets lanceolate, multifaceted and sharply pointed. Microstylets arranged in a double row distally, forming a cluster near point of hook. Micropoints located on ventral valve rim and on lateral surfaces of hook, leaving narrow unsculptured distal hook margin.

Remarks

The glochidium of *S. subvexus* resembles that of *S. u. undulatus*, except the former has a roundish outline and a more centrally positioned ventral terminus. The near symmetrical outline of this glochidium is overemphasized slightly by Lea (1874: Pl. 21, fig. 15, as *Margaritana spillmanii*, = *S. subvexus*, fide Johnson, 1967), but his figure correctly shows the broadly curving margins.

Alasmidonta viridis (Rafinesque, 1820)
(Fig. 18A–F)

Material Examined

OSUM 47518 – Horse Lick Creek, 0.3 mi. below mouth of Raccoon Creek at Dango, 7.6 mi. SW of Mckee, Jackson Co., Kentucky, 28 February 1980, S. Call et al.

Description

Glochidium depressed pyriform, only slightly asymmetric, length of 300 to 319 μm ($307 \pm 7.59 \mu\text{m}$, $n = 7$), height 245 to 260 μm ($251 \pm 4.35 \mu\text{m}$, $n = 7$). Dorsal margin straight, 245 to 258 μm ($250 \pm 3.93 \mu\text{m}$, $n = 7$) long. Posterior margin gently and evenly curved; anterior margin only slightly more produced than posterior margin. Ventral terminus roundly pedicellate, about 45% from posterior to anterior. Exterior surface of valve finely malleated and pitted, except along valve margin (Fig. 18C) and at umbo. Pit density reduced in area of adductor muscle scar (Fig. 18F), and loose-looped sculpture covering the exterior surface of valve (Fig. 18E). Central ligament 73 to 81 μm ($76 \pm 4.16 \mu\text{m}$, $n = 3$) in length, centered about 42% from posterior to anterior. Posterior ligament 65 to 71 μm ($68 \pm 4.04 \mu\text{m}$, $n = 3$) long; anterior ligament 100 to 109 μm ($105 \pm 4.93 \mu\text{m}$, $n = 3$) in length. Hook styliform, broadly connected to ventral terminus and covered with about 30 microstylets and numerous micropoints. Microstylets lanceolate, arranged in three proximal rows

and reduced to two distal rows. Six to eight microstylets and micropoints forming a cluster near point of hook. Micropoints extend over nipple-like ventral terminus beyond edge of ventral margin of valve, leaving wide unsculptured distal hook margin.

Remarks

Clarke (1981a) described many of the glochidia of the Alasmidontini using SEM, including this species. Our descriptions of this glochidium are almost identical, except for the location of the hook. Actually, we agree here as well, but Clarke confused the anterior-posterior orientation of the glochidium. He stated, "apices are located slightly anterior of center (about 47%)." Actually the apices (= ventral termini) are located about that distance from the posterior margin.

Ortmann (1912) reported length and height measurements of 300 $\mu\text{m} \times$ 250 μm , and Surber (1912) gave 300 $\mu\text{m} \times$ 255 μm for this glochidium. Clarke (1981a) gave the following ranges: length 286–292 μm , height 232–235 μm , and hinge length 205 μm . The figure for hinge length is probably a typographical error and should read 250 μm (approximate hinge length taken from his fig. 6b, c).

This glochidium is distinguished from that of *Strophitus* by its finer exterior valve sculpture and its broadly connected hook. This glochidium is figured by Lea (1858: pl. 5, fig. 30, as *Margaritana deltoidea*, = *A. viridis*, fide Simpson, 1900), Surber (1912: pl. 1, fig. 1, as *Alasmidonta calceola*, = *A. viridis*, fide Clarke, 1981a), Ortmann (1912: pl. 19, fig. 4, as *Alasmidonta minor*, = *A. viridis*, fide Clarke, 1981a), Clarke (1981a: fig. 6), and Zale & Neves (1982: fig. 1, as *A. minor*).

Alasmidonta heterodon (Lea, 1829)
(Fig. 19A–F)

Material Examined

OSUM 25106.2 – Canoe River at old Newland St. bridge, 2.45 mi. NNE of Norton, Bristol Co., Massachusetts, 2 June 1969, H. D. Athearn.

Description

Glochidium depressed-pyriform, symmetric when immature (Fig. 18A), becoming asymmetric with the development of hook (Fig.

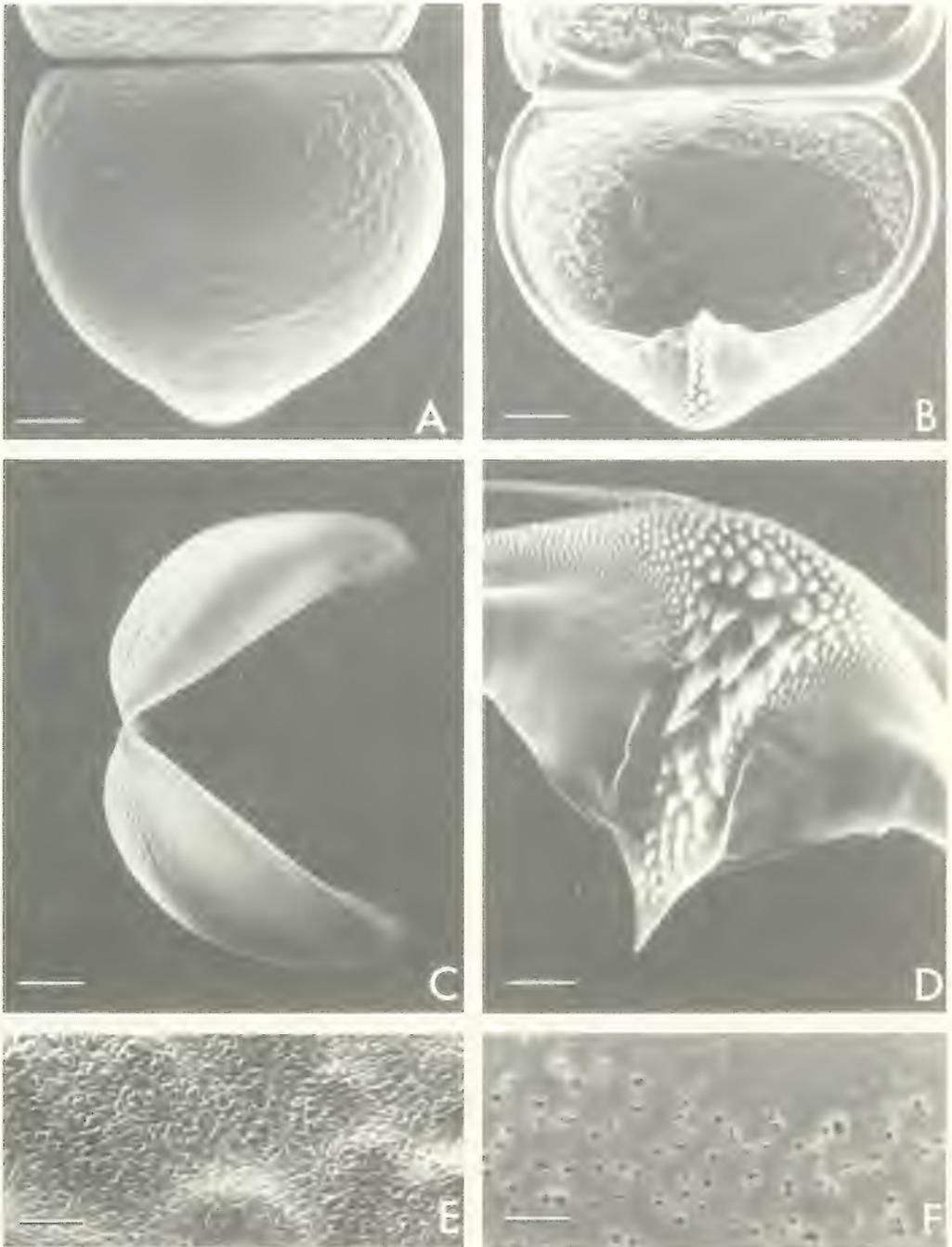


FIG. 18. Glochidium of *Alasmidonta viridis*, OSUM 47518; A. exterior valve, bar length = 45 μ m; B. interior valve, bar length = 45 μ m; C. lateral view, bar length = 45 μ m; D. styliform hook, bar length = 15 μ m; E. exterior valve sculpture, bar length = 2 μ m; F. interior valve pitting, bar length = 10 μ m.

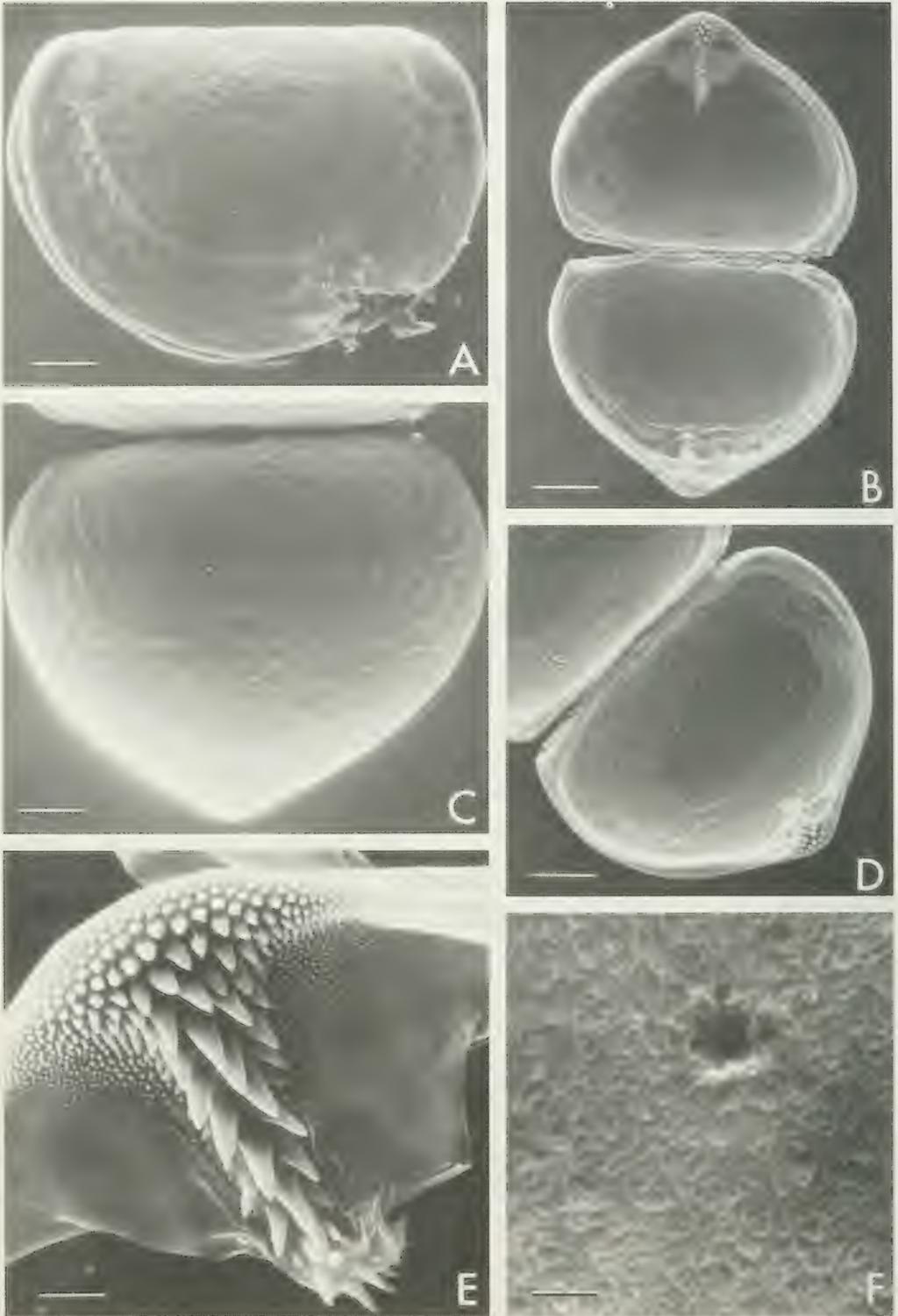


FIG. 19. Glochidium of *Alasmidonta heterodon*, OSUM 25106.2; A. exterior valve of immature glochidium, bar length = 45 μm ; B. interior valve, bar length = 70 μm ; C. exterior valve, bar length = 45 μm ; D. interior valve, bar length = 60 μm ; E. styliform hook, bar length = 10 μm ; F. exterior valve sculpture, bar length = 1 μm .

18B, C, D), length 330 to 338 μm ($334 \pm 3.65 \mu\text{m}$, $n = 4$), height 258 to 268 μm ($265 \pm 4.86 \mu\text{m}$, $n = 4$). Dorsal margin straight, 253 to 266 μm ($261 \pm 5.94 \mu\text{m}$, $n = 4$) in length. Posterior margin slightly and evenly curved; anterior margin more broadly curved, especially near dorsal margin. Point of maximum lateral inflation at between 40–50% from dorsal to ventral. Ventral terminus curved, slightly outwardly produced. Exterior valve surface finely malleated and pitted, except along its margins and at umbo. Interior pitting not uniform; pit density reduced in adductor muscle scar. Loose-looped sculpture occurring on exterior surface of valves (Fig. 19F). Central ligament about 80 μm long, centered about 42% from posterior to anterior. Posterior ligament about 75 μm long; anterior ligament about 110 μm long. A styliform hook extending dorsally from ventral terminus of each valve. Hook covered with about 40 microstylets and many micropoints, located about 42% from posterior to anterior. Microstylets arranged in four to six proximal rows and reduced to two rows distally. Micropoints extending over edge of ventral terminus, along ventral margin of valve, and for a short distance on lateral surfaces of hook, leaving wide unsculptured distal hook margin.

Remarks

Clarke's (1981a) and my descriptions of this glochidium are, not surprisingly, very similar. We both examined glochidia removed the same adult female. He reported length, height, and hinge length measurements of 325 $\mu\text{m} \times 255 \mu\text{m} \times 267 \mu\text{m}$, respectively. All of these are within the ranges found during this study. This glochidium is very similar to that of *A. viridis*, but differs from that species in being more inflated toward the anterior end and in having a less broadly attached styliform hook. This glochidium has been figured by Clarke (1981a: fig. 9).

Alasmidonta undulata (Say, 1817)
(Fig. 20A–F)

Material Examined

OSUM 52434.4 – Merrimack River just below Sewalls Fall's Dam, 2.7 mi. SE of Penacook, 3.7 mi. NNW of Concord, Concord Twp., Merrimack Co., New Hampshire, 31 October 1982, K. E. Wright.

Description

Glochidium pyriform, much higher than long, asymmetric, length 343 to 358 μm ($353 \pm 5.89 \mu\text{m}$, $n = 5$), height 365 to 378 μm ($371 \pm 5.13 \mu\text{m}$, $n = 5$). Dorsal margin straight, 247 to 260 μm ($254 \pm 5.59 \mu\text{m}$, $n = 5$) in length. Posterior margin outwardly curved dorsally, becoming straight to slightly incurved before ventral terminus. Anterior margin broadly curved. Ventral terminus slightly incurved. Maximum anterior and posterior inflation at about 30% from dorsal to ventral, with ventral terminus rounded, located about 40% from posterior to anterior. Valve surface coarsely malleated, uniformly pitted, except at the umbo and along valve margin, where pits and malleations are absent. Exterior surface covered with sparse rosette sculpture, separated by areas of beaded sculpture (Fig. 20E). Larval thread present (Fig. 20D). Central ligament 89 to 98 μm ($92 \pm 5.19 \mu\text{m}$, $n = 3$) in length, centered about 40% from posterior to anterior. Posterior ligament 53 to 55 μm ($54 \pm 1.15 \mu\text{m}$, $n = 3$) in length; anterior ligament 103 to 105 μm ($104 \pm 1.00 \mu\text{m}$, $n = 3$) long. Hook styliform, covered with numerous (about 120) microstylets and micropoints. Microstylets pyramidal, multifaceted, arranged in about six proximal rows, reduced to about four rows distally. Micropoints present along ventral valve margin, at lateral margins of microstylets and for a short distance down lateral surfaces of hook, leaving wide unsculptured distal hook margin.

Remarks

The glochidium of *A. undulata* can be distinguished by its large size, pyriform valve shape, complex hook and unique exterior valve sculpture. Ortmann (1912) described this glochidium as, "moderately large, higher than long, with strong hooks. Length 0.34 mm; height 0.36 mm." Clarke (1981a) reported length and height measurements of 310 $\mu\text{m} \times 370 \mu\text{m}$ and figured the glochidium (his fig. 13). This glochidium also is figured by Wiles (1975: fig. 6, as *Anodonta cataracta*).

Alasmidonta marginata Say, 1818
(Fig. 21A–I)

Material Examined

MAH 277.1 – Big Darby Creek at and above McLean Mill Rd. bridge, 0.2 mi. SW of Fox, 4.7

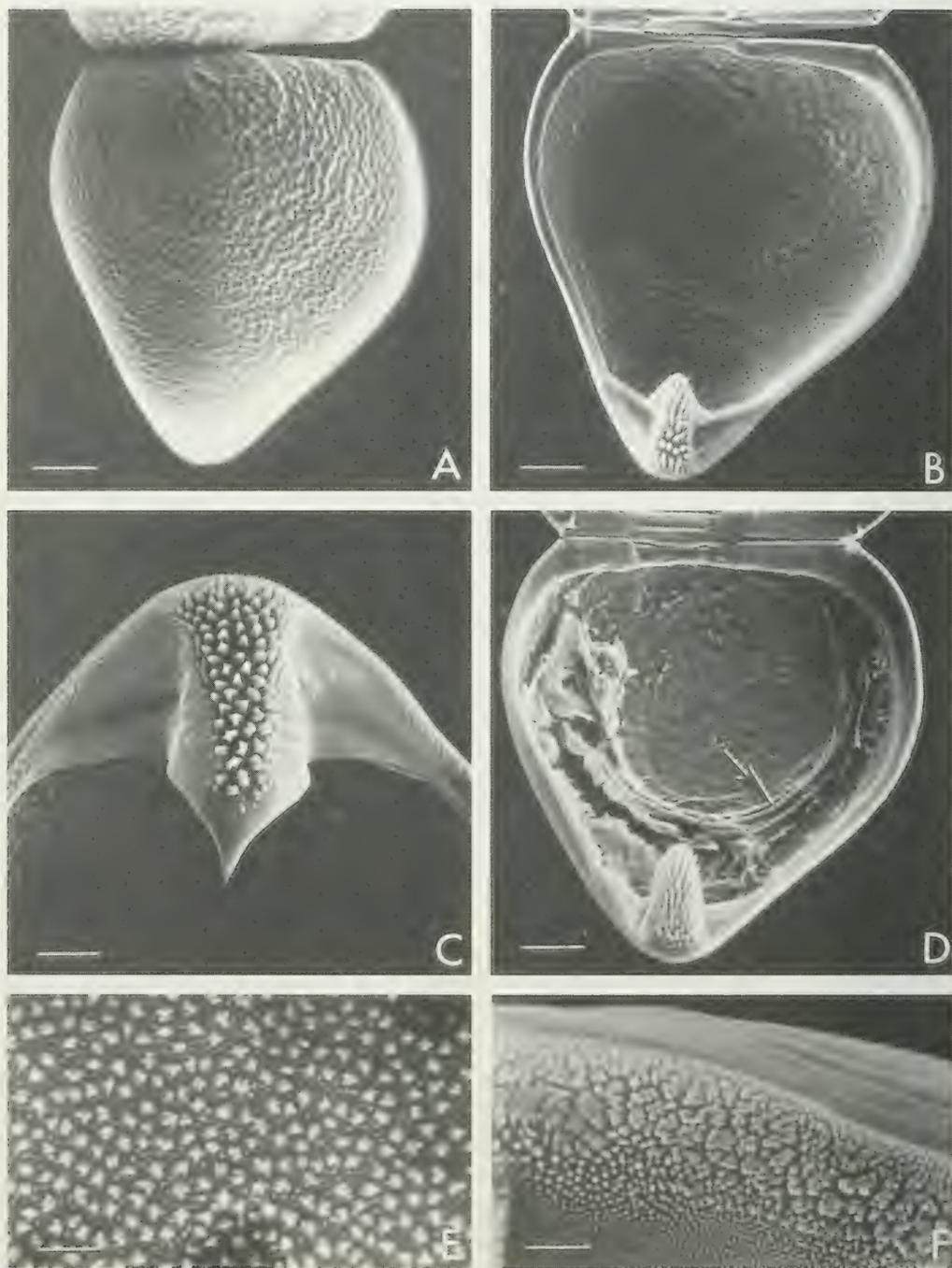


FIG. 20. Glochidium of *Alasmidonta undulata*, OSUM 52434.4; A. exterior valve, bar length = 60 μm ; B. interior valve, bar length = 55 μm ; C. styliform hook, bar length = 20 μm ; D. interior valve, bar length = 50 μm ; E. exterior valve sculpture, bar length = 1 μm ; F. micropoints, bar length = 10 μm .

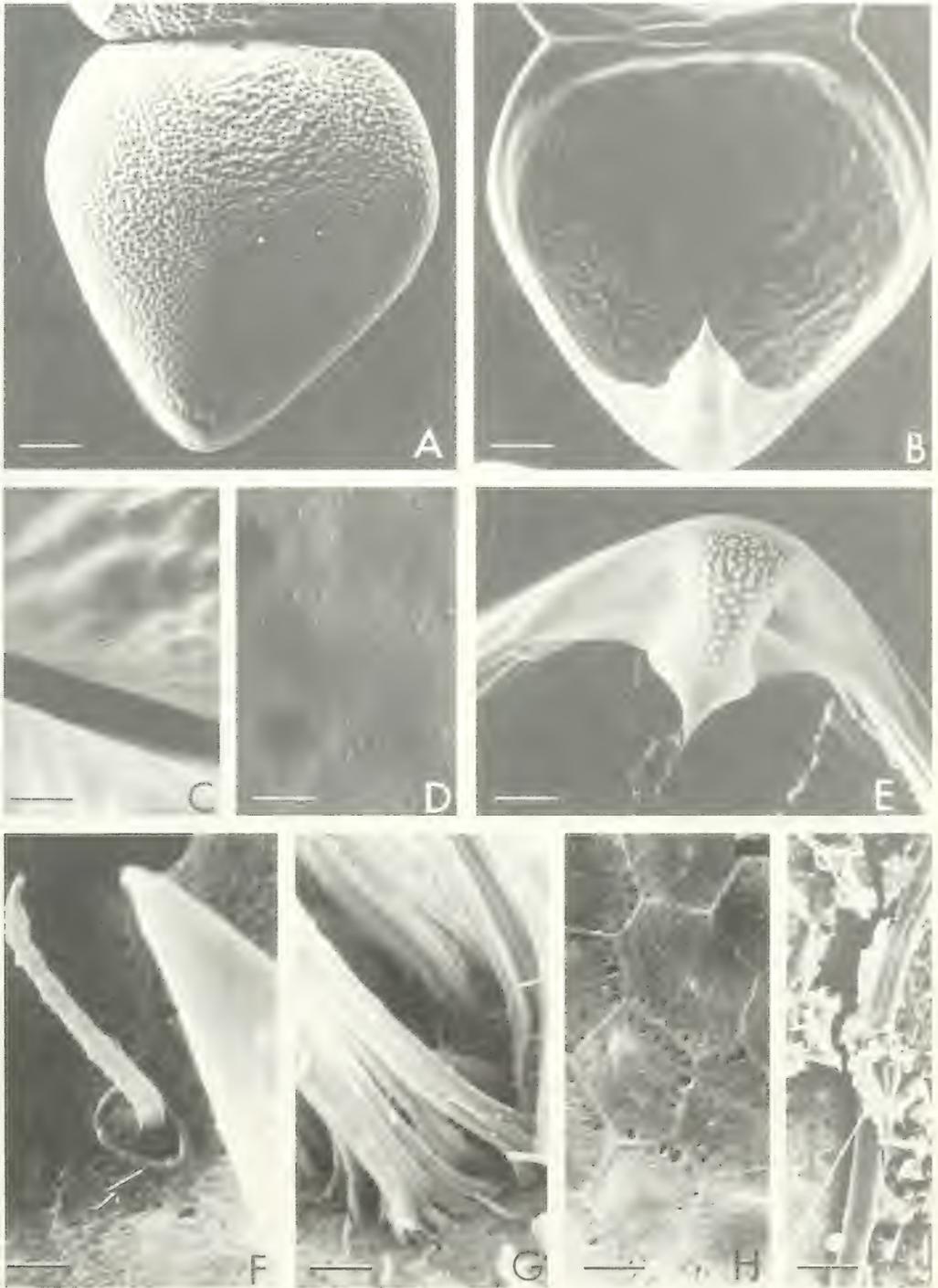


FIG. 21. Glochidium of *Alasmidonta marginata*: A. exterior valve, MAH 724.1, bar length = 55 μm ; B. interior valve, MAH 724.1, bar length = 45 μm ; C. exterior valve sculpture, MAH 724.1, bar length = 1 μm ; D. exterior valve sculpture, MAH 277.1, bar length = 1 μm ; E. styliform hook, MAH 277.1, bar length = 25 μm ; F. hair cell, MAH 724.1, bar length = 5 μm ; G. adductor muscle, MAH 724.1, bar length = 5 μm ; H. mantle cells, MAH 724.1, bar length = 10 μm ; I. larval thread, MAH 724.1, bar length = 10 μm .

mi. NW of Circleville, Jackson Twp., Pickaway Co., Ohio, 1 October 1982, M. A. Hoggarth et al.. MAH 724.1 – Fish Creek above and below Edon Rd. bridge, 1.9 mi. NW of Edgerton, St. Joseph Twp., Williams Co., Ohio, 2 October 1985, M. A. Hoggarth & D. Rice.

Description

Glochidium pyriform, higher than long, asymmetric, length 335 to 341 μm ($339 \pm 2.87 \mu\text{m}$, $n = 4$), height 360 to 372 μm ($365 \pm 5.74 \mu\text{m}$, $n = 4$). Dorsal margin straight, 230 to 235 μm ($233 \pm 0.96 \mu\text{m}$, $n = 4$) in length. Posterior margin broadly curved (especially dorsally), with maximum inflation at about 30% from dorsal to ventral. Anterior margin more broadly curved than posterior margin. Maximum anterior inflation at about 40% from dorsal margin. Lateral margins slightly incurved ventrally, producing a narrowly rounded ventral terminus. Ventral terminus about 40% from posterior to anterior. Exterior surface of valve coarsely malleated and densely pitted, except at valve margins and at umbo. Exterior surface covered with beaded sculpture (Fig. 21D), although fine loose-looped sculpture present near umbo of one glochidium (Fig. 21C). Larval thread present (Fig. 21I). Central ligament 67 to 73 μm ($70 \pm 3.54 \mu\text{m}$, $n = 2$) in length, centered about 40% from posterior to anterior. Posterior ligament 59 to 61 μm ($60 \pm 1.41 \mu\text{m}$, $n = 2$) long; anterior ligament 100 to 107 μm ($104 \pm 4.95 \mu\text{m}$, $n = 2$) in length. Hook styliform, with many microstylets (about 120) and many micropoints. As in *A. undulata*, distal margin of hook parallel to ventral margin, except at its center, where it becomes strongly curved to a sharp distal point. Microstylets pyramidal, multifaceted, arranged in six to seven proximal rows, reduced to four rows distally. Micropoints occur along rim of ventral terminus, at lateral margins of microstylets and for a short distance down lateral surfaces of hook. Distal hook margin with wide unsculptured band.

Sensory hair cells (Fig. 21F) have been described using light microscopy (Lillie, 1895; Wood, 1974), SEM (Giusti et al., 1975; Rand & Wiles, 1982) and transmission electron microscopy (Zs.-Nagy & Labos, 1969). It is generally thought that during attachment to the host, the tissues of the host push down on the hair cells and that the response to this stimulus is prolonged muscle contraction. It appears that when the hairs are bent, their movement stimulates a ring of tissue, possibly

composed of nerve tissue that encompass the base of the cell (at arrow).

The adductor muscle (Fig. 21G) is composed of long cells of contractile elements that attach to the crystalline matrix of the valves. The adductor muscle scar is often visible as a rough elliptical area or by its reduced number of pits. There is no evidence that the muscle cells actually insert within these pits. The larval mantle cells are five to seven sided (Fig. 21H) and do not appear to be pitted, as suggested by Rand & Wiles (1982). They suggested that the larval mantle was pitted to correspond to the pits in the valve and that this might facilitate gas exchange, nutrient uptake, or waste elimination. This was not observed, and therefore it is suggested that the pits are simply a result of the absence of stress traversing the body of the shell. Where stress is transferred from ventral margin to hinge (the lateral margins), pitting is absent and ridges (providing additional strength) occur. The pits may serve no function and may simply be a consequence of glochidial valve morphology (Hoggarth & Gaunt, 1988).

Remarks

This glochidium can be distinguished from that of *A. undulata* by differences in exterior valve sculpturing. Otherwise they are very similar. Lea (1858) described this glochidium as subtriangular, with a long, straight dorsal line and inflated side margins. He described the hook as terminating in an arrowhead point. Clarke (1981a) also described the point of the hook as arrowhead-like. The arrowhead effect is probably due to the collapse of the lateral surfaces of the hook and is therefore an artifact of drying. This glochidium has a wide size range: (length \times height) 330 $\mu\text{m} \times$ 360 μm (Ortmann, 1912); 350 $\mu\text{m} \times$ 380 μm (Surber, 1912); 300 $\mu\text{m} \times$ 350 μm (Utterback, 1915–1916); 341 $\mu\text{m} \times$ 346 μm (Clarke, 1981a) and is figured by Lea (1858:pl. 5, fig. 27), Surber (1912:pl. 3, fig. 42, as *A. truncata*, = *A. marginata*, *fide* Stansbery et al., 1985), and Clarke (1981a: fig. 20).

Pegias fabula (Lea, 1838)
(Fig. 22A–F)

Material Examined

OSUM 41308.3 – Little South Fork Cumberland River at Freedom Church Ford, 2.0 mi. ENE of Ritner, 14.3 mi. E of Monticello, Wayne

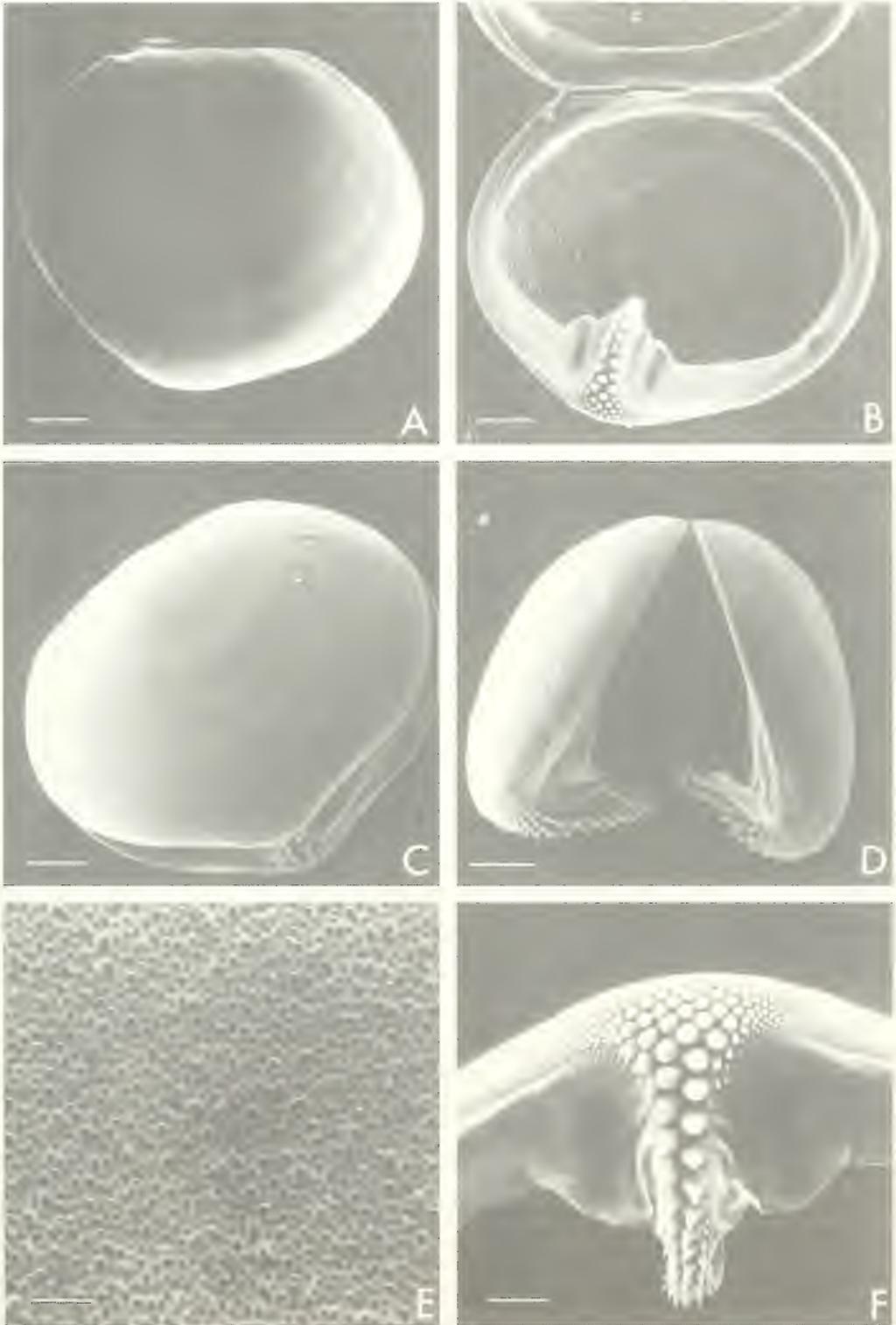


FIG. 22. Glochidium of *Pegias fabula*; A. exterior valve, OSUM 41308.3, bar length = 55 μ m; B. interior valve, OSUM 41309.1, bar length = 55 μ m; C. exterior valve, OSUM 41308.3, bar length = 55 μ m; D. lateral view, OSUM 41308.3, bar length = 55 μ m; E. exterior valve sculpture, OSUM 41308.3, bar length = 2 μ m; F. styli-form hook, OSUM 41309.1, bar length = 20 μ m.

Co., Kentucky, 22 October 1977, W. & L. Starnes. OSUM 41309.1 – Little South Fork Cumberland River at Freedom Church Ford, 2.0 mi. ENE of Ritner, 14.3 mi. E of Monticello, Wayne Co., Kentucky, 22 October 1977, A. Bogan.

Description

Glochidium oval quadrate to roundly trapezoidal, length 385 to 388 μm ($386 \pm 1.53 \mu\text{m}$, $n = 3$), height 319 to 325 μm ($322 \pm 3.06 \mu\text{m}$, $n = 3$). Dorsal margin straight, 200 to 210 μm ($205 \pm 5.03 \mu\text{m}$, $n = 3$) long. Posterior margin roundly arcuate. Anterior margin inflated dorsally, slightly incurved near ventral terminus. Ventral terminus slightly rounded, about 35% from posterior to anterior. Exterior surface pitted but not malleated, smooth in area of adductor muscle scar and at margin of valve. Adductor muscle scar very large (Fig. 22B-D). Exterior sculpturing tight-looped (Fig. 22E). Central ligament 68 to 73 μm ($71 \pm 2.89 \mu\text{m}$, $n = 3$) long, centered about 45% from posterior to anterior. Posterior ligament 56 to 63 μm ($60 \pm 3.51 \mu\text{m}$, $n = 3$) in length; anterior ligament 69 to 79 μm ($74 \pm 5.03 \mu\text{m}$, $n = 3$) long. Hook, styliform, sharply pointed, broadly connected to ventral valve margin. Hook with about 75 lanceolate microstylets arranged three abreast near proximal end of hook and in a double row distally. About 15 microstylets forming a cluster near distal end of hook. Micropoints limited to ventral rim of valve and along margins of microstylets, leaving very wide unsculptured distal hook margin.

Remarks

The glochidium of *P. fabula* cannot be confused with that of any other species. Its quadrate shape, broadly connected styliform hook, tight looped exterior valve sculpturing and extremely large adductor muscle scar distinguish it. Clarke (1981a) figured this glochidium (his fig. 3) and provided length and height measurements for two specimens: 354 $\mu\text{m} \times 309 \mu\text{m}$ and 380 $\mu\text{m} \times 310 \mu\text{m}$.

Arcidens confragosus (Say, 1829)
(Fig. 23A–G)

Material Examined

OSUM 52015 – Green River at Glenmore below lock 5 dam, 12 mi. N of Bowling Green,

Warren Co., Kentucky, 2 November 1977, D. H. Stansbery et al.

Description

Glochidium pyriform, about as long as high, asymmetric, length 352 to 363 μm ($359 \pm 5.32 \mu\text{m}$, $n = 4$), height 353 to 355 μm ($354 \pm 1.41 \mu\text{m}$, $n = 2$). Dorsal margin straight, 237 to 252 μm ($246 \pm 7.16 \mu\text{m}$, $n = 5$) in length. Posterior margin produced dorsally, incurved ventrally, with its maximum inflation between 30–40% from dorsal to ventral. Anterior margin broadly rounded dorsally, incurved just before ventral terminus, maximum inflation at about 50% from dorsal margin. Ventral terminus narrowly rounded, located about 40% from posterior to anterior. Exterior surface coarsely malleated and densely pitted, except at umbo and along valve margin. Dense rosette sculpturing covering exterior surface of valve (Fig. 23E). Central ligament 63 to 78 μm ($71 \pm 6.40 \mu\text{m}$, $n = 4$) long, centered about 42% from posterior to anterior. Posterior ligament 59 to 72 μm ($68 \pm 5.91 \mu\text{m}$, $n = 4$) long; anterior ligament 100 to 115 μm ($110 \pm 7.14 \mu\text{m}$, $n = 4$) long. Styliform hook extending from ventral terminus as a very strongly biconcave triangular plate with about 80 pyramidal microstylets arranged in about five rows (Fig. 23D, F). Number of rows of microstylets same from proximal to distal ends of hook. Micropoints occur on rim of valve, at ventral terminus and along microstylet border, leaving wide unsculptured distal hook margin.

Remarks

The glochidium of *A. confragosus* is pyriform and about as long as high. Anterior and posterior margins are greatly inflated dorsally and slightly incurved ventrally. The ventral terminus is narrowly rounded, and the number of rows of microstylets remain constant from proximal to distal ends of the styliform hook.

This glochidium is similar to that of some members of the genera *Alasmidonta* and *Las-migona* but can be distinguished by its equal height and length, its exterior valve sculpture, and the arrangement of microstylets on the hook. Length and height measurements are given by Surber (1912: pl. 1, fig. 5) 355 $\mu\text{m} \times 350 \mu\text{m}$, and Clarke (1981a: fig. 31) 359 $\mu\text{m} \times 360 \mu\text{m}$, both of whom also figure the glochidium.

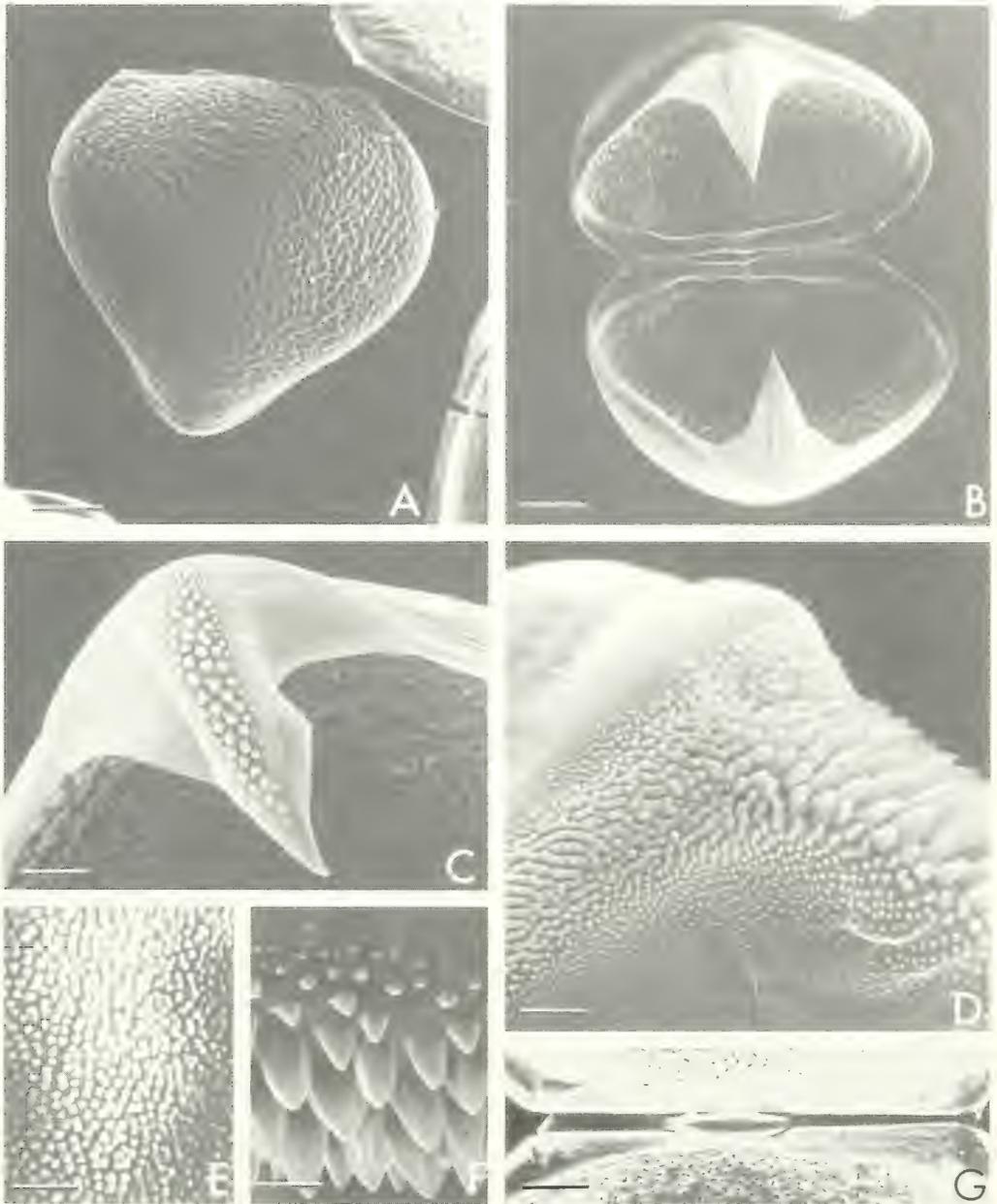


FIG. 23. Glochidium of *Arcidens confragosus*, OSUM 52015; A. exterior valve, bar length = 65 μ m; B. interior valve, bar length = 65 μ m; C. styliform hook, bar length = 25 μ m; D. styliform hook, bar length = 5 μ m; E. exterior valve sculpture, bar length = 1 μ m; F. microstylets, bar length = 5 μ m; G. hinge, bar length = 40 μ m.

Lasmigona compressa (Lea, 1829)
(Fig. 24A–F)

Material Examined

OSUM 23179.1 – Little Darby Creek above

Rosedale-Plain City Rd. bridge, 2.8 mi. E of Rosedale, Pike Twp., Madison Co., Ohio, 20 October 1969, C. B. Stein et al. MAH 702 – Big Darby Creek below access point within Battelle-Darby Metro Park, 0.6 mi. S of Georgesville, 3.5 mi. SW of Galloway, Pleas-

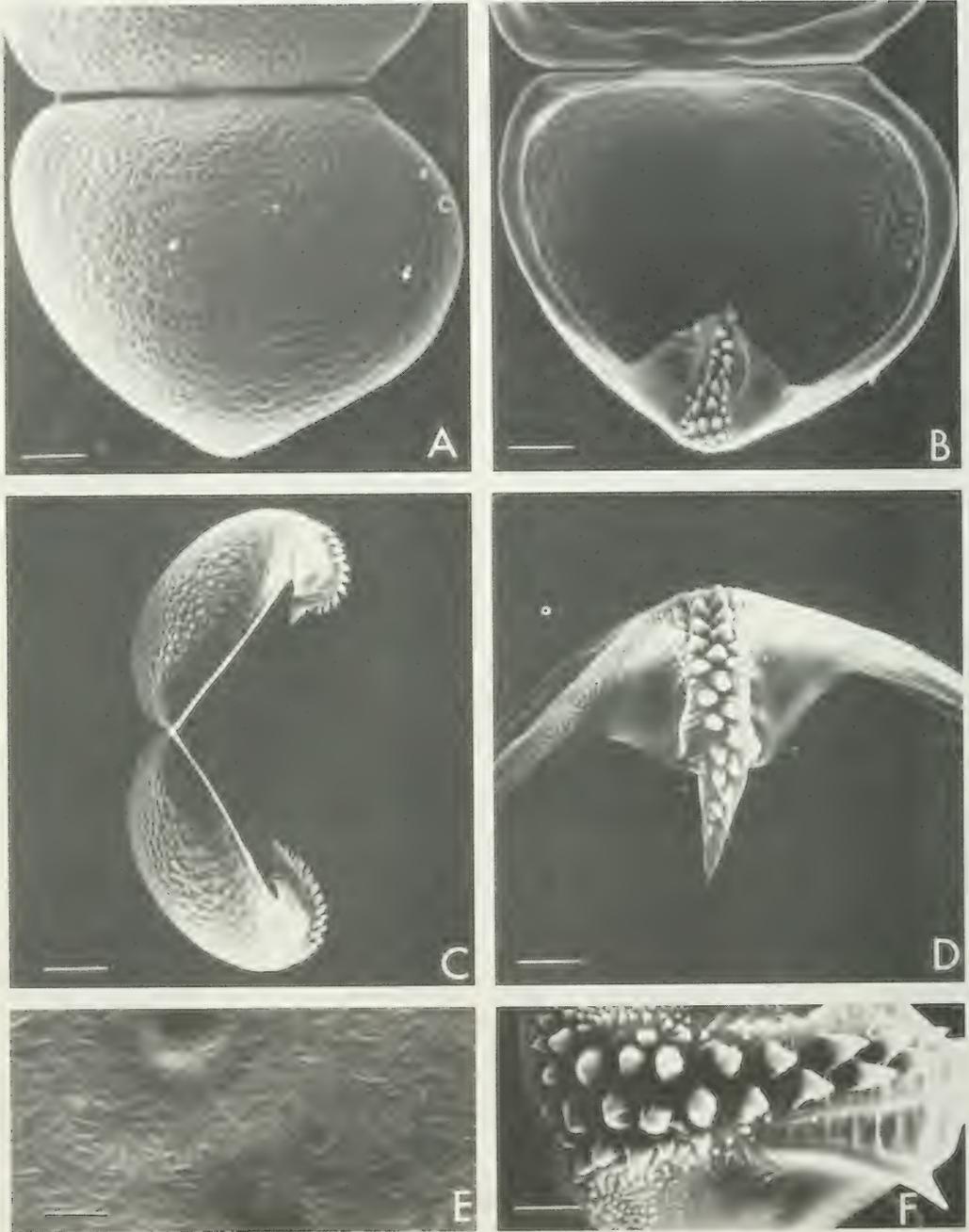


FIG. 24. Glochidium of *Lasmigona compressa*; A. exterior valve, OSUM 23179.1, bar length = 45 μ m; B. interior valve, OSUM 23179.1, bar length = 45 μ m; C. lateral view, MAH 702, bar length = 70 μ m; D. styliform hook, MAH 702, bar length = 30 μ m; E. exterior valve sculpture, MAH 702, bar length = 1 μ m; F. microstylets, MAH 702, bar length = 10 μ m.

ant Twp., Franklin Co., Ohio, 9 September 1985, H. T. Albin. MAH 727 – Fish Creek above and below Edon Rd. bridge, 1.9 mi. NW of Edgerton, St. Joseph Twp., Williams Co., Ohio, 2 October 1985, M. A. Hoggarth & D. Rice.

Description

Glochidium depressed pyriform, longer than high, strongly asymmetric, length 317 to 327 μm ($323 \pm 4.39 \mu\text{m}$, $n = 5$), height 283 to 288 μm ($286 \pm 2.51 \mu\text{m}$, $n = 5$). Dorsal margin straight, 230 to 239 μm ($234 \pm 3.36 \mu\text{m}$, $n = 5$) long. Posterior margin strongly curved; anterior margin greatly inflated dorsally, becoming more gently curved ventrally. Posterior and anterior margins joining at a gently rounded, nipple-like ventral terminus. Exterior valve surface coarsely malleated and pitted throughout, except at umbo and at margin of valve (Fig. 24C). Loose-looped sculpture covering exterior surface of valve (Fig. 24E). Central ligament 83 to 84 μm ($84 \pm 0.71 \mu\text{m}$, $n = 2$) in length, centered about 46% from posterior margin. Posterior ligament 67 to 68 μm ($68 \pm 0.71 \mu\text{m}$, $n = 2$) long; anterior ligament 81 to 82 μm ($82 \pm 0.71 \mu\text{m}$, $n = 2$) in length. Styliform hook armed with about 25 stout microstylets arranged in three proximal rows reduced to two widely off-set distal rows, extending from ventral terminus and located approximately 45% from posterior to anterior. Micropoints extend over edge of valve at ventral terminus and along valve margin, leaving very wide unsculptured distal hook margin.

Remarks

Surber (1912), Ortmann (1912), Tompa (1979), and Clarke (1985) gave length and height measurements for this glochidium; 353 $\mu\text{m} \times 313 \mu\text{m}$, 340 $\mu\text{m} \times 280 \mu\text{m}$, 320 $\mu\text{m} \times 260 \mu\text{m}$, and 344 $\mu\text{m} \times 275 \mu\text{m}$. This glochidium can be distinguished by its widely offset double row of microstylets and its wide unsculptured distal hook margin. Clarke (1985) described the hook of this species as having a single distal row of microstylets; however, his micrographs show only collapsed hooks that are very difficult to interpret. This glochidium was figured by Lea (1858: pl. 5, fig. 23, as *Unio pressus*, = *L. compressa*, *vide* Simpson 1900), Ortmann (1911: pl. 89, fig. 10), Surber (1912: pl. 3, fig. 44), and Clarke (1985: fig. 13).

Lasmigona subviridis (Conrad, 1835)
(Fig. 25A–D)

Material Examined

OSUM 27131.66, 27131.68 – Little River at U.S. Rt. 221 bridge, at Woods Store, 5.3 mi. NE of Floyd, 33.7 mi. SW of Roanoke, Floyd Co., Virginia, 3 October 1970, D. H. Stansbery & W. J. Clench.

Description

Glochidium depressed pyriform, 368 to 383 μm ($376 \pm 5.81 \mu\text{m}$, $n = 5$) in length, 309 to 318 μm ($312 \pm 3.71 \mu\text{m}$, $n = 5$) in height. Posterior margin, strongly curved; anterior margin broadly rounded. Point of maximum posterior inflation at about 30% from dorsal to ventral; point of maximum anterior inflation at about 40–50% from dorsal margin. Dorsal margin straight, 245 to 264 μm ($254 \pm 6.82 \mu\text{m}$, $n = 5$) in length. Exterior valve surface coarsely malleated and uniformly pitted, except within a narrow marginal band. Ventral terminus broadly rounded, not produced or nipple-like as in the other members of the genus, located about 40% from posterior to anterior. Exterior surface sculpturing loose-looped (Fig. 25C). Central ligament 87 to 89 μm ($88 \pm 1.00 \mu\text{m}$, $n = 3$) in length, centered about 40% from posterior to anterior. Posterior ligament 57 to 68 μm ($63 \pm 5.51 \mu\text{m}$, $n = 3$) long; anterior ligament 100 to 107 μm ($104 \pm 3.61 \mu\text{m}$, $n = 3$) long. Styliform hook armed with about 25 microstylets and numerous micropoints. Microstylets lanceolate, about five to six abreast proximally, arranged in a double off-set row distally. Micropoints located on ventral margin of valve and on the lateral surfaces of hook but not over the edge of valve at the ventral terminus (Clarke 1985, Fig. 16b), leaving wide unsculptured distal hook margin.

Remarks

This glochidium can be distinguished by its more broadly curved lateral margins and the absence of micropoints extending onto the valve at the ventral terminus. Ortmann (1912) gave 360 $\mu\text{m} \times 300 \mu\text{m}$ for the length and height of this glochidium, and Clarke (1985) gave 350–372 $\mu\text{m} \times 285$ –303 μm . This glochidium was figured by Lea (1874: pl. 21, fig. 14, as *Unio tappanianus*, = *L. subviridis*, *vide* Ortmann & Walker, 1922) and Clarke

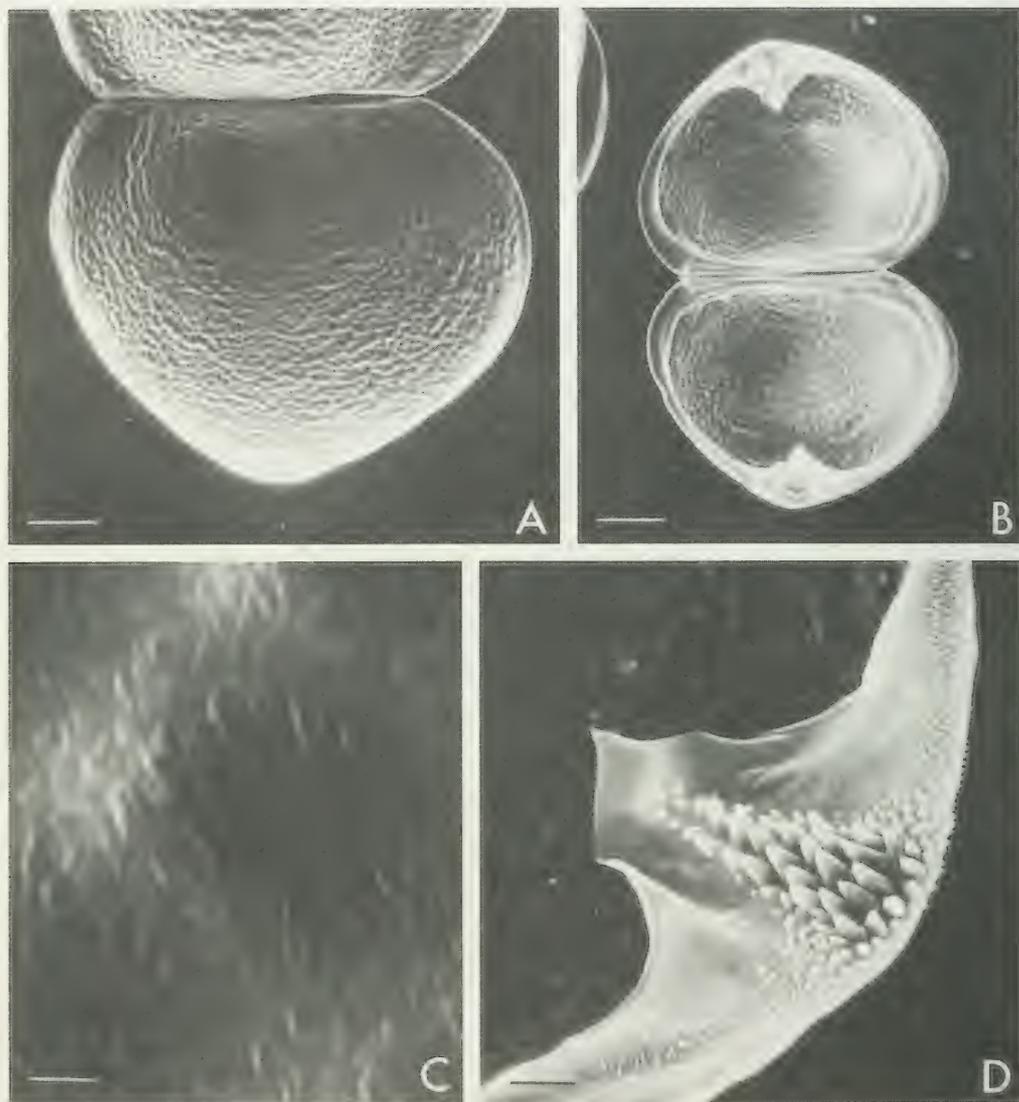


FIG. 25. Glochidium of *Lasmigona subviridis*; A. exterior valve, OSUM 27131.68, bar length = 55 μ m; B. interior valve, OSUM 27131.68, bar length = 80 μ m; C. exterior valve sculpture, OSUM 27131.66, bar length = 1 μ m; D. styliform hook, OSUM 27131.68, bar length = 15 μ m.

(1985: fig. 16). Lea's figure does not show the morphologically depression of the glochidium.

Lasmigona holstonia (Lea, 1838)
(Fig. 26A–D)

Material Examined

OSUM 55826.6, 55826.7 – South Fork Clinch River at St. Rt. 61 bridge, E edge of

Tazewell, Tazewell Co., Virginia, 13 October 1985, D. H. Stansbery.

Description

Glochidium subtriangular, length and height about equal, 281 to 291 μ m ($286 \pm 3.65 \mu$ m, $n = 5$) in length, 275 to 294 μ m ($282 \pm 7.76 \mu$ m, $n = 5$) in height. Dorsal margin straight, 221 to 235 μ m ($228 \pm 5.32 \mu$ m, $n = 5$) long. Posterior

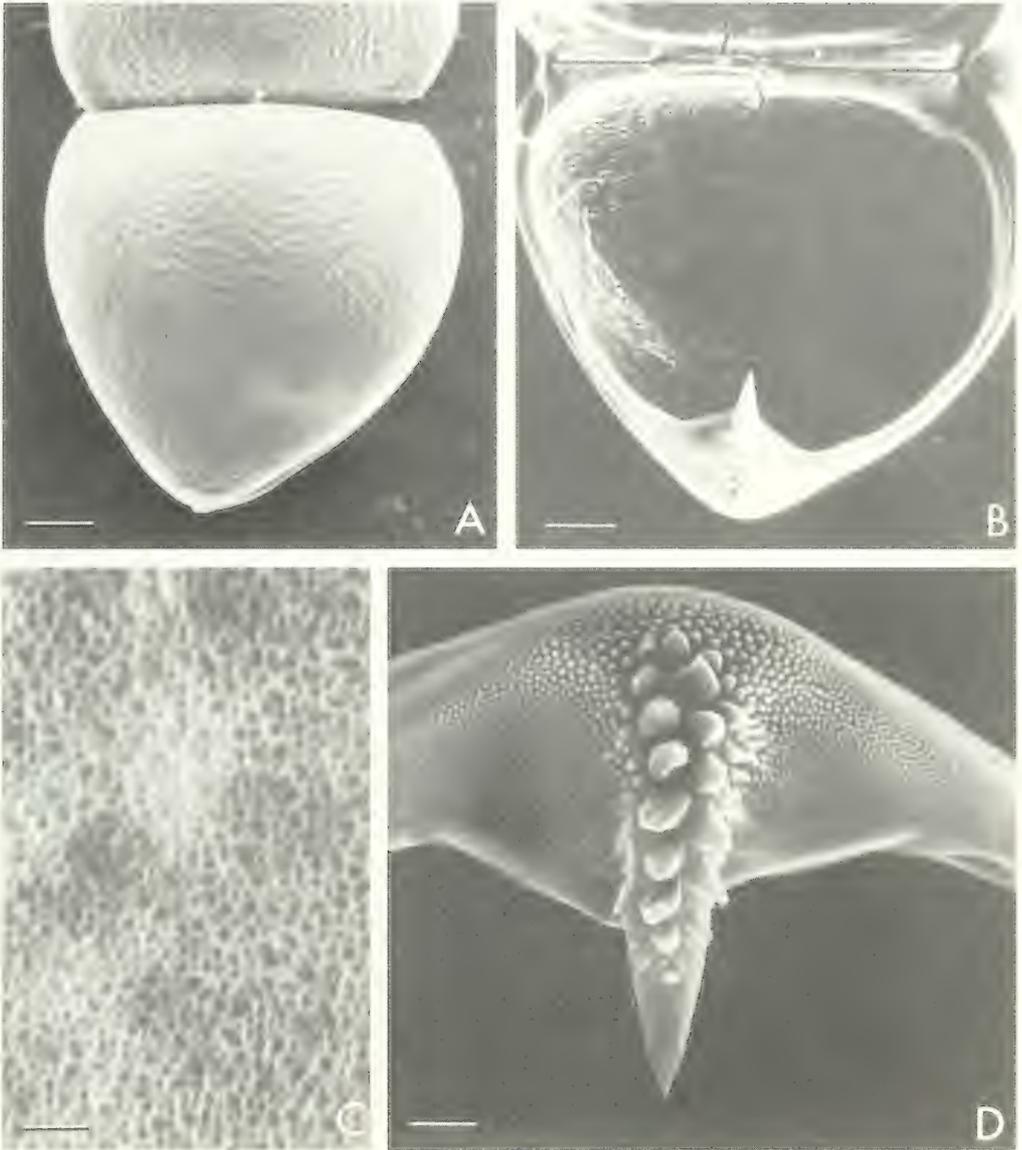


FIG. 26. Glochidium of *Lasmigona holstonia*, OSUM 55826.6: A. exterior valve, bar length = 45 μ m; B. interior valve, bar length = 40 μ m; C. exterior valve sculpture, bar length = 1 μ m; D. styliform hook, bar length = 10 μ m.

margin slightly and evenly curved, with its point of maximum inflation about 20–30% from dorsal margin. Anterior margin broadly curved, with a tendency to be incurved before ventral terminus, with its maximum inflation at about 40–50% from dorsal to ventral. Ventral terminus narrowly rounded, located about 40% from posterior to anterior. Exterior surface finely malleated, uniformly pitted, except

along valve margin. Tight-looped exterior valve sculpture covering surface of valve (Fig. 26C). Clarke (1985) reported a central ligament length of 65 μ m centered 35% from the anterior margin (actually the posterior margin). A central ligament length of 63 to 73 μ m ($68 \pm 7.07 \mu$ m, $n = 2$) was found during this study, with a midpoint about 38% from posterior to anterior. Posterior and anterior ligaments 52 to

55 μm ($54 \pm 2.12 \mu\text{m}$, $n = 2$), and 109 to 110 μm ($110 \pm 0.71 \mu\text{m}$, $n = 2$) long, respectively. Hook styliform, with about 20 microstylets and many micropoints. Micropoints located on ventral rim and lateral surfaces of hook, leaving narrow unsculptured distal hook margin. Microstylets lanceolate, arranged in a double offset rows distally.

Remarks

The shape of this glochidium resembles that of *Anodonta*, except for the slightly incurved margins prior to ventral terminus. The hook structure also differs from that of *Anodonta*. These two characters, as well as the far posterior position of the hook, the broadly rounded anterior margin and looped exterior valve sculpture ally this glochidium with some members of the genera *Alasmidonta* and *Lasmigona*. It is easily distinguished from these, however, by its shape and the tightness of its looped sculpture.

Lasmigona costata (Rafinesque, 1820)
(Fig. 27A–E)

Material Examined

MAH 279.1 – Big Darby Creek at and above McLean Mill Rd. bridge, 0.2 mi. SW of Fox, 4.7 mi. NW of Circleville, Jackson Twp., Pickaway Co., Ohio, 1 October 1982, M. A. Hoggarth et al. MAH 585 – Big Darby Creek at access, 0.9 mi. N of Harrisburg, 1.7 mi. NW of Orient, Pleasant Twp., Franklin Co., Ohio, 27 September 1983, M. A. Hoggarth. MAH 882.1 – Fish Creek at bridge 0.7 mi. W of Arctic, 3.8 mi. NE of Butler, Sec. 20/29, Troy Twp., Dekalb Co., Indiana, 30 October 1985, D. H. Stansbery et al.

Description

Glochidium pyriform, asymmetric, length 340 to 348 μm ($344 \pm 2.73 \mu\text{m}$, $n = 7$), height 363 to 377 μm ($369 \pm 5.68 \mu\text{m}$, $n = 7$). Dorsal margin 239 to 245 μm ($241 \pm 2.23 \mu\text{m}$, $n = 7$) long. Posterior and anterior margins broadly rounded dorsally, slightly incurved ventrally. Maximum inflation of posterior margin at about 30% from dorsal to ventral; maximum inflation of anterior margin at about 40% from dorsal margin. Exterior valve surface coarsely malleated and densely pitted, except at valve margins and at umbo. Exterior surface cov-

ered with densely beaded sculpture (Fig. 27C). Central ligament 78 to 84 μm ($80 \pm 2.49 \mu\text{m}$, $n = 5$) long, centered at about 45% from posterior to anterior (Fig. 27B). Posterior ligament 61 to 68 μm ($64 \pm 2.77 \mu\text{m}$, $n = 5$) in length; anterior ligament 90 to 105 μm ($99 \pm 7.05 \mu\text{m}$, $n = 5$) long. Hook styliform, arising from ventral terminus as a broadly incurved triangular plate. Microstylets lanceolate, multifaceted, arranged in about seven proximal rows, reduced to five distal rows and numbering about 100. Micropoints on proximal border of hook but ending abruptly at ventral rim of valve, not extending onto exterior valve surface (Fig. 27D) nor very far onto lateral surface of the hook, leaving wide unsculptured distal hook margin.

Remarks

The glochidium of *L. costata* can be distinguished by its distinctly pear-shaped outline, its exterior valve sculpture, and hook structure. Glochidia of this species were figured by Lea (1858: pl. 5, fig. 26, as *Margaritana rugosa*, = *L. costata*, fide Ortmann & Walker, 1922), Lefevre & Curtis (1910: fig. B, 1912: fig. 1B), Surber (1912: pl. 1, fig. 7), Arey (1924: pl. 1, fig. 2), and Clarke (1985: fig. 5). This glochidium varies greatly in size: (length \times height) Lea, 368 $\mu\text{m} \times$ 400 μm ; Surber, 385 $\mu\text{m} \times$ 390 μm ; Lefevre & Curtis, 350 $\mu\text{m} \times$ 390 μm ; Ortmann, 340 $\mu\text{m} \times$ 370 μm ; Clarke, 333 $\mu\text{m} \times$ 364 μm .

Lasmigona complanata (Barnes, 1823)
(Fig. 28A–F)

Material Examined

MAH 278.2 – Big Darby Creek at and above McLean Mill Rd. bridge, 0.2 mi. SW of Fox, 4.7 mi. NW of Circleville, Jackson Twp., Pickaway Co., Ohio, 1 October 1982, M. A. Hoggarth et al.

Description

Glochidium pyriform, almost symmetrical, length 289 to 296 μm ($293 \pm 2.90 \mu\text{m}$, $n = 6$), height 293 to 310 μm ($300 \pm 6.90 \mu\text{m}$, $n = 6$). Dorsal margin straight, 193 to 208 μm ($200 \pm 4.69 \mu\text{m}$, $n = 7$) in length. Maximum inflation of anterior and posterior margins at about 40% from dorsal to ventral. Exterior surface coarsely malleated, densely pitted except along valve margin. Umbo malleated but not

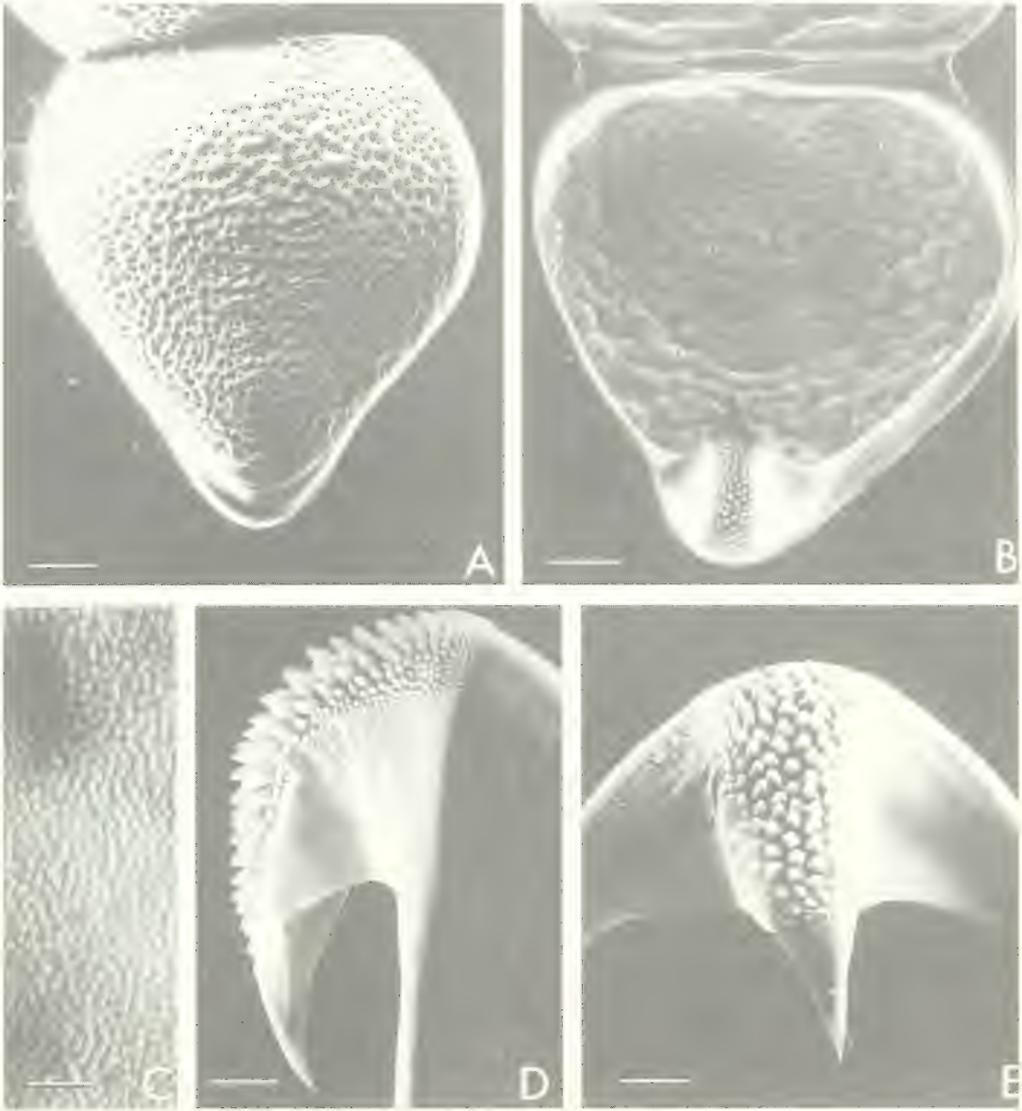


FIG. 27. Glochidium of *Lasmigona costata*: A. exterior valve, MAH 585, bar length = 50 μ m; B. interior valve, MAH 279.1, bar length = 50 μ m; C. exterior valve sculpture, MAH 585, bar length = 1 μ m; D. styliform hook, MAH 585, bar length = 20 μ m; E. styliform hook, MAH 585, bar length = 20 μ m.

pitted. Dense rosette sculpture covering exterior surface of valves (Fig. 28E, F). Central ligament 60 to 68 μ m ($64 \pm 3.32 \mu$ m, $n = 4$) long, centered about 42% from posterior to anterior. Posterior ligament 50 to 53 μ m ($52 \pm 1.29 \mu$ m, $n = 4$) in length; anterior ligament 75 to 88 μ m ($81 \pm 5.48 \mu$ m, $n = 4$) long. Hook styliform, very similar to that of *L. costata*. Microstylets (about 100) lanceolate, multifaceted, arranged in six proximal rows reduced to four rows distally. Micropoints restricted to the proximal margin of

hook, leaving wide unsculptured distal hook margin. Ventral terminus located about 40% from posterior to anterior.

Remarks

This glochidium can be distinguished by its nearly equal length and height, few micropoints and exterior valve sculpture. It is figured by Lea (1858: pl. 5, fig. 29), Lefevre & Curtis (1910: fig. A, 1912: fig. 1A), Ortmann (1911: pl.

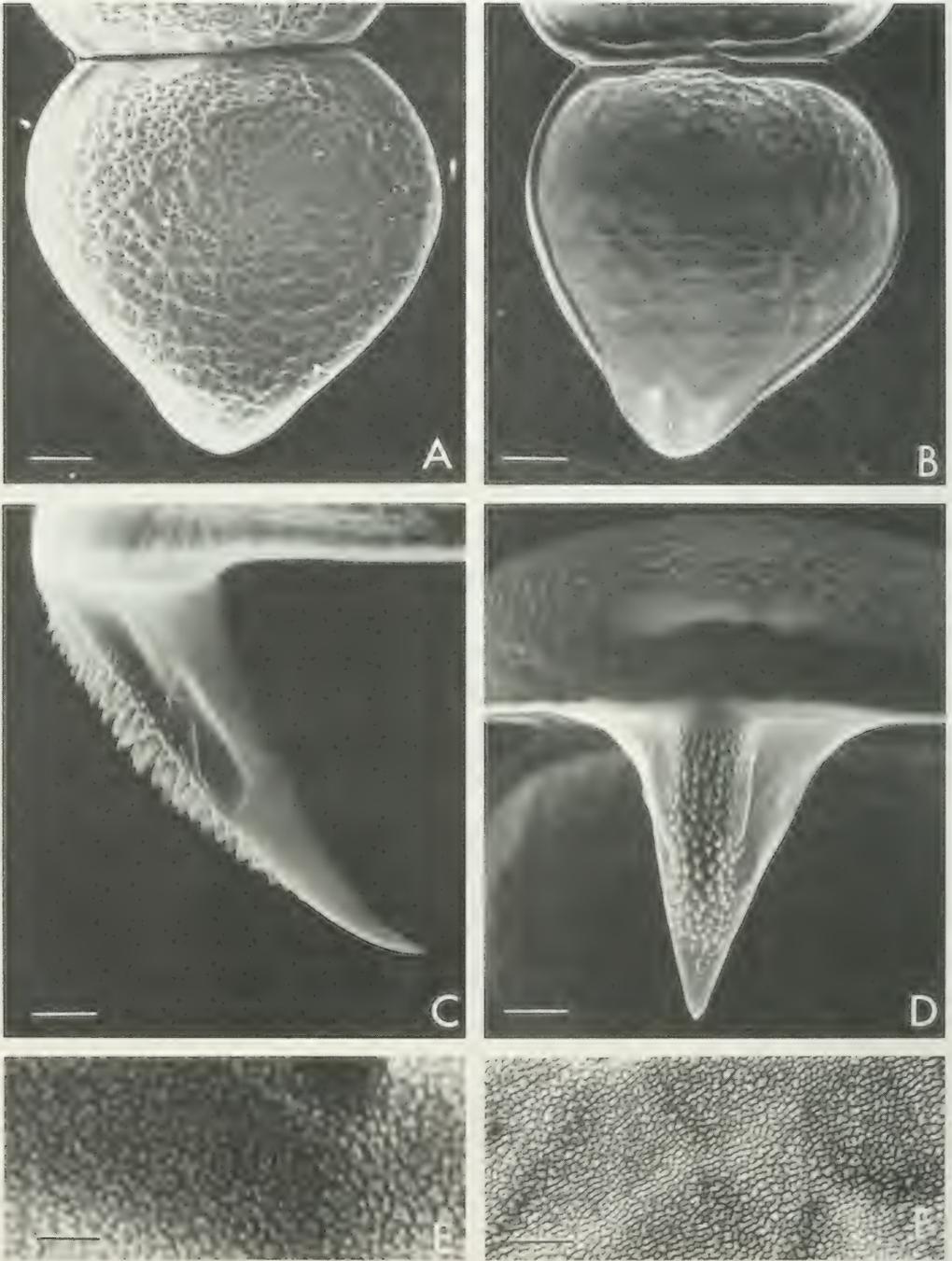


FIG. 28. Glochidium of *Lasmigona complanata*, MAH 278.2; A. exterior valve, bar length = 45 μ m; B. interior valve, bar length = 45 μ m; C. styliform hook, bar length = 15 μ m; D. styliform hook, bar length = 20 μ m; E. exterior valve sculpture, bar length = 1 μ m; F. exterior valve sculpture, bar length = 2 μ m.

89, fig. 11), Surber (1912: pl. 1, fig. 6), Arey (1921: pl. 1, fig. 1, 2), and Clarke (1985: fig. 8). Measurements given for this glochidium are: $290\ \mu\text{m} \times 300\ \mu\text{m}$ (Lefevre & Curtis, 1910), $340\ \mu\text{m} \times 340\ \mu\text{m}$ (Ortmann, 1911), $310\ \mu\text{m} \times 320\ \mu\text{m}$ (Surber, 1912), and $337\ \mu\text{m} \times 337\ \mu\text{m}$ (Clarke, 1985). This glochidium appears to vary a great deal in size, but its relative dimensions remain fairly constant (i.e., length = height).

Subfamily Ambleminiæ

Megaloniais nervosa (Rafinesque, 1820)
(Fig. 29A–G)

Material Examined

OSUM – 13032.66 St. Francis River Bay halfway between Wynne and Perkin, 1.0 mi. S of Rt. 64 bridge, Cross Co., Arkansas, 25 October 1964, C. B. Stein. OSUM 54178 – Mississippi River, R.Ml. 299.8–301.1, 7.8–9.0 mi. SE of Hannibal (MO), Pike/Ralls Co., Illinois/Missouri, 16 October 1979, R. B. Lewis et al. OSUM 178–Licking River, 1.6 mi. E of Butler, immediately above mouth of Flour Creek, 22 mi. SE of Cincinnati, Pendleton Co., Kentucky, 31 October 1986, D. H. Stansbery et al. OSUM – 1986:22 Green River at Glenmore, below Lock 5 Dam, 12.0 mi. N of Bowling Green, Warren Co., Kentucky, 1 November 1986, D. H. Stansbery et al.

Description

Glochidium subelliptical, length 254 to 268 μm ($261 \pm 6.58\ \mu\text{m}$, $n = 4$), height 340 to 350 μm ($346 \pm 4.78\ \mu\text{m}$, $n = 4$). Dorsal margin straight, 145 to 155 μm ($150 \pm 3.27\ \mu\text{m}$, $n = 6$) in length. Lateral margins gently curved but unequal. Maximum inflation of posterior margin at about 70% from dorsal to ventral; maximum inflation of anterior margin at about 40% from dorsal margin. Ventral margin narrowly rounded. Tight-looped sculpture covering exterior surface of valve (Fig. 29F). Coiled larval thread present (Figs. 29C, D). Central ligament 49 to 52 μm ($50 \pm 1.53\ \mu\text{m}$, $n = 3$) long, centered about 44% from posterior to anterior. Anterior ligament 60 to 63 μm ($61 \pm 2.00\ \mu\text{m}$, $n = 3$) long, posterior ligament 43 to 44 μm ($43 \pm 0.58\ \mu\text{m}$, $n = 3$) in length. Lanceolate micropoints occurring in broken vertical rows on a narrow ventral flange, and along rim of ventral margin of valve, covering most of ventral flange, leaving very narrow unsculptured distal flange margin.

Remarks

Surber (1915) stated, "notwithstanding its great variation in size, and even outline, this species cannot be readily confused with any other, even though the larval gland may have been absorbed. . ." Surber (1912), Howard (1914c), and Surber (1915) gave length and height measurements of $260\ \mu\text{m} \times 340\ \mu\text{m}$, $250\text{--}260\ \mu\text{m} \times 316\text{--}340\ \mu\text{m}$, and $250\text{--}280\ \mu\text{m} \times 300\text{--}380\ \mu\text{m}$ (as *Quadrula heros* = *M. nervosa*, fide Stansbery et al., 1985). This glochidium has been figured by Lea (1858: pl. 5, fig. 3, as *Unio multiplicatus*, = *M. nervosa* fide Stansbery et al., 1985), Surber (1912: pl. 2, fig. 32), Howard (1914c: pl. 3, fig. 21; pl. 5, fig. 35), Surber (1915: pl. 1, fig. 10), and Utterback (1915–1916: fig. 3a, b). Surber's, Howard's, and Utterback's figures agree with mine, whereas Lea's does not.

Megaloniais boykiniana (Lea, 1840)
(Fig. 30A–D)

Material Examined

OSUM 51107.5 – Apalachicola River below U.S. Rt. 90 bridge, 1.0 mi. W of Chattahoochee, 17.8 mi. WNW of Quincy, T4N, R6W, Sec. 32, Gadsden Co., Florida, 29 October 1981, D. H. Stansbery et al.

Description

Glochidium subelliptical, with a straight dorsal margin, a narrowly rounded ventral margin, and gently but unequally curved lateral margins. A single specimen gave the following measurements for length, height, and hinge length: $245\ \mu\text{m} \times 350\ \mu\text{m} \times 150\ \mu\text{m}$. Tight-looped sculpture covering exterior surface of valve (Fig. 30C), lanceolate micropoints cover a narrow ventral flange (Fig. 30D), and a larval thread is coiled around adductor muscle, not supercoiled as in *M. nervosa* (Fig. 30B).

Remarks

The glochidium of this species can be distinguished from that of *M. nervosa* by its larval thread and from all other glochidia by its dimensions and outline. No published figure of this glochidium was found.

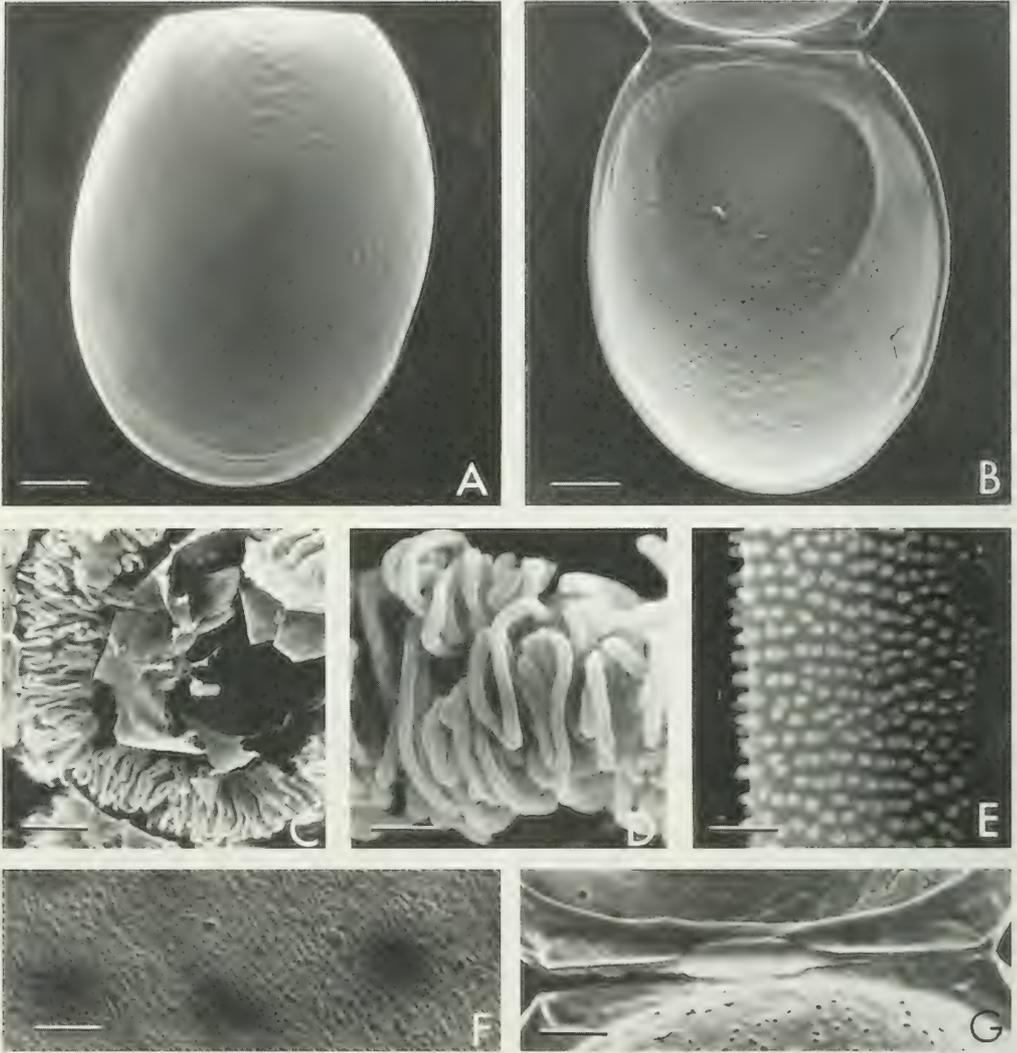


FIG. 29. Glochidium of *Megaloniais nervosa*; A. exterior valve, OSUM 54178, bar length = 50 μ m; B. interior valve, OSUM 178, bar length = 50 μ m; C. larval thread, OSUM 54178, bar length = 25 μ m; D. larval thread, OSUM 54178, bar length = 5 μ m; E. micropoints, OSUM 54178, bar length = 5 μ m; F. exterior valve sculpture, OSUM: 1986:22, bar length = 2 μ m; G. hinge, OSUM 178, bar length = 20 μ m.

Plectomerus dombeyana
(Valenciennes, 1827)
(Fig. 31A–H)

Material Examined

OSUM 42011 – Black Warrior River at Hall Shoals, below Eutaw Dam, 5.8 mi. SE of Eutaw, Sec. 25, T21N, R2E, Green Co., Alabama, 28–30 July 1975, J. D. Williams et al.. OSUM 53273.2, 53273.3 – Calcasieu River at

Nevel's Bluff, 2.0 mi. NE of Indian Village, 6.9 mi. WSW of Kinder, 22.0 mi. NE of Lake Charles, Allen Parish, Louisiana, 25 July 1982, D. H. Stansbery & M. A. Hoggarth.

Description

Glochidium subelliptical, length 223 to 231 μ m ($226 \pm 2.99 \mu$ m, $n = 10$), height 238 to 259 μ m ($246 \pm 7.07 \mu$ m, $n = 10$). Dorsal margin straight, 130 to 135 μ m ($133 \pm 1.36 \mu$ m, $n =$

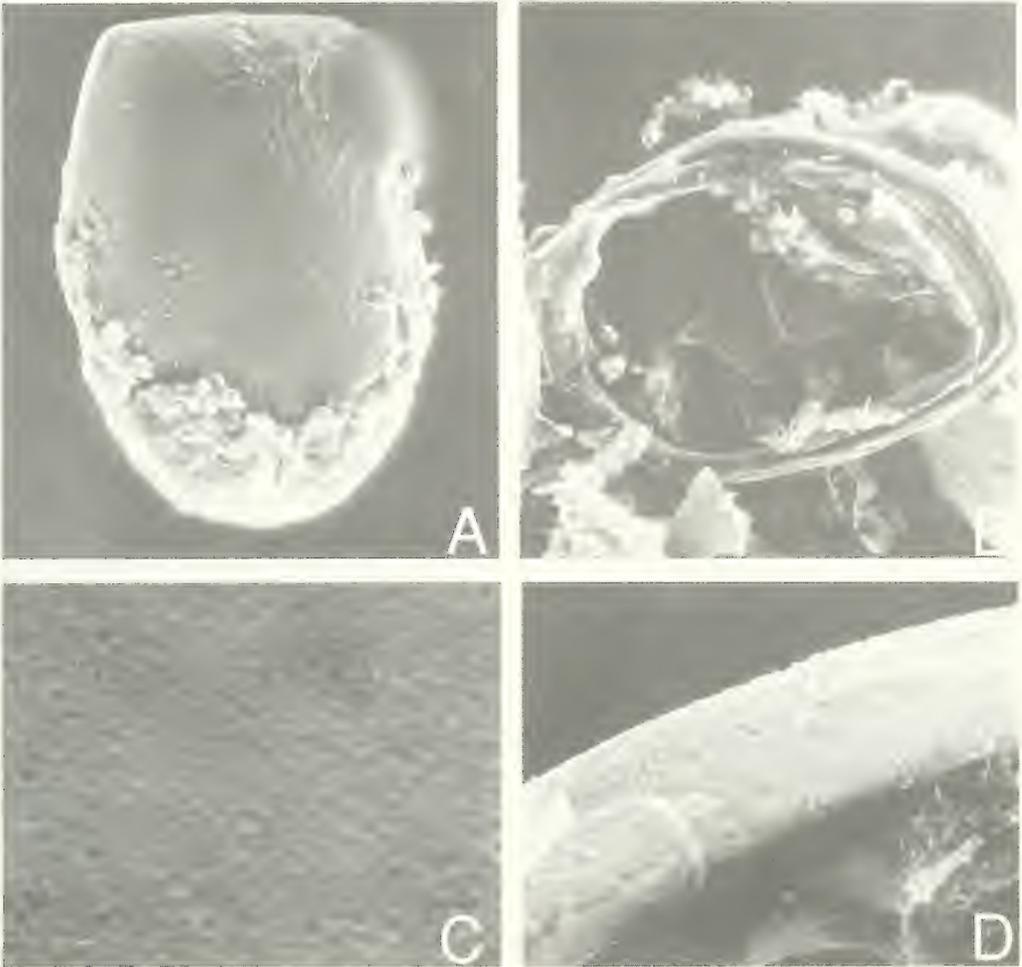


FIG. 30. Glochidium of *Megaloniais boykiniana*, OSUM 51107.5; A. exterior valve, bar length = 50 μm ; B. larval thread, bar length = 25 μm ; C. exterior valve sculpture, bar length = 1 μm ; D. micropoints, bar length = 5 μm .

11) long. Lateral margins gently and equally curved, symmetrical. Ventral margin semicircular. Valve surface finely malleated, with many pits (Fig. 31E). Loose-looped sculpture covers exterior valve surface (Fig. 31C,H). Figure 31C demonstrates that the larval valve has an exterior membrane that is at least analogous, if not homologous, with the periostracum of the adult. It is also evident from this micrograph that the exterior valve sculpture occurs within this membrane. Central ligament 42 to 47 μm ($44 \pm 2.06 \mu\text{m}$, $n = 4$) long, centered about 40% from posterior to anterior. Anterior ligament 51 to 59 μm ($56 \pm 3.59 \mu\text{m}$,

$n = 4$) long; posterior ligament 31 to 35 μm ($32 \pm 1.89 \mu\text{m}$, $n = 4$) in length. Lanceolate micropoints in broken rows on rim of ventral margin of valve and on a narrow ventral flange. Surface of ventral flange mostly covered with micropoints, leaving narrow unsculptured distal flange margin.

Remarks

This glochidium is distinguished from others examined by its shape and dimensions. No published figure of this glochidium was found.

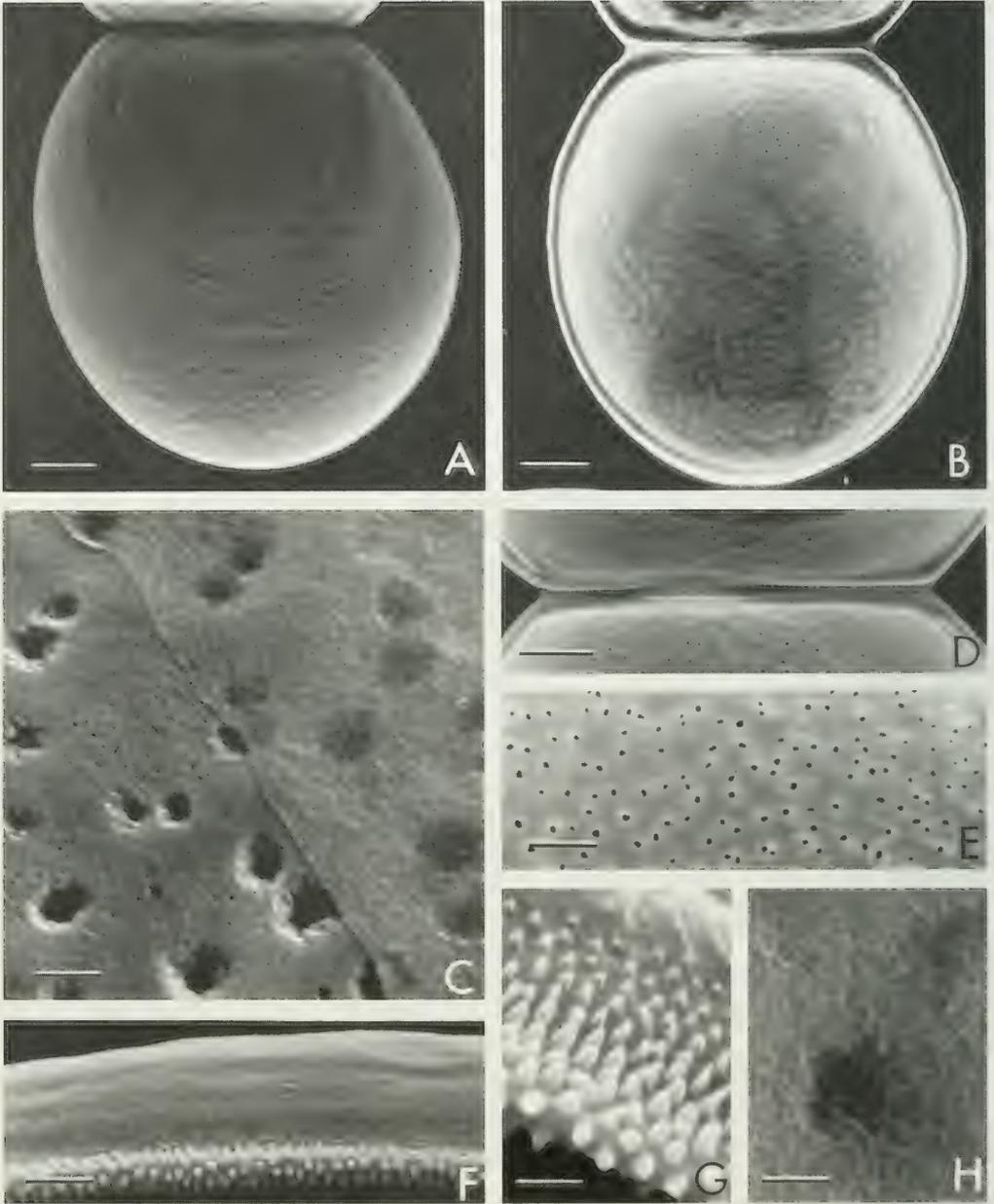


FIG. 31. Glochidium of *Plectomerus dombeyana*; A. exterior valve, OSUM 53273.3, bar length = 35 μm ; B. interior valve, OSUM 53273.3, bar length = 35 μm ; C. exterior valve sculpture and torn exterior valve membrane, OSUM 53273.3, bar length = 2 μm ; D. hinge, OSUM 53273.2, bar length = 25 μm ; E. interior valve pitting, OSUM 42011, bar length = 10 μm ; F. micropoints, OSUM 53273.3, bar length = 5 μm ; G. micropoints, OSUM 42011, bar length = 3 μm ; H. exterior valve sculpture, OSUM 42011, bar length = 1 μm .

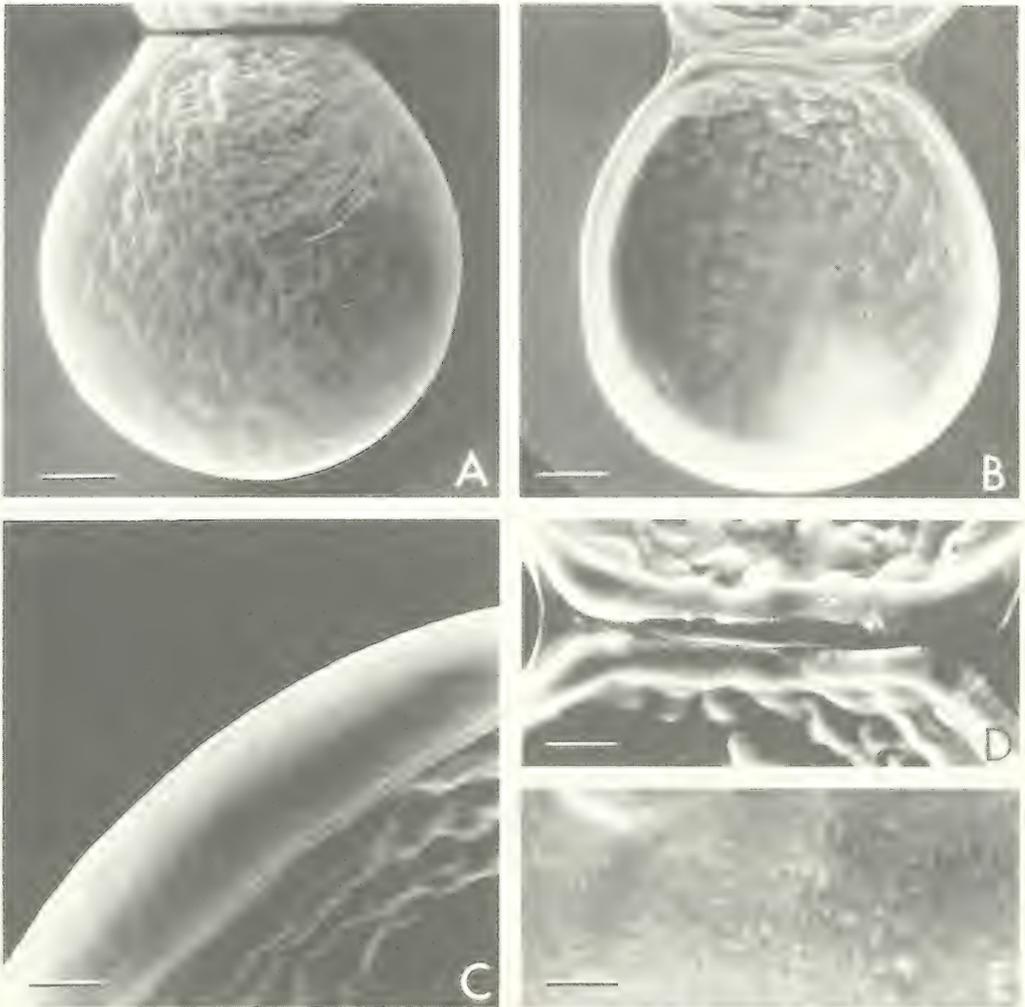


FIG. 32. Glochidium of *Tritogonia verrucosa*, MAH 654.1; A. exterior valve, bar length = 15 μ m; B. interior valve, bar length = 15 μ m; C. micropoints, bar length = 5 μ m; D. hinge, bar length = 7 μ m; E. exterior valve sculpture, bar length = 2 μ m.

Tritogonia verrucosa (Rafinesque, 1820)
(Fig. 32A-E)

Material Examined

MAH 654.1 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. NW of Circleville, Scioto/Darby Twp., Pickaway Co., Ohio, 18 May 1984, M. A. Hoggarth & G. T. Watters.

Description

Glochidium subelliptical to subrotund, length 85 to 94 μ m ($90 \pm 3.38 \mu$ m, $n = 6$), height 97 to 101 μ m ($100 \pm 1.75 \mu$ m, $n = 6$). Dorsal margin slightly curved, 43 to 46 μ m ($44 \pm 1.11 \mu$ m, $n = 7$) long. Lateral margins gently and equally curving throughout their lengths. Ventral margin semicircular. Exterior surface rough, sparsely pitted (Fig. 32E). Central ligament 30 to 34 μ m ($32 \pm 1.47 \mu$ m,

$n = 6$) long, centered about 45% from posterior to anterior. Anterior ligament 6 to 9 μm ($8 \pm 1.51 \mu\text{m}$, $n = 6$) long; posterior ligament 3 to 5 μm ($4 \pm 0.82 \mu\text{m}$, $n = 6$) in length; central ligament comprising about 75% of total hinge length. Micropoints extremely small, almost undetected even at high magnification, unorganized (Fig. 32C), on rim of ventral valve margin and on exterior surface of valve. Ventral flange not observed.

Remarks

This glochidium is figured by Surber (1912: pl. 2, fig. 31, as *T. tuberculata*, = *T. verrucosa*, *fide* Ortmann, 1919), who gave length and height measurements of $85 \mu\text{m} \times 90 \mu\text{m}$. It can be distinguished from all other glochidia by its very small size and the outline of its valve.

Quincuncina infucata (Conrad, 1834)
(Fig. 33A–G)

Material Examined

OSUM 48537.1, 48537.2 – Suwannee River at Fl. Rt. 51 bridge, 8.4 mi. SSE of Jasper, Sec. 17, Hamilton/Suwannee Co., Florida, 14 May 1978, W. J. Clench et al.

Description

Glochidium subelliptical, with a short hinge line, equally curved lateral margins, a broadly curved ventral margin. Glochidium 234 to 242 μm ($240 \pm 4.00 \mu\text{m}$, $n = 4$) in length, 275 to 287 μm ($283 \pm 5.68 \mu\text{m}$, $n = 4$) in height. Dorsal margin straight, 102 to 105 μm ($103 \pm 1.34 \mu\text{m}$, $n = 5$) in length. Valve surface densely pitted, except along valve margin, and rough sculpture covering exterior valve surface (Fig. 33F). Central ligament 54 to 58 μm ($56 \pm 2.83 \mu\text{m}$, $n = 2$) long, centered about 48% from posterior to anterior. Anterior ligament 27 to 28 μm ($28 \pm 0.71 \mu\text{m}$, $n = 2$) long, posterior ligament 20 to 22 μm ($21 \pm 1.54 \mu\text{m}$, $n = 2$) in length. Micropoints coronal, with fused bases and lanceolate points, not extending onto ventral rim or on ventral margin of valve, covering about 90% of ventral flange, leaving narrow unsculptured distal flange margin.

Remarks

This glochidium will not be confused with that of any other species examined. The most striking feature is its unusual micropoint struc-

ture. The more proximal micropoints resemble crowns. The bases of the micropoints are fused with their points extending outward. The number of points in each "crown" range from seven along the proximal margin of the flange to two points distally. The furthest micropoints on the flange are simple lanceolate points. No published figure of this glochidium was found.

Elliptio dilatata (Rafinesque, 1820)
(Fig. 34A–F)

Material Examined

MAH 946.9 – Kalamazoo River above St. Rt. 60 bridge, 3.0 mi. WSW of Spring Arbor, 12.0 mi. WSW of Jackson, Jackson Co., Michigan, 13 May 1986, M. A. Hoggarth.

Description

Glochidium subelliptical, length 210 to 219 μm ($216 \pm 4.27 \mu\text{m}$, $n = 4$), height 219 to 225 μm ($221 \pm 3.00 \mu\text{m}$, $n = 4$). Dorsal margin straight, 140 to 147 μm ($143 \pm 2.99 \mu\text{m}$, $n = 4$) in length. Ventral margin semicircular. Lateral margins subequal, with anterior margin slightly more produced than posterior margin. Valve pitting eliminated in region of adductor muscle scar (Fig. 34D) and sparse throughout remainder of valve. Loose-looped sculpture covering exterior surface of valve (Fig. 34C). Central ligament 45 to 50 μm ($48 \pm 3.54 \mu\text{m}$, $n = 2$) long, centered about 43% from posterior to anterior. Anterior ligament about 56 μm long; posterior ligament 36 to 39 μm ($38 \pm 2.12 \mu\text{m}$, $n = 2$) in length. Micropoints lanceolate, located on ventral rim of valve and on narrow ventral flange, arranged in broken vertical rows, covering most of the area of flange. Unsculptured distal flange margin narrow.

Remarks

This glochidium is figured by Lea (1874: pl. 21, fig. 10, as *Unio gibbosus*, = *E. dilatata*, *fide* Ortmann & Walker, 1922), Lefevre & Curtis (1910: fig. N; 1912: fig. 10, as *U. gibbosus*), Ortmann (1911: pl. 89, fig. 7, as *E. gibbosus*), and Surber (1912: pl. 2, fig. 38, as *U. gibbosus*). Lefevre & Curtis (1910, 1912) gave length and height measurements of $220 \mu\text{m} \times 190 \mu\text{m}$ for this glochidium, Surber (1912) gave $200 \mu\text{m} \times 215 \mu\text{m}$, Ortmann (1912) gave $200 \mu\text{m} \times 220 \mu\text{m}$, and Ortmann (1919, as *E. cupreus*, = *E. dilatata*, *fide* Johnson, 1970) gave $200 \mu\text{m} \times 200 \mu\text{m}$.

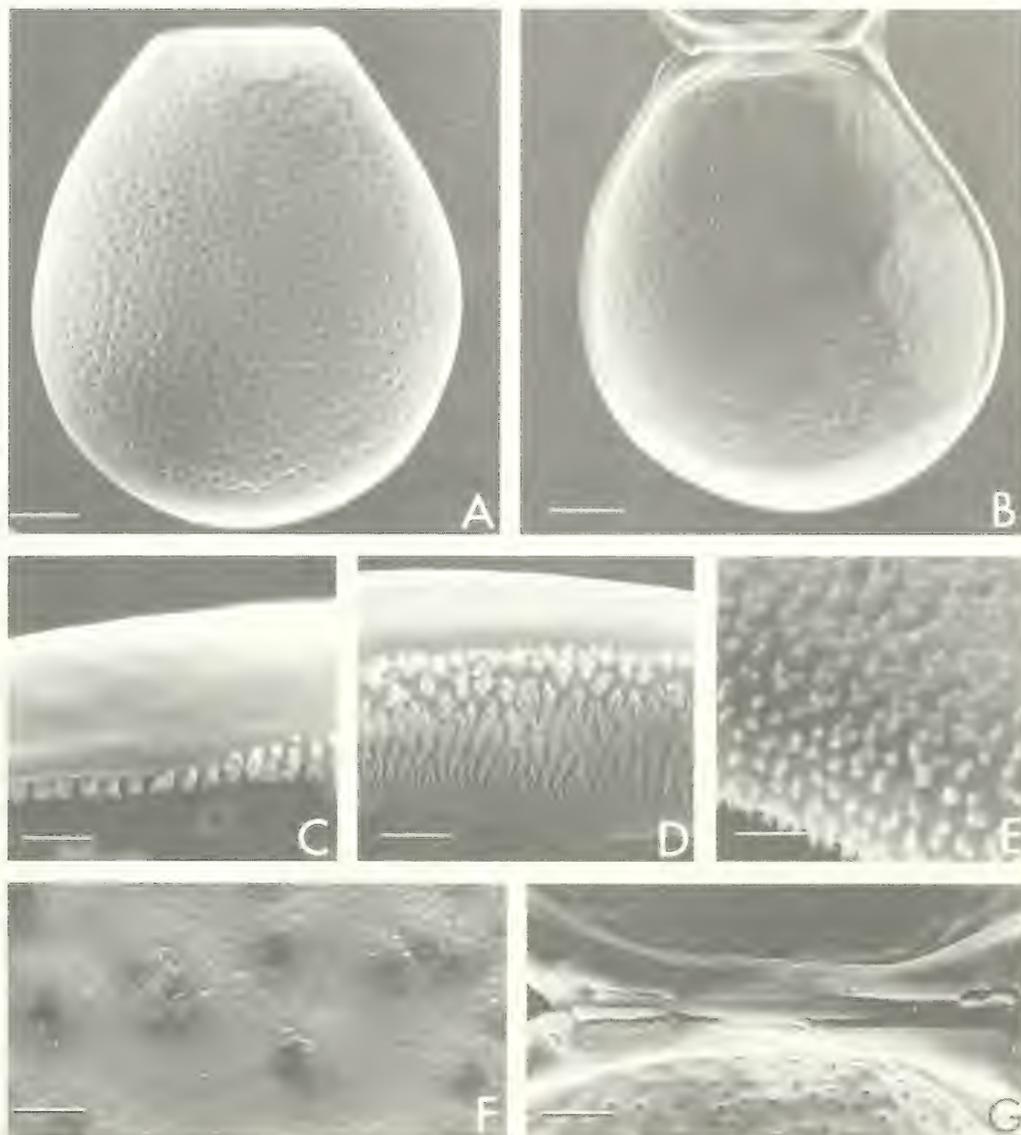


FIG. 33. Glochidium of *Quincuncina infucata*: A. exterior valve, OSUM 48537.2, bar length = 40 μ m; B. interior valve, OSUM 48537.2, bar length = 40 μ m; C. micropoints, OSUM 48537.2, bar length = 5 μ m; D. micropoints, OSUM 48537.2, bar length = 5 μ m; E. micropoints, OSUM 48537.1, bar length = 2 μ m; F. exterior valve sculpture, OSUM 48537.1, bar length = 2 μ m; G. hinge, OSUM 48537.1, bar length = 15 μ m.

Subfamily Lampsilinae
Ptychobranchus fasciolaris
 (Rafinesque, 1820)
 (Fig. 35A–E)

Material Examined

MAH 640.1 – Little Darby Creek at Co. Rt. 131 bridge (Grewell Rd.), 1.8 mi. E of

Plumwood, 7.7 mi. NW of West Jefferson, Monroe Twp., Madison Co., Ohio, 11 May 1984, M. A. Hoggarth & G. T. Watters. MAH 651 – Little Darby Creek at Little Darby Rd. access, 2.7 mi. SE of Plumwood, 6.4 mi. NW of West Jefferson, Monroe Twp., Madison Co., Ohio, 17 May 1984, M. A. Hoggarth & G. T. Watters.

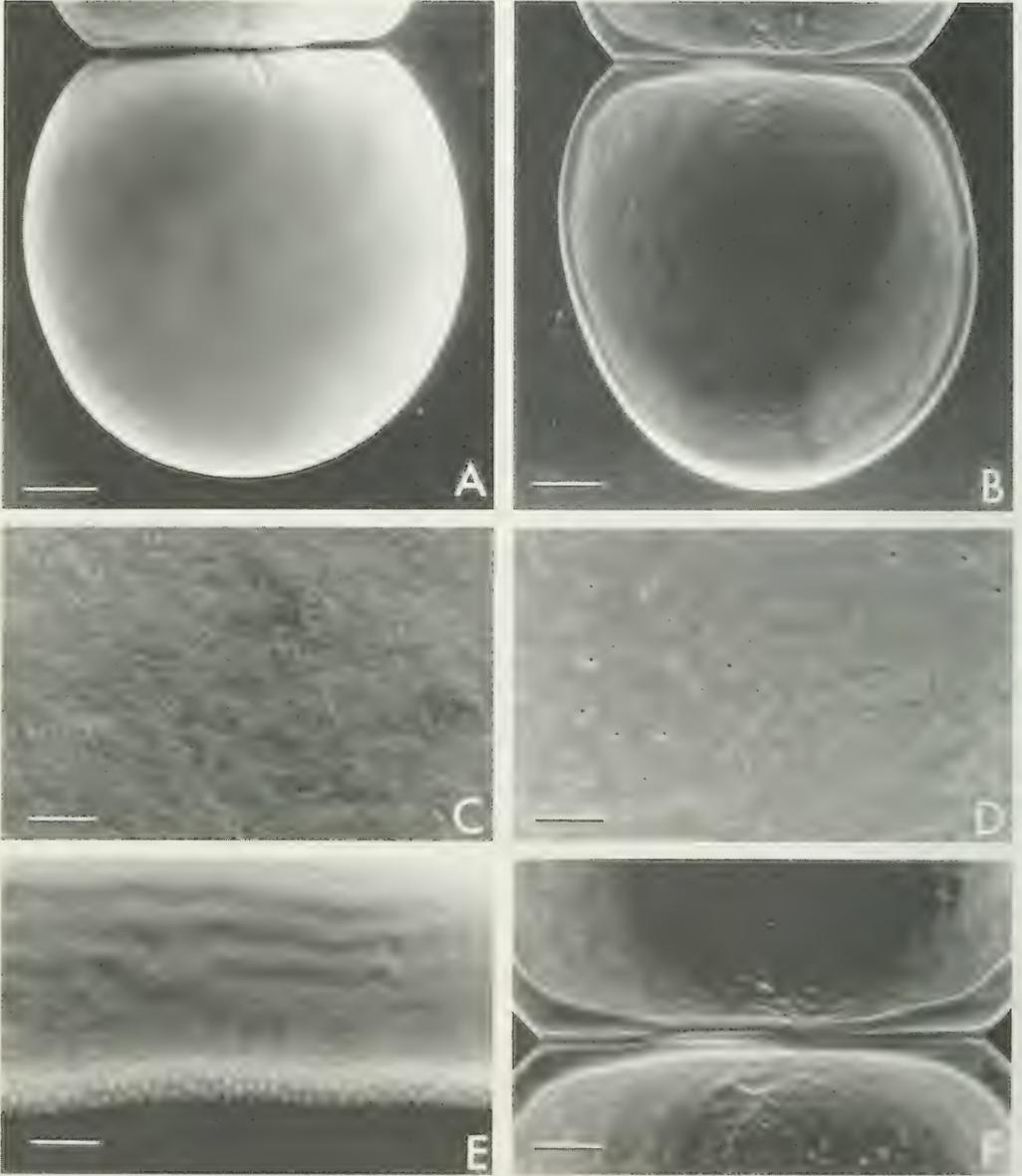


FIG. 34. Glochidium of *Elliptio dilatata*, MAH 946.9; A. exterior valve, bar length = 35 μm ; B. interior valve, bar length = 35 μm ; C. exterior valve, bar length = 1 μm ; D. interior valve, bar length = 10 μm ; E. micropoints, bar length = 5 μm ; F. hinge, bar length = 20 μm .

Description

Glochidium subelliptical, higher than long, length 170 to 175 μm ($173 \pm 2.89 \mu\text{m}$, $n = 3$), height 182 to 195 μm ($187 \pm 6.81 \mu\text{m}$, $n = 3$). Dorsal margin slightly curved, 80 to 89 μm ($83 \pm 4.93 \mu\text{m}$, $n = 3$) in length. Anterior and pos-

terior margins equally curved to a maximum inflation at about 60% from dorsal to ventral; ventral margin broadly rounded. Surface of the valve smooth, with only a few pits. Dorsal alae absent. Loose-looped sculpture covering exterior surface of valve (Fig. 35E). Central ligament 36 to 40 μm ($38 \pm 2.83 \mu\text{m}$, $n = 2$)

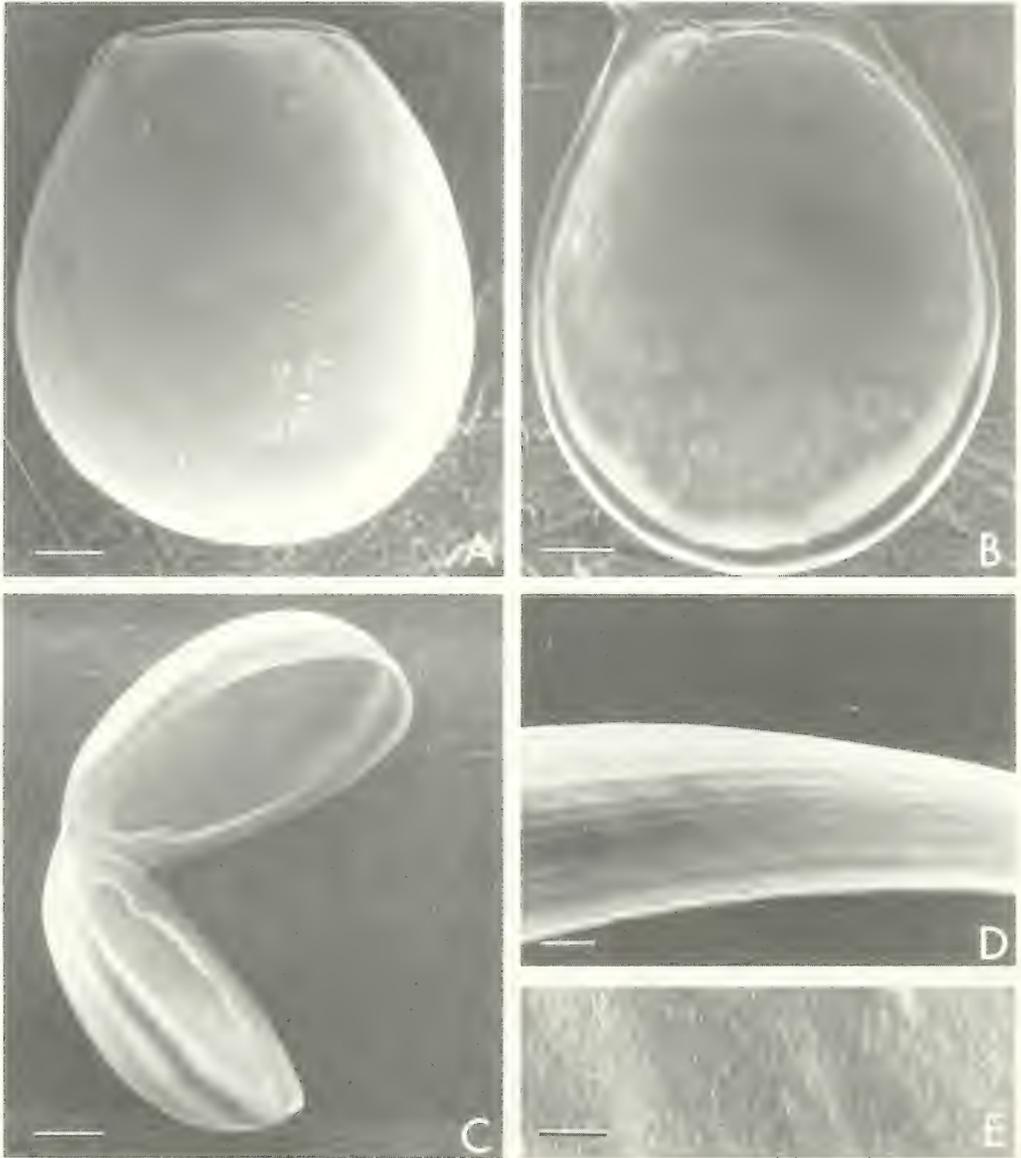


FIG. 35. Glochidium of *Ptychobranchus fasciolaris*; A. exterior valve, MAH 640.1, bar length = 25 μ m; B. interior valve, MAH 640.1, bar length = 25 μ m; C. lateral view, MAH 640.1, bar length = 35 μ m; D. ventral valve edge, MAH 651, bar length = 5 μ m; E. exterior valve sculpture, MAH 651, bar length = 1 μ m.

long, centered about 49% from posterior to anterior. Posterior ligament 19 to 22 μ m (21 ± 2.12 μ m, $n = 2$) long; anterior ligament 22 to 26 μ m (24 ± 2.83 μ m, $n = 2$) in length. Micro-points very small, few in number, located on ventral margin of valve rather than on broad ventral flange.

Remarks

This glochidium can be distinguished by its small size, central ligament position, and its very simple ventral margin. It was figured by Lea (1858: pl. 5, fig. 12, as *Unio phaseolus*, = *P. fasciolaris*, fide Ortmann & Walker, 1922)

and Ortmann (1911: pl. 89, fig. 14, as *P. phaseolus*). Ortmann (1912) gave $170\ \mu\text{m} \times 190\ \mu\text{m}$ for length and height of this glochidium and noted that Lea's figure does not represent its shape or size accurately. The glochidium of *P. fasciolaris* is much smaller than that of *Ligumia recta* rather than, as pictured by Lea (1858), larger than that species.

Ptychobranthus occidentalis (Conrad, 1836)
(Fig. 36A–D)

Material Examined

OSUM – 45361.14, 45361.17 Current River “between Mo. Rt. 106 and Van Buren,” about 19 mi. of stream, Shannon/Carter Co., Missouri, 24 October 1977, R. D. Oesch.

Description

Glochidium subelliptical, higher than long, length 197 to 203 μm ($200 \pm 4.24\ \mu\text{m}$, $n = 2$), height 234 to 241 μm ($238 \pm 4.95\ \mu\text{m}$, $n = 2$). Dorsal margin slightly curved, 101 to 104 μm ($102 \pm 1.53\ \mu\text{m}$, $n = 3$) in length. Anterior and posterior margins equally and gently curving to their maximum inflation at about 60% from dorsal to ventral; ventral margin broadly curved. Dorsal alae absent; valve surface sparsely pitted. Loose-looped sculpture covering exterior valve surface (Fig. 36C). Central ligament 44 to 48 μm ($46 \pm 2.83\ \mu\text{m}$, $n = 2$) in length, centered about 47% from the posterior margin. Anterior ligament 30 to 34 μm ($32 \pm 2.83\ \mu\text{m}$, $n = 2$) in length; posterior ligament 23 to 26 μm ($25 \pm 2.12\ \mu\text{m}$, $n = 2$) long. Ventral flange poorly developed, very narrow, covered with micropoints. Micropoints lanceolate and unorganized.

Remarks

This glochidium is slightly larger than that of *P. fasciolaris* and is further distinguished from that species by its larger and more numerous micropoints. No published figure of this glochidium was found.

Ptychobranthus greeni (Conrad, 1834)
(Fig. 36E–H)

Material Examined

OSUM 19025.2 – Conasauga River above lower Kings Bridge, 5 mi. WNW of Eton, Mur-

ray/Whitfield Co., Georgia, 25 September 1966, D. H. Stansbery & H. D. Athearn.

Description

Glochidium subelliptical, higher than long, symmetrical, length 183 to 190 μm ($187 \pm 3.61\ \mu\text{m}$, $n = 3$), height 226 to 228 μm ($227 \pm 1.15\ \mu\text{m}$, $n = 3$). Dorsal margin slightly curved, 90 to 96 μm ($93 \pm 3.06\ \mu\text{m}$, $n = 3$) in length. Anterior and posterior margins equal and slightly curved to their point of maximum inflation at about 70% from dorsal to ventral. Ventral margin broadly rounded. Surface of valve sparsely pitted. Loose-looped sculpture covering exterior surface of valve (Fig. 36G). Dorsal alae present but very small, oriented almost perpendicular to hinge. Central ligament about 49 μm long centered about 48% from posterior to anterior. Anterior ligament about 26 μm long; posterior ligament 21 μm in length. Micropoints occur on ventral rim of valve, small, lanceolate, unorganized. Ventral flange not observed.

Remarks

This glochidium is about the same size as that of *P. fasciolaris*; however, it can be distinguished from that species by its dorsal alae and micropoints. This glochidium is figured by Lea (1858: pl. 5, fig. 16, as *Unio woodwardianus*, = *P. greeni*, *vide* Ortmann, 1923–1924). His figure is essentially correct except for size. Glochidia of *Lampsilis ovata* and *L. fasciola* are larger than those of *P. greeni* rather than smaller as figured by Lea.

Ptychobranthus subtentum (Say, 1825)
(Fig. 37A–F)

Material Examined

OSUM 43156.5 – Clinch River at mouth of Copper Creek, 1.3 mi. S of Clinchport, 9.3 mi. W of Gate City, Scott Co., Virginia, 21 October 1978, D. H. Stansbery et al.

Description

Glochidium subelliptical, higher than long, symmetrical, length 181 to 195 μm ($190 \pm 5.54\ \mu\text{m}$, $n = 5$), height 236 to 251 μm ($241 \pm$

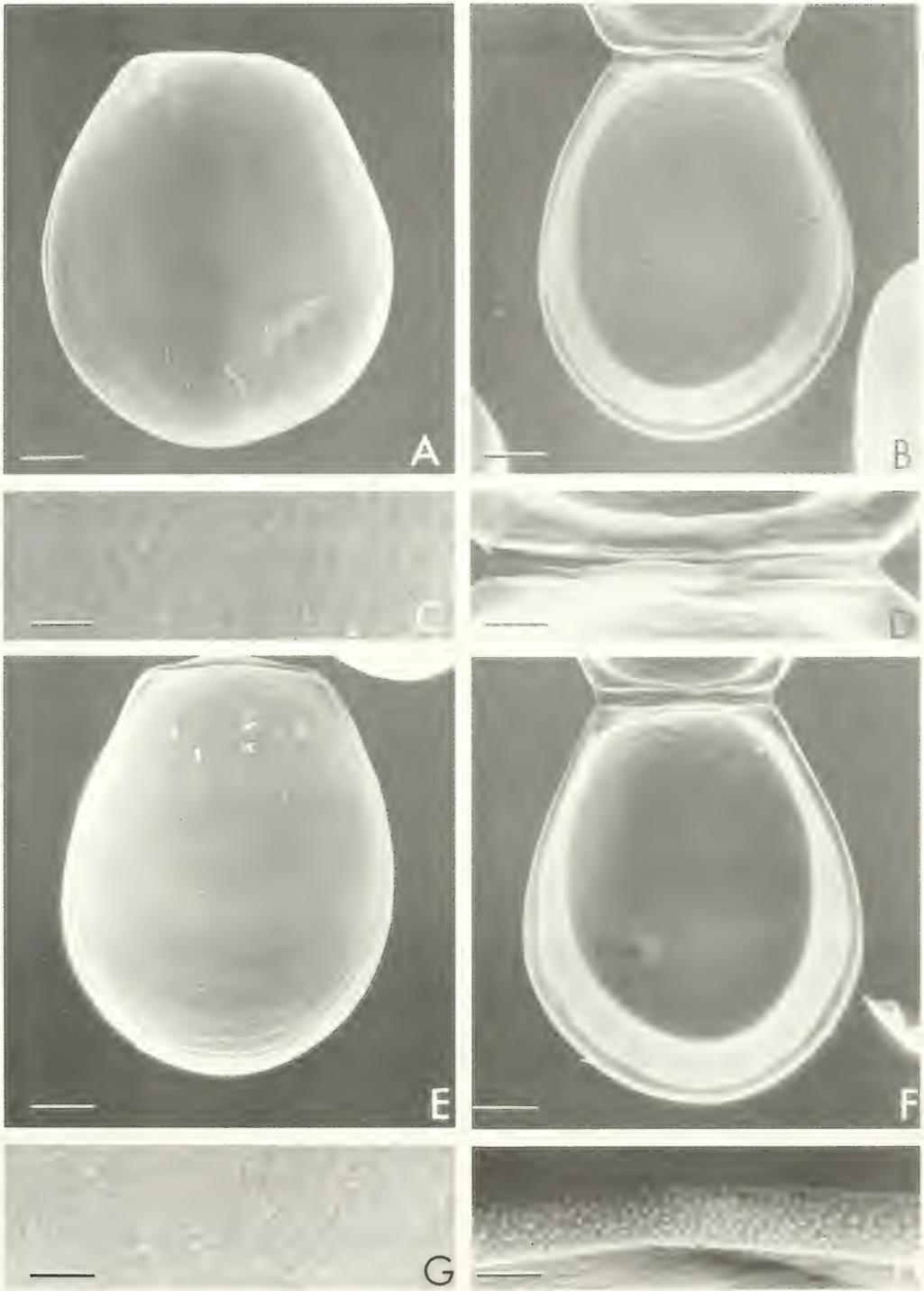


FIG. 36. Glochidium of *Ptychobranchus occidentalis* (A–D) and *Ptychobranchus greeni* (E–H); A. exterior valve OSUM 45361.17, bar length = 35 μ m; B. interior valve, OSUM 45361.14, bar length = 35 μ m; C. exterior valve sculpture, OSUM 45361.17, bar length = 1 μ m; D. hinge, OSUM 45361.17, bar length = 15 μ m; E. exterior valve, OSUM 19025.2, bar length = 35 μ m; F. interior valve, OSUM 19025.2, bar length = 35 μ m; G. exterior valve sculpture, OSUM 19025.2, bar length = 1 μ m; H. micropoints, OSUM 19025.2, bar length = 2 μ m.

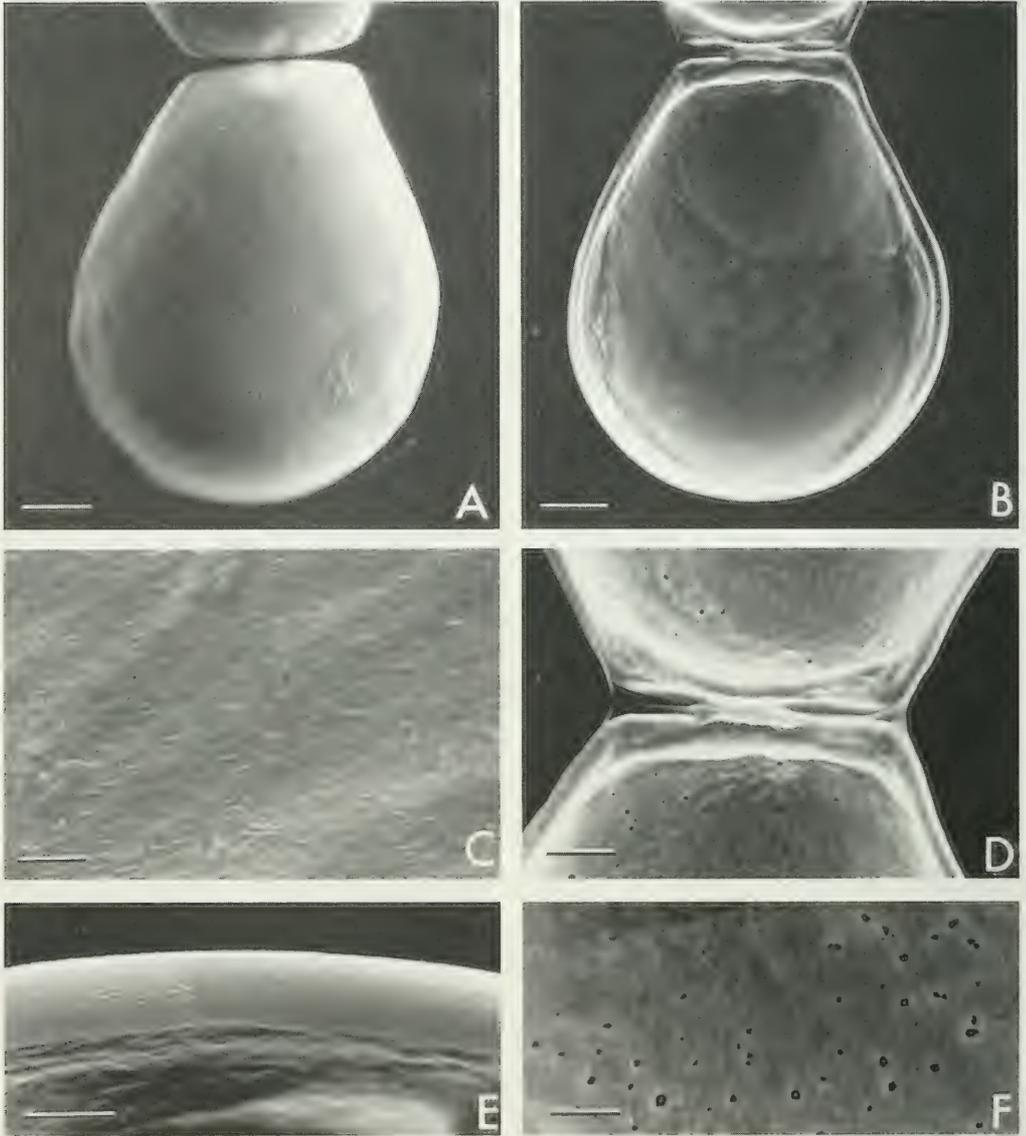


FIG. 37. Glochidium of *Ptychobranchus subtentum* OSUM 43156.5; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. exterior valve sculpture, bar length = 1 μ m; D. hinge, bar length = 20 μ m; E. micropoints, bar length = 5 μ m; F. interior valve pitting, bar length = 10 μ m.

5.76 μ m, $n = 5$). Dorsal margin slightly curved, 82 to 90 μ m ($85 \pm 2.88 \mu$ m, $n = 6$) in length. Anterior and posterior margins about straight to their maximum inflation at about 60% from dorsal to ventral, where they begin to curve to form a broadly rounded ventral margin. Valve surface sparsely pitted, loose-looped sculp-

ture covering exterior surface of valve (Fig. 37C). Adductor muscle scar present (Fig. 36B,D,F). Dorsal alae short, poorly developed. Central ligament 36 to 38 μ m ($37 \pm 1.15 \mu$ m, $n = 3$) in length, centered about 48% from posterior to anterior. Anterior ligament 25 to 26 μ m ($25 \pm 0.58 \mu$ m, $n = 3$) long; posterior

ligament 20 to 24 μm ($22 \pm 2.08 \mu\text{m}$, $n = 3$) in length. Micropoints small, numerous, unorganized, covering a large portion of ventral rim, leaving very narrow unsculptured distal margin.

Remarks

This glochidium can be distinguished by its relatively short hinge line, simple micropoints and small dorsal alae. Ortmann (1912) noted that the glochidium of *P. subtentum* is larger than that of *P. fasciolaris*. He gave 180 μm \times 220 μm for the length and height of *P. subtentum* glochidia. This glochidium is figured by Ortmann (1912: p1. 29, fig. 5).

Obliquaria reflexa Rafinesque, 1820
(Fig. 38A–D)

Material Examined

OSUM 54361.1 – Mississippi River, R.Mi. 564.5-566.1, about 3.5 mi. SW of Galena (IL), Jo Daviess/Jackson Co., Illinois/Iowa, 7–8 August 1979, R. B. Lewis et al.

Description

Glochidium subrotund, symmetrical, length 213 to 219 μm ($217 \pm 3.46 \mu\text{m}$, $n = 3$), height 206 to 221 μm ($215 \pm 8.14 \mu\text{m}$, $n = 3$). Dorsal margin slightly curved outward, 119 to 127 μm ($122 \pm 4.16 \mu\text{m}$, $n = 3$) in length. Lateral and ventral valve margins more or less round in outline, with maximum inflation of both side margins at about 50% from dorsal to ventral. Exterior surface malleated and pitted, except along valve margin, where shell fairly smooth. Within this smooth marginal area, longitudinal ridges present (Fig. 38C). Dorsal alae absent. Central ligament 52 to 54 μm ($53 \pm 1.41 \mu\text{m}$, $n = 2$) in length, centered about 46% from posterior to anterior. Anterior ligament 37 to 44 μm ($41 \pm 4.95 \mu\text{m}$, $n = 2$) long; posterior ligament 30 to 31 μm ($31 \pm 0.71 \mu\text{m}$, $n = 2$) in length. Large, bluntly pyramidal micropoints occurring on ventral rim and on narrow ventral flange, decreasing in size distally, arranged in broken vertical rows that extend two thirds length of flange, leaving a narrow unsculptured distal flange edge. Micropoints extending laterally to about point of maximum lateral inflation of valves.

Remarks

Lefevre & Curtis (1910, 1912), Surber (1912), and Ortmann (1912) gave the following measurements for this glochidium; 230 μm \times 225 μm , 225 μm \times 235 μm , and 220 μm \times 220 μm . Ortmann (1919) noted that the shape of this glochidium, "may best be compared with a circle a small section of which is cut off." This glochidium can be distinguished by its shape and micropoints. It was figured by Lefevre & Curtis (1910: fig. M, 1912: fig. 1N), Surber (1912: p1. 2, fig. 39), and Ortmann (1912: p1. 20, fig. 1).

Cyrogenia stegaria (Rafinesque, 1820)
(Fig. 39A–D)

Material Examined

OSUM 6298.21 – Green River at Munfordville, Hart Co., Kentucky, 22 October 1961, D. H. Stansbery.

Description

Glochidium elongate-oval, subrotund, length 204 to 208 μm ($206 \pm 2.08 \mu\text{m}$, $n = 3$), height 164 to 170 μm ($167 \pm 3.06 \mu\text{m}$, $n = 3$). Dorsal margin straight, long, with a length of 113 to 120 μm ($116 \pm 3.00 \mu\text{m}$, $n = 5$). Anterior and posterior margins curving greatly to create mirror images of each other, with maximum lateral inflation at about 50% from dorsal to ventral. Exterior surface smooth, lacking malleations, with only a few pits. As pointed out by Sterki (1898), concentric ridges occur near margins of valve. Valve disc smooth, except for loose-looped exterior valve sculpture (Fig. 39C). Dorsal alae absent. Central ligament 35 to 40 μm ($38 \pm 2.65 \mu\text{m}$, $n = 3$) in length, centered about 45% from posterior to anterior. Posterior ligament 30 to 33 μm ($32 \pm 1.53 \mu\text{m}$, $n = 3$) in length; anterior ligament 43 to 47 μm ($45 \pm 2.08 \mu\text{m}$, $n = 3$) long. Micropoints lanceolate, bluntly pointed, located on the ventral rim of valve. Ventral flange not observed.

Remarks

This glochidium can be distinguished from other species by its more broadly rounded valve outline. Sterki (1898), Surber (1912), and Ortmann (1912) gave the following mea-

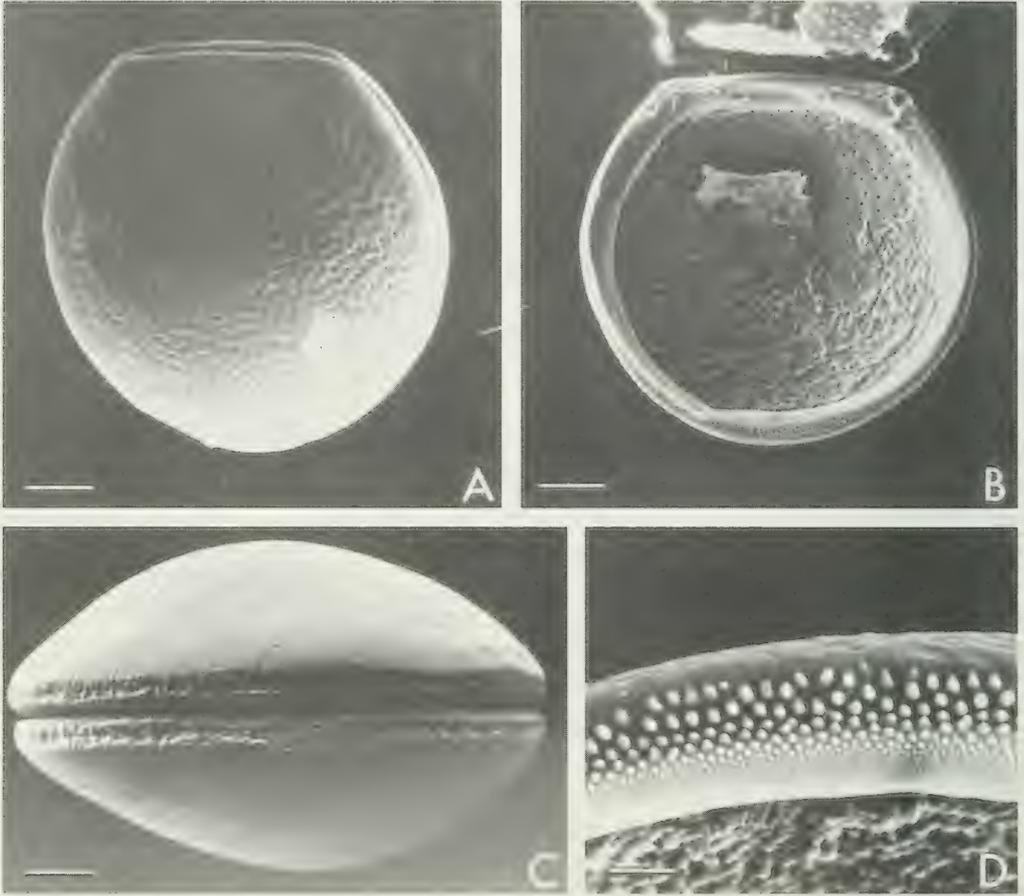


FIG. 38. Glochidium of *Obliquaria reflexa*, OSUM 54361.1; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. ventral view, bar length = 25 μ m; D. micropoints, bar length = 7 μ m.

measurements for length and height; 210 μ m \times 170 μ m, 210 μ m \times 185 μ m, and 180 μ m \times 150 μ m. This glochidium was figured by Ortmann (1911: p1. 19, fig. 6, as *C. irrorata*) and Surber (1912: p1. 1, fig. 11, as *C. irrorata*).

Cyrogenia aberti (Conrad, 1850)
(Fig. 39E-I)

Material Examined

OSUM - 48067 St. Francis River along Mo. Rt. E, 2.5 mi. NNE of French Mills, 11.5 mi. SW of Fredericktown, SE 1/4, Sec. 3, T32N, R5E, Madison Co., Missouri, 5 October 1977, W. L. Pflieger & T. Grace.

Description

Glochidium elongate oval, subrotund, length 200 to 218 μ m ($208 \pm 7.03 \mu$ m, $n = 6$), height 143 to 161 μ m ($154 \pm 7.79 \mu$ m, $n = 6$). Dorsal margin straight, 125 to 136 μ m ($131 \pm 4.18 \mu$ m, $n = 7$) long. Anterior and posterior margins equally curved to their maximum lateral inflation at about 50% from dorsal to ventral. Ventral margin flatly curved, dorsal alae absent. Central ligament 28 to 36 μ m ($31 \pm 3.11 \mu$ m, $n = 5$) long, centered about 46% from posterior to anterior. Anterior ligament 48 to 61 μ m ($54 \pm 6.04 \mu$ m, $n = 5$) in length; posterior ligament 41 to 49 μ m ($44 \pm 3.13 \mu$ m, $n = 5$) long. Micropoints lanceolate, bluntly

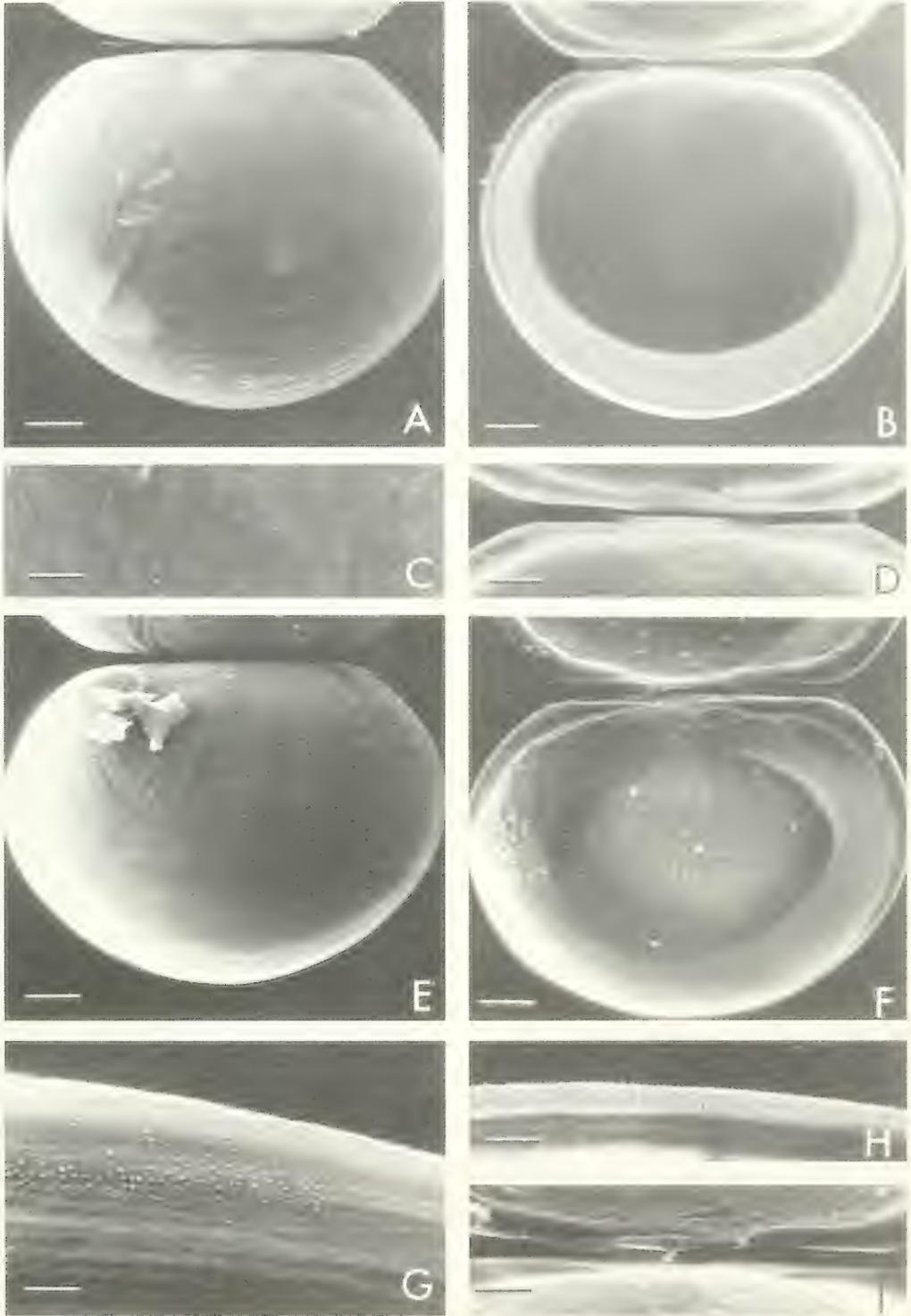


FIG. 39. Glochidium of *Cyprogenia stegaria* (A–E) OSUM 6298.21, and *Cyprogenia aberti* (F–I) OSUM 48067; A. exterior valve, bar length = 30 μm; B. interior valve, bar length = 30 μm; C. exterior valve sculpture, bar length = 2 μm; D. hinge, bar length = 20 μm; E. exterior valve, bar length = 30 μm; F. interior valve, bar length = 30 μm; G. micropoints, bar length = 5 μm; H. micropoints, bar length = 5 μm; I. hinge, bar length = 20 μm.

pointed, unorganized on ventral margin. Ventral flange only poorly developed.

Remarks

This glochidium is essentially like that of *C. stegaria*, although it is slightly more depressed than that species. The only other glochidium that approaches this shape is that of *Dromus dromas*. However, the glochidium of *D. dromas* would not be confused with either of these because of its extreme valve depression. No published figure of the glochidium of *C. aberti* was found.

Dromus dromas (Lea, 1834)
(Fig. 40A–E)

Material Examined

OSUM 20407.1 – Powell River 2.5 mi. ENE of Hoop, 11.5 mi. NE of Tazewell, Clairborne Co., Tennessee, 20 October 1968, G. F. Ahrens. OSUM 23200.9 – Powell River at Hoop ("Brooks Bridge"), 9.5 mi. NE of Tazewell, Clairborne Co., Tennessee, 19 October 1969, D. H. Stansbery et al.

Description

Glochidium fabelliform or bean-shaped, much longer than high, with a straight dorsal margin, narrowly rounded anterior and posterior margins, broadly curved ventral margin. Glochidium 219 to 230 μm ($224 \pm 5.09 \mu\text{m}$, $n = 5$) long, 114 to 120 μm ($118 \pm 2.49 \mu\text{m}$, $n = 5$) in height. Dorsal margin 160 to 182 μm ($174 \pm 8.65 \mu\text{m}$, $n = 6$) long. Anterior and posterior margins equal, with their points of maximum inflation occurring about 30% from dorsal to ventral. Exterior valve surface smooth, with pits only evident in internal view (Figs. 40B–D). Pits absent at valve margins, but present in central portion of valve (Fig. 40C). Dorsal alae absent. Central ligament 39 to 40 μm ($39 \pm 0.58 \mu\text{m}$, $n = 3$), centered at about 45% from posterior to anterior. Anterior ligament 76 to 82 μm ($79 \pm 3.06 \mu\text{m}$, $n = 3$) in length; posterior ligament 51 to 61 μm ($56 \pm 4.58 \mu\text{m}$, $n = 3$) in length. Numerous bluntly pointed micropoints present on valve margin. Ventral flange only poorly developed.

Remarks

This glochidium is easily distinguished by its shape. It represents the end of an evolutionary line from the nearly round glochidium of *O. reflexa*, through the progressively more depressed valve of *C. stegaria* and *C. aberti*, to the extremely depressed valves of this species. Surber (1912), Lefevre & Curtis (1912), and Ortmann (1912) gave 190 $\mu\text{m} \times 100 \mu\text{m}$ for length and height. This glochidium was figured by Surber (1912: p1. 1, fig. 13), Lefevre & Curtis (1912: fig. 1M) and Ortmann (1912: p1. 29, fig. 7).

Actinonaias pectorosa (Conrad, 1834)
(Fig. 41A–G)

Material Examined

A. pectorosa: OSUM 24337 – Middle Fork Holston River, 3.7 mi. S of Glade Spring at Va. Rt. 91 bridge (Craigs Bridge), Washington Co., Virginia, 16 September 1968, D. H. Stansbery & W. J. Clench. OSUM 48748.3 – Clinch River below footbridge at Slant, 7.1 mi. NNW of Gate City, 8.1 mi. ENE of Clinchport, Dekalb Twp., Scott Co., Virginia, 22 December 1980, C. C. Coney. *A. l. carinata*: MAH 842.1 – Fish Creek at bridge 2.0 mi. NW of Edgerton, 11.9 mi. W of Bryan, Sec. 20, T6N, R1E, St. Joseph Twp., Williams Co., Ohio, 29 October 1985, D. H. Stansbery et al.

Description

Glochidium subelliptical, nearly symmetrical, length 244 to 253 μm ($248 \pm 4.58 \mu\text{m}$, $n = 3$), height 260 to 270 μm ($267 \pm 5.77 \mu\text{m}$, $n = 3$). Dorsal margin straight, 139 to 151 μm ($144 \pm 5.20 \mu\text{m}$, $n = 3$) in length. Anterior and posterior margins gently and evenly curving; ventral margin semicircular. Exterior surface of valve malleated and pitted, except at its margin, where longitudinal ridges occur (Fig. 41C, D). Dorsal alae well developed, about 38 μm in length (Fig. 41A, C). Beaded exterior valve sculpture covering surface of valve (Fig. 41G). Central ligament 58 to 62 μm ($60 \pm 3.21 \mu\text{m}$, $n = 3$) in length, centered about 46% from posterior to anterior. Anterior ligament 46 to 52 μm ($48 \pm 3.21 \mu\text{m}$, $n = 3$) long; posterior ligament 31 to 39 μm ($35 \pm 3.61 \mu\text{m}$, $n = 3$) in length. Micropoints, lanceolate, located on ventral margin and on a narrow ventral flange.

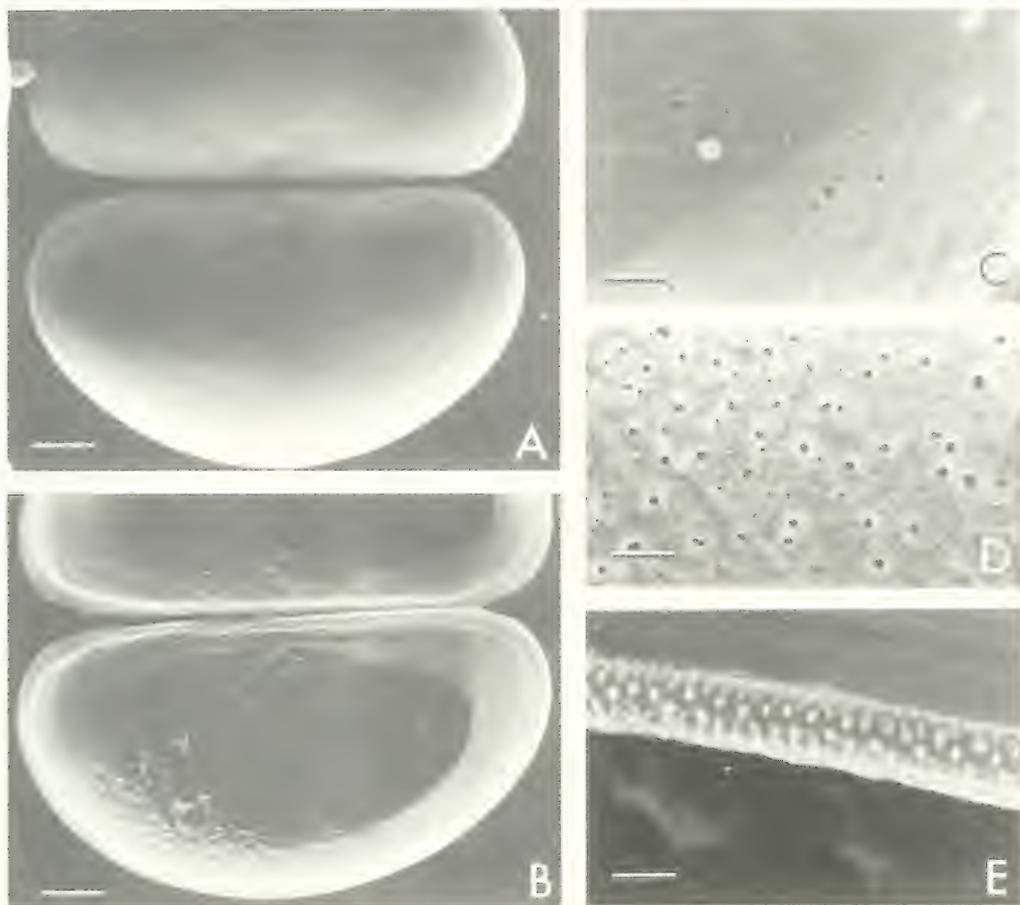


FIG. 40. Glochidium of *Dromus dromas*; A. exterior valve, OSUM 20407.1, bar length = 30 μ m; B. interior valve, OSUM 20407.1, bar length = 30 μ m; C. interior valve pitting, OSUM 23200.9, bar length = 5 μ m; D. interior valve pitting, OSUM 20407.1, bar length = 3 μ m; E. micropoints, OSUM 23200.9, bar length = 2 μ m.

Ventral flange slightly produced centrally to give the abducted valve a beak-like appearance (Fig. 41C, D); this region of flange densely covered with micropoints and probably facilitates attachment by digging deeply into host tissue.

Remarks

The glochidium of *A. l. carinata* is nearly identical to that of *A. pectorosa*, except it is smaller. A single glochidium of *A. l. carinata* gave the following measurements: length, 220 μ m; height, 243 μ m; hinge length, 125 μ m; anterior ligament length, 38 μ m; central ligament length, 57 μ m; and posterior ligament length, 30 μ m. Ortmann (1912) gave 220 μ m \times 240 μ m

for length and height measurements for *A. ligamentina* and 250 μ m \times 290 μ m for *Nephronajas pendix* (= *A. pectorosa*, *vide* Ortmann & Walker, 1922). The glochidium of *A. ligamentina* was figured by Lea (1858: p1. 5, fig. 18), Ortmann (1911: p1. 89, fig. 16), and Surber (1912: p1. 2, fig. 18). The glochidium of *A. pectorosa* was figured by Ortmann (1912: p1. 19, fig. 12, as *N. pendix*).

Obovaria retusa (Lamarck, 1819)
(Fig. 42A–D)

Material Examined

UMMZ Uncataloged Tennessee River (Kentucky Lake), U.S. Rt. 70 bridge, New John-

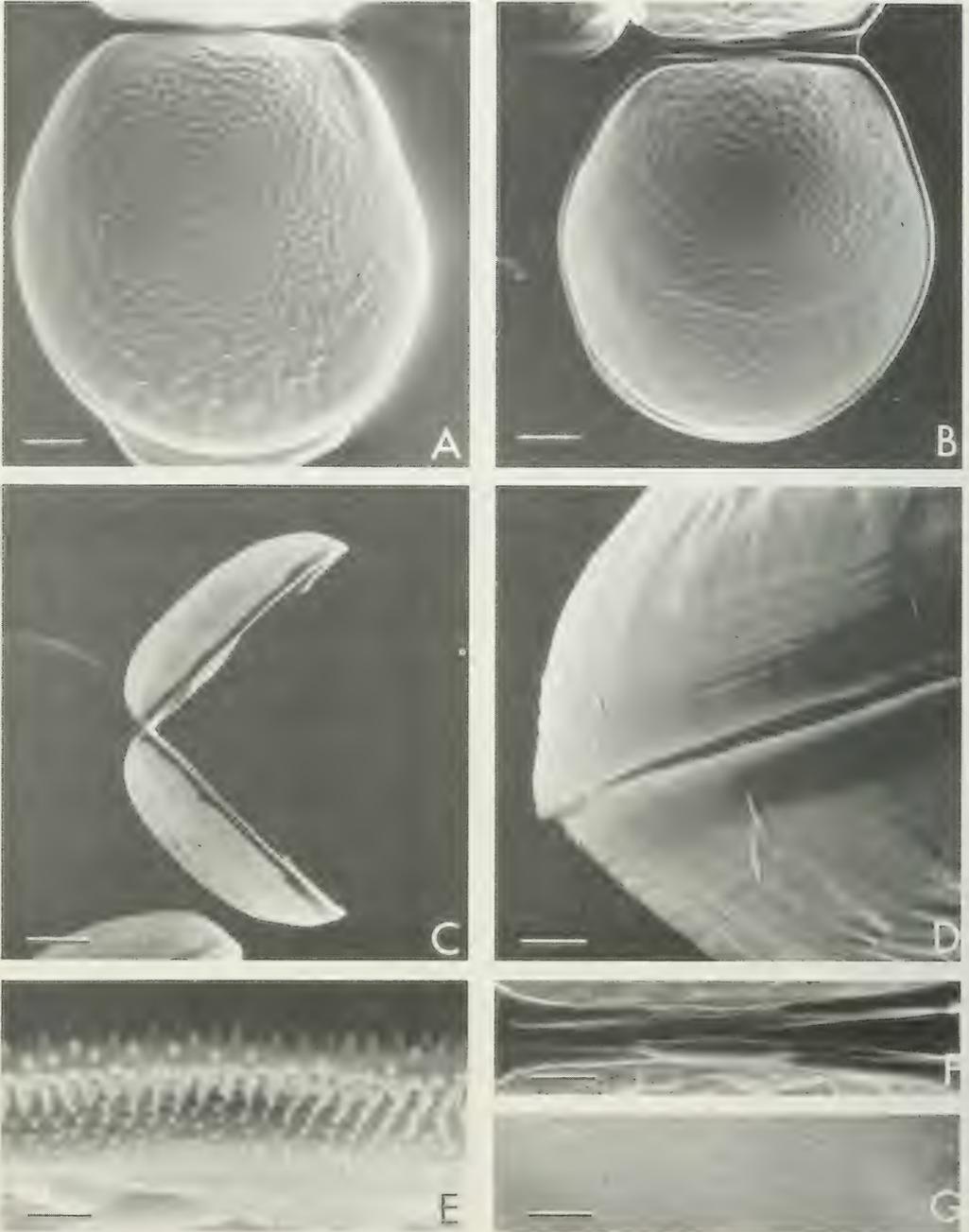


FIG. 41. Glochidium of *Actinonaias pectorosa*; A. exterior valve, OSUM 48748.3, bar length = 35 μm ; B. interior valve, OSUM 48748.3, bar length = 40 μm ; C. lateral view, OSUM 24337, bar length = 60 μm ; D. micropoints, OSUM 24337, bar length = 5 μm ; E. micropoints, OSUM 24337, bar length = 2 μm ; F. hinge, OSUM 48748.3, bar length = 20 μm ; G. exterior valve sculpture, OSUM 48748.3, bar length = 1 μm .

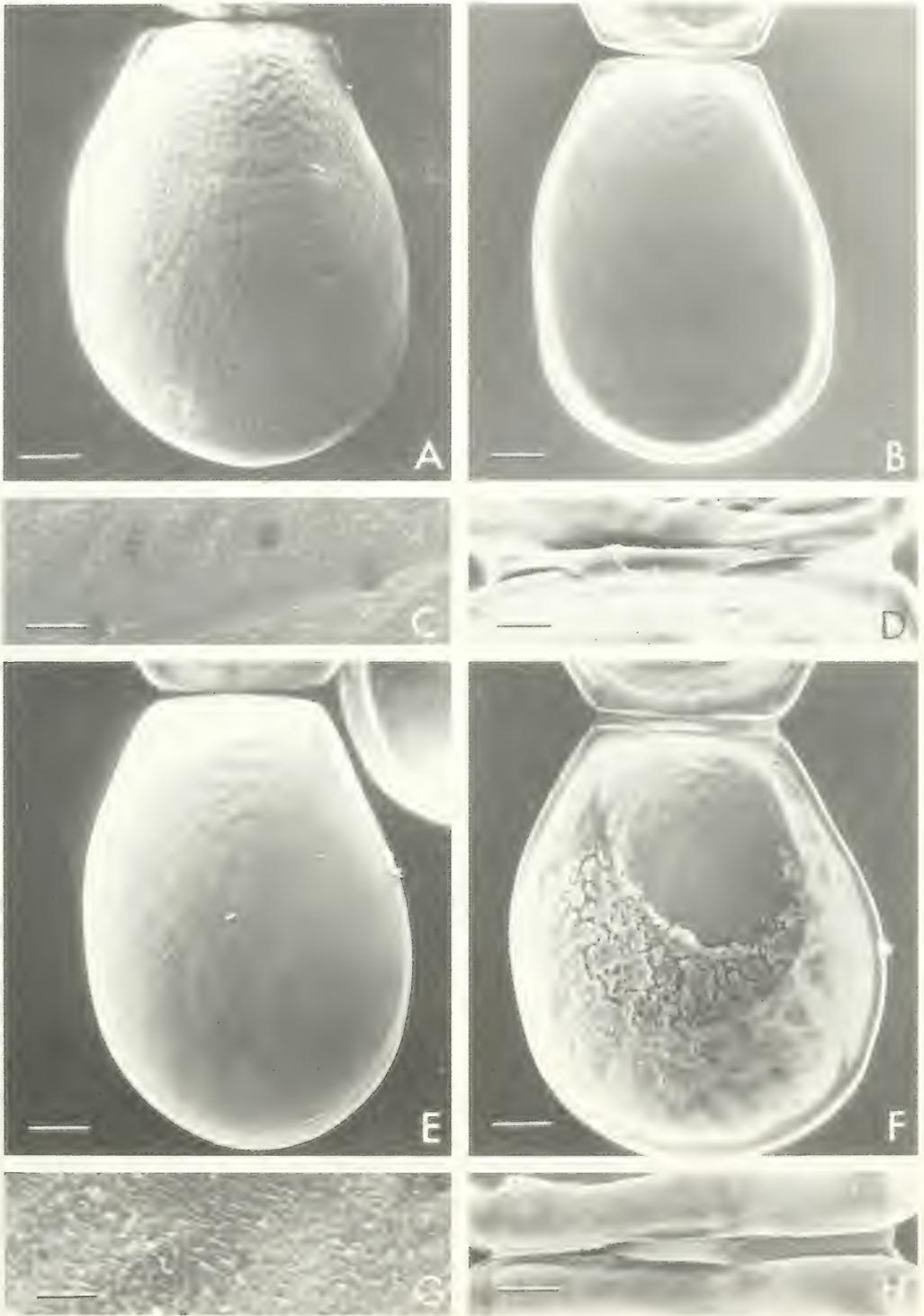


FIG. 42. Glochidium of *Obovaria retusa* UMMZ Uncataloged (A–D) and *Obovaria olivaria* OSUM 51282.2 (E–H); A. exterior valve, bar length = 40 μ m; B. interior valve, bar length = 40 μ m; C. exterior valve sculpture, bar length = 1 μ m; D. hinge, bar length = 20 μ m; E. exterior valve, bar length = 40 μ m; F. interior valve, bar length = 40 μ m; G. exterior valve sculpture, bar length = 1 μ m; H. hinge, bar length = 15 μ m.

sonville, Humphreys Co., Tennessee, October 1958, J. Bates.

Description

Glochidium subelliptical, slightly asymmetric, length 218 to 223 μm ($221 \pm 3.54 \mu\text{m}$, $n = 2$), height 272 to 278 μm ($275 \pm 4.24 \mu\text{m}$, $n = 2$). Dorsal margin slightly curved, 115 to 119 μm ($117 \pm 2.08 \mu\text{m}$, $n = 3$) in length. Dorsal half of posterior margin straight, oblique to the dorsal line. Ventral half of posterior margin gently curved initially, then straight to run more or less perpendicular to dorsal line. Anterior margin gently curved throughout its length, with maximum lateral inflation occurring at about 70–80% from dorsal to ventral. Ventral margin semicircular in outline. Exterior valve surface finely malleated and evenly pitted, except at valve margin, where surface is smooth. Dorsal alae about 39 μm in length. Beaded to loose-looped sculpture covering exterior surface of valve (Fig. 42C). Central ligament about 45 μm in length, centered at about 45% from posterior to anterior. Posterior ligament about 36 μm long; anterior ligament about 38 μm long. Micropoints lanceolate, arranged in broken vertical rows on ventral rim of valve and on narrow ventral flange.

Remarks

Surber (1912) gave measurements for length and height of 240 $\mu\text{m} \times 295 \mu\text{m}$, whereas Ortmann (1912) gave 220 $\mu\text{m} \times 270 \mu\text{m}$. This rather wide discrepancy is not addressed by either author and demonstrates the difficulty of trying to determine the species of a glochidium based solely on size. In the case of *O. retusa*, the moderately sized dorsal alae and valve shape distinguish the glochidium from species other than those of *Obovaria*. Its beaded to loose-looped sculpture distinguish this species from the other members of the genus. This glochidium was figured by Lea (1858: pl. 5, fig. 7), Surber (1912: pl. 3, fig. 47), and Ortmann (1912: pl. 19, fig. 9). Lea's figure shows the anterior and posterior margins evenly curved, whereas Surber's figure shows the correct outline.

Obovaria olivaria (Rafinesque, 1820)
(Fig. 42E–H)

Material Examined

OSUM 51282.2 Mississippi River, R.Mi. 634.7, at U.S. Rt. 18 bridge, main channel,

1.5 mi. W of Prairie du Chien, Crawford Co., Wisconsin, 15 May 1981, M. E. Havlik et al.

Description

Glochidium subelliptical, slightly asymmetric, length 198 to 206 μm ($202 \pm 4.00 \mu\text{m}$, $n = 3$), height 254 to 261 μm ($258 \pm 3.51 \mu\text{m}$, $n = 3$). Dorsal margin slightly curved, 101 to 118 μm ($109 \pm 5.81 \mu\text{m}$, $n = 5$) long. As in *O. retusa*, posterior margin is mostly straight sided, with a bend about half the distance from dorsal to ventral. Anterior margin gently curved; ventral margin semicircular. Dorsal alae well developed, about 37 μm in length. Loose-looped sculpture covering exterior surface of valve (Fig. 42G). Central ligament 34 to 44 μm ($39 \pm 5.03 \mu\text{m}$, $n = 3$) in length, centered about 45% from posterior to anterior. Posterior ligament 26 to 29 μm ($27 \pm 1.53 \mu\text{m}$, $n = 3$) long; anterior ligament 32 to 43 μm ($39 \pm 5.86 \mu\text{m}$, $n = 3$) long. Micropoints lanceolate, located on ventral rim and on narrow ventral flange.

Remarks

This glochidium is very similar to that of *O. retusa*. However, it is smaller and it has a different exterior valve sculpture. This glochidium was figured by Surber (1912: pl. 2, fig. 25, as *O. ellipsis*, = *O. olivaria*, *fide* Ortmann & Walker, 1922) and Ortmann (1912: pl. 19, fig. 11). Surber gave length and height measurements of 210 $\mu\text{m} \times 265 \mu\text{m}$ and Ortmann (1912) recorded length and height measurements of 190 $\mu\text{m} \times 220 \mu\text{m}$. Ortmann's figures are considerably smaller than Surber's or mine.

Obovaria subrotunda (Rafinesque, 1820)
(Fig. 43A–E)

Material Examined

MAH 659.2 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. NW of Circleville, Scioto/Darby Twp., Pickaway Co., Ohio, 18 May 1984, M. A. Hoggarth & G. T. Watters. MAH 805.1 – Fish Creek at Co. Rt. 49 bridge, 0.4 mi. above its mouth, 1.1 mi. N of Edgerton, 10.4 mi. W of Bryan, St. Joseph Twp., Williams Co., Ohio, 29 October 1985, D. H. Stansbery et al.

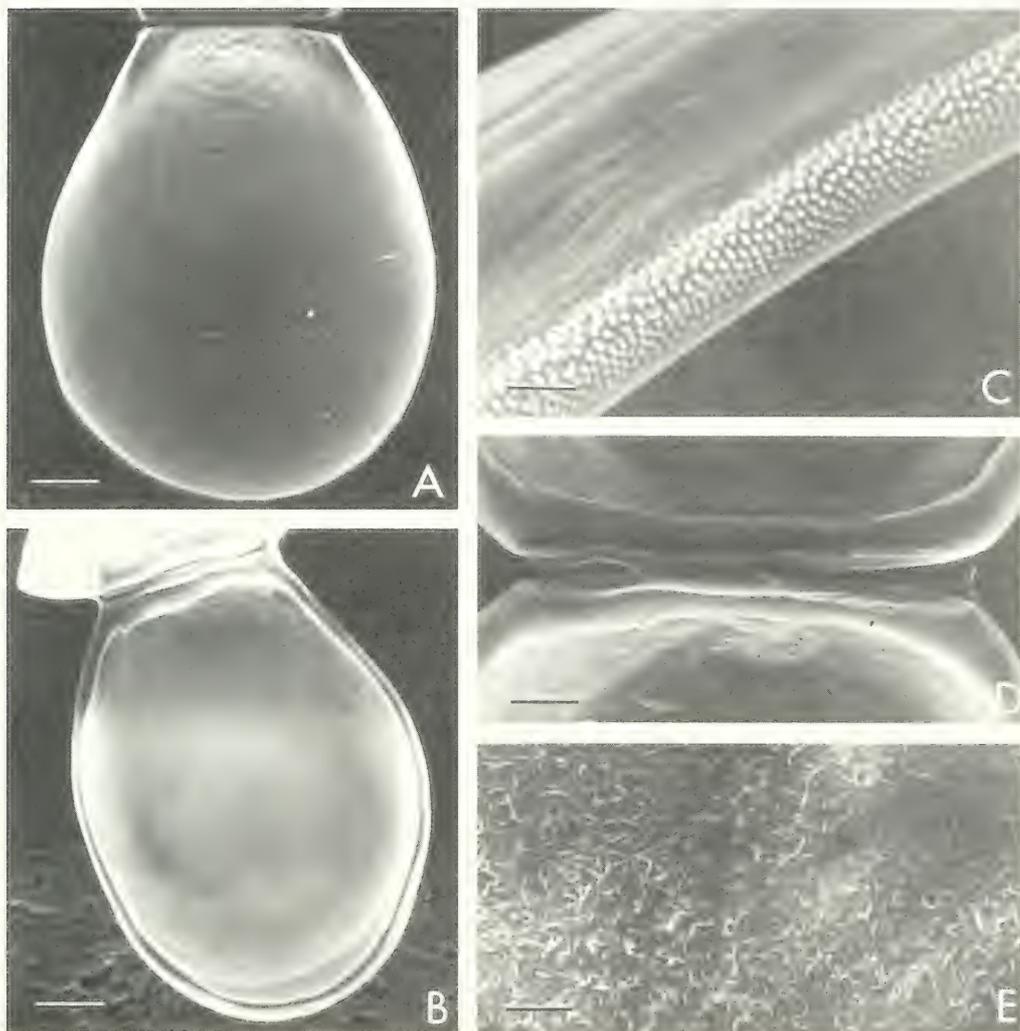


FIG. 43. Glochidium of *Obovaria subrotunda*; A. exterior valve, MAH 805.1, bar length = 30 μm ; B. interior valve, MAH 805.1, bar length = 35 μm ; C. micropoints, MAH 805.1, bar length = 3 μm ; D. hinge, MAH 659.2, bar length = 15 μm ; E. exterior valve sculpture, MAH 805.1, bar length = 1 μm .

Description

Glochidium subelliptical, length 174 to 180 μm ($177 \pm 2.70 \mu\text{m}$, $n = 5$), height 197 to 210 μm ($204 \pm 5.63 \mu\text{m}$, $n = 5$). Dorsal margin slightly curved, 85 to 95 μm ($91 \pm 3.50 \mu\text{m}$, $n = 6$) in length. Posterior margin straight, oblique dorsally; ventral portion of posterior margin curved. Anterior margin gently curved throughout its length, with maximum lateral inflation at about 60% from dorsal to ventral. Ventral margin broadly rounded. Dorsal alae about 29 μm in length, and loose-looped valve sculpture covering exterior surface of the

valve (Fig. 43E). Central ligament 40 to 43 μm ($42 \pm 2.12 \mu\text{m}$, $n = 2$) in length, centered about 48% from posterior to anterior. Anterior ligament 24 to 29 μm ($27 \pm 3.54 \mu\text{m}$, $n = 2$) long; posterior ligament 21 to 25 μm ($23 \pm 2.83 \mu\text{m}$, $n = 2$) in length. Micropoints lanceolate, arranged in broken vertical rows on ventral rim and on short ventral flange. Unsculptured distal flange margin narrow.

Remarks

Surber (1915) described this glochidium as, "semielliptical in shape; ventral margin

rounded; hinge line long and slightly depressed near center; size medium." He suggested that this glochidium is intermediate between those of *O. retusa* and *O. olivaria* but differs from both by its smaller size. This study supports Surber's observations. This glochidium shares moderately long dorsal alae with *O. retusa*, and moderately dense loose-looped sculpture with *O. olivaria*. Ortmann (1912) gave $200\ \mu\text{m} \times 230\ \mu\text{m}$ for length and height, whereas Surber (1915) gave $170\ \mu\text{m} \times 215\ \mu\text{m}$. This glochidium was figured by Ortmann (1911: pl. 89, fig. 15, as *O. circulus*, = *O. subrotunda*, *vide* Ortmann & Walker, 1922), and Surber (1915: pl. 1, fig. 8, as *O. circulus*).

Obovaria jacksoniana (Frierson, 1912)
(Fig. 44A–D,G)

Material Examined

OSUM 50233.8 – Sipsey River below Rt. 21 bridge, 1.3 mi. W of Brownville, 16.8 mi. NW of Tuscaloosa, Tuscaloosa Co., Alabama, 10 October 1981, L. M. Koch.

Description

Glochidium subelliptical, almost symmetrical, length 175 to 187 μm ($182 \pm 5.74\ \mu\text{m}$, $n = 4$), height 230 to 243 μm ($236 \pm 6.56\ \mu\text{m}$, $n = 4$). Dorsal margin slightly curved, 89 to 100 μm ($95 \pm 4.57\ \mu\text{m}$, $n = 4$) long. Posterior and anterior margins about equal, except posterior margin slightly more produced as a result of a larger angle of divergence. Posterior margin curving ventrally at about 50% from dorsal to ventral, becoming almost perpendicular to dorsal margin, anterior margin gently rounded. Exterior surface smooth with just a few pits and very little malleation, except at the umbo. Loose-looped sculpture covering exterior surface (Fig. 44C). Dorsal alae with a length of about 34 μm . Central ligament 41 to 42 μm ($42 \pm 0.71\ \mu\text{m}$, $n = 2$) in length, centered 46% from posterior to anterior. Anterior ligament 26 to 31 μm ($29 \pm 3.54\ \mu\text{m}$, $n = 2$) long; posterior ligament 21 to 25 μm ($23 \pm 2.83\ \mu\text{m}$, $n = 2$) long. Micropoints lanceolate, located on ventral rim of valve and on short ventral flange.

Remarks

This glochidium is very similar to the other members of the genus. It is intermediate in size between *O. olivaria* and *O. subrotunda*, and much smaller than *O. retusa*. No published figure of this glochidium was found.

Obovaria unicolor (Lea, 1845)
(Fig. 44E, F, H)

Material Examined

OSUM 33158 – Sabine River at La. Rt. 8 and Tex. Rt. 3 bridge, 1.3 mi. W of Burr Ferry, Vernon Parish/Newton Co., Louisiana/Texas, 14 July 1968, D. H. Stansbery et al. OSUM 47696.6–Pearl River, 0.8 mi. SSE of Drysdale, 6.7 mi. SW of Carthage, Sec. 25/36, T10N, R6E, Lake Co., Mississippi, 5 October 1979, R. G. Rummel & P. Hartfield.

Description

Glochidium subelliptical, almost symmetrical, length 168 to 180 μm ($172 \pm 4.36\ \mu\text{m}$, $n = 5$), height 218 to 234 μm ($225 \pm 5.86\ \mu\text{m}$, $n = 5$). Dorsal line slightly curved, 85 to 96 μm ($90 \pm 4.22\ \mu\text{m}$, $n = 6$) long. Anterior and posterior margins about equal, straight sided dorsally, slightly curved ventrally, as in *O. jacksoniana*. Ventral margin broadly rounded. Exterior surface mostly smooth, with malleations at umbo and only a few pits. Loose-looped sculpture covering exterior surface of valve Dorsal alae about 32 μm in length. Central ligament 32 to 33 μm ($32 \pm 0.58\ \mu\text{m}$, $n = 3$) in length, centered about 45% from posterior to anterior. Anterior ligament 32 to 41 μm ($35 \pm 4.62\ \mu\text{m}$, $n = 3$) long; posterior ligament 23 to 28 μm ($25 \pm 2.65\ \mu\text{m}$, $n = 3$) long. Micropoints lanceolate, arranged in incomplete vertical rows, located on ventral rim of valve and on short ventral flange.

Remarks

This glochidium is essentially identical to that of *O. jacksoniana*. My measurements would indicate that the glochidium of *O. unicolor* is smaller than that of *O. jacksoniana*, but even here there is overlap. This glochidium is figured by Ortmann (1912: pl. 19, fig. 10), who gave length and height measurements of $160\ \mu\text{m} \times 210\ \mu\text{m}$.

Ellipsaria lineolata (Rafinesque, 1820)
(Fig. 45A–F)

Material Examined

OSUM 1984: 14 – Ohio River, R.Mi. 443.0–445.0, from 0.4 mi. NW of Moscow, Ohio, to Point Pleasant, Ohio, 5.0–6.8 mi. SE of New Richland, Ohio, Pendleton/Campbell Co.,

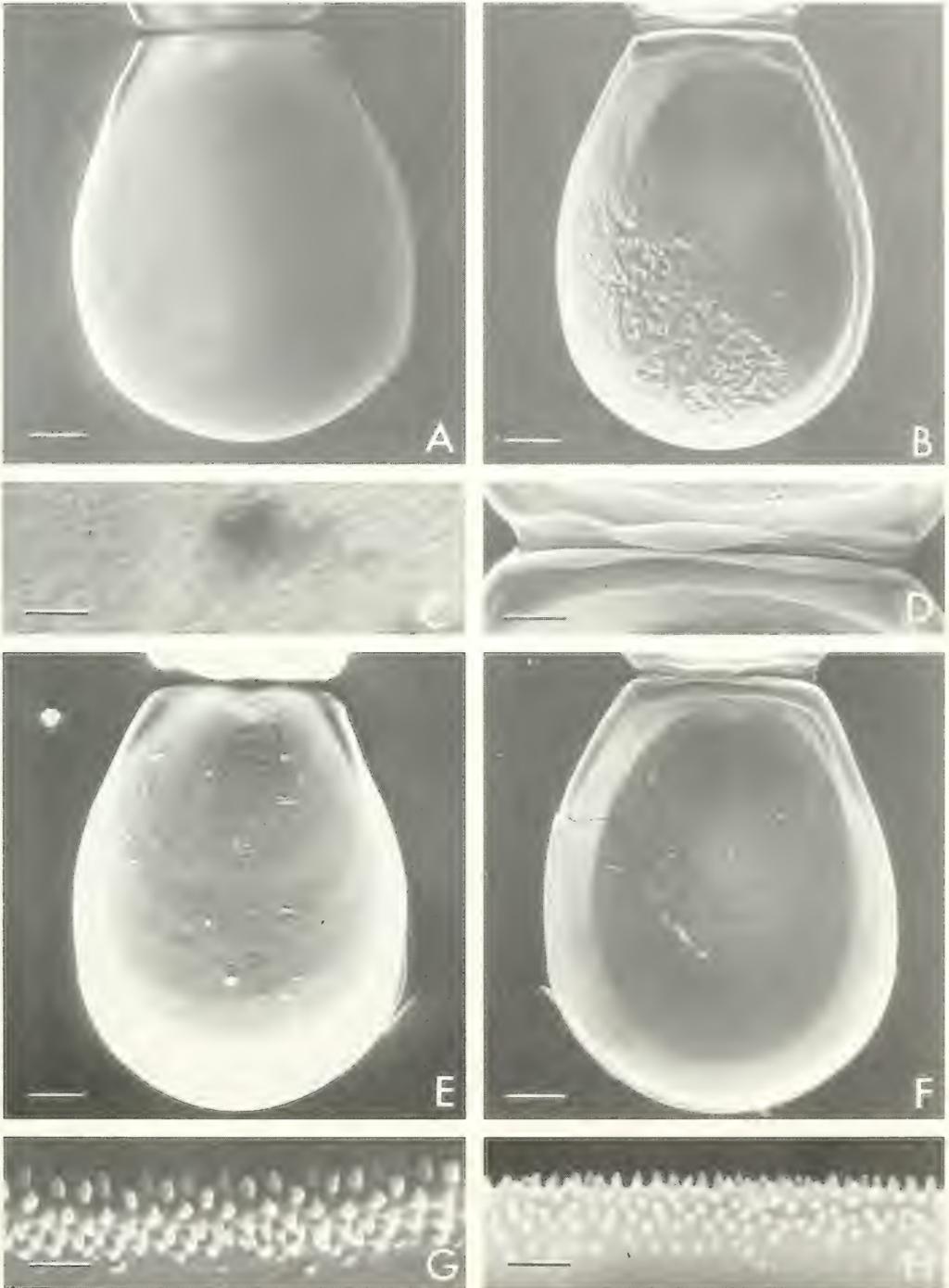


FIG. 44. Glochidium of *Obovaria jacksoniana* (A–D, G) OSUM 50233.8 and *Obovaria unicolor* (E, F, H): A. exterior valve, bar length = 30 μ m; B. interior valve, bar length = 30 μ m; C. exterior valve sculpture, bar length = 1 μ m; D. hinge, bar length = 15 μ m; E. exterior valve, OSUM 33158, bar length = 30 μ m; F. interior valve, OSUM 33158, bar length = 30 μ m; G. micropoints, OSUM 33158, bar length = 2 μ m; H. micropoints, OSUM 47696.6, bar length = 3 μ m.

Kentucky, 16 November 1984, D. H. Stansbery et al.

Description

Glochidium subligulate, much higher than long, length 229 to 245 μm ($237 \pm 5.06 \mu\text{m}$, $n = 7$), height 310 to 325 μm ($321 \pm 5.15 \mu\text{m}$, $n = 7$). Dorsal margin slightly curved, 87 to 96 μm ($91 \pm 3.55 \mu\text{m}$, $n = 7$) long. Anterior and posterior margins more or less straight to slightly incurved dorsally. At about 40% from dorsal to ventral, margins again becoming straight, slightly divergent. Point of maximum lateral inflation at about 80% from dorsal to ventral. Ventral margin broad, over twice length of hinge margin, but more narrowly curved than found in *Obovaria*, *Actinonaias* or *Ptychobranthus*. External valve surface finely malleated and evenly pitted, except along valve margins, where pits and malleations are absent (Fig. 45C). Vermiculate sculpture covering exterior surface of valve (Fig. 45F); dorsal alae very small and oriented at a 45° angle to hinge. Central ligament 35 to 51 μm ($43 \pm 8.42 \mu\text{m}$, $n = 4$) long, centered about 48% from posterior to anterior. Posterior ligament 20 to 24 μm ($22 \pm 1.63 \mu\text{m}$, $n = 4$) long; anterior ligament 23 to 29 μm ($26 \pm 2.75 \mu\text{m}$, $n = 4$) in length. Micropoints lanceolate, arranged in complete vertical rows on ventral valve margin and on wide ventral flange, decreasing in size distally, covering proximal two thirds of ventral flange, leaving a narrow unsculptured distal flange margin. The ventral flange becomes folded laterally so that it forms small hook-like points (Fig. 45C).

Remarks

Lefevre & Curtis (1910), Surber (1912), and Ortmann (1912) gave the following measurements for length and height: 230 $\mu\text{m} \times 310 \mu\text{m}$, 230 $\mu\text{m} \times 330 \mu\text{m}$, and 260 $\mu\text{m} \times 350 \mu\text{m}$. This glochidium can be distinguished from all others by its fan-shaped outline, small dorsal alae, exterior valve sculpture and its distinct hook-like folding of the ventral flange. This glochidium is figured by Lea (1858: pl. 5, fig. 6, as *Unio securis*, = *E. lineolata*, *fide* Ortmann & Walker, 1922), Lefevre & Curtis (1910: fig. H, 1912: fig. 1H, as *Plagiola securis*), Ortmann (1911: pl. 89, fig. 17, as *P. securis*), and Surber (1912: pl. 2, fig. 14, as *P. securis*).

Leptodea fragilis (Rafinesque, 1820)
(Fig. 46A–E)

Material Examined

MAH 626.1 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp., Pickaway Co., Ohio, 13 April 1984, M. A. Hoggarth & G. T. Watters.

Description

Glochidium subelliptical, very small, length 72 to 73 μm ($72 \pm 0.58 \mu\text{m}$, $n = 3$), height 80 to 83 μm ($83 \pm 1.53 \mu\text{m}$, $n = 3$). Dorsal margin straight, 30 to 35 μm ($33 \pm 2.89 \mu\text{m}$, $n = 3$) long. Anterior and posterior margins about equally divergent dorsally, ventrally curved. Ventral margin broadly rounded, joining lateral margins at their point of maximum inflation, near 70% from dorsal to ventral. Exterior surface only malleated near umbo; however, valve surface otherwise densely pitted, except along valve margin (Fig. 46A, C) and at adductor muscle insertion (Fig. 46A, B). Exterior surface lightly covered with loose-looped valve sculpture (Fig. 46E); dorsal alae very small. When fully adducted, valves gape in lateral view (Fig. 46C). Central ligament about 11 μm long, centered about 45% from posterior to anterior. Posterior ligament 8 to 10 μm ($9 \pm 1.41 \mu\text{m}$, $n = 2$) in length; anterior ligament 11 to 14 μm ($13 \pm 2.12 \mu\text{m}$, $n = 2$) long. Micropoints wide, lamellate plates arranged in complete vertical rows on ventral valve margin and on narrow ventral flange. Unsculptured distal flange margin narrow.

Remarks

This glochidium will not be confused with any other. Its size, round subelliptical shape, small dorsal alae, and lamellate micropoints distinguish it from all others. Lefevre & Curtis (1910, 1912), Surber (1912), and Ortmann (1912) gave the following measurements for length and height: 75 $\mu\text{m} \times 85 \mu\text{m}$, 70 $\mu\text{m} \times 95 \mu\text{m}$, and 80 $\mu\text{m} \times 90 \mu\text{m}$. This glochidium is figured by Lefevre & Curtis (1910: fig. K; 1912: fig. 1K, as *Lampsilis gracilis*, = *L. fragilis*, *fide* Ortmann & Walker, 1922), Ortmann (1911: pl. 89, fig. 19, as *Paraptera gracilis*), Coker & Surber (1911: pl. 1, fig. 2, 2a, as *L. gracilis*), and Surber (1912: pl. 2, fig. 28, as *L. gracilis*).

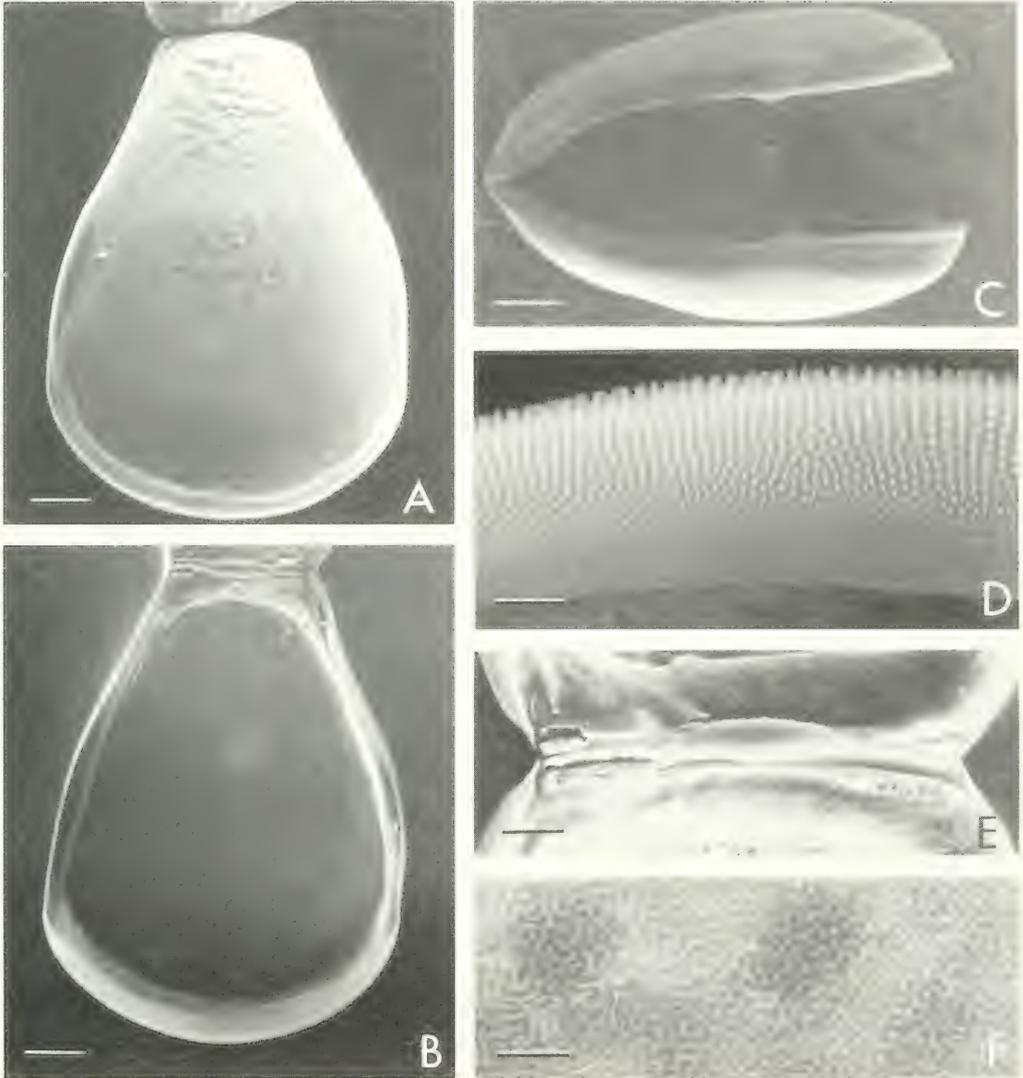


FIG. 45. Glochidium of *Ellipsaria lineolata*. OSUM:1984:14; A. exterior valve, bar length = 45 μm ; B. interior valve, bar length = 45 μm ; C. lateral view, bar length = 35 μm ; D. micropoints, bar length = 5 μm ; E. hinge, bar length = 15 μm ; F. exterior valve sculpture, bar length = 1 μm .

Leptodea ochracea (Say, 1817)
(Fig. 47 A-E)

Material Examined

MAH 896 – Great Herring Pond at Carter's Brook Rd., 4.0 mi. NNE of Buzzards Bay, 3.0 mi. NW of Sagamore, Plymouth Co., Massachusetts, 31 July 1985, G.T. Watters et al.

Description

Glochidium subelliptical to subligulate, length 241 to 246 μm ($243 \pm 1.95 \mu\text{m}$, $n = 5$), height 289 to 294 μm ($291 \pm 1.92 \mu\text{m}$, $n = 5$). Dorsal margin straight, short, 102 to 110 μm ($107 \pm 3.90 \mu\text{m}$, $n = 6$) in length. Anterior and posterior margins equal, more or less straight to slightly incurved dorsally, bending ventrally

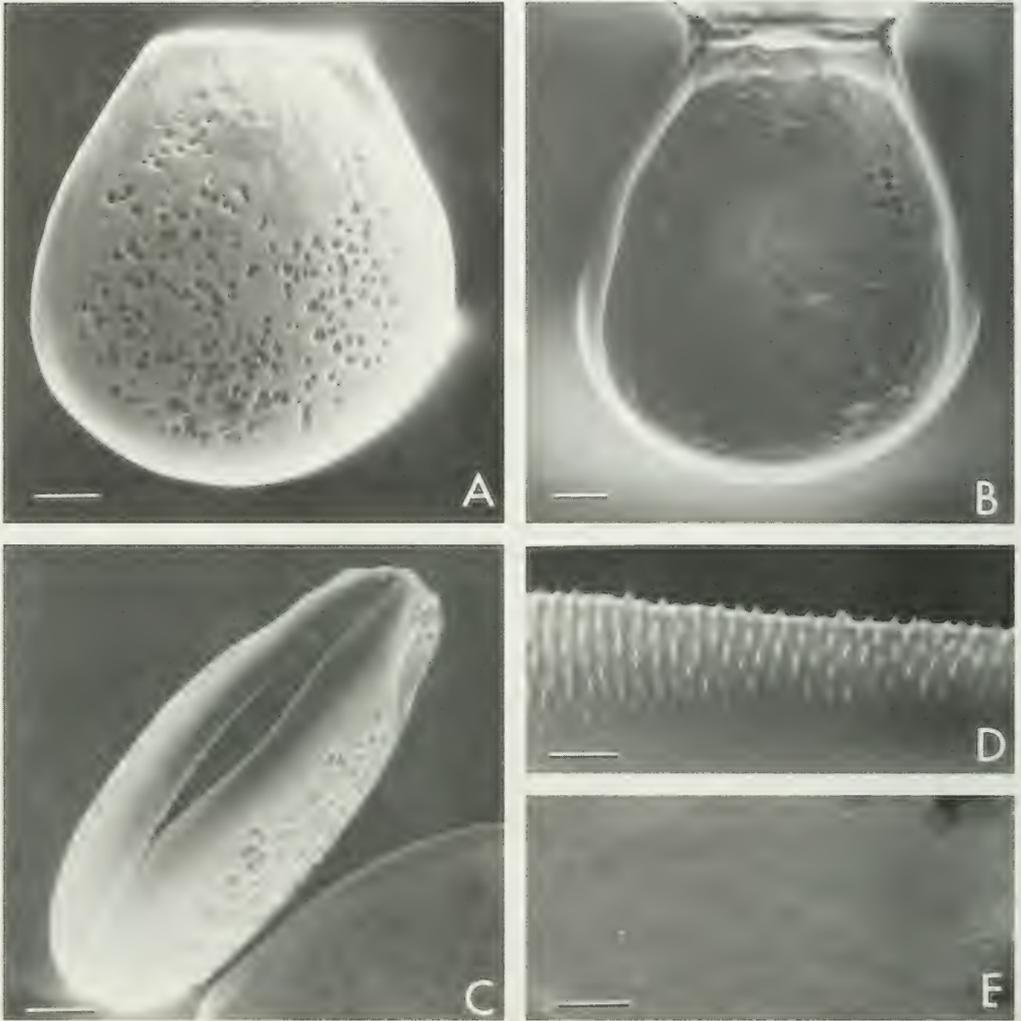


FIG. 46. Glochidium of *Leptodea fragilis*, MAH 626.1; A. exterior valve, bar length = 10 μ m; B. interior valve, bar length = 10 μ m; C. lateral view, bar length = 10 μ m; D. micropoints, bar length = 2 μ m; E. exterior valve sculpture, bar length = 1 μ m.

about 50% from dorsal margin, slightly curved to their maximum inflation at about 80% from dorsal to ventral. Ventral margin gently and evenly curved throughout its length. Exterior surface malleated and pitted, except along valve margins where longitudinal ridges occur (Fig. 47A, C). Small dorsal alae occur at dorsal lateral margin of valve. Lateral valve gape moderate. Central ligament 39 to 47 μ m ($43 \pm 3.65 \mu$ m, $n = 4$) in length, centered about 48% from posterior to anterior. Anterior ligament 32 to 37 μ m ($35 \pm 2.22 \mu$ m, $n = 4$) long; posterior ligament 28 to 34 μ m ($31 \pm 2.58 \mu$ m, $n = 4$) in length. Micropoints narrow lamellate plates

arranged in complete vertical rows on ventral flange and ventral valve rim, becoming smaller, more lanceolate distally covering about four fifths of the surface of flange. Unsculptured distal flange edge very narrow.

Remarks

The adult shell of *L. ochracea* is so similar to that of *Lampsilis cariosa* that the adults are often confused. The glochidia are very dissimilar, however, and are easily distinguished by valve outline, development of dorsal alae, presence of lateral valve gape, and micro-

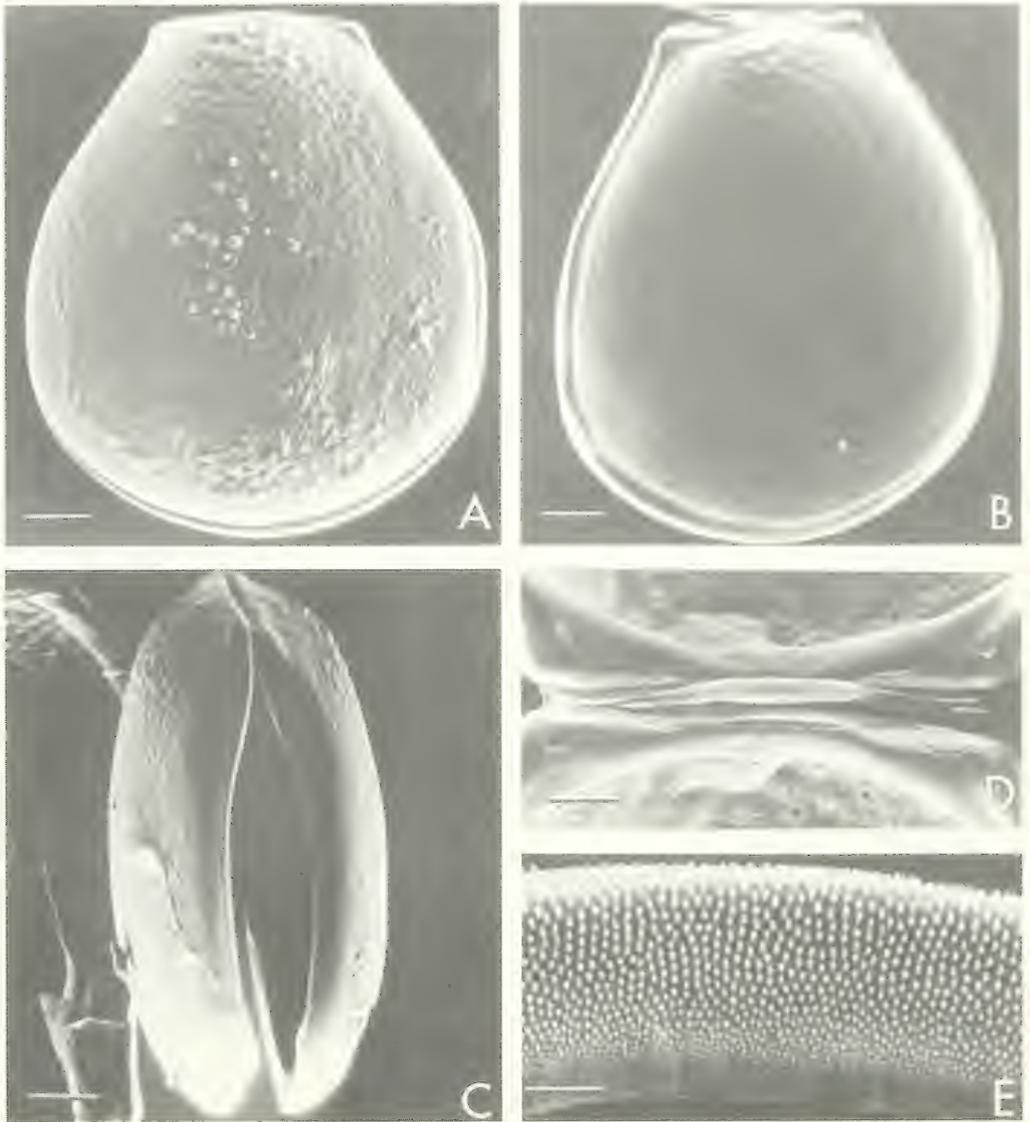


FIG. 47. Glochidium of *Leptodea ochracea*. MAH 896: A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. lateral view, bar length = 40 μ m; D. hinge, bar length = 15 μ m; E. micropoints, bar length = 5 μ m.

point structure (see glochidium of *L. cariosa* below). The glochidium of *L. ochracea* was figured by Porter & Horn (1981: fig. 7).

This species is often included in the genus *Lampsilis*, primarily because the adult shell resembles that of other members of that genus and because of the pronounced sexual dimorphism of the shell (Simpson, 1900, 1914; Johnson, 1947, 1970; Burch, 1975; Clarke, 1981b). However, Morrison (1975) was unable

to find mantle flaps in female *L. ochracea*. Nonetheless, Fuller & Bereza (1975) suggested that this was not sufficient evidence to re-classify *L. ochracea*, and Porter & Horn (1981) concluded that because the shape and size of the glochidium of *ochracea* is similar to that of *cariosa*, the species should remain in *Lampsilis*. In fact, the glochidium of this species does not ally *ochracea* with any species of *Lampsilis* examined (see below).

Because the anatomy of this species is not that of *Lampsilis*, and the glochidium, although larger, is only closely allied to *L. fragilis*, I agree with Morrison (1975) that this species belongs in *Leptodea*.

Potamilus ohiensis (Rafinesque, 1820)
(Fig. 48A–E)

Material Examined

OSUM 54520.1 – Big Blue River below bridge, 3.0 mi. NNW of Barneston, 4.0 mi. SE of Wymore, T2N, R7E, Gage Co., Nebraska, 7 September 1981, F. E. Hoke.

Description

Glochidium ax-head shaped, dorsally ligulate, becoming very broad ventrally (Fig. 47A), length 120 to 126 μm ($123 \pm 3.05 \mu\text{m}$, $n = 3$), height 175 to 187 μm ($181 \pm 6.03 \mu\text{m}$, $n = 3$). Dorsal margin straight, very short, 42 to 50 μm ($45 \pm 3.42 \mu\text{m}$, $n = 5$) in length. Anterior and posterior margins equal, straight sided dorsally, crescent shaped ventrally, outwardly curved to their maximum inflation at about 90% from dorsal to ventral. Exterior surface smooth, without malleations or pits, and loose-looped exterior valve sculpture covering the valve (Fig. 48D). Dorsal alae small, lateral valve gape very large (Fig. 48B). Central ligament 16 to 21 μm ($19 \pm 3.54 \mu\text{m}$, $n = 2$) in length, centered about 50% from both lateral margins. Anterior ligament, 13 to 15 μm ($14 \pm 1.41 \mu\text{m}$, $n = 2$) long; posterior ligament 13 to 14 μm ($14 \pm 0.71 \mu\text{m}$, $n = 2$) long. Lamellate micropoints present on ventral rim and on narrow ventral flange. Lanceolate hooks absent.

Remarks

Lea (1858, 1863) described this glochidium as wedge shaped, with a hook-like process at each corner of the ventral margin. However, Coker & Surber (1911) reported that the spines or hooks were absent, and Surber (1912) reported, "Glochidium without spines (?)." This glochidium can be distinguished from that of most other members of the genus *Potamilus* by the absence of spines. They are further distinguished by micropoint structure, exterior valve sculpture and shape, especially the roundness of the ventral margin. This glochidium shares these characters with *Leptodea fragilis*, but can be distinguished from that species by its ax-head shape. The glochidium

of *P. ohiensis* was figured by Lea (1858: pl. 5, fig. 24), Coker & Surber (1911: pl. 1, fig. 1, 1a), Surber (1912: pl. 1, fig. 10), and Arey (1921: pl. 2, figs. 5, 6; 1924: pl. 1, fig. 3). Lea incorrectly figured the glochidium with hooks, whereas Coker & Surber (1911), Surber (1912), and Arey (1921) did not. The shape of the glochidium is correctly drawn in each figure.

Potamilus amphichaena (Frierson, 1898)
(Fig. 49A–E)

Material Examined

OSUM 33163.13 – Sabine River at La. Rt. 8 and Tx. Rt. 9 bridge, 1.3 mi. W of Burr Ferry, Vernon Parish / Newton Co., Louisiana/Texas, 14 July 1968, D. H. Stansbery et al.

Description

Glochidium ax-head shaped, length 111 to 113 μm ($112 \pm 1.41 \mu\text{m}$, $n = 2$), height 170 to 171 μm ($171 \pm 0.71 \mu\text{m}$, $n = 2$) in length; anterior and posterior margins lie parallel dorsally, becoming strongly curved ventrally. Anterior and posterior margins meeting broadly rounded ventral margin at point of maximum lateral inflation at about 90% from dorsal to ventral. Dorsal alae small. Exterior surface of valve with loose-looped sculpture (Fig. 49D). The hinge of a single specimen gave the following measurements: anterior ligament, 11 μm ; central ligament, 20 μm ; posterior ligament, 10 μm . The central ligament is centered about 49% from posterior to anterior. Micropoints lamellate, as in *L. fragilis* and *P. ohiensis*, arranged in vertical rows on ventral valve margin and on short ventral flange. Lanceolate hooks absent.

Remarks

This glochidium is almost identical with that of *P. ohiensis* but can be distinguished by its slightly more broadly rounded ventral margin. No previously published figure of this glochidium was found.

Potamilus alatus (Say, 1817)
(Fig. 50A–F)

Material Examined

OSUM: 1983:58 – Muskingum River, R.Mi. 31.8–33.4, 1.4 mi. N of Luke Chute Lock and

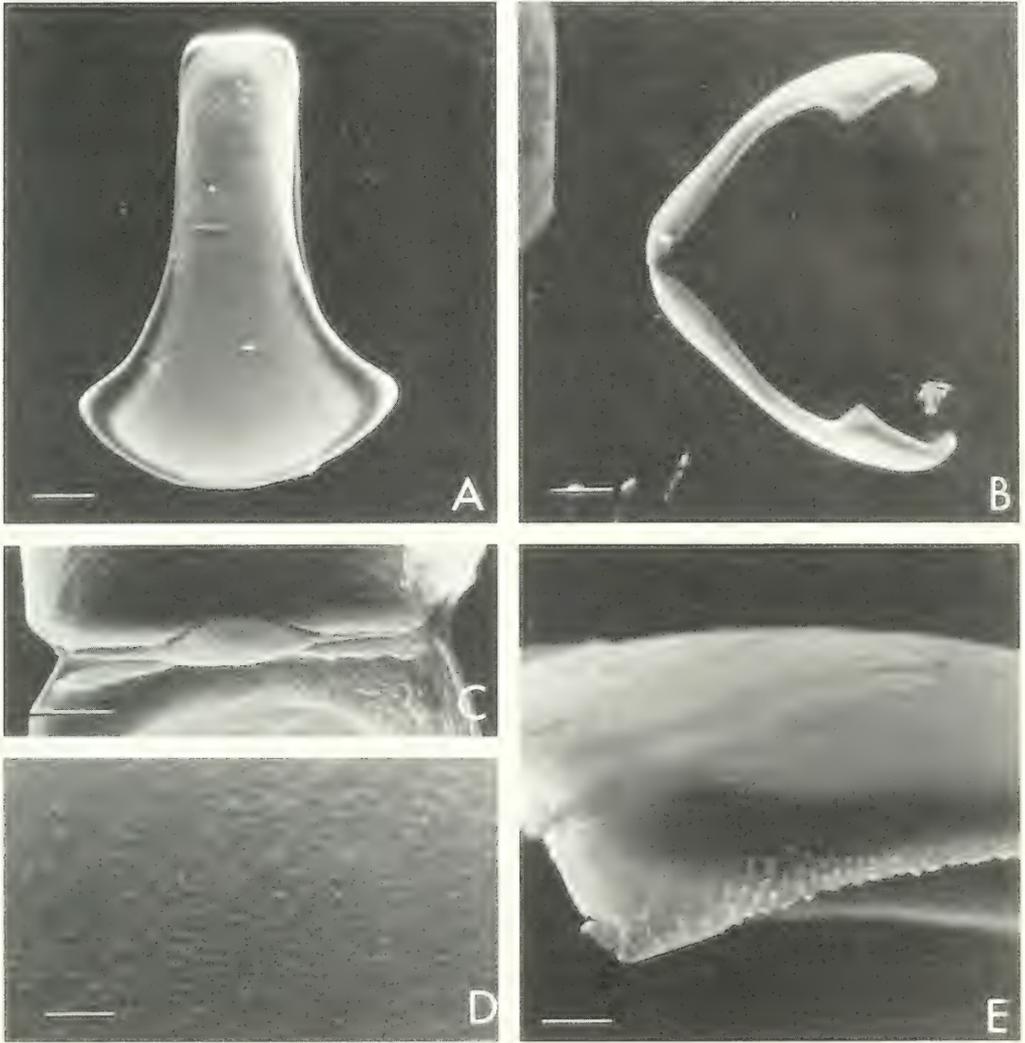


FIG. 48. Glochidium of *Lastena ohioensis*, OSUM 54520.1; A. exterior valve, bar length = 25 μm ; B. lateral view, bar length = 30 μm ; C. hinge, bar length = 10 μm ; D. exterior valve sculpture, bar length = 2 μm ; E. micropoints, bar length = 5 μm .

Dam, 4.3 mi. W of Beverly, Windsor/Waterford Twp., Morgan/Washington Co., Ohio, 25 September 1983, W. N. Kasson & K. Perkins. OSUM 55465 – Clinch River, R.Mi. 213.0 at Clinchport, just above swinging bridge, Scott Co., Virginia, 5 October 1985, G. T. Watters.

Description

Glochidium ligulate, ax-head shaped, much higher than long, length 206 to 227 μm ($216 \pm 9.15 \mu\text{m}$, $n = 5$), height 371 to 386 μm ($378 \pm 7.66 \mu\text{m}$, $n = 5$). Dorsal margin straight, al-

though appearing slightly curved in exterior view due to deep umbo cavity (Fig. 50C), 96 to 109 μm ($102 \pm 5.93 \mu\text{m}$, $n = 5$) in length, or less than half total length of valve. Anterior and posterior margins equal, parallel sided for about 70% their length, gently outwardly curved to their point of maximum inflation at about 95% from dorsal to ventral. Ventral margin slightly curved. Adductor muscle scar very rough, with numerous ridges; however, pit density not appearing to be reduced. Dorsal alae small (Fig. 50A); lateral valve gape pronounced (Fig. 50C); exterior surface covered with vermiculate sculpture (Fig. 50E). Central

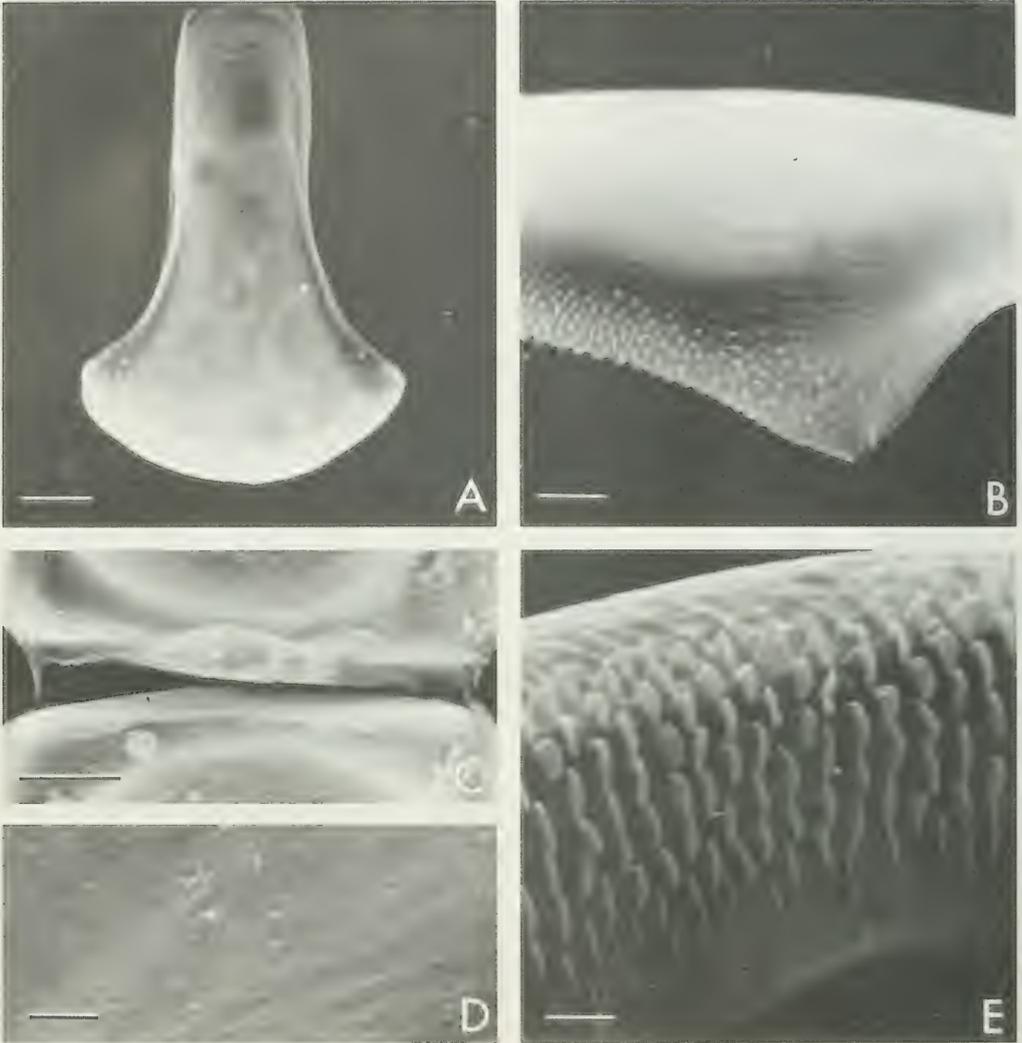


FIG. 49. Glochidium of *Lastena amphichaena*, OSUM 33163.13; A, exterior valve, bar length = 25 μ m; B, micropoints, bar length = 5 μ m; C, hinge, bar length = 10 μ m; D, exterior valve sculpture, bar length = 2 μ m; E, micropoints, bar length = 2 μ m.

ligament 42 μ m in the two specimens measured, centered about 44% from posterior to anterior. Anterior ligament 35 to 38 μ m (37 ± 2.12 μ m, $n = 2$) in length; posterior ligament 19 to 29 μ m (24 ± 7.07 μ m, $n = 2$) long. Lanceolate hooks present at lateral margins of ventral flange, long, slightly inwardly curved, attenuate. Micropoints arranged in complete vertical rows on the ventral rim of valve and on rather wide ventral flange, bluntly pointed, becoming gradually smaller toward distal edge of flange,

covering most of flange, leaving narrow to absent unsculptured distal flange edge.

Remarks

This glochidium was figured by Lea (1858: pl. 5, fig. 25), Lefevre & Curtis (1910: fig. D; 1912: fig. 1D), Ortmann (1911: pl. 89, fig. 18), Coker & Surber (1911: pl. 1, fig. 3), Surber (1912: pl. 1, fig. 8), and Utterback (1915-6: figs. 9a, b). Length and height measurements

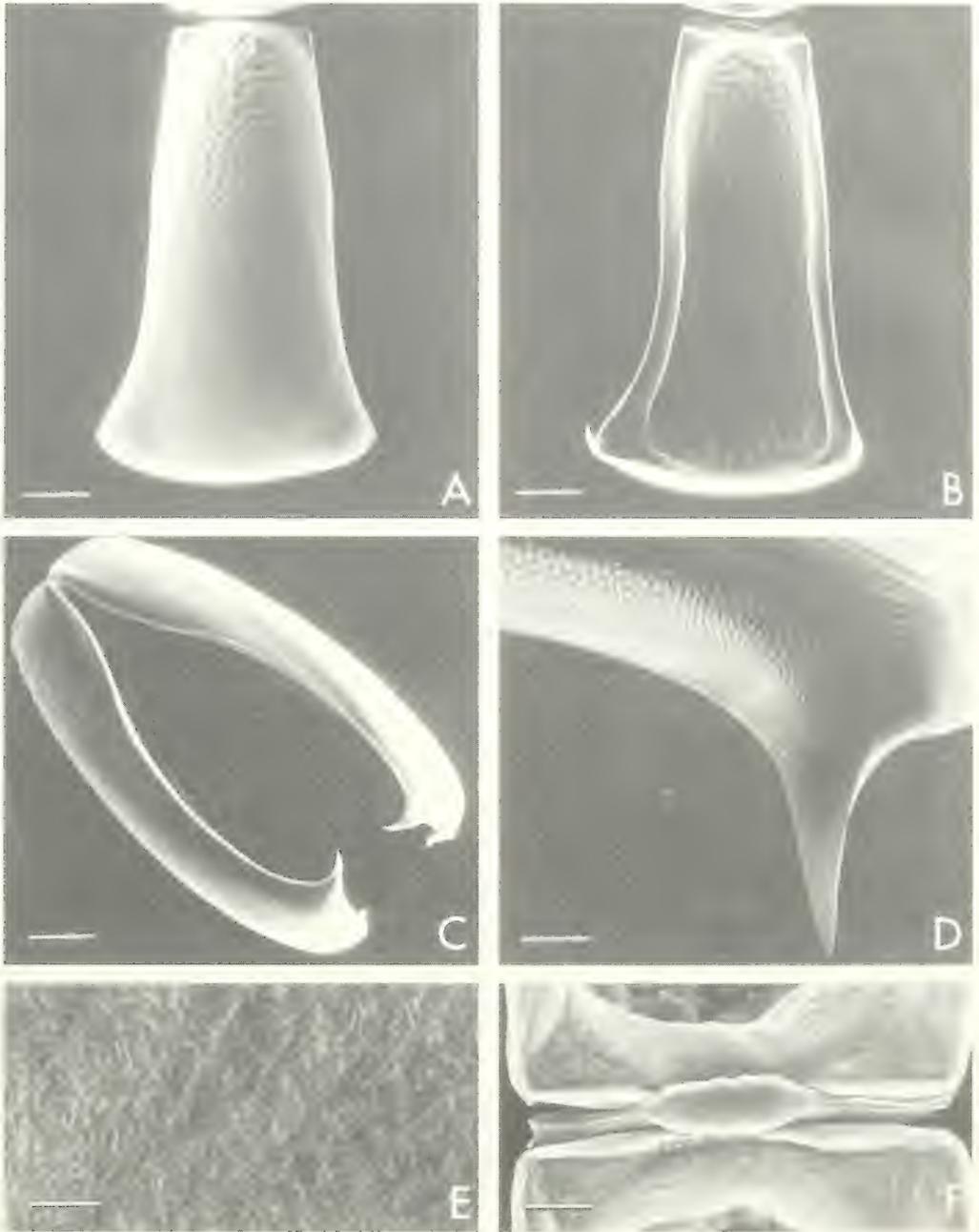


FIG. 50. Glochidium of *Potamilus alatus*; A. exterior valve, OSUM 55465, bar length = 50 μ m; B. interior valve, OSUM 55465, bar length = 50 μ m; C. lateral view, OSUM 55465, bar length = 50 μ m; D. lanceolate hook, OSUM:1983:58, bar length = 5 μ m; E. exterior valve sculpture, OSUM 55465, bar length = 1 μ m; F. hinge, OSUM 55465, bar length = 15 μ m.

were given by Lefevre & Curtis (1910, 1912), Surber (1912), and Ortmann (1912), 230 μm \times 410 μm , 220 μm \times 380 μm , and 200 μm \times 380 μm . This glochidium can be distinguished from other species by its size, shape, and micropoint structure.

The glochidium of *Potamilus capax* is also similar to that of *P. alatus* (Cummings et al., 1990). The glochidium of *P. capax* is about the same size as that of *P. ohioensis* (105 μm in length and 185 μm in height, Coker & Surber, 1911). This glochidium possess lanceolate hooks, vermiculate exterior valve sculpture, and a narrowly curved ventral margin. The glochidium of *P. capax* is figured by Cummings et al. (1990: fig. 6).

Potamilus purpuratus (Lamarck, 1819)
(Fig. 51A–F)

Material Examined

OSUM 15738.2 – Brazos River at foot of Whitney Dam, about 20 mi. NW of Waco, Bosque/Hill Co., Texas, 20 March 1966, C. B. Stein.

Description

Glochidium ligulate, ax-head shaped, much higher than long, length 190 to 200 μm (195 ± 5.00 μm , $n = 3$), height 347 to 356 μm (350 ± 4.93 μm , $n = 3$). Dorsal margin short, straight, 100 to 109 μm (105 ± 3.87 μm , $n = 4$) long. Anterior and posterior margins parallel to about 80% from dorsal to ventral, becoming evenly curved to point of maximum lateral inflation at about 95% from the dorsal margin. Ventral margin only slightly curved. Exterior surface of valve malleated, pitted dorsally, ventrally smooth, with sparse pitting in area of adductor muscle scar compared to remainder of valve. Dorsal alae small (Fig. 51A, C), lateral valve gape wide, and exterior surface covered with vermiculate sculpture (Fig. 51E). Central ligament 37 to 41 μm (39 ± 2.83 μm , $n = 2$) long, centered about 46% from posterior to anterior. Anterior ligament 37 μm in length; posterior ligament 28 to 29 μm (29 ± 0.71 μm , $n = 2$) long. Lanceolate hooks located at lateral margins of ventral flange. Micropoints arranged in complete vertical rows on ventral rim of valve and on wide ventral flange. Micropoints bluntly pointed (Fig. 51D), becoming gradually smaller toward distal edge of flange, covering a large portion of

flange, leaving narrow unsculptured distal edge.

Remarks

This glochidium can be distinguished from that of *P. alatus* by its micropoint structure and its size. This glochidium was figured by Lea (1874: pl. 21, fig. 13) and Surber (1915: pl. 1, fig. 5).

Ligumia recta (Lamarck, 1819)
(Fig. 52A–E)

Material Examined

OSUM:1984:2 – Mississippi River, R.Mi. 635.6, E channel, 1.1 mi. NW of Prairie du Chien, Crawford Co., Wisconsin, 29 April 1984, D. H. Stansbery et al.

Description

Glochidium subelliptical, length 205 to 219 μm (211 ± 6.02 μm , $n = 5$), height 257 to 265 μm (260 ± 3.08 μm , $n = 5$). Dorsal margin slightly curved, 105 to 115 μm (109 ± 3.24 μm , $n = 7$) in length. Anterior and posterior margins equally curved, valve outline symmetrical. Exterior surface of valve malleated and pitted, except along valve margin, where shell is smooth. Dorsal alae long, well developed, about 52 μm in length. Rough exterior surface sculpture covering valve (Fig. 52E). Central ligament 35 to 40 μm (38 ± 3.54 μm , $n = 2$) long, centered about 45% from posterior to anterior. Anterior ligament 41 to 45 μm (43 ± 2.83 μm , $n = 2$) in length; posterior ligament 30 to 34 μm (32 ± 2.83 μm , $n = 2$) long. Micropoints numerous, lanceolate, located on ventral margin of valve and on rather wide ventral flange (not figured). Center of ventral margin almost beak-like, similar to that of *A. pectorosa* (Fig. 41C).

Remarks

This glochidium has the same valve outline as that of *A. pectorosa*. However, the glochidium of *L. recta* can be distinguished from that glochidium by its larger size, broader ventral flange, and longer dorsal alae. The glochidium of *L. recta* was figured by Lea (1858: pl. 5, fig. 11), Lefevre & Curtis (1910: fig. L, 1912: fig. 1L), Ortmann (1911: pl. 89, fig. 21), Surber (1912: pl. 2, fig. 17), Isom & Hudson (1982: fig. 1), and Isom (1983: figs. 1a, b).

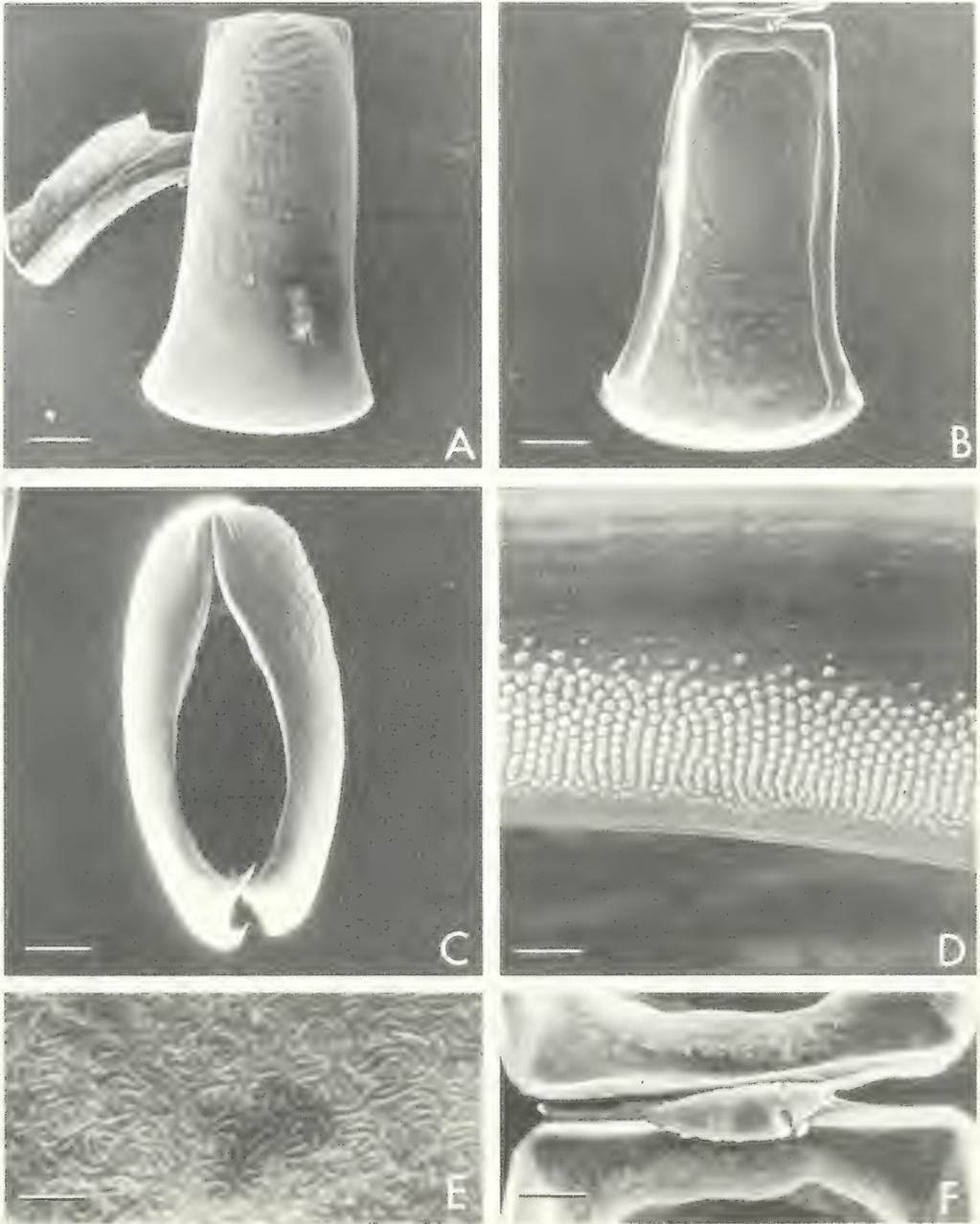


FIG. 51. Glochidium of *Potamilus purpuratus*, OSUM 15738.2; A. exterior valve, bar length = 50 μm ; B. interior valve, bar length = 50 μm ; C. lateral view, bar length = 50 μm ; D. micropoints, bar length = 5 μm ; E. exterior valve sculpture, bar length = 1 μm ; F. hinge, bar length = 15 μm .

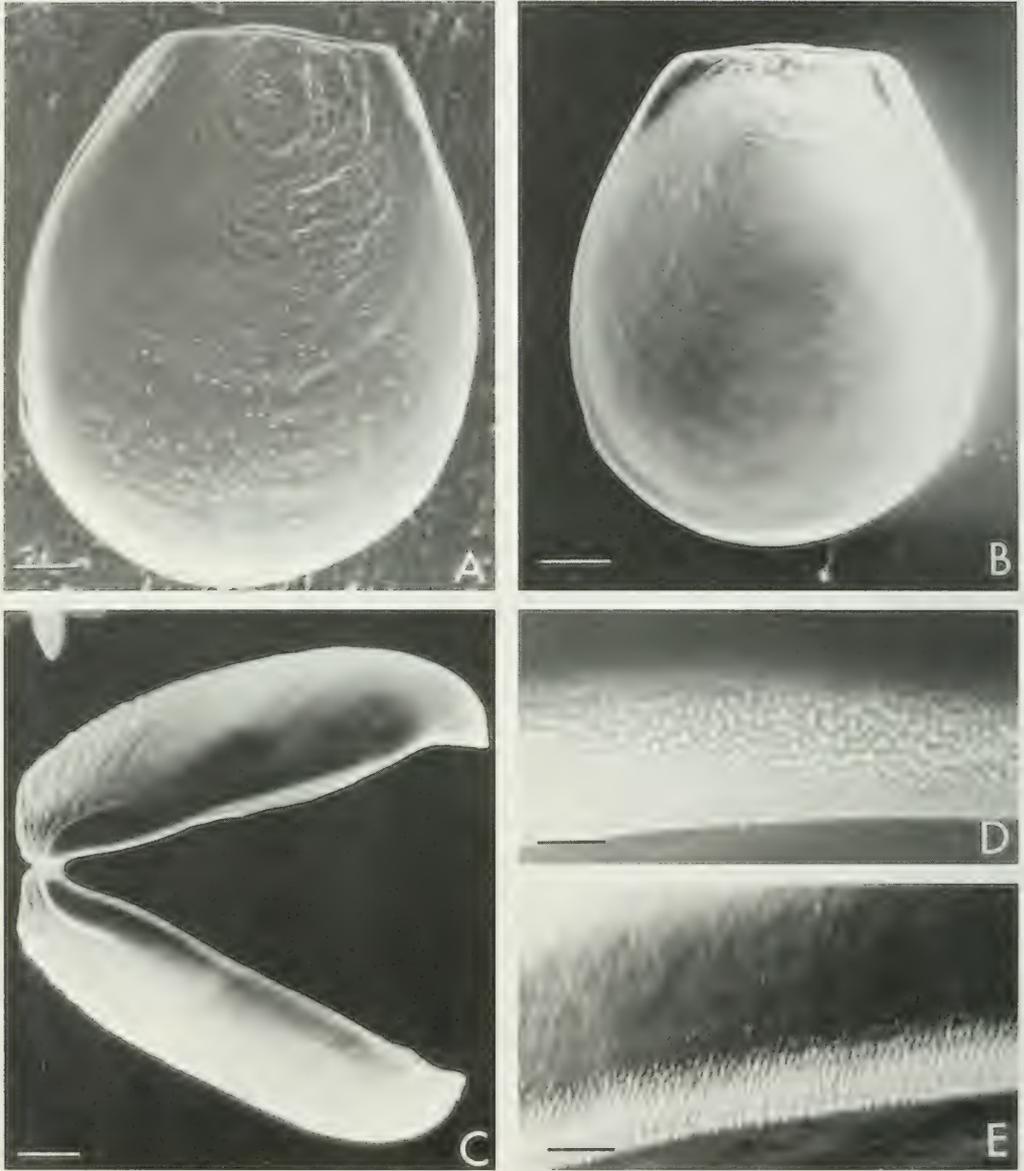


FIG. 52. Glochidium of *Ligumia recta*, OSUM:1984:2; A. exterior valve, bar length = 30 μm ; B. exterior valve, bar length = 35 μm ; C. lateral view, bar length = 35 μm ; D. micropoints, bar length = 3 μm ; E. micropoints, bar length = 5 μm .

Venustaconcha ellipsiformis ellipsiformis
(Conrad, 1834)
(Fig. 53A-G)

12.0 mi. WSW of Jackson, Jackson Co.,
Michigan, 13 May 1986, M. A. Hoggarth.

Material Examined

MAH 947.2 - Kalamazoo River above St.
Rt. 60 bridge, 3.0 mi. WSW of Spring Arbor,

Description

Glochidium subelliptical, symmetrical,
length 223 to 230 μm ($226 \pm 2.94 \mu\text{m}$, $n = 4$),
height 280 to 287 μm ($285 \pm 3.32 \mu\text{m}$, $n = 4$).

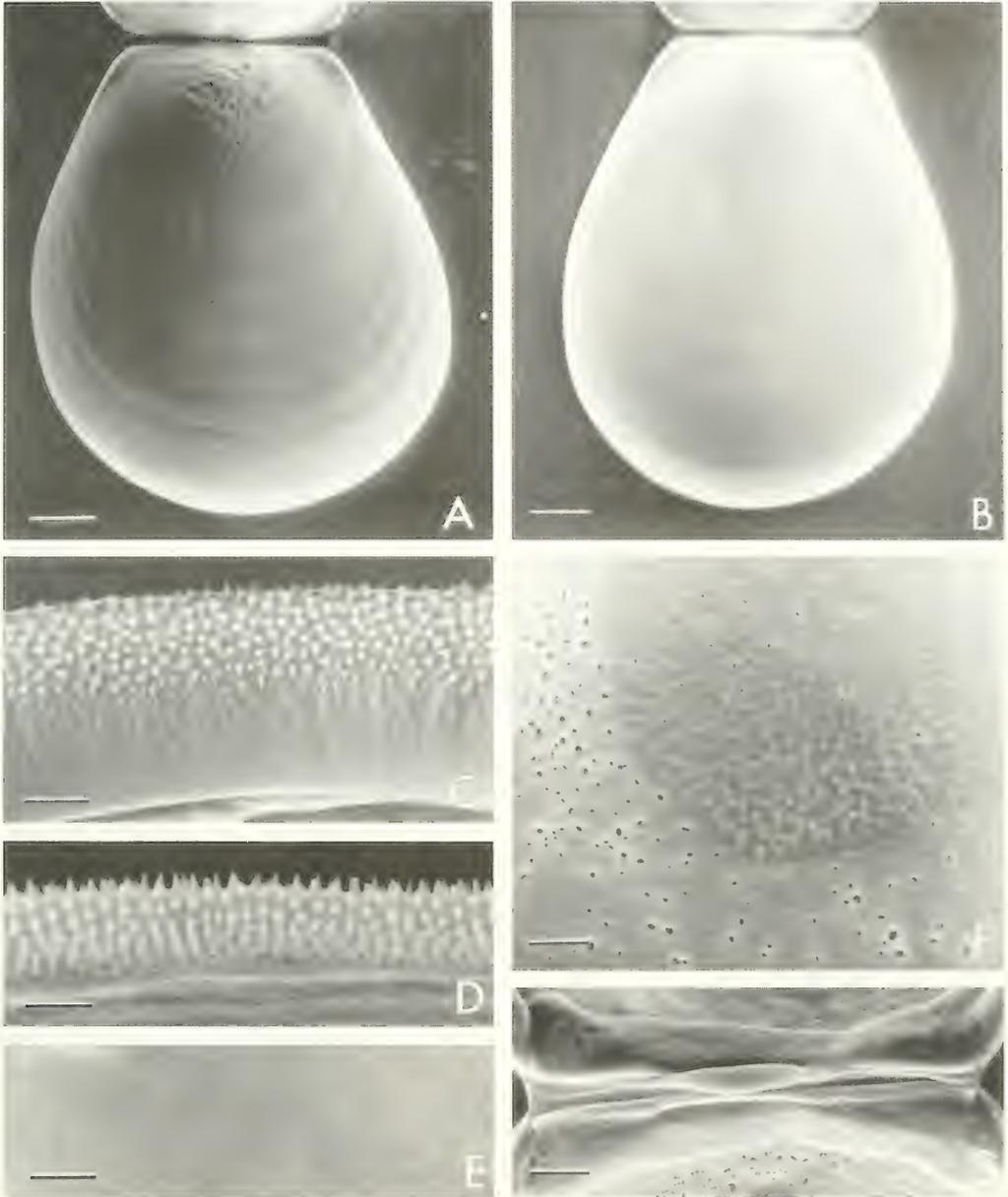


FIG. 53. Glochidium of *Venustaconcha e. ellipsiformis*, MAH 947.2; A. exterior valve, bar length = 35 μ m; B. exterior valve, bar length = 35 μ m; C. micropoints, bar length = 2 μ m; D. micropoints, bar length = 2 μ m; E. exterior valve sculpture, bar length = 1 μ m; F. interior valve pitting and adductor muscle scar, bar length = 10 μ m; G. hinge, bar length = 15 μ m.

Dorsal margin straight, 102 to 110 μ m (104 ± 2.48 μ m, $n = 6$) in length. Anterior and posterior margins equally and gently curving to their maximum inflation at about 70% from dorsal to ventral. Exterior surface of valve finely mal-

leated near umbo, with fine loose-looped exterior valve sculpture (Fig. 53E). Adductor muscle scar indicated by numerous small ridges and reduced pitting (Fig. 53F). Central ligament 38 to 40 μ m (40 ± 3.21 μ m, $n = 3$)

long, centered 47% from posterior to anterior. Posterior ligament 28 to 32 μm ($30 \pm 2.08 \mu\text{m}$, $n = 3$) long; anterior ligament 34 to 39 μm ($36 \pm 2.65 \mu\text{m}$, $n = 3$) in length. Micropoints lanceolate, located on ventral rim of valve and on moderately wide ventral flange, arranged in broken vertical rows that extend about half distance of flange, leaving a wide unsculptured distal flange margin.

Remarks

This glochidium is identical to that of *L. recta*, except it is larger. It was figured by Lea (1858: pl. 5, fig. 9, as *Unio spatulatus*, = *V. elipsiformis*, *vide* Simpson, 1900) and van der Schalie (1963: pl. 1, center, left).

Villosa trabalis (Conrad, 1834)
(Fig. 54A, B, D, E)

Material Examined

V. trabalis: OSUM 9516.49 – Rockcastle River, Rt. 80 W of London, Rockcastle/Laurel Co., Kentucky, 26 October 1963, C. B. Stein & D. H. Stansbery. *V. perpurpurea* (Fig. 53C): OSUM 16262 – Clinch River at St. Rt. 460 bridge at Richlands, Tazewell Co., Virginia, 6 October 1965, D. H. Stansbery & J. J. Jenkinson.

Description

Glochidium subelliptical, higher than long, with a short, straight dorsal margin, gently curved, almost equal lateral margins and broadly rounded ventral margin. Glochidium 211 to 216 μm ($214 \pm 2.36 \mu\text{m}$, $n = 4$) in length, 277 to 280 μm ($278 \pm 1.50 \mu\text{m}$, $n = 4$) in height. Dorsal margin 94 to 99 μm ($96 \pm 2.17 \mu\text{m}$, $n = 5$) in length. Exterior valve surface mostly smooth, with relief occurring at pits, which are surrounded by smooth, circular discs (Fig. 54E). Dorsal alae moderate in length. Central ligament 31 to 36 μm ($33 \pm 2.89 \mu\text{m}$, $n = 3$) long, centered about 45% from posterior to anterior. Posterior ligament 25 to 28 μm ($27 \pm 1.53 \mu\text{m}$, $n = 3$) long; anterior ligament 36 to 40 μm ($37 \pm 2.31 \mu\text{m}$, $n = 3$) in length. Micropoints lanceolate, numerous, unorganized, found on ventral rim of valve and on distal half of wide ventral flange, leaving wide unsculptured distal flange margin.

Remarks

Surber (1912) gave $193 \mu\text{m} \times 255 \mu\text{m}$ for the length and height of this glochidium, and Ortmann (1912) gave $220 \mu\text{m} \times 270 \mu\text{m}$. This glochidium is similar in shape, size and dorsal alae structure to *Obovaria*, but it can be distinguished by its rather wide ventral flange. The glochidium of *Villosa perpurpurea* (Lea, 1861) is virtually identical to that of *V. trabalis*, although smaller: length, 165 μm ; height, 241 μm ; hinge length, 88 μm . The glochidium of *V. trabalis* was figured by Surber (1912: pl. 3, fig. 40) and Ortmann (1912: pl. 20, fig. 4).

Villosa villosa (Wright, 1898)
(Fig. 55A–G)

Material Examined

OSUM 45940.3, 45940.7 – Santa Fe River at U.S. Rt. 41/441 bridge, 2.0 mi. NNW of High Spring, 27.3 mi. NW of Gainesville, Sec. 27/28, Alachua/Columbia Co., Florida, 4 August 1975, J. M. Condit & E. P. Keferl.

Description

Glochidium subelliptical (Fig. 54C) to subspatulate (Fig. 55A, B, D), length 240 to 250 μm ($245 \pm 3.89 \mu\text{m}$, $n = 7$), height 296 to 308 μm ($303 \pm 4.26 \mu\text{m}$, $n = 7$). Dorsal margin straight, 105 to 116 μm ($111 \pm 3.65 \mu\text{m}$, $n = 7$) in length. Glochidium usually characterized by a gently curved ventral margin and lateral margins that are oblique to the dorsal margin dorsally and perpendicular ventrally. Dorsal alae long, extending about half distance of dorsal oblique section of lateral margins. Valve finely malleated, densely pitted, except along margins; exterior sculpturing rough but not quite beaded, best described as very fine pustules (Fig. 55G). Central ligament 37 to 42 μm ($40 \pm 3.54 \mu\text{m}$, $n = 2$) long, centered about 49% from posterior to anterior. Anterior ligament 35 to 43 μm ($38 \pm 4.24 \mu\text{m}$, $n = 2$) long; posterior ligament about 33 μm long (both specimens had identical posterior ligament lengths). Micropoints lanceolate, arranged in more or less complete vertical rows on ventral flange and on the rim of ventral margin of valve. Micropoints becoming smaller from proximal to distal portion of flange, covering about 75% of flange surface, leaving narrow unsculptured distal flange margin.

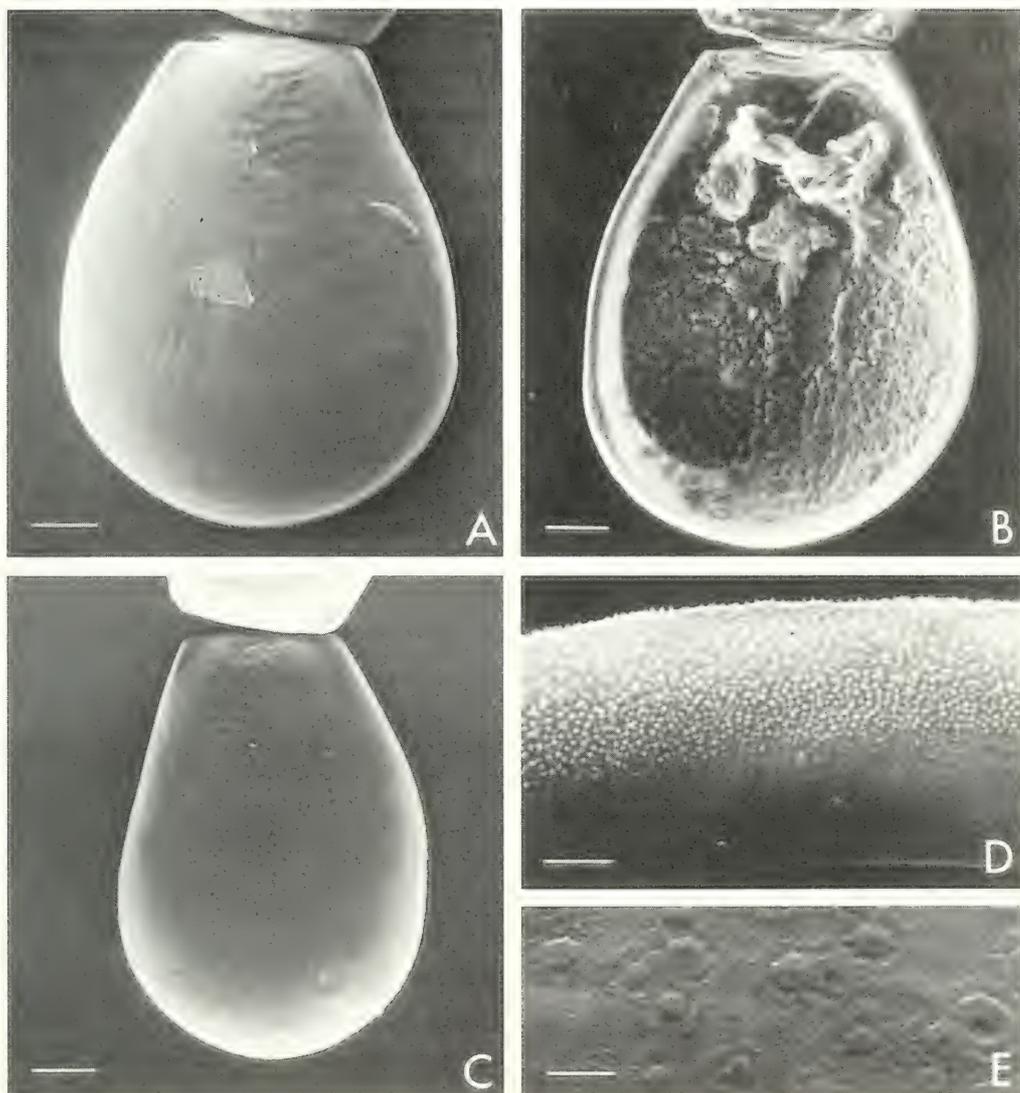


FIG. 54. Glochidium of *Villosa trabalis* (A, B, D, E) OSUM 9516.49, and *Villosa perpurpurea* (C) OSUM 16262; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. exterior valve, bar length = 40 μ m; D. micropoints, bar length = 2 μ m; E. exterior valve sculpture, bar length = 1 μ m.

Remarks

The outline of the valve of this glochidium distinguish it from the glochidia previously described, but not from other members of this genus (see *Villosa vibex* and *Villosa iris iris* below) and all members of the genus *Lampsilis*. This appears to be the final change in glochidial shape within this lineage. This glochidium is figured here for the first time.

Villosa vibex (Conrad, 1834) (Fig. 56A–F)

Material Examined

OSUM 54631 – Yellow River at U.S. Rt. 84 bridge, 3.0 mi. SE of Sanford, 5.0 mi. W of Opp, Sec. 33, T4N, R17E, Covington Co., Alabama, 2 June 1969, H. Harima & B. Wall.
OSUM 24124 – Santa Fe River at U.S. Rt. 27

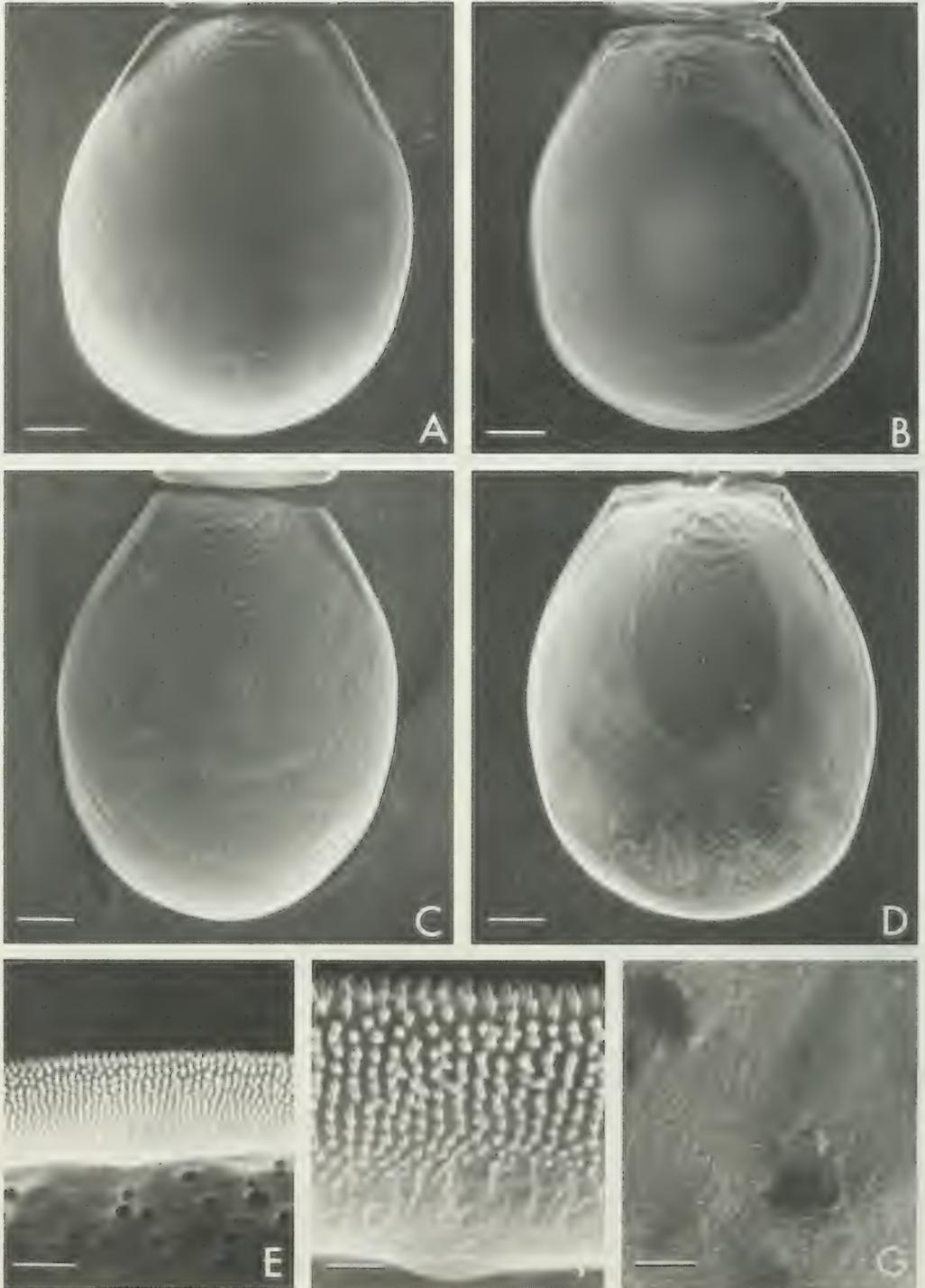


FIG. 55. Glochidium of *Villosa villosa*: A. exterior valve, OSUM 45940.7, bar length = 40 μm ; B. interior valve, OSUM 45940.3, bar length = 40 μm ; C. exterior valve, OSUM 45940.7, bar length = 40 μm ; D. interior valve, OSUM 45940.7, bar length = 40 μm ; E. micropoints, OSUM 45940.3, bar length = 5 μm ; F. micropoints, OSUM 45940.3, bar length = 2 μm ; G. exterior valve sculpture, OSUM 45940.7, bar length = 1 μm .

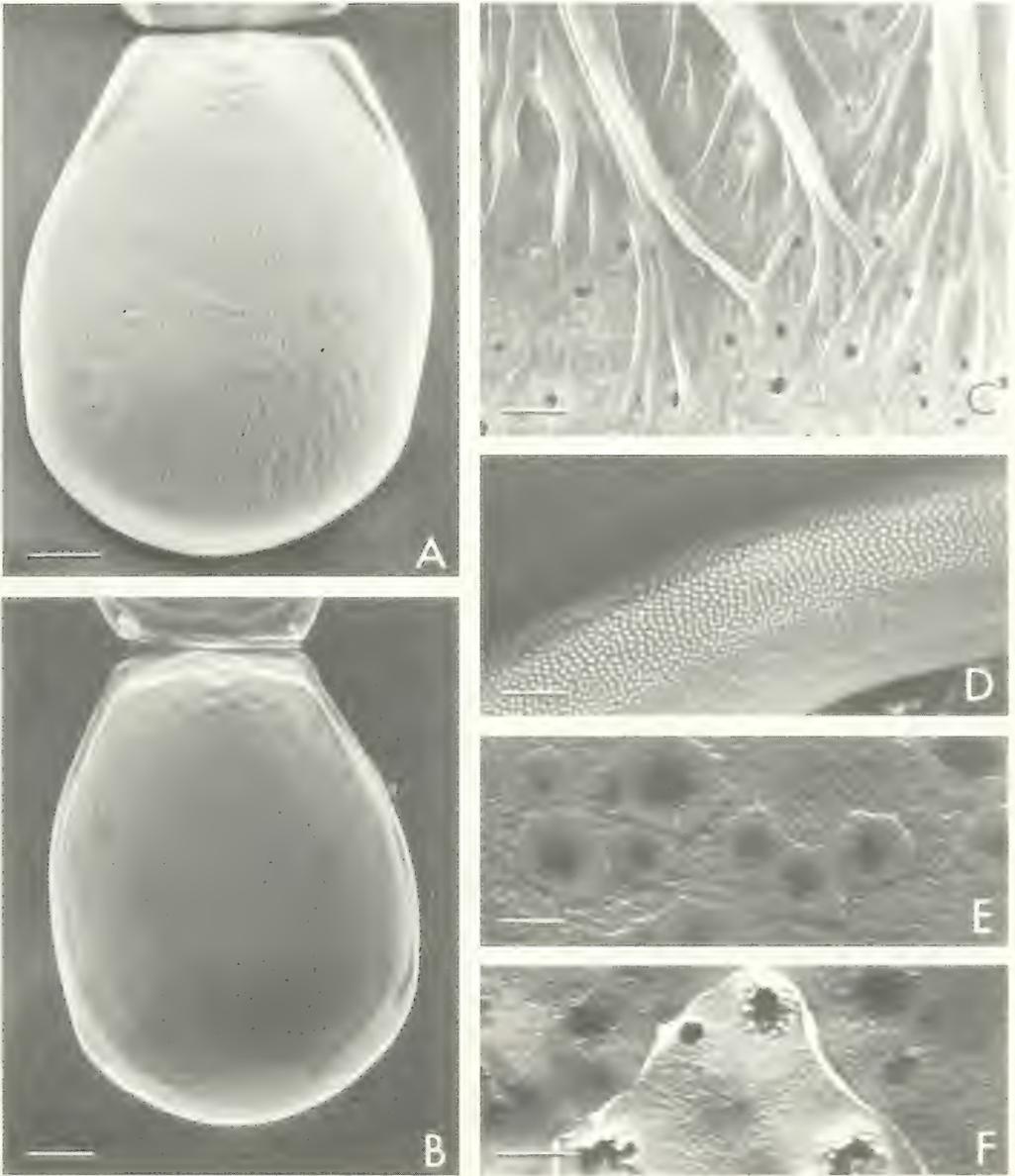


FIG. 56. Glochidium of *Villosa vibex*; A. exterior valve, OSUM 24124, bar length = 40 μ m; B. interior valve, OSUM 24124, bar length = 40 μ m; C. adductor muscle insertion, OSUM 54631, bar length = 5 μ m; D. micropoints, OSUM 54631, bar length = 5 μ m; E. exterior valve sculpture, OSUM 54631, bar length = 2 μ m; F. exterior valve sculpture, OSUM 24124, bar length = 2 μ m.

bridge, 2.5 mi. NW of High Springs, 7.5 mi. SE of Fort White, Columbia/Alachua Co., Florida, 24 February 1970, J. J. Jenkinson. OSUM 48628 – "Nichols Cr." Monroe Co., Mississippi, in the Tombigbee River drainage (Mobile River), 18 March 1972, collector unknown, ex J. D. Williams.

Description

Glochidium subspatulate, length 224 to 239 μ m ($230 \pm 5.42 \mu$ m, $n = 8$), height 295 to 304 μ m ($300 \pm 4.17 \mu$ m, $n = 8$). Dorsal margin straight, 100 to 120 μ m ($110 \pm 7.79 \mu$ m, $n = 8$) long. Lateral margins straight, divergent dor-

sally, ventrally parallel; ventral margin gently curved. Malleations and pits more or less uniform over surface of valve; fine sculpture of exterior surface rough (Fig. 56E, F). Membrane covering exterior surface torn in Figure 56F, intact in Figure 56E. No adductor muscle scar evident, but just as in *Alasmidonta marginata*, adductor muscle inserting on interior surface of valve, rather than on walls of pits (Fig. 56C). Long dorsal alae like those in *V. villosa*. Central ligament 44 to 45 μm ($45 \pm 0.71 \mu\text{m}$, $n = 2$) long, centered about 48% from posterior to anterior. Anterior ligament 29 to 32 μm ($31 \pm 2.12 \mu\text{m}$, $n = 2$) in length; posterior ligament 28 to 29 μm ($29 \pm 0.71 \mu\text{m}$, $n = 2$) long. Micropoints pyramidal, arranged in broken vertical rows on wide ventral flange and on ventral rim of valve, covering proximal half of ventral flange and leaving wide unsculptured distal flange margin.

Remarks

This glochidium was figured by Lea (1858: pl. 5, fig. 4, as *Unio rutilans*, = *V. vibex*, *vide* Johnson, 1970; 1874: pl. 21, fig. 7, as *Unio sudus*, = *V. vibex*, *vide* Johnson, 1970). The 1874 figure is closer to my figure of this glochidium than the 1858 figure, except that the ventral half of the figure is much too round. This glochidium is very close to that of *V. villosa*, but it can be distinguished by its bluntly pyramidal micropoints and its wide unsculptured distal flange margin.

Villosa iris iris (Lea, 1829)
(Fig. 57A–F)

Material Examined

MAH 641.1 – Little Darby Creek at Co. Rt. 131 bridge (Grewell Rd.), 1.8 mi. E of Plumwood, 7.7 mi. NW of West Jefferson, Monroe Twp., Madison Co., Ohio, 11 May 1984, M. A. Hoggarth & G. T. Watters. OSUM 55828.3 55828.5 – South Fork Clinch River at Fourway, at E edge of Tazewell, at U.S. Rt. 19 bridge, Tazewell Co., Virginia, 13 October 1985, D. H. Stansbery.

Description

Glochidium subspatulate, length 217 to 232 μm ($225 \pm 5.84 \mu\text{m}$, $n = 8$), height 289 to 305 μm ($296 \pm 6.64 \mu\text{m}$, $n = 8$). Dorsal margin straight, 107 to 115 μm ($113 \pm 2.51 \mu\text{m}$, $n = 8$) in length. Anterior and posterior margins sub-

equal, dorsally divergent, ventrally parallel. Anterior margin may be slightly more rounded ventrally than the posterior margin; ventral margin gently curved. Dorsal alae long, but only about half as long as the dorsal portion of lateral margins. Valve malleated and pitted; umbo deeply folded (Fig. 57A, C, D). Concentric ridges extending from umbo ventrally for a short distance; loose-looped sculpture covering exterior surface of valve (Fig. 57E). Central ligament 41 to 42 μm ($42 \pm 0.58 \mu\text{m}$, $n = 3$) long, centered 49% from posterior to anterior. Anterior ligament 38 to 40 μm ($39 \pm 1.00 \mu\text{m}$, $n = 3$) long; posterior ligament 33 to 34 μm ($33 \pm 0.58 \mu\text{m}$, $n = 3$) long. Micropoints lanceolate, arranged in broken rows on ventral rim of valve and on wide ventral flange, covering proximal half of flange, leaving wide unsculptured distal flange margin.

Remarks

This glochidium can be distinguished from all other subspatulate glochidia examined by its loose-looped exterior valve sculpture, its wide unsculptured distal flange border, and smaller dorsal alae. Surber (1912) gave length and height measurement of 240 μm \times 300 μm for this glochidium, and Ortmann (1912) gave 220 μm \times 280 μm . This glochidium was figured by Lea (1858: pl. 5, fig. 14, as *Unio novi-eboraci*, = *V. iris*, *vide* Simpson, 1900), Ortmann (1911: pl. 89, fig. 20), and Surber (1912: pl. 3, fig. 46).

Lampsilis teres teres (Rafinesque, 1820)
(Fig. 58A–E)

Material Examined

OSUM 36409 – Tombigbee River 0.5 mi. below mouth of Luxaplilla Creek, 3.2 mi. S of Columbus, Sec. 32/5, T18S, R18W, Lowndes Co., Mississippi, 5 October 1974, R. Grace & T. Whitfields. OSUM 36531.1 – Whitewater River at Bollinger Mill Dam, at Burfordville, T31N, R11E, Cape Girardeau Co., Missouri, 17 October 1974, F. Schilling & H. Kemper. OSUM 51669.2 – Mississippi River, R.Mi. 632.1, E. Shore main channel, 2.9 mi. SW of Prairie du Chien, Craford Co., Wisconsin, 10 June 1981, D. J. Heath & M. C. Weisensel.

Description

Glochidium subspatulate, length 189 to 194 μm ($191 \pm 1.87 \mu\text{m}$, $n = 9$), height 255 to 265

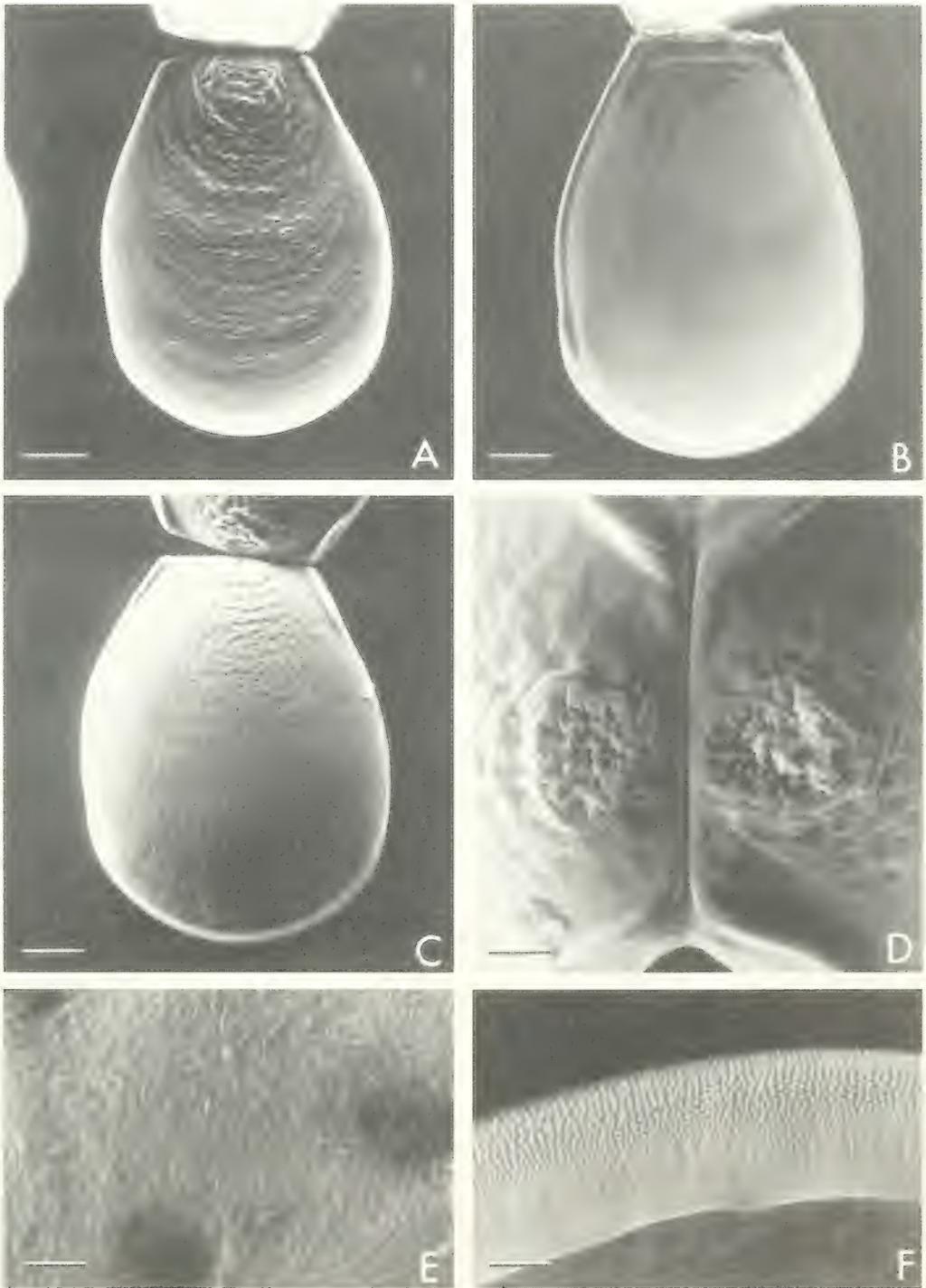


FIG. 57. Glochidium of *Villosa i. iris*: A. exterior valve, OSUM 55828.4, bar length = 50 μm ; B. interior valve, MAH 641.1, bar length = 40 μm ; C. exterior valve, MAH 641.1, bar length = 40 μm ; D. umbo, MAH 641.1, bar length = 15 μm ; E. exterior valve sculpture, MAH 641.1, bar length = 1 μm ; F. micropoints, MAH 641.1, bar length = 5 μm .

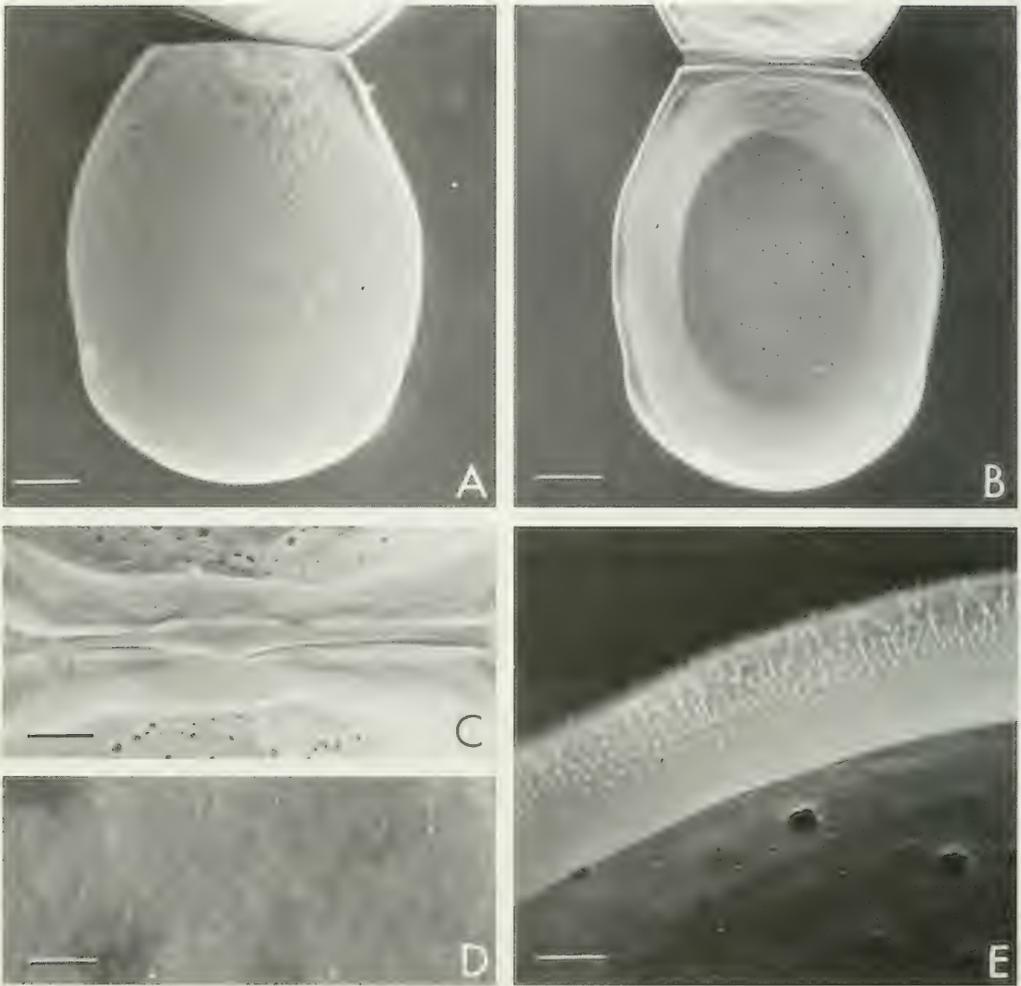


FIG. 58. Glochidium of *Lampsilis t. teres*: A. exterior valve, OSUM 51669.2, bar length = 35 μ m; B. interior valve, OSUM 51669.2, bar length = 40 μ m; C. hinge, OSUM 36409, bar length = 15 μ m; D. exterior surface sculpture, OSUM 51669.2, bar length = 1 μ m; E. micropoints, OSUM 51669.2, bar length = 5 μ m.

μ m ($258 \pm 3.71 \mu\text{m}$, $n = 9$). Dorsal margin straight, 100 to 116 μ m ($110 \pm 3.64 \mu\text{m}$, $n = 14$) long. Anterior and posterior margins equal, dorsally divergent, parallel to slightly convergent ventrally. Ventral margin gently curved. Exterior valve surface malleated and pitted; exterior surface rough (Fig. 58D). Dorsal alae about half as long as divergent portion of lateral margins. Central ligament 27 to 39 μ m ($34 \pm 4.09 \mu\text{m}$, $n = 6$) long, centered about 45% from posterior to anterior. Anterior ligament 41 to 54 μ m ($46 \pm 5.08 \mu\text{m}$, $n = 6$) long; posterior ligament 30 to 32 μ m ($31 \pm 0.82 \mu\text{m}$, $n = 6$) in length. Micropoints lanceo-

late (deformed in Fig. 58E), sharply pointed, restricted to proximal half of ventral flange and ventral rim of valve. This leaves a wide unsculptured distal flange margin.

Remarks

Surber figured this glochidium (1912: pl. 2, fig. 22, as *L. fallaciosa*, = *L. teres*, *vide* Johnson, 1972) and gave length and height measurements of 200 μ m \times 240 μ m. This glochidium is much smaller than any other recorded for *Lampsilis*, except *L. t. anodontoides* (see below).

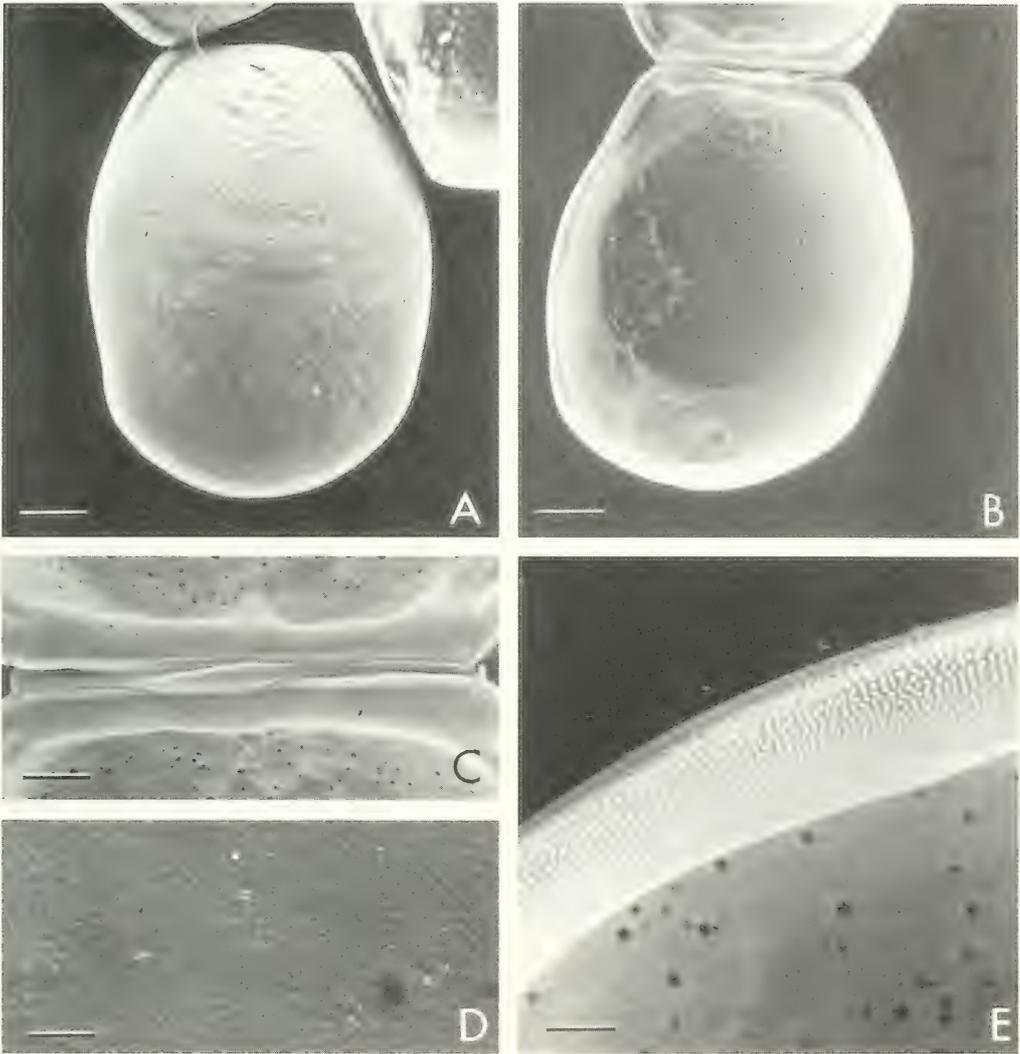


FIG. 59. Glochidium of *Lampsilis t. anodontoides*: A. exterior valve, OSUM 35612, bar length = 40 μ m; B. interior valve, OSUM 35612, bar length = 35 μ m; C. hinge, OSUM 41762.2, bar length = 15 μ m; D. exterior valve sculpture, OSUM 41762.2, bar length = 1 μ m; E. micropoints, OSUM 41762.2, bar length = 5 μ m.

Lampsilis teres anodontoides (Lea, 1831)
(Fig. 59A-E)

Sec. 21, T8N, R5E, Cross Co., Arkansas, 14
March 1978, D. H. Stansbery et al.

Material Examined

OSUM 35612 – Uphapee Creek at I-85
bridge, 3.4 mi. N of Tuskegee, 6.3 mi. S of No-
tasulga, Sec. 7, T17N, R24E, Macon Co., Al-
abama, 4 April 1968, J. S. Ramsey et al.
OSUM 41762.2 – St. Francis River 2.2 mi. N
of Parkin, 33.0 mi. WNW of Memphis (TN),

Description

Glochidium subspatulate, length 187 to 207
 μ m ($199 \pm 7.19 \mu$ m, $n = 7$), height 249 to 255
 μ m ($251 \pm 2.87 \mu$ m, $n = 7$). Dorsal margin
straight, 105 to 118 μ m ($111 \pm 4.42 \mu$ m, $n = 9$)
long. Valve outline like that of *L. t. teres*; lat-
eral margins dorsally divergent, becoming

parallel, convergent ventrally; ventral margin gently curved. Exterior surface of valve malleated, especially near umbo; pits numerous. As in *L. t. teres*, dorsal alae about half as long as dorsal portion of lateral margins. Exterior surface rough under high magnification (Fig. 59D). Central ligament 36 to 42 μm ($38 \pm 2.45 \mu\text{m}$, $n = 5$) long, centered about 45% from posterior margin. Anterior ligament 44 to 50 μm ($46 \pm 2.88 \mu\text{m}$, $n = 5$) long; posterior ligament 30 to 33 μm ($31 \pm 1.14 \mu\text{m}$, $n = 5$) long. Micropoints lanceolate, located on ventral rim of valve and on wide ventral flange, arranged in incomplete vertical rows; unsculptured distal flange margin moderate in length.

Remarks

This glochidium is identical to that of *L. t. teres*. Surber (1912) gave length and height measurements of 185 $\mu\text{m} \times 210 \mu\text{m}$ for this glochidium, and Ortmann (1912) gave 200 $\mu\text{m} \times 260 \mu\text{m}$. Surber's measurements seem too small, even for this glochidium, and his figure (1912: pl. 2, fig. 21) shows the ventral margin as semicircular rather than gently curved. These discrepancies suggest that his material was not mature. This glochidium was also figured by Lea (1858: pl. 5, fig. 2) and Ortmann (1912: pl. 20, fig. 9). Lea's figure is much closer to Ortmann's and mine than to Surber's figure.

Lampsilis radiata radiata (Gmelin, 1791)
(Fig. 60A–E)

Material Examined

L. r. radiata: MAH 897.1 – Great Herring Pond at Carter's Brook Rd. 4.0 mi. NNE of Buzzards Bay, 3.0 mi. NW of Sagamore, Plymouth Co., Massachusetts, 31 July 1985, G. T. Watters et al. *L. r. luteola*: MAH 321.6 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp., Pickaway Co., Ohio, 7 January 1983, M. A. Hoggarth & G. T. Watters. MAH 587.1 – Big Darby Creek at access 0.9 mi. N of Harrisburg, 1.7 mi. NW of Orient, Pleasant Twp., Franklin Co., Ohio, 27 September 1983, M. A. Hoggarth. MAH 628.1 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp.,

Pickaway Co., Ohio, 13 April 1984, M. A. Hoggarth & G. T. Watters. MAH 652 – Little Darby Creek at Little Darby Rd access, 2.7 mi. SE of Plumwood, 6.4 mi. NW of West Jefferson, Monroe Twp., Madison Co., Ohio, 17 May 1984, M. A. Hoggarth & G. T. Watters. MAH 721.2 – Fish Creek at St. Rt. 49 bridge, 0.4 mi. above its mouth, 1.1 mi. N of Edgerton, 10.4 mi. W of Bryan, St. Joseph Twp., Williams Co., Ohio, 1 October 1985, M. A. Hoggarth & D. Rice.

Description

Glochidium subspatulate, length 250 to 260 μm ($255 \pm 4.43 \mu\text{m}$, $n = 4$), height 295 to 311 μm ($303 \pm 6.58 \mu\text{m}$, $n = 4$). Dorsal margin straight, 121 to 129 μm ($125 \pm 3.78 \mu\text{m}$, $n = 4$) in length. Lateral margins dorsally divergent, ventrally parallel, ventral margin gently curved. Dorsal alae long; fine structure of exterior surface of valve rough (Fig. 60D). Exterior surface finely malleated. Valve at umbo deeply folded. Central ligament 45 to 47 μm ($46 \pm 1.00 \mu\text{m}$, $n = 3$) long, centered about 42% from posterior to anterior. Anterior ligament 46 to 50 μm ($48 \pm 2.08 \mu\text{m}$, $n = 3$) long; posterior ligament 27 to 32 μm ($29 \pm 2.52 \mu\text{m}$, $n = 3$) long. Micropoints lanceolate, located on rim of ventral margin and on wide ventral flange, arranged in broken vertical rows and covering proximal half of ventral flange. Unsculptured distal flange margin wide.

Morphometrics from the subspecies *L. r. luteola* are: length, 227 to 235 μm ($231 \pm 2.39 \mu\text{m}$, $n = 8$); height, 280 to 295 μm ($285 \pm 6.63 \mu\text{m}$, $n = 8$); hinge length 107 to 123 μm ($115 \pm 5.23 \mu\text{m}$, $n = 12$); anterior ligament length 37 to 42 μm ($39 \pm 1.94 \mu\text{m}$, $n = 6$); central ligament length 40 to 46 μm ($42 \pm 2.58 \mu\text{m}$, $n = 6$); posterior ligament length 32 to 40 μm ($36 \pm 3.39 \mu\text{m}$, $n = 6$).

Remarks

Surber (1912) and Ortmann (1912) gave length and height measurements for the glochidium of *L. r. luteola*; Ortmann's figures are 230 $\mu\text{m} \times 280 \mu\text{m}$ (almost identical to mine), and Surber gave 250 $\mu\text{m} \times 290 \mu\text{m}$ (almost the same as my measurements for *L. r. radiata*). This species is as far ranging as *Pyganodon grandis* and appears to have just as wide a range in glochidial size. The glochidium of *L. r. radiata* was figured by Lea (1858: pl. 5, fig. 20), Wiles (1975: fig. 5), and

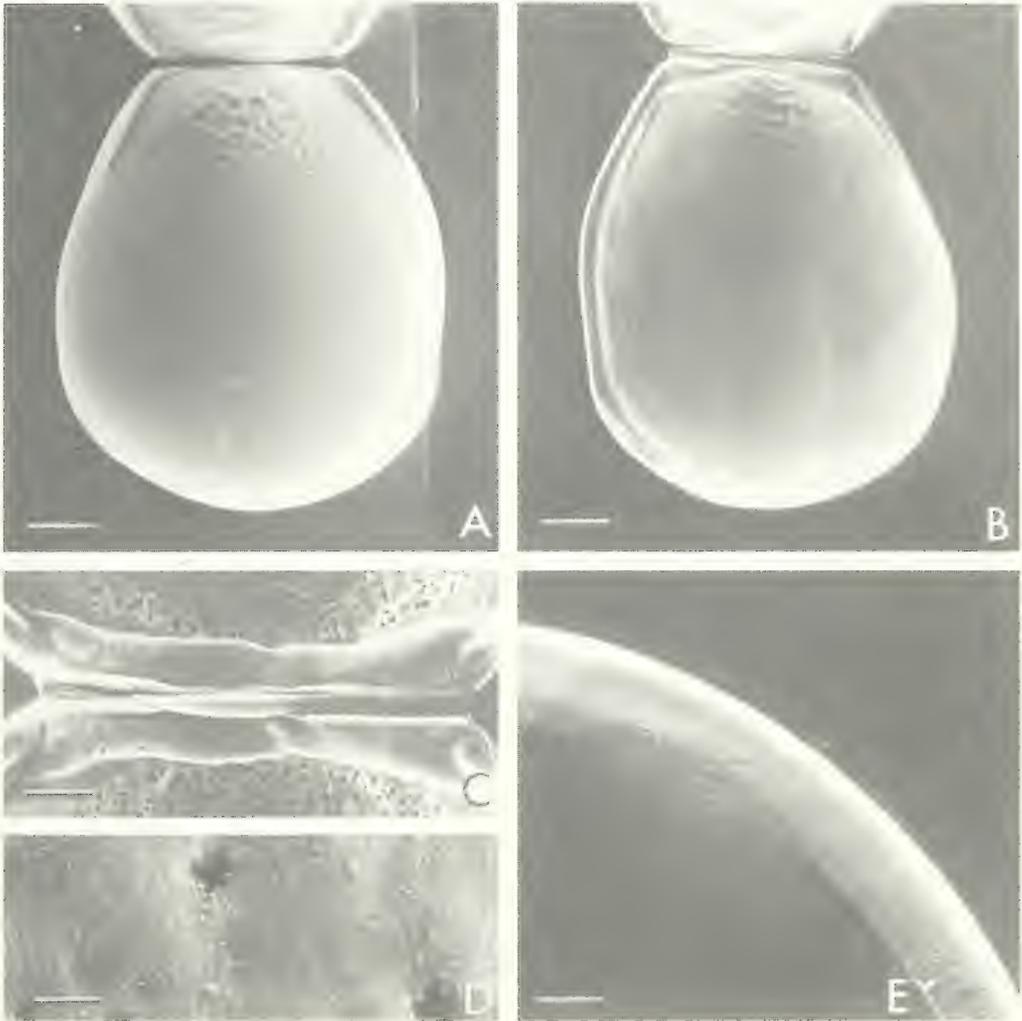


FIG. 60. Glochidium of *Lampsilis r. radiata*, MAH 897.1: A. exterior valve, bar length = 45 μ m; B. interior valve, bar length = 45 μ m; C. hinge, bar length = 20 μ m; D. exterior valve sculpture, bar length = 1 μ m; E. micro-points, bar length = 10 μ m.

Calloway & Turner (1979: pl. 3, figs. 2, 4, 6, 9). The glochidium of *L. r. luteola* was figured by Lea (1958; pl. 5, fig. 10), Surber (1912: pl. 2, fig. 15), and Arey (1921: pl. 2, fig. 3, 4; 1924: pl. 1, fig. 1).

Lampsilis abrupta (Say, 1831)
(Fig. 61A–F)

Material Examined

OSUM 13303 – Gasconade River 3 mi. N of Mount Sterling, Gasconade Co., Missouri, 17

September 1964, D. H. Stansbery & J. J. Jenkinson. OSUM 38841 – Kanawha River immediately below Kanawha Falls, 0.3 mi. SSE of Glen Ferris, 1.4 mi. SW of Gauley Bridge, Falls Twp., Fayette Co., West Virginia, 27 November 1976, K. G. Borror et al.

Description

Glochidium subspatulate, length 207 to 214 μ m ($210 \pm 2.66 \mu$ m, $n = 6$), height 251 to 259 μ m ($254 \pm 3.13 \mu$ m, $n = 6$). Dorsal margin straight, 96 to 112 μ m ($102 \pm 5.62 \mu$ m, $n = 7$)

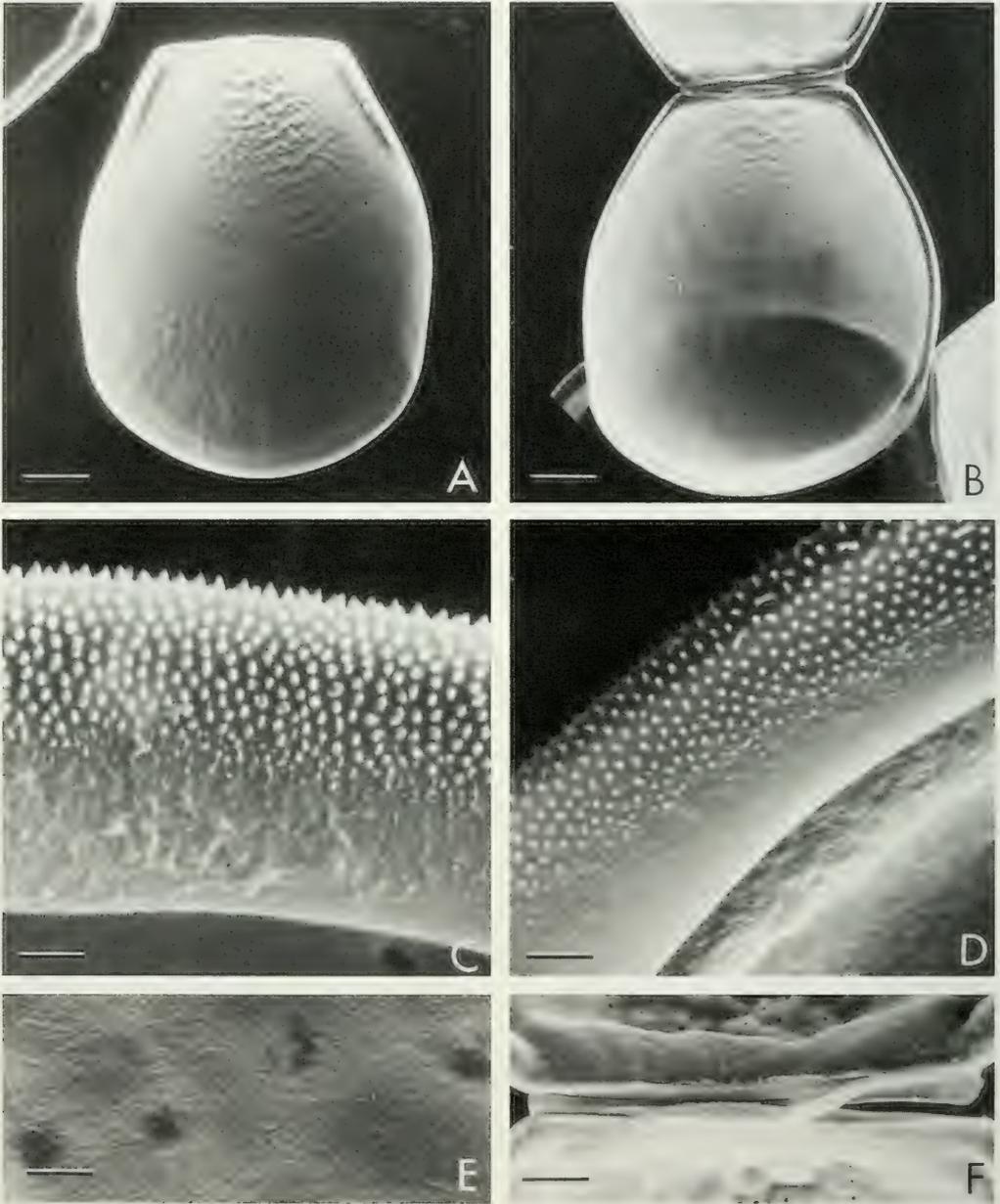


FIG. 61. Glochidium of *Lampsilis abrupta*; A. exterior valve, OSUM 13303, bar length = 40 μ m; B. interior valve, OSUM 38841, bar length = 40 μ m; C. micropoints, OSUM 13303, bar length = 2 μ m; D. micropoints, OSUM 38841, bar length = 2 μ m; E. exterior valve sculpture, OSUM 13303, bar length = 1 μ m; F. hinge, OSUM 13303, bar length = 15 μ m.

in length. Exterior surface finely malleated, wrinkled near umbo. Fine structure of exterior surface of valve rough (Fig. 61E). Pits few in number, more or less evenly distributed throughout valve. Dorsal alae long, extending about one fifth height of valve. Hinge of a sin-

gle specimen with the following ligament lengths: anterior ligament, 46 μ m; central ligament, 38 μ m; posterior ligament, 27 μ m. Central ligament centered about 44% from posterior to anterior. Micropoints lanceolate, arranged in broken vertical rows on wide ven-

tral flange and on rim of ventral margin, covering proximal half of ventral flange, leaving wide unsculptured distal flange margin.

Remarks

Lea (1863) stated that this glochidium is, "almost exactly the same with *multiradiatus*," [= *Lampsilis fasciola*, *fide* Ortmann & Walker, 1922]; however, the glochidium of *L. abrupta* is much smaller than that of *L. fasciola*. Ortmann (1912) gave length and height measurements of $190\ \mu\text{m} \times 210\ \mu\text{m}$ and $200\ \mu\text{m} \times 250\ \mu\text{m}$ for the glochidium of *L. orbiculata* (= *L. abrupta*, *fide* Stansbery et al., 1985). My figures are nearer his second set of measurements, and the glochidia were all about the same size. There is often differential maturation of glochidia and Ortmann's smaller individuals may have been immature. This glochidium was figured by Ortmann (1911: pl. 89, fig. 22, as *L. orbiculata*).

Lampsilis higginsi (Lea, 1857)
(Fig. 62A–F)

Material Examined

OSUM 49024.1 Mississippi River, R.Mi. 633.3, E channel, N end of Indian Isle, 2.1 mi. SW of Prairie du Chien, Sec. 1/2, T6N, R7W, Craford Co., Wisconsin, 11 November 1980, A. Reed, donor: M. E. Havlik.

Description

Glochidium subspatulate, length 214 to 217 μm ($216 \pm 2.12\ \mu\text{m}$, $n = 2$), height 254 to 257 μm ($256 \pm 2.12\ \mu\text{m}$, $n = 2$). Dorsal margin straight, 108 to 118 μm ($111 \pm 4.72\ \mu\text{m}$, $n = 4$) in length. Anterior and posterior margins dorsally divergent, subparallel ventrally. Ventral margin gently curved. Surface of valve finely malleated, with concentric ridges near umbo. Dorsal alae strongly curved, extending about half distance of dorsally divergent portions of lateral margins. Fine structure of exterior surface rough (Fig. 62E). Central ligament 39 to 47 μm ($43 \pm 5.66\ \mu\text{m}$, $n = 2$) long, centered about 47% from posterior to anterior. Anterior ligament 38 to 40 μm ($39 \pm 1.41\ \mu\text{m}$, $n = 2$) in length; posterior ligament about 31 μm long. Micropoints lanceolate, arranged in incomplete rows on ventral flange and on rim of ventral margin. Micropoints decreasing in size distally, covering about three quarters of flange

surface. Unsculptured distal flange margin moderate in width.

Remarks

Waller et al. (1988) gave almost identical morphometric data for this glochidium. They also found that the glochidium of *L. recta* had similar morphometrics. They suggest, however, that the glochidia are also the same shape and can be distinguished only by the extent of the development of dorsal alae and placement of the central hinge ligament. Their conclusion regarding shape was based on their inability to distinguish any differences in outline upon overlaying light microscopy transparencies. I found that the glochidium of *L. recta* can best be described as subelliptical, with a rounded ventral border and equally curved lateral margins (similar to that of *A. pectorosa*), whereas the glochidium of *L. higginsi* is subspatulate, with a gently curved ventral margin and lateral margins that are dorsally divergent and ventrally parallel. The anterior margin of *L. higginsi* is slightly more rounded than the posterior margin, and the margins are clearly not equal. These additional differences help distinguish these glochidia. This glochidium was figured by Waller et al. (1988: figs. 2, 4), who gave length, height and hinge length measurements of $215\ \mu\text{m} \times 259\ \mu\text{m} \times 110\ \mu\text{m}$.

Lampsilis ovata (Say, 1817)
(Fig. 63A–F)

Material Examined

L. ovata: OSUM 43164.1 – Clinch River above mouth of Copper Creek, 1.3 mi. S of Clinchport, 9.3 mi. W of Gate City, Scoot Co., Virginia, 21 October 1978, D. H. Stansbery et al. *L. ornata*: OSUM 54661–Amite River 1 mi. NNW of Denham Springs, about 13 mi. WNW of Livingston, T6S, R3E, Ward 2, Livingston Parish, Louisiana, 1 October 1967, E. N. Lambremont et al. *L. satura*: OSUM 38973.51, 38973.52 – West Fork San Jacinto River below I-45 bridge, 4.5 mi. S of Conroe, 36 mi. N of Houston, Montgomery Co., Texas, 20 July 1968, D. H. Stansbery et al.

Description

Glochidium broadly subspatulate, length about 232 μm , height 271 to 276 μm ($274 \pm$

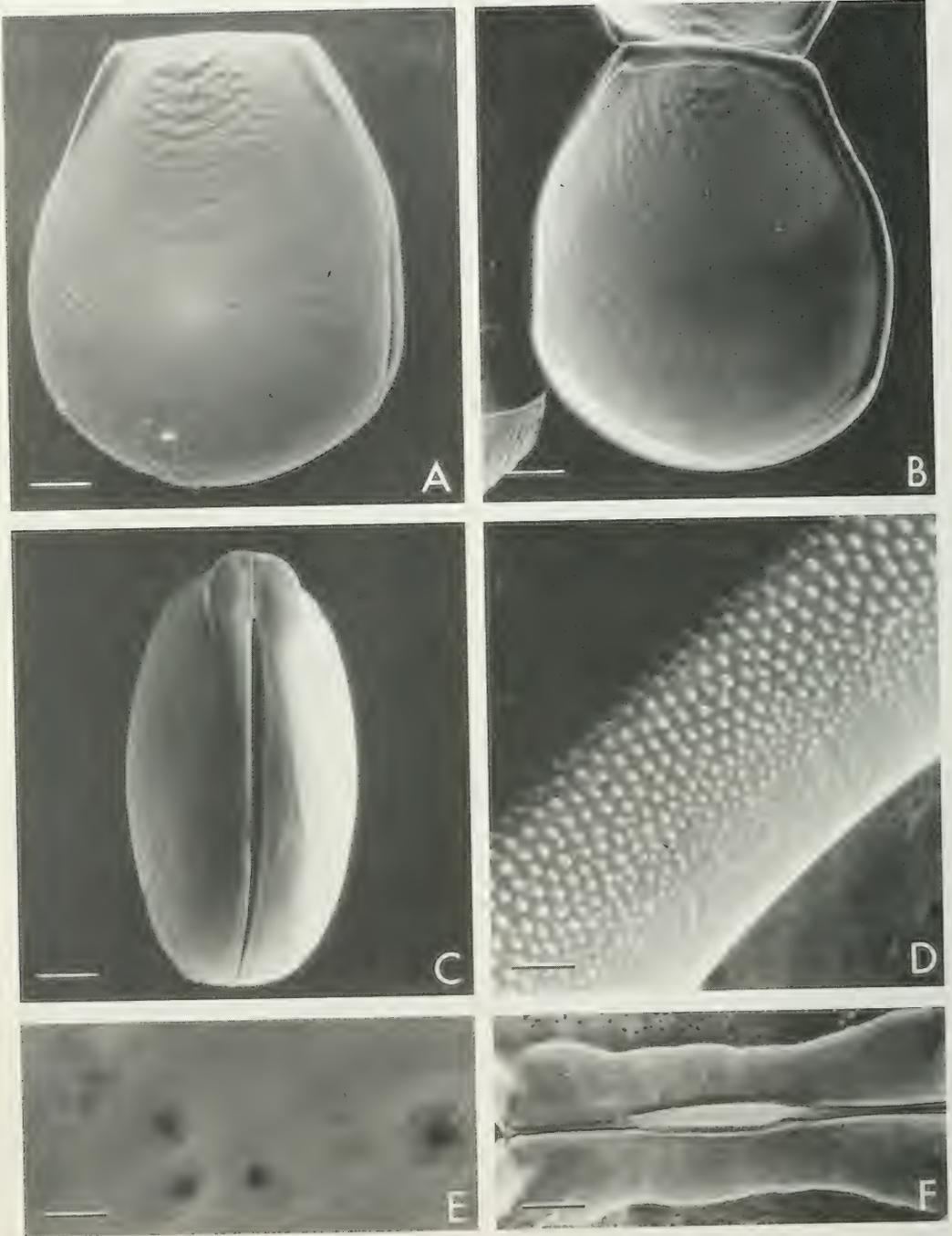


FIG. 62. Glochidium of *Lampsilis higginsi*, OSUM 49024.1; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. lateral valve view, bar length = 35 μ m; D. micropoints, bar length = 2 μ m; E. exterior valve sculpture, bar length = 1 μ m; F. hinge, bar length = 15 μ m.

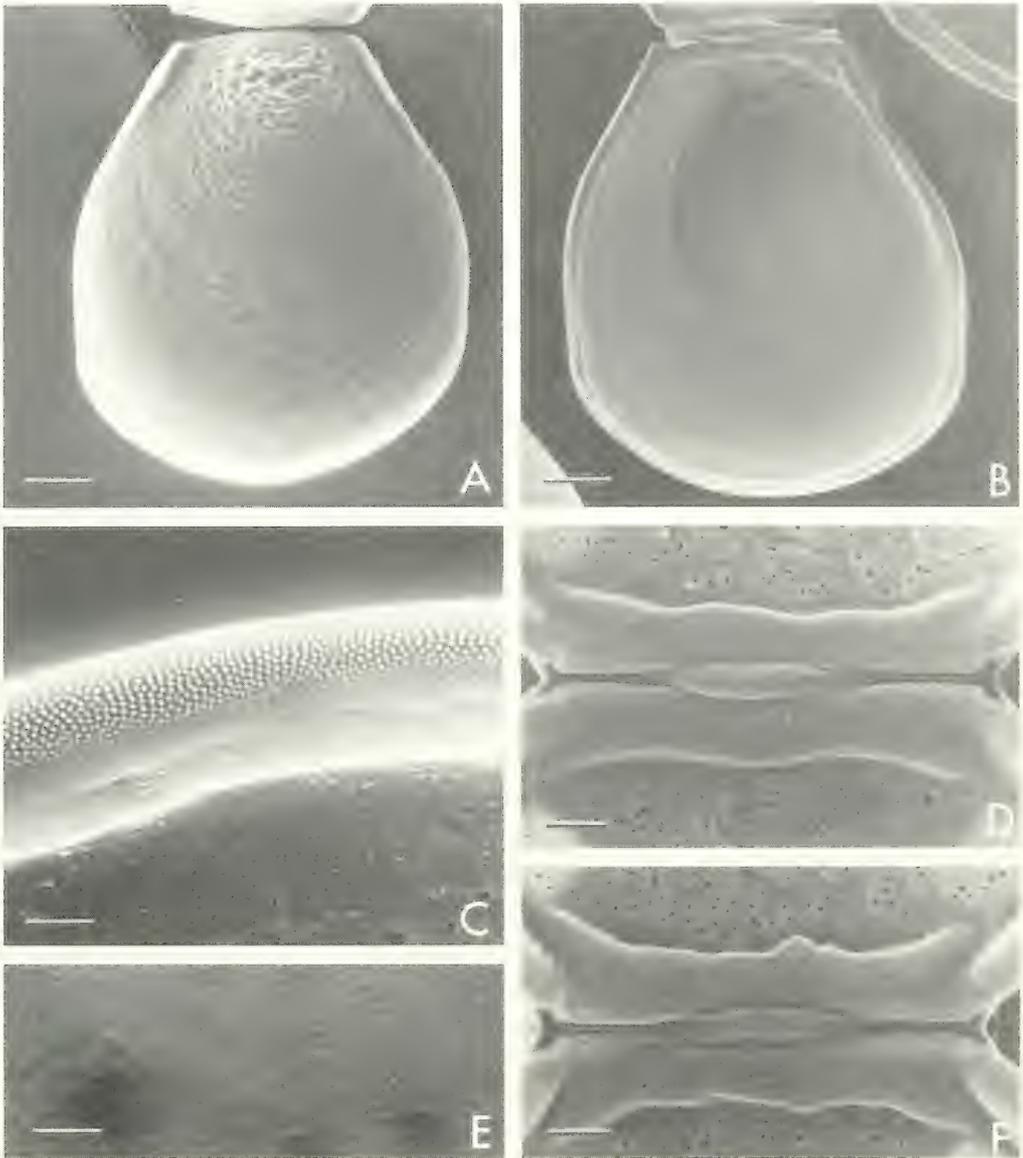


FIG. 63. Glochidium of *Lampsilis ovata*, OSUM 43164.1; A. exterior valve, bar length = 40 μm ; B. interior valve, bar length = 40 μm ; C. micropoints, bar length = 5 μm ; D. exterior valve sculpture, bar length = 1 μm ; E. hinge, bar length = 20 μm ; F. hinge, bar length = 20 μm .

3.54 μm , $n = 2$). Dorsal margin straight, 113 to 119 μm ($116 \pm 2.58 \mu\text{m}$, $n = 4$) in length. Dorsal portions of lateral margins strongly divergent; ventral portions of these margins subparallel; anterior margin slightly curved. Dorsal alae extending about three quarters length of dorsal divergent portion of lateral

margins, ventral margin gently curved. Fine structure of exterior surface of valve rough (Fig. 63E). Central ligament 43 to 44 μm ($44 \pm 0.71 \mu\text{m}$, $n = 2$) long, centered about 49% from posterior to anterior. Anterior ligament 39 to 40 μm ($40 \pm 0.71 \mu\text{m}$, $n = 2$) long; posterior ligament 34 to 35 μm ($35 \pm 0.71 \mu\text{m}$, $n = 2$) in

length. Micropoints bluntly pyramidal to lanceolate, arranged in broken vertical rows on ventral flange and on rim of ventral margin, covering three quarters of ventral flange, leaving moderately wide unsculptured distal flange margin.

Remarks

The glochidium of *L. ovata* is distinguished from those previously described by its broadly subspatulate shape. However, this shape is also found in the glochidia of the other members of the *L. ovata* complex (i.e., *L. ventricosa*, *L. satura* and *L. ornata*). Relative size may be the only way to distinguish these species. Morphometric data for *Lampsilis satura* is: length, 220 to 223 μm ($222 \pm 2.12 \mu\text{m}$, $n = 2$); height, 268 to 269 μm ($269 \pm 0.71 \mu\text{m}$, $n = 2$); hinge length, 113 to 117 μm ($115 \pm 1.82 \mu\text{m}$, $n = 4$); anterior ligament length, 42 to 43 μm ($43 \pm 0.71 \mu\text{m}$, $n = 2$); central ligament length, 40 μm ; and posterior ligament length, 33 to 35 μm ($34 \pm 1.41 \mu\text{m}$, $n = 2$). The position of the central ligament is about 48% from posterior to anterior. Morphometric data for *Lampsilis ornata*: length, 198 to 204 μm ($202 \pm 3.21 \mu\text{m}$, $n = 3$); height, 257 to 260 μm ($258 \pm 1.53 \mu\text{m}$, $n = 3$); hinge length, 96 to 100 μm ($98 \pm 2.08 \mu\text{m}$, $n = 3$). A single specimen gave the following ligament lengths: anterior ligament, 38 μm ; central ligament, 33 μm ; posterior ligament, 29 μm . The midpoint of the central ligament is about 46% from posterior to anterior. The glochidium of *L. ovata* was figured by Lea (1858: p1.5, fig. 15) and Isom (1983: fig. 1c).

Lampsilis ventricosa (Barnes, 1823)
(Fig. 64A–G)

Material Examined

OSUM 44619 – Green River below Lock 5 dam at Glenmore, 12 mi. N of Bowling Green, Warren Co., Kentucky, 21 October 1979, D. H. Stansbery et al. MAH 846.1 – Fish Creek at bridge 2.0 mi. NW of Edgerton, 11.9 mi. W of Bryan, Sec. 20, T6N, R1E, St. Joseph Twp., Williams Co., Ohio, 29 October 1985, D. H. Stansbery et al. MAH 954.1, 954.2, 954.3, 954.4, 954.5 & 954.6–Sugar River 300 m downstream of Lake Belle View Dam, 150 m upstream of St. Rt. 69 bridge, at Belleville, Sec. 34, T5N, R8E, Dane Co., Wisconsin, 15 May 1986, M. A. Hoggarth & D. J. Heath.

Description

Glochidium broadly subspatulate, length of 240 to 255 μm ($249 \pm 4.46 \mu\text{m}$, $n = 18$), height 274 to 293 μm ($283 \pm 6.30 \mu\text{m}$, $n = 18$). Dorsal margin straight, 104 to 118 μm ($111 \pm 4.06 \mu\text{m}$, $n = 21$) in length. Shape very much like that of *L. ovata*. Straight dorsal portion of lateral margin strongly divergent; ventral portion of lateral margin almost parallel; ventral margin gently curved. Dorsal alae long, strongly arched (Figs. 64A, C). Fine structure of exterior valve sculpture rough (Fig. 64F). Central ligament 37 to 42 μm ($39 \pm 1.98 \mu\text{m}$, $n = 8$) in length, centered about 47% from posterior to anterior. One female produced glochidia with a more posterior central ligament (43%) whereas another produced glochidia with a more anterior central ligament (50%). Anterior ligament 38 to 50 μm ($44 \pm 4.82 \mu\text{m}$, $n = 8$) long; posterior ligament 29 to 33 μm ($32 \pm 1.41 \mu\text{m}$, $n = 8$) in length. Micropoints lanceolate to bluntly pyramidal, arranged in broken vertical rows on wide ventral flange and on rim of ventral margin, covering about 75% of flange and leaving moderately wide unsculptured distal flange margin.

Remarks

The glochidium of *L. ventricosa* is longer and higher than that of *L. ovata*, but both species have about the same hinge length. This glochidium was figured by Lea (1858: p1.5, fig. 13, as *Unio occidentens*, = *L. ventricosa*, *vide* Johnson, 1970), Ortmann (1911: p1.89, fig. 23), Surber (1912: p1. 2, fig. 24), and Waller et al. (1988: figs. 5, 6). Surber gave length and height measurements of 200 $\mu\text{m} \times 250 \mu\text{m}$ for this glochidium; Ortmann (1912) gave 250 $\mu\text{m} \times 290 \mu\text{m}$; and Waller et al. gave 216 $\mu\text{m} \times 257 \mu\text{m}$. My measurements are 249 $\mu\text{m} \times 283 \mu\text{m}$. Waller et al. and Surber collected their material from the Mississippi River, whereas Ortmann's and my material came from smaller streams.

Lampsilis reeviana brevicula (Call, 1887)
(Fig. 65A–E)

Material Examined

OSUM 45363.20, 45363.22, 45363.35 & 45363.50 – Current River “between Mo. Rt. 106 and Van Buren,” about 9 mi. of stream, Shannon/Carter Co., Missouri, 24 October 1981, R. D. Oesch.

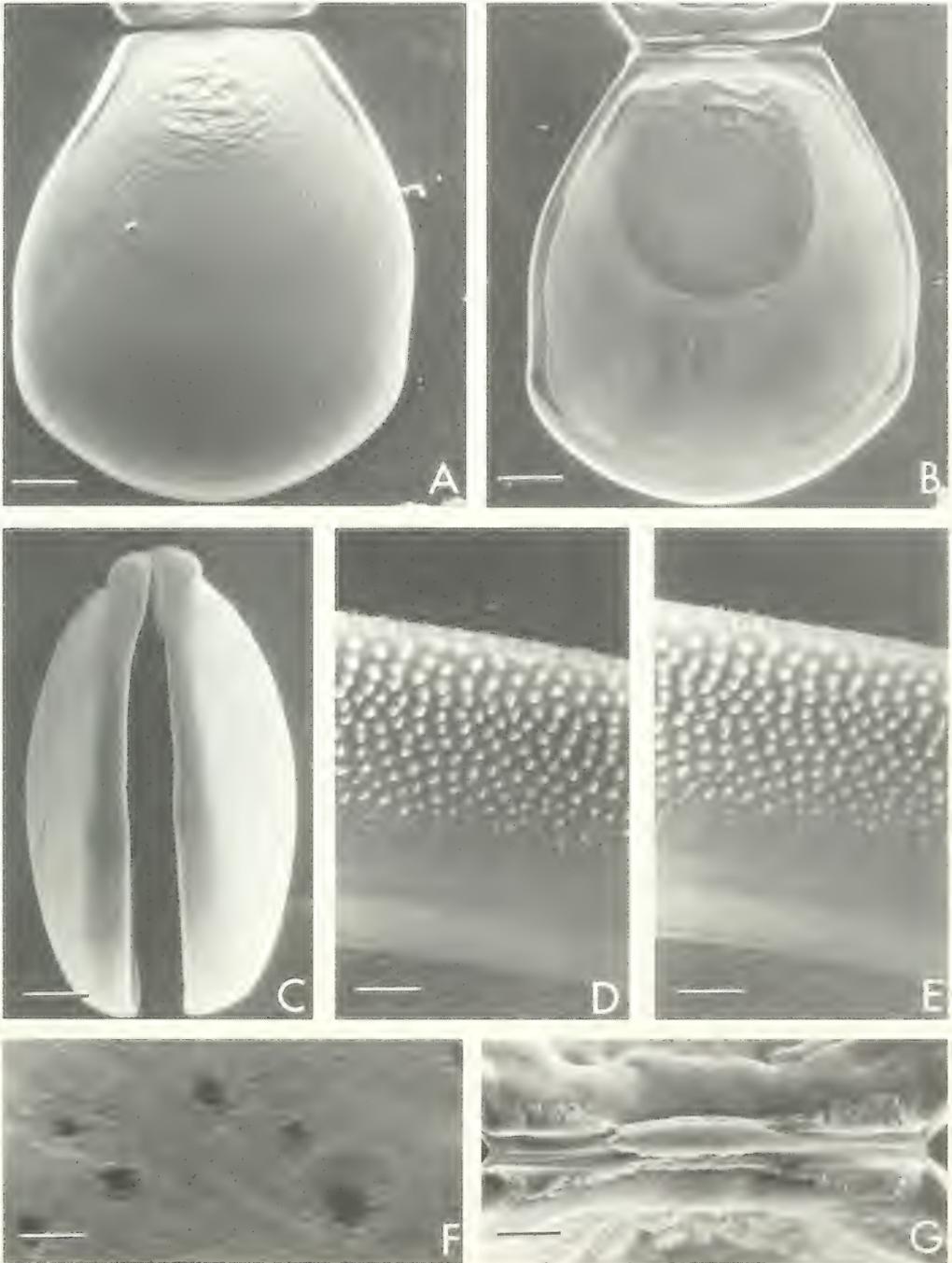


FIG. 64. Glochidium of *Lampsilis ventricosa*; A. exterior valve, MAH 954.6, bar length = 40 μm ; B. interior valve, MAH 954.3, bar length = 40 μm ; C. lateral view, OSUM 44619, bar length = 40 μm ; D. micropoints, MAH 954.3, bar length = 2 μm ; E. micropoints, MAH 954.4, bar length = 2 μm ; F. exterior valve sculpture, MAH 954.1, bar length = 1 μm ; G. hinge, MAH 954.6, bar length = 15 μm .

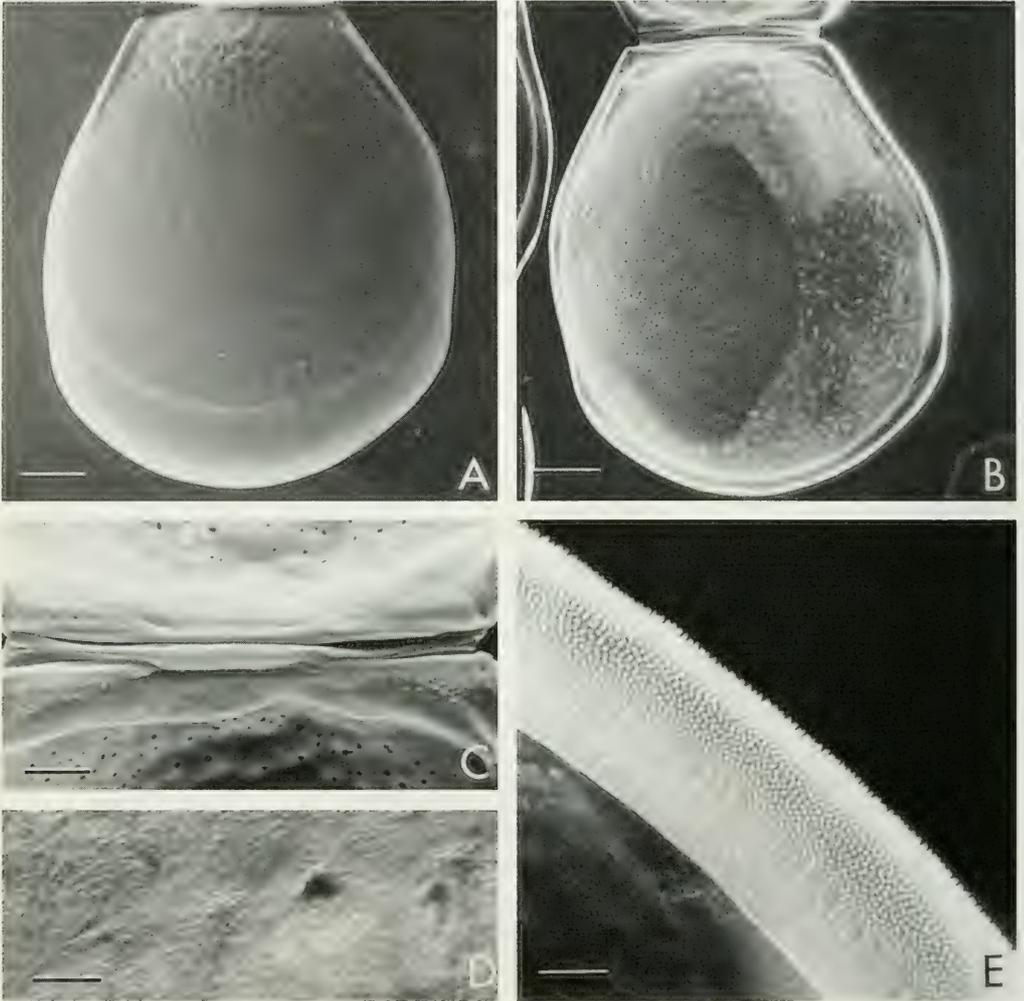


FIG. 65. Glochidium of *Lampsilis breviculata*; A. exterior valve, OSUM 45363.35, bar length = 40 μ m; B. interior valve, OSUM 45363.20, bar length = 40 μ m; C. hinge, OSUM 45363.50, bar length = 15 μ m; D. exterior valve sculpture, OSUM 45363.50, bar length = 1 μ m; E. micropoints, OSUM 45363.35, bar length = 5 μ m.

Description

Glochidium broadly subspatulate length 230 to 245 μ m ($235 \pm 6.05 \mu$ m, $n = 8$), height 286 to 297 μ m ($290 \pm 4.37 \mu$ m, $n = 8$). Dorsal margin straight, 113 to 127 μ m ($119 \pm 4.50 \mu$ m, $n = 9$) long. Lateral margins straight, strongly divergent dorsally, subparallel ventrally. Anterior margin slightly more curved ventrally than the posterior margin; ventral margin gently curved throughout its length. Dorsal alae about as long as the divergent dorsal portion of lateral margins. Fine structure of the exterior surface rough (Fig. 65D). Central ligament 37 to 48 μ m ($43 \pm 4.39 \mu$ m,

$n = 5$) in length, centered about 47% from posterior to anterior. Anterior ligament 38 to 50 μ m ($43 \pm 5.45 \mu$ m, $n = 5$) long; posterior ligament 32 to 36 μ m ($34 \pm 2.19 \mu$ m, $n = 5$) in length. Micropoints lanceolate, arranged in broken vertical rows on ventral rim of valve and on a wide ventral flange, covering proximal half of ventral flange, leaving a wide unsculptured distal flange margin.

Remarks

This glochidium was figured by Surber (1915: p1. 1, fig. 14), who gave length and height measurements of 230 μ m \times 290 μ m.

Lampsilis crocata (Lea, 1841)
(Fig. 66A–F)

Material Examined

OSUM 54485.1, 54485.2 – Tar River at U.S. Rt. 64 bridge, 2.4 mi. SW of Spring Hope, 10.8 mi. WSW of Nashville, Nash Co., Tennessee, 1973, M. Imlay. OSUM 42060.1 – Waccamaw River just below Lake Waccamaw Dam, 6.2 mi. SE of Hallsboro, 11.7 mi. ESE of Whiteville, Columbus Co., North Carolina, 21 July 1978, D. H. Stansbery et al.

Description

Glochidium narrowly subspatulate, length 235 to 249 μm ($242 \pm 6.23 \mu\text{m}$, $n = 8$), height 287 to 303 μm ($293 \pm 5.03 \mu\text{m}$, $n = 8$). Dorsal margin straight, 110 to 125 μm ($118 \pm 4.81 \mu\text{m}$, $n = 8$) in length. Lateral margins weakly divergent dorsally, ventrally subparallel. Ventral margin evenly and broadly curved; dorsal alae long, strongly arched (Fig. 66A, C). Fine structure of exterior surface of valve rough (Fig. 66E). Central ligament 52 to 56 μm ($54 \pm 2.83 \mu\text{m}$, $n = 2$) long, centered about 46% from posterior to anterior. Anterior ligament 36 to 39 μm ($38 \pm 2.12 \mu\text{m}$, $n = 2$) long; posterior ligament about 32 μm long. Micropoints bluntly lanceolate, covering about 75% of ventral flange, arranged in broken vertical rows on ventral flange and on rim of ventral margin, remaining about equal in length from proximal to distal. Unsculptured distal flange margin narrow.

Remarks

The shape of this glochidium is like that of *L. teres*, but it is much larger. No published figure of this glochidium was found.

Lampsilis cariosa (Say, 1817)
(Fig. 67A–D)

Material Examined

OSUM 54500 – Tar River at U.S. Rt. 15 bridge, 2.7 mi. SW of Clay, 6.7 mi. SSW of Oxford, Granville Co., North Carolina, 1973, M. Imlay.

Description

Glochidium narrowly subspatulate, length about 241 μm , height about 314 μm , hinge length about 109 μm . Dorsal margin straight;

ventral margin evenly and broadly curved; lateral margins about equally divergent dorsally and ventrally parallel. Dorsal alae long. Fine structure of exterior surface of valve rough (Fig. 67C). Valve pitted, except along margins; pitting reduced in adductor muscle scar (Fig. 67B). Umbo deeply folded and the center of numerous concentric ridges. Hinge ligaments not seen. Micropoints lanceolate, restricted to proximal half of ventral flange and on rim of ventral valve margin, arranged in broken vertical rows. Unsculptured distal edge of ventral flange wide.

Remarks

It is clear that this glochidium is very different from that of *Leptodea ochracea*. The long dorsal alae, lanceolate micropoints, and rough exterior valve sculpture place the glochidium of *L. cariosa* firmly in *Lampsilis*, whereas the small dorsal alae, lamellate micropoints, loose-looped exterior sculpture, and lateral valve gape separate the glochidium of *Leptodea ochracea* from *Lampsilis*. No published figure of this glochidium was found.

Lampsilis fasciola Rafinesque, 1820
(Fig. 68A–F)

Material Examined

OSUM 25467 – Kanawha River immediately below Kanawha Falls, 1.2 mi. below Gauley Bridge, Falls Twp., Fayette Co., West Virginia, 1 October 1970, D. H. Stansbery & W. J. Clench. MAH 562.3 – Big Darby Creek at and above Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp., Pickaway Co., Ohio, 19 August 1983, M. A. Hoggarth. OSUM 55977 – Olentangy River just above Powell Rd. bridge, 1.6 mi. E of Powell, 9.8 mi. S of Delaware, T3N, R19W, Delaware Co., Ohio, 29 September 1983, C. B. Stein et al. OSUM 55033.2 – Clinch River, R.Mi. 269.7, 1.0 mi. above Carbo, 1.8 mi. W of Cleveland, 6.2 mi. NW of Lebanon, Castlewood Twp., Russell Co., Virginia, 26 July 1985, D. H. Stansbery & C. B. Stein.

Description

Glochidium narrowly subspatulate, length 240 to 250 μm ($247 \pm 4.47 \mu\text{m}$, $n = 5$) height 287 to 295 μm ($290 \pm 3.08 \mu\text{m}$, $n = 5$). Dorsal

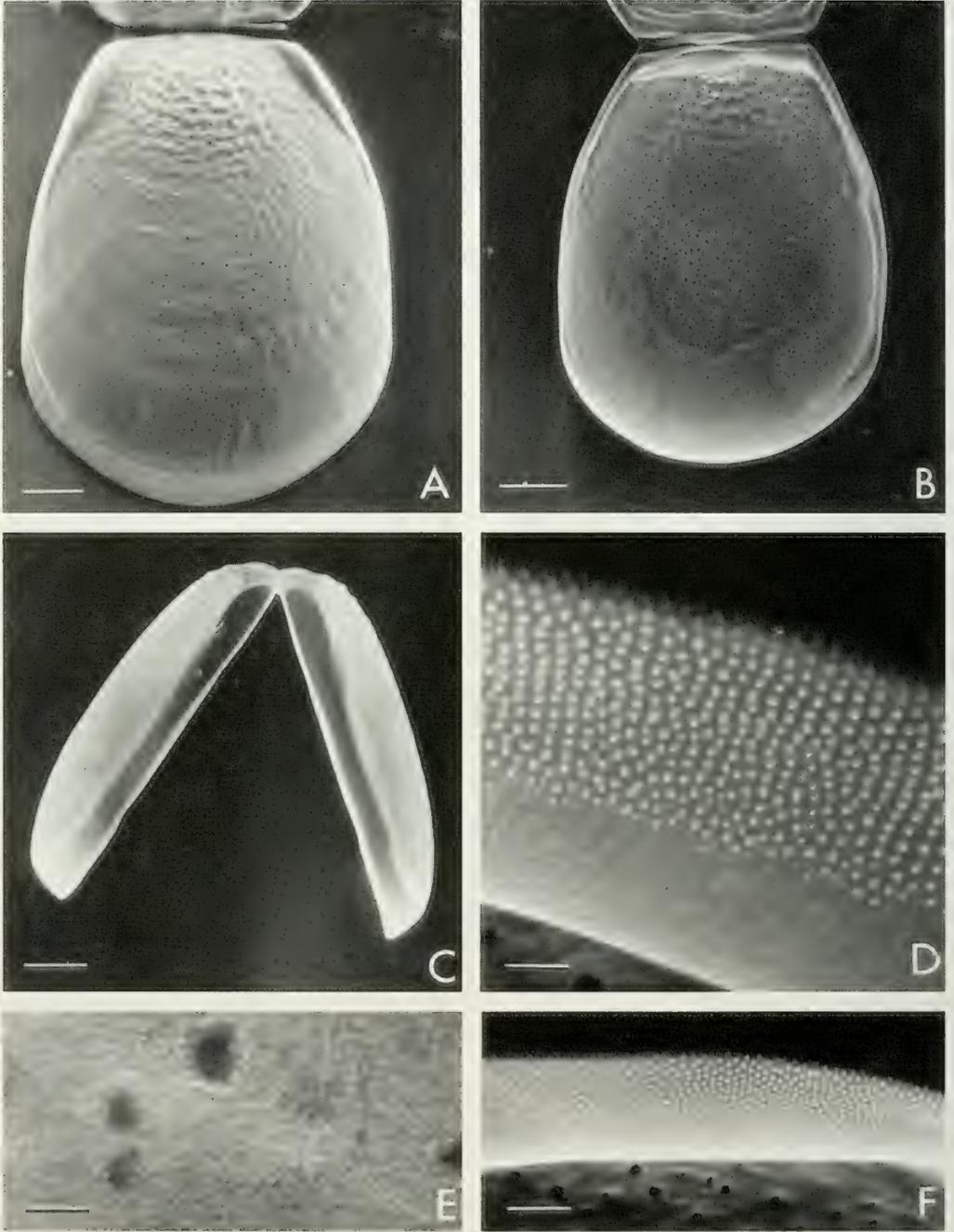


FIG. 66. Glochidium of *Lampsilis crocata*; A. exterior valve, OSUM 42060.1, bar length = 40 μm ; B. interior valve, OSUM 42060.1, bar length = 50 μm ; C. lateral view, OSUM 54485.1, bar length = 50 μm ; D. micropoints, OSUM 54485.1, bar length = 2 μm ; E. exterior valve sculpture, OSUM 54485.1, bar length = 1 μm ; F. micropoints, OSUM 42060.1, bar length = 5 μm .

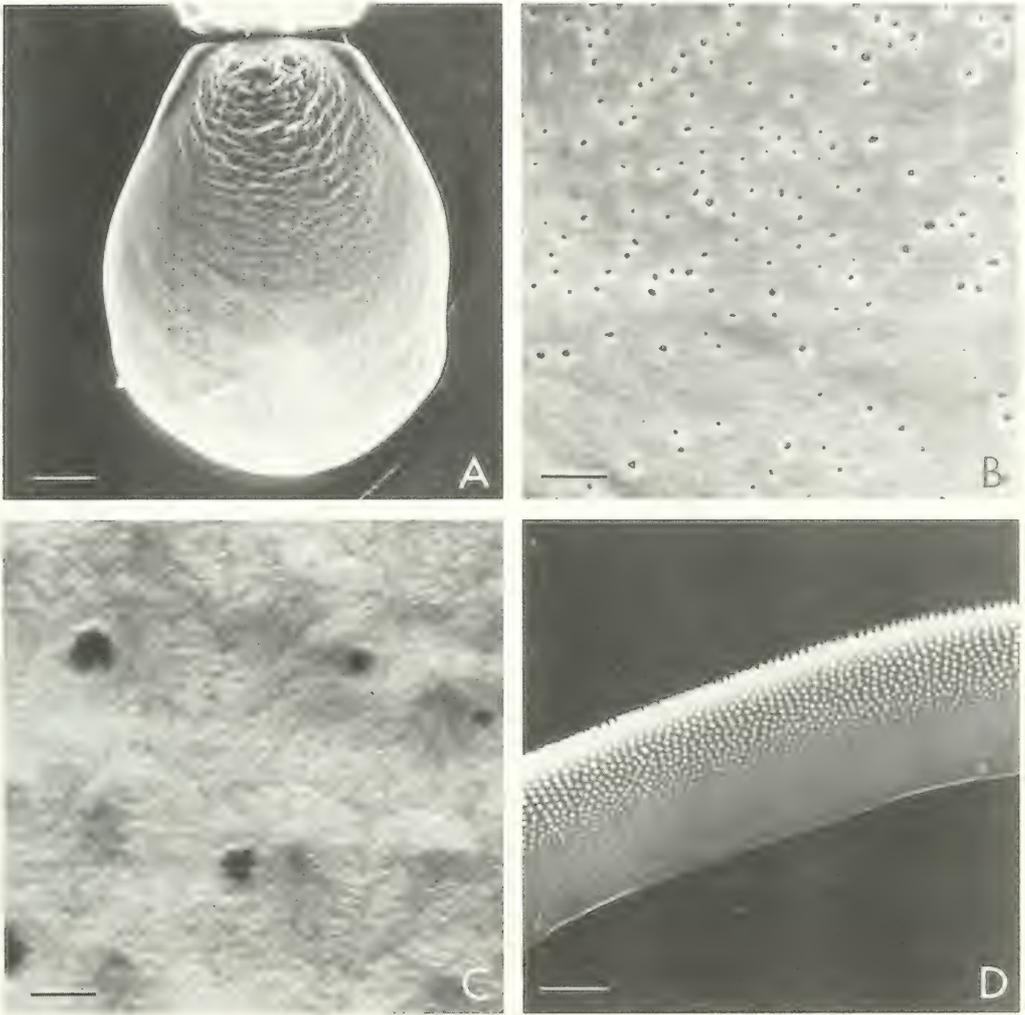


FIG. 67. Glochidium of *Lampsilis cariosa*, OSUM 54500; A. exterior valve, bar length = 45 μm ; B. interior valve pitting and adductor muscle scar, bar length = 15 μm ; C. exterior valve sculpture, bar length = 1 μm ; D. micropoints, bar length = 5 μm .

margin straight, 107 to 115 μm (110 ± 2.95 μm , $n = 5$) in length. Posterior margin strongly divergent from dorsal margin for about half its length. Near its midpoint, posterior margin bending ventrally, straightening out to run more or less perpendicular to hinge. Anterior margin diverging from dorsal margin at a lesser angle, straight for about three quarters of its length, curving continuously to ventral margin. Ventral margin gently and evenly curved. Dorsal alae long, with a strong arch (Fig. 68A, C). Fine structure of exterior surface of the valve rough (Fig. 68E). Central ligament about 44 μm long, centered about

48% from posterior margin. Anterior ligament about 38 μm long; posterior ligament about 33 μm long. Micropoints lanceolate, arranged in broken vertical rows on ventral rim of valve and on a wide ventral flange. Micropoints decreasing in size distally on flange covering about three quarters of its proximal surface, leaving narrow unsculptured distal flange margin.

Remarks

The glochidium of *L. fasciola* was figured by Lea (1858: p1. 5, fig. 17, as *Unio multiradia-*

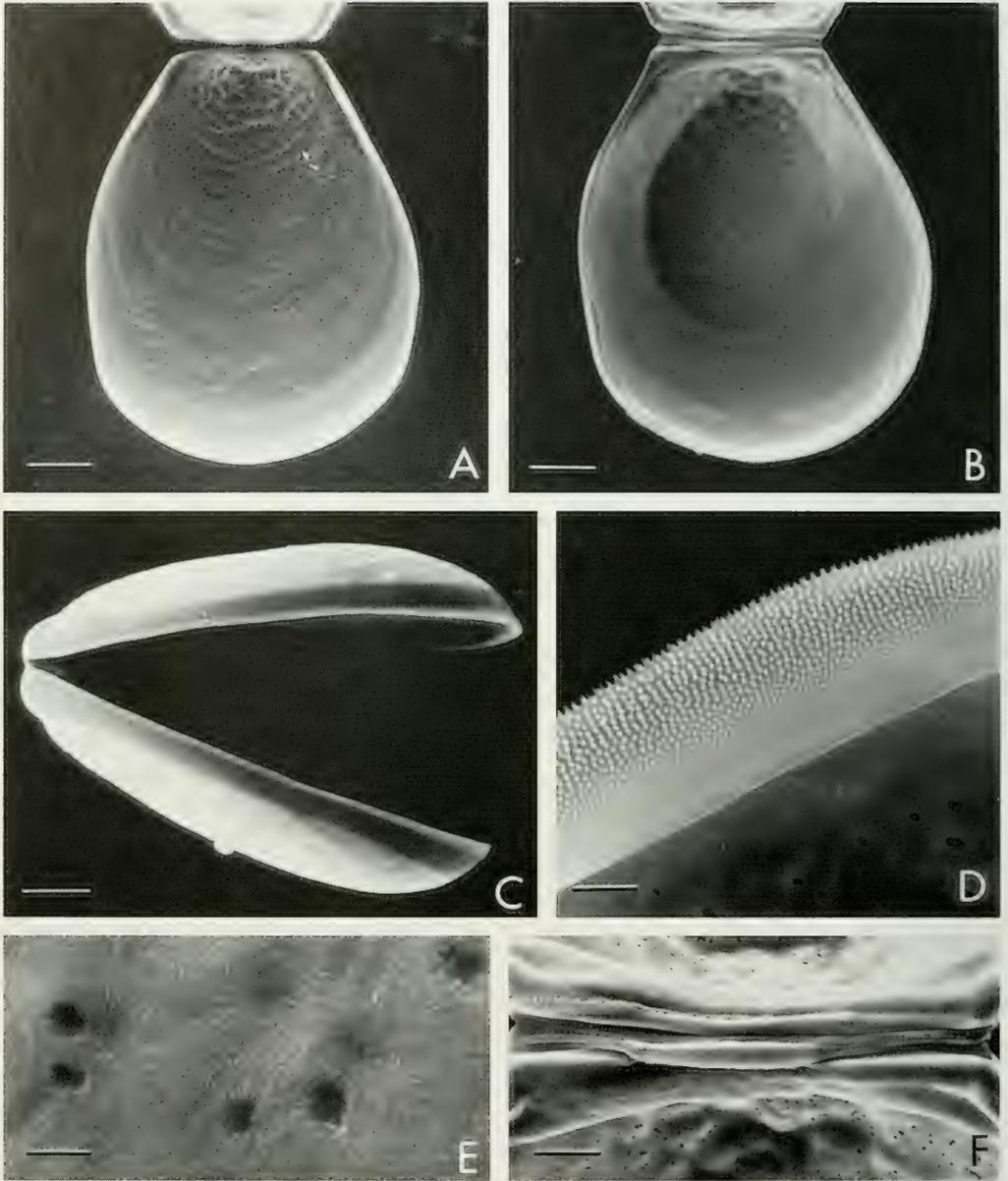


FIG. 68. Glochidium of *Lampsilis fasciola*; A. exterior valve, OSUM 55033.2, bar length = 45 μ m; B. interior valve, OSUM 55033.2, bar length = 45 μ m; C. lateral view, OSUM 25467, bar length = 40 μ m; D. micropoints, OSUM 55033.2, bar length = 5 μ m; E. exterior valve sculture, OSUM 55033.2, bar length = 1 μ m; F. hinge, OSUM 55033.2, bar length = 15 μ m.

tus, = *L. fasciola*, fide Ortmann & Walker, 1922) and Surber (1915: p1. 1, fig. 2, as *L. multiradiatus*). This glochidium is like other narrowly subspatulate glochidia, except that the anterior margin is slightly more rounded. In this regard, this glochidium resembles that

of *Obovaria*, but it will not be confused with *Obovaria* because of its posterior margin, wide ventral flange, and rough exterior valve sculture. The glochidium of *Obovaria* has a curved posterior margin, narrow ventral flange, and loose-looped exterior valve sculture.

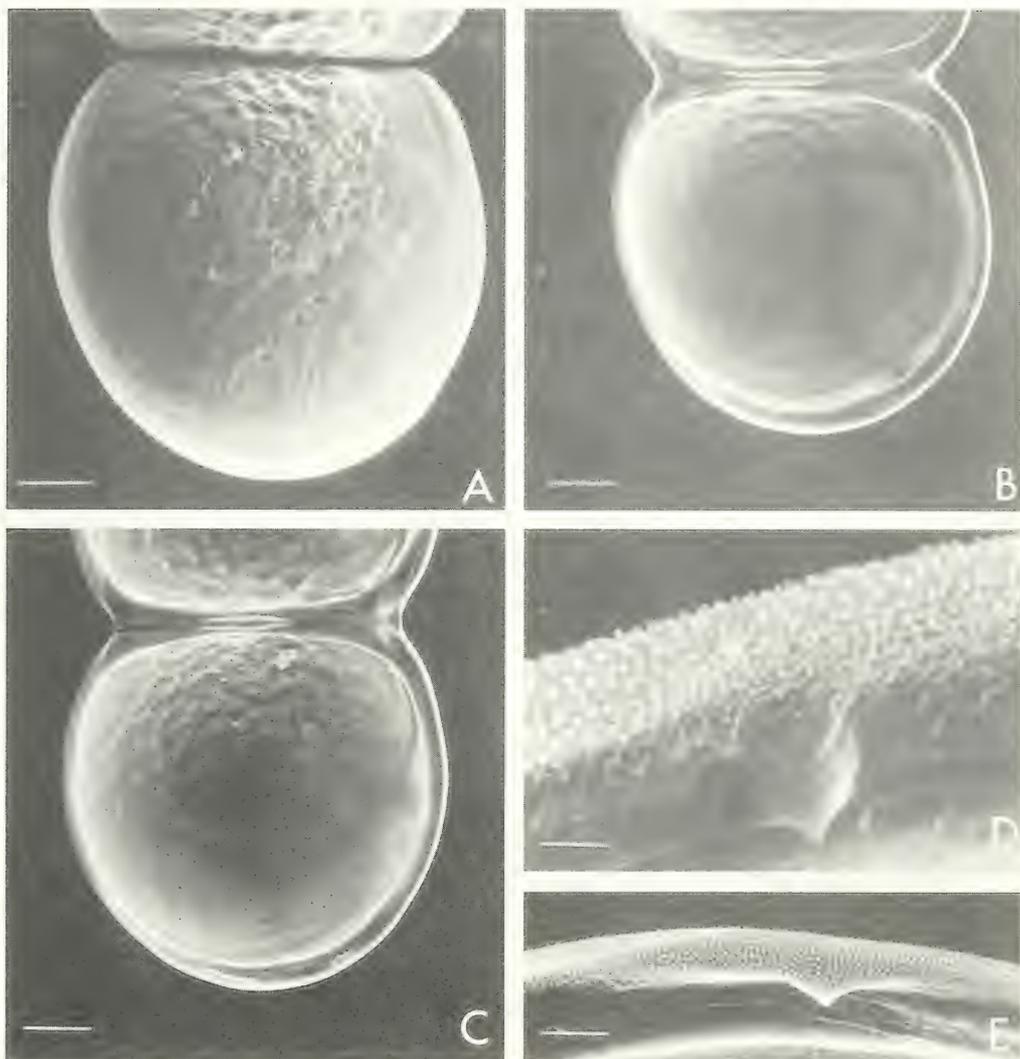


FIG. 69. Glochidium of *Epioblasma triquetra*. MAH 588.1; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 40 μ m; C. interior valve, bar length = 40 μ m; D. supernumerary hook and micropoints, bar length = 2 μ m; E. micropoints, bar length = 5 μ m.

Epioblasma triquetra (Rafinesque, 1820)
(Fig. 69A-E)

Darby Twp., Pickaway Co., Ohio, 13 April
1984, M. A. Hoggarth & G. T. Watters.

Material Examined

MAH 588.1 – Big Darby Creek at access 0.9 mi. N of Harrisburg, 1.7 mi. NW of Orient, Pleasant Twp., Franklin Co., Ohio, 27 September 1983, M. A. Hoggarth. MAH 631.1 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/

Description

Glochidium depressed subelliptical, length 208 to 217 μ m ($214 \pm 3.54 \mu$ m, $n = 5$), height 205 to 214 μ m ($211 \pm 4.09 \mu$ m, $n = 5$). Dorsal margin straight, 149 to 156 μ m ($152 \pm 3.05 \mu$ m, $n = 5$) in length. Lateral margins equally and gently curved; ventral margin broadly curved. Exterior surface sparsely malleated,

pits few. Exterior valve sculpture loose-looped. Dorsal alae absent (Fig. 69A). Central ligament 48 to 53 μm ($50 \pm 2.89 \mu\text{m}$, $n = 3$) in length, centered about 44% from posterior to anterior. Anterior ligament 56 to 65 μm ($60 \pm 4.51 \mu\text{m}$, $n = 3$) long; posterior ligament 39 to 48 μm ($43 \pm 4.73 \mu\text{m}$, $n = 3$) in length. Micropoints blunt, irregular in shape. Ventral flange narrow; distal unsculptured flange edge wide. Supernumerary hooks (Fig. 69D) about 2 μm in length, on unsculptured portion of flange.

Remarks

This glochidium was figured by Lea (1858: pl. 5, fig. 19, as *Unio triangularis*, = *E. triquetra*, *vide* Ortmann & Walker, 1922) and Ortmann (1911: pl. 89, fig. 24). Ortmann (1919) note that Lea's figure was incorrect and gave length and height measurements of 210 μm \times 210 μm (Ortmann, 1912). Lea's figure does not show the morphological depression of the valve.

Epioblasma brevidens (Lea, 1831) (Fig. 70A–F)

Material Examined

OSUM 16173 – Cedar Creek about 1 mi. SE of Mingo at bridge, Tishomingo Co., Mississippi, 5 November 1965, P. Yokley & B. G. Isom.

Description

Glochidium depressed subelliptical, length 213 to 220 μm ($216 \pm 3.11 \mu\text{m}$, $n = 4$), height 205 to 214 μm ($210 \pm 4.24 \mu\text{m}$, $n = 4$). Dorsal margin straight, 141 to 153 μm ($147 \pm 5.06 \mu\text{m}$, $n = 4$) long. Lateral margins equally and evenly curved; ventral margin broadly curved. Exterior surface sparsely malleated, pits few. Valve pitting reduced in adductor muscle scar; however, irregular ridges, probably to increase surface area for attachment of large adductor muscle, found within muscle scar (Fig. 70B, D). Fine structure of exterior surface of valve rough (Fig. 70E), Dorsal alae absent. Central ligament 48 to 49 μm ($49 \pm 0.71 \mu\text{m}$, $n = 2$) in length, centered about 46% from posterior margin. Anterior ligament 49 to 52 μm ($51 \pm 2.12 \mu\text{m}$, $n = 2$) in length; posterior ligament 41 to 47 μm ($44 \pm 4.24 \mu\text{m}$, $n = 2$) long. Micropoints small, triangular, arranged in horizontal rows on ventral flange and on

ventral rim of valve. Supernumerary hooks triangular (Fig. 70B) to lanceolate (Fig. 70C). Unsculptured distal margin of ventral flange narrow.

Remarks

This glochidium is similar to that of *E. triquetra*. They are of about the same shape and size and have similar micropoints and central ligament positions. However, the glochidium of *E. brevidens* has rough exterior valve sculpture, whereas that of *E. triquetra* has loose-looped exterior valve sculpture.

Epioblasma capsaeformis (Lea, 1834) (Fig. 71A–G)

Material Examined

OSUM 42007 – Clinch River at Clinchport, above swinging bridge, 2.3 mi. N of Speer's Ferry, Scoot Co., Virginia, 2 July 1978, C. R. Ciola & K. L. Ciola.

Description

Glochidium depressed subelliptical, length 240 to 252 μm ($246 \pm 3.88 \mu\text{m}$, $n = 8$), height 226 to 238 μm ($234 \pm 4.34 \mu\text{m}$, $n = 8$). Dorsal margin straight, 154 to 173 μm ($162 \pm 6.33 \mu\text{m}$, $n = 8$) long. Lateral margins about equal and gently curved (Fig. 71A, B) to more broadly curved (Fig. 71E). Ventral margin broadly curved. Dorsal alae absent. Loose-looped sculpture covering valve exterior (Fig. 71F). Adductor muscle scar large, with only a few pits and with numerous small ridges (Fig. 71B, E). Central ligament 51 to 60 μm ($57 \pm 3.43 \mu\text{m}$, $n = 6$) in length, centered about 46% from posterior to anterior. Anterior ligament 52 to 64 μm ($59 \pm 4.45 \mu\text{m}$, $n = 6$) long; posterior ligament 40 to 57 μm ($47 \pm 5.89 \mu\text{m}$, $n = 6$) in length. Micropoints blunt, irregular (Fig. 71C), arranged in broken horizontal rows on a narrow ventral flange. Near proximal border, micropoints coalescing to form broken ridges. Distal unsculptured margin narrow. Attenuate supernumerary hooks present on otherwise unsculptured distal flange margin (Fig. 71D).

Remarks

This glochidium is slightly larger than that of *E. triquetra* or *E. brevidens*, but it is otherwise very similar. No published figure of this glochidium was found.

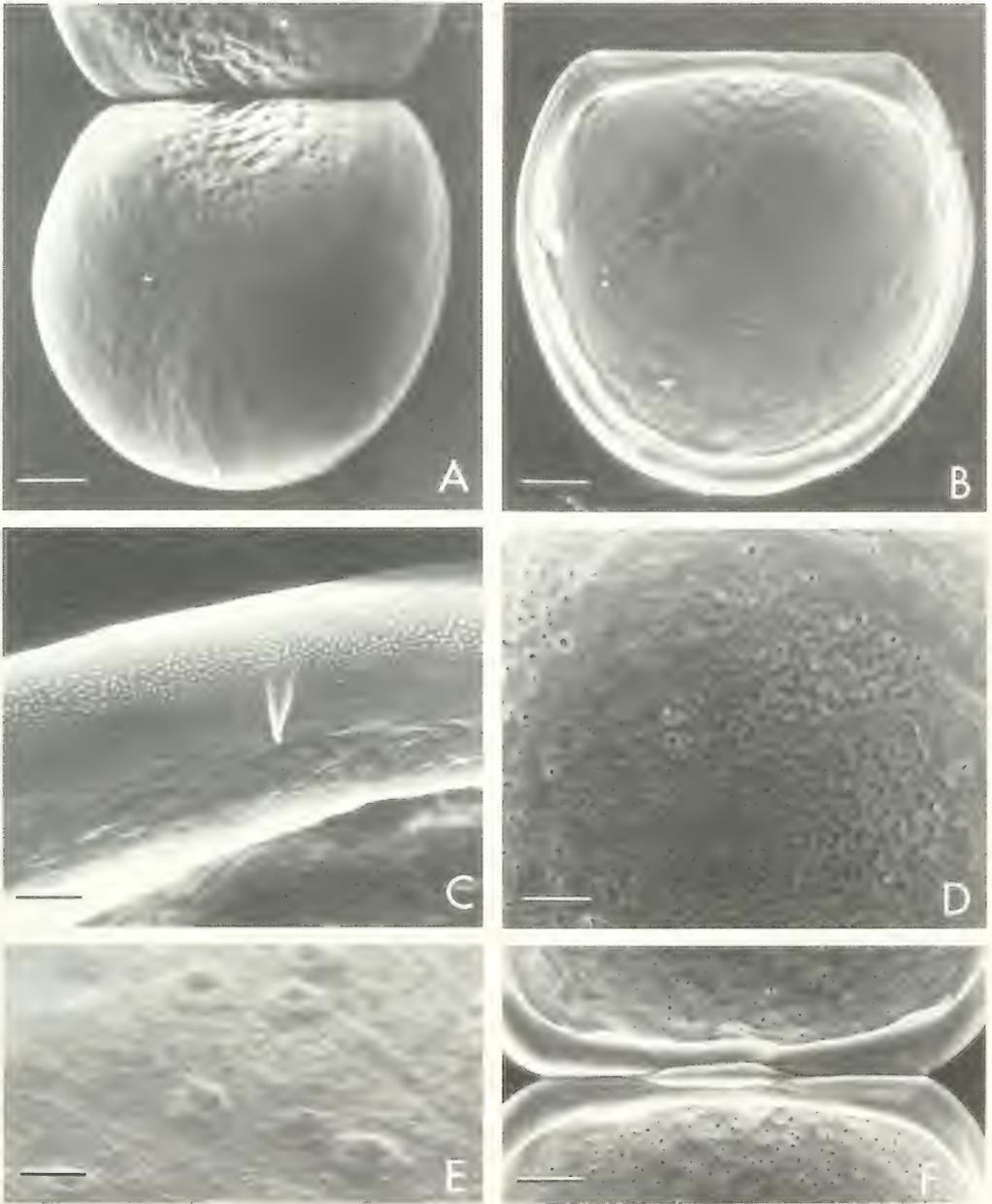


FIG. 70. Glochidium of *Epioblasma brevidens*. OSUM 16173; A. exterior valve, bar length = 40 μm ; B. interior valve, bar length = 40 μm ; C. supernumerary hook and micropoints, bar length = 5 μm ; D. adductor muscle scar, bar length = 20 μm ; E. exterior valve sculpture, bar length = 1 μm ; F. hinge, bar length = 25 μm .

Epioblasma rangiana (Lea, 1839)
(Fig. 72A-F)

Material Examined

MAH 632.1 – Big Darby Creek at Scioto-

Darby (Mt. Sterling-Commercial Pt.) Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp., Pickaway Co., Ohio, 13 April 1983, M. A. Hoggarth & G. T. Watters. MAH 701 – Big Darby Creek at Scioto-Darby (Mt. Sterling-Commercial Pt.)

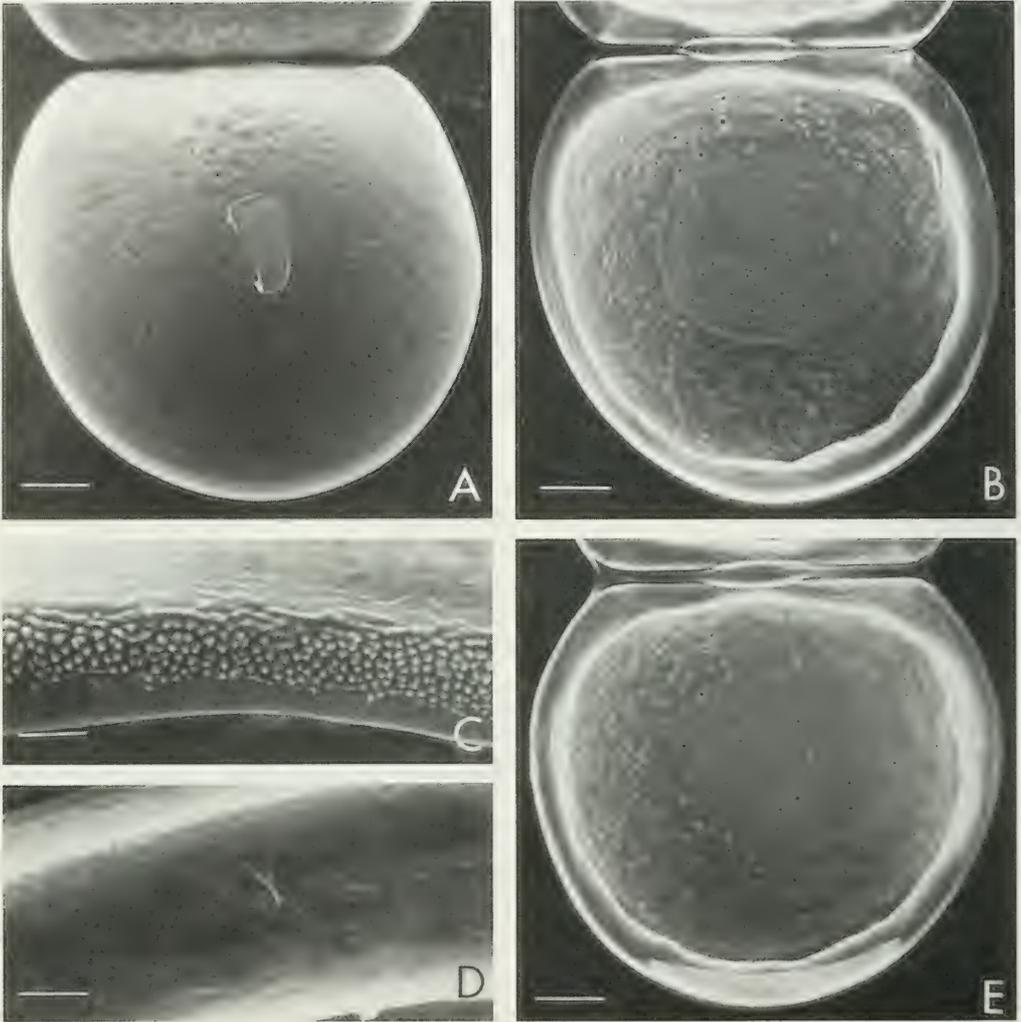


FIG. 71. Glochidium of *Epioblasma capsaeformis*, OSUM 42007; A. exterior valve, bar length = 35 μ m; B. interior valve, bar length = 35 μ m; C. micropoints, bar length = 5 μ m; D. supernumerary hook, bar length = 5 μ m; E. interior valve, bar length = 35 μ m; F. exterior valve sculpture, bar length = 1 μ m; G. hinge, bar length = 25 μ m.

Rd. bridge, 3.4 mi. S of Orient, 15.3 mi. SW of Columbus, Scioto/Darby Twp., Pickaway Co., Ohio, 6 September 1985, M. A. Hoggarth.

Description

Glochidium depressed subelliptical, length 238 to 258 μ m ($249 \pm 7.34 \mu$ m, $n = 8$), height 210 to 238 μ m ($224 \pm 9.34 \mu$ m, $n = 8$). Dorsal margin straight, 160 to 188 μ m ($170 \pm 6.30 \mu$ m, $n = 8$) in length. Lateral margins and ventral margin are about equally rounded. Shape of this glochidium approaching that of *Obli-*

quaria reflexa (subrotund). Dorsal alae absent. Surface sculpturing loose-looped (Fig. 72F). Central ligament 53 to 59 μ m ($57 \pm 3.21 \mu$ m, $n = 3$) long, centered about 46% from posterior to anterior. Anterior ligament 60 to 77 μ m ($68 \pm 8.62 \mu$ m, $n = 3$) long; posterior ligament 50 to 55 μ m ($52 \pm 5.21 \mu$ m, $n = 3$) in length. Micropoints blunt, located on a narrow ventral flange and on ventral rim of valve (Fig. 72E), covering about 50% of flange, leaving a wide unsculptured distal flange margin. Supernumerary hooks present as triangular extensions of this unsculptured area of flange.

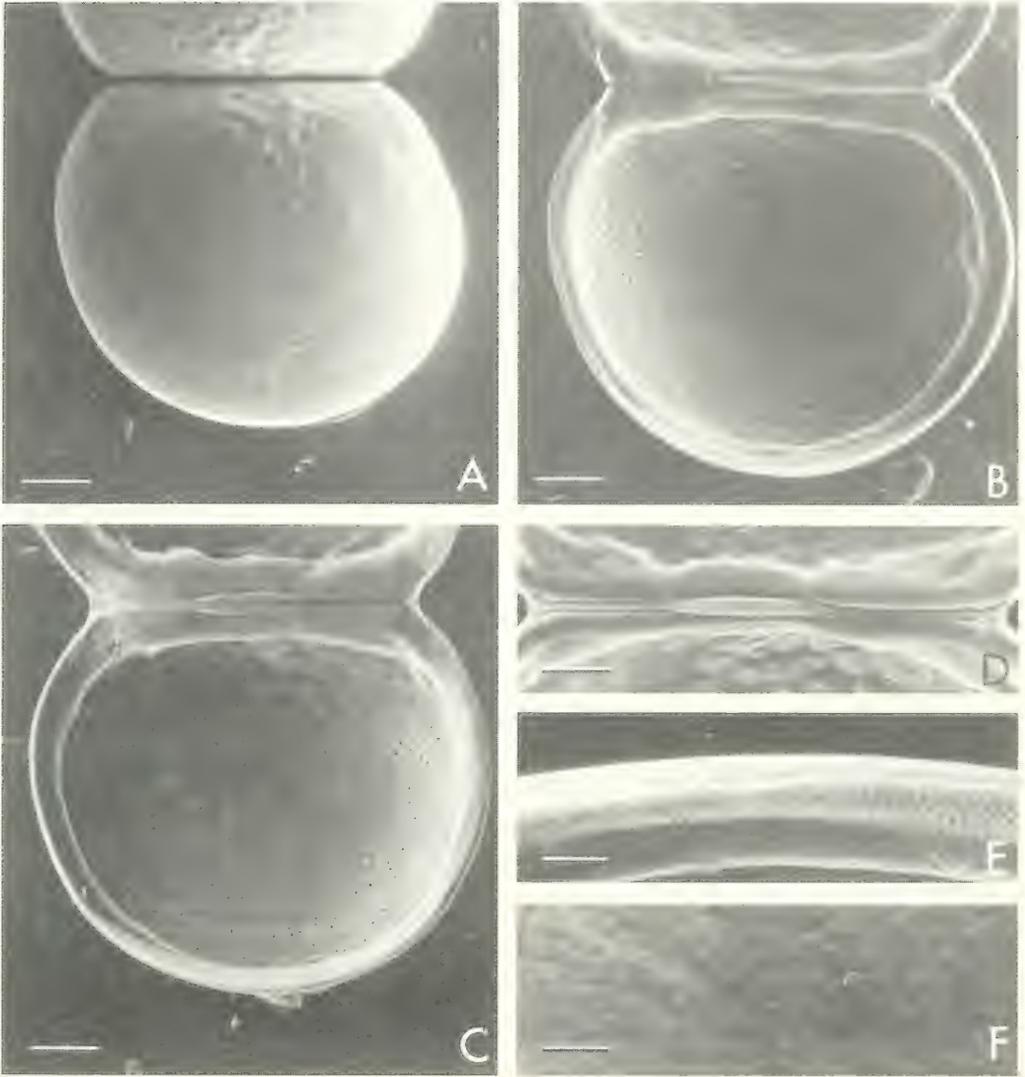


FIG. 72. Glochidium of *Epioblasma torulosa rangiana*; A. exterior valve, MAH 632.1, bar length = 40 μm ; B. interior valve, MAH 632.1, bar length = 35 μm ; C. interior valve, MAH 632.1, bar length = 35 μm ; D. hinge, MAH 701, bar length = 25 μm ; E. micropoints and supernumerary hooks, MAH 701, bar length = 5 μm ; F. exterior valve sculpture, MAH 632.1, bar length = 1 μm .

Remarks

This glochidium can be distinguished by its extreme morphological depression. It was figured by Lea (1858: pl. 5, fig. 21, as *Unio perplexus*, = *E. rangiana*, fide Ortmann & Walker, 1922), who provided length and height measurements of 224 $\mu\text{m} \times 240 \mu\text{m}$. Lea's figure is correctly drawn, so it must be assumed that his valve length equals my valve height and that his valve height equals my valve length.

His measurements would then agree with mine and Ortmann's (1912) 260 $\mu\text{m} \times 230 \mu\text{m}$.

DISCUSSION

Anyone interested in the literature dealing with the glochidium of a particular species is referred to the remarks section above for that species, and this information will not be repeated here. Instead, this discussion will re-

late glochidial structure to an understanding of relationships within the Unionidae and provide some suggestions for future investigations. Larval stages have been instrumental in our understanding of relationships among higher taxa of invertebrates (phyla, classes and orders) and have played increasing roles in lower level taxonomic decisions, even within the Unionidae (Sterki, 1903; Ortmann, 1912; Morrison, 1955).

Hoeh (1990) proposed a radically new arrangement of the genus *Anodonta*, which has resulted in the splitting of the that genus into *Anodonta s.s.*, *Utterbackia*, and *Pyganodon* in North America. The genus *Anodonta* was restricted to *A. cygena* and related species found in Europe and along the east coast of North America. Glochidial characters, support this reclassification of the genus *Anodonta*, with one exception. Hoeh (1990) includes the species *implicata* in the genus *Anodonta*, but glochidial valve shape, fine structure of the styliform hook, and exterior valve sculpture appear to separate this species from from all other species of *Anodonta*, *Utterbackia* and *Pyganodon* and suggests that additional work to refine the relationship between this species and the other members of this group is warranted. Also, it might be noted that glochidial morphology confirms the close relationship between the members of these genera and *A. ferrusianus*.

Glochidial characters assist in confirming the relationship between the Ambleminae and the Lampsilinae. Davis & Fuller (1981) conclude that the Ambleminae and Lampsilinae are monophyletic, and the lack of a clean morphological break in glochidial structure appears to agree with that conclusion. Glochidial morphology of the more primitive lampsiline species, such as *P. fasciolaris*, agree almost completely with that of some members of the amblemine species, such as *E. dilatata* and *P. dombeyana*. It is suggested that an examination of more of the glochidia of the amblemine species would be helpful to confirm this conclusion.

Perhaps, however, the most powerful demonstration of the use of glochidial characters in the determination of relationship is the confirmation of *ochracea* in the genus *Leptodea*. The species has been assigned to the genus *Lampsilis* because of adult shell morphology, including pronounced sexual dimorphism (Simpson, 1900, 1914; Johnson, 1947, 1970; Burch, 1975; Fuller & Bereza, 1975;

Clarke, 1981b; Porter & Horn 1981). Morrison (1975), on the other hand, was unable to locate mantle flaps on gravid females and placed the species in *Leptodea*. The size and shape of the glochidium of this species confirm Morrison's conclusion, which is also in agreement with most modern arrangements of unionid taxa (e.g., Turgeon et al. 1988).

The glochidia described above suggest other taxonomic relationships within the Unionidae which have not been proposed previously. Within the tribe Alasmidontini, two types of glochidia occur. One has a depressed, pyriform shape, with two row of microstylets on the styliform hook and looped exterior valve sculpture. Furthermore, these glochidia posses exceptionally large adductor muscles in cross-sectional area and dorsal placement of the adductor muscle (Hoggarth & Gaunt, 1988). Species with this type of glochidium included all members of the genus *Strophitus* examined, *A. viridis*, *A. heterodon*, *L. compressa*, *L. subviridis*, *L. holstonia* and *P. fabula*. The other type of glochidium is high, pyriform, with at least four rows of microstyletes on the hook and beaded to rosette exterior valve sculpture. These species, which include *A. undulata*, *A. marginata*, *A. confrigosus*, *L. costata* and *L. complanata*, have a smaller adductor muscle in cross-sectional area and the adductor muscle is placed further from the dorsal shell margin (Hoggarth & Gaunt, 1988). Additional study using anatomical or molecular methods might confirm closer relationships among the members of these two groups and thereby support a different taxonomy of the tribe.

Glochidial structures suggest a much different arrangement of the species of *Potamilus* and *Leptodea* than currently accepted (e.g., Turgeon et al., 1988). Furthermore, *E. lineolata* is shown to belong to this group of taxa as it posses lateral valve gape and small, obliquely oriented dorsal alae. Ortmann (1912) was the first to suggest this association based on the presence of lateral valve gape in the glochidia of *Potamilus* and *Ellipsaria*.

The genus *Potamilus* is currently defined on the basis of a single character; the presence of a ligulate glochidium. Ortmann (1912) stated, ". . .this genus [*Potamilus*] stands in all characters except the glochidia, by the side of *Paraptera* [= *Leptodea*]." The current study suggests that there are many other, more subtle, glochidial characters that can be used to define the genus. If the genus is restricted

to *alatus*, *purpuratus*, and *capax* (the glochidium of *capax* was described by Cummings et al., 1990), then it can be defined as having ligulate glochidia with lanceolate hooks at the lateral margins of the ventral flange. The glochidia of these species are generally large (210 μm \times 380 μm in length and height) (except *capax*), and they possess vermiculate exterior valve sculpture. Two species currently in the genus, *P. ohioensis* and *P. amphichaena*, lack these characters. The glochidia of these two species, although possessing lateral valve gape, lack lanceolate hooks at the ventral margin of the valve and possess looped rather than vermiculate exterior valve sculpture. They are much more rounded at the ventral margin than any other member of the genus; they are smaller (about the same size as *capax*); and they have lamellate rather than lanceolate micropoints on the ventral flange. These two species share these glochidial characters with *L. fragilis* and *L. ochracea*. I believe a study of adult anatomical characters could provide useful data that would support a different relationship among these taxa. Roe & Lydeard (1998) found molecular genetic characters support the separation of *ohioensis* and *amphichaena* from *alatus* and *purpuratus*.

Finally, it is suggested that an examination of the glochidia of additional species would suggest other taxonomic questions and/or provide support for currently accepted views of unionid taxonomy. An examination of the glochidia of the Ambleminae would be particularly helpful. Fewer amblemine glochidia have been examined with SEM than any other group primarily, because it is more difficult to tell when amblemine females are gravid and because they hold mature glochidia for a shorter period of time. Still, such structures as the coronal micropoints of *Q. infucata* and the exaggerated larval threads of *M. nervosa* and *M. boykiniana* suggest a fruitful area of investigation.

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GROWTH PATTERN AND DYNAMICS OF A SOUTHERN PERIPHERAL
POPULATION OF *PISIDIUM AMNICUM* (MÜLLER, 1774)
(BIVALVIA: SPHAERIIDAE) IN SPAIN

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ABSTRACT

The dynamics of a Spanish population of *P. amnicum*, one of the southernmost populations of the species in the world, is presented. *P. amnicum* is the only living European species of the subgenus *Pisidium s. s.* The study is based on monthly samples, from June 1990 to May 1991, from the Miño River, Galicia, northwestern Spain. Specimens from all size classes were dissected and all embryo/larval stages were counted and measured. Studies were conducted to find out the growth pattern of this population using the von Bertalanffy model. *Pisidium amnicum* in Spain is semelparous and univoltine. Only two cohorts coexisted from late spring to late summer. The life span of the species is about 15 months. The parental generation disappear in August. Juvenile recruitment occurs in April-May when water temperature ranges between 15–20°C. The minimum observed size (shell length) of a gravid specimen was 3–4 mm. Fertilized eggs were brooded for approximately nine months in the inner demibranches until they reached up to 2 mm. The bigger clams had, in the same month, more and bigger embryos (or larvae), than the smaller ones. Not all the initial embryos completed their development. Nevertheless, this phenomenon of intramarsupial suppression does not seem to be very important in the Spanish population. A main feature of the Spanish population of *P. amnicum* is the high number of larvae incubated during the months immediately before birth.

Key words: *Pisidium amnicum*, population dynamics, growth, Spain, peripheral population.

INTRODUCTION

The natural species range is the area in which it is well adapted both morphologically as ecologically. However, it is known that environmental factors may be extreme at the edge of a species distribution and may prevent it from extending its range. Under such conditions peripheral populations may show restriction to particular biotopes (Ford, 1964), tendency to isolation (Mayr, 1963), changes in the genetic structure of the populations (Ramos, 1985), as well as in other physiological aspects, such as development, fecundity or life span (Möller & Swaddle, 1997).

All known species of the family *Sphaeriidae* are hermaphroditic, and incubate fertilized eggs in brood sacs developed in the inner gill. Therefore, they are an excellent material for studying reproductive biology. Taking into account that some reproductive aspects have been mentioned as very important not only in the subgeneric characterization (Heard, 1965) but also at the specific level, it seems impor-

tant to know the specific variation in these characters along a geographical range.

Pisidium amnicum is the only European species of the subgenus *Pisidium s. s.* Only a few papers include data on the biology and reproduction of this species (Odhner, 1929; Meier-Brook, 1970; Holopainen, 1979; Holopainen & Hanski, 1986) or deal specifically with its population dynamics and growth (Daneel & Hinz, 1976; Bass, 1979; Vincent et al., 1981), but they all are referred to northern or central palearctic populations and do not include data on larval stages prior to shell formation.

This paper describes the dynamics of a Spanish isolated population, the southernmost known of the species in Europe and in the world, with the exception of the North African population cited by Kuiper (1972). Therefore, its reproductive characteristics can also be used as a sensitive measure to ascertain the extent to which this peripheral population has adapted to local conditions. Data such as life span, birth period, size at sexual

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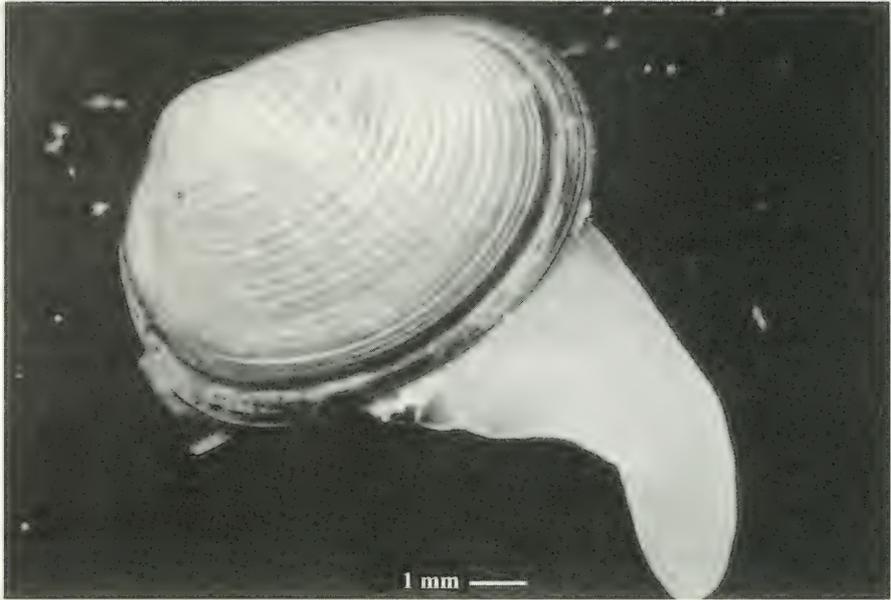


FIG. 1. Live *Pisidium amnicum* specimen from the Miño River, Galicia, Spain.

maturity, and percentage and size of incubated larvae, when compared with the available data from central and northern populations, should allow us to determine if the geographical position of this population at the edge of the species range represents marginality in the ecological and/or physiological sense, or whether the response remains essentially similar in all the populations. Studies were conducted for the first time to discover and describe the growth pattern of a population of *P. amnicum*, dissecting specimens from all size classes, and counting and measuring all embryo/larval stages.

MATERIAL AND METHODS

Specimens of *Pisidium amnicum* (Fig. 1) were collected monthly between June 1990 and May 1991 in the Miño River in north-western Iberian Peninsula (Fig. 2). From June to August the sample locality was the Miño River near Goian, Pontevedra, close to the Spain-Portugal ferryboat docks. As no more live specimens of the species were found after September (Araujo, et al., 1993), the site was changed to 15 km upstream on the same river (Fig. 2). The two sites are on the same shore of the river, which is 400 m wide, and are exposed to tidal influences as

they are 10 and 25 km, respectively, from the Atlantic Ocean.

The sampling method consisted of dragging the bottom with a dredge in which sand and mud were retained so that specimens of all size classes were collected. Animals were sorted from the sediment using a 1-mm mesh sieve. They were carried alive on ice to the laboratory in plastic jars containing water from the sample site. Artificial aeration was provided for five sec every eight h. In the laboratory, specimens of each monthly sample were measured (maximum shell length) and sorted into 1-mm size classes.

To discover the ratio of gravid animals in each size class and month, ten animals (when possible) from each class and month were dissected. To obtain data on number and size of incubated embryos (or larvae), ten specimens from each size class per month, from June to December, were prepared in the field using the method developed by Eggleton (Heard, 1965), which consists of isolating each animal in a glass tube filled with water. When the tissues decayed, the larval shells were counted and measured. Once it was realized that this technique does not permit the recovery of embryonic phases prior to shell formation, data for all months were obtained directly from the animals. After dissection, embryos and larvae present in each gill were

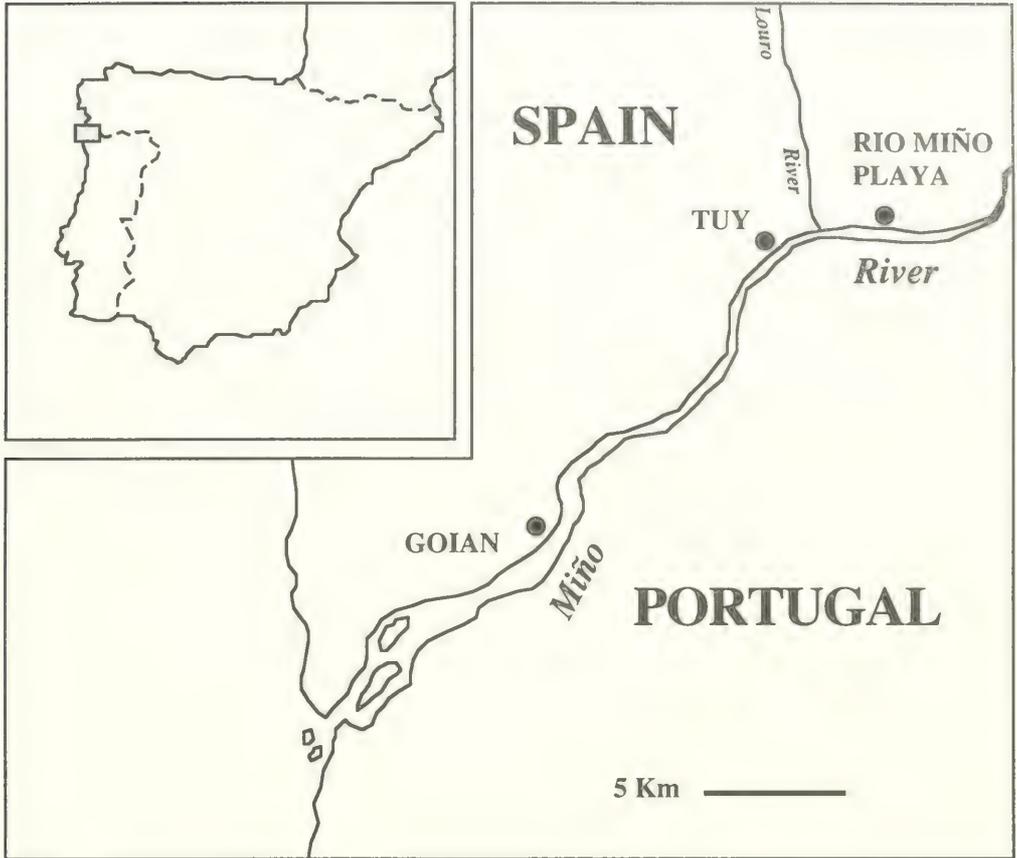


FIG. 2. Map of sample sites.

counted and measured (maximum length). The sizes of the embryos and larvae were obtained by recording the largest and smallest in each gill. Observations, dissections, counting and measuring were carried out under a Bausch and Lomb stereomicroscope with 10× oculars, 1× to 7× zoom and micrometric ocular. Due to the extremely small size of the incubated phases after fertilization, values for June to September are less reliable than those for the rest of the year.

The frequency of gravid animals was calculated from the data of dissected animals, and the number and mean size of the incubated embryos and larvae refer to the number of gravid animals. In order to check if there was lateralization, that is differences in the number and sizes of the incubated phases between gills, and as these variables are not normally distributed, a repeated measures ANOVA test

was performed using SuperAnova. Descriptive statistics were carried out using StatView 4.1. Both software packages are from Abacus Concepts by Macintosh. The possible influence on the specimens' gravidity, coded as gravid (1) and non-gravid (0), of both month and the specimen size, as well as the interaction between these two factors, was explored by means of a logistic regression analysis using the SPSS 6.0 statistical package. The effect of these factors (specimen size and month) on the number of incubated embryos for each of the gravid specimens was tested by an ANCOVA performed with the Statistica 4.1 package from Statsoft. The variances of the dependent variable (number of embryos) were not homogeneous, so the squared-root transformation was used in the analysis. In the analysis, data from June to August were excluded due to very low sample size ($n < 10$).

The same statistical procedure was used to study the relation of these factors to the size of the incubated embryos.

The von Bertalanffy model (Bertalanffy, 1934) was used for analysis of growth. Although this model was designed specifically for fisheries, it has been applied to freshwater bivalves (Morton, 1969, 1977). This mathematical model expresses length (L) as a function of age (t) as follows: $L_t = L_{inf} (1 - e^{-K(t-t_0)})$, where L_{inf} = the mean length of an "infinitely" old animal, K = the curvature parameter showing the speed at which the animal becomes L_{inf} and t_0 = the parameter of initial condition, is the time at which the animal has zero length. It does not have biological significance.

As no data about age were available, formula parameter values were estimated from size class composition of the monthly samples. With these data, the Bhattacharya (1967) method was used to identify both the different cohorts alive during the year and the mean of the normal curve adjusted to each generational group. These values were the basis to calculate the parameters of the von Bertalanffy formula. They were estimated using ELEFAN (Gayani et al., 1988) software. As the time between samples was constant (one month) the value of L_{inf} was estimated using the graphic method of Ford (1933) and Walford (1946), as discussed in Sparre et al., (1989).

While collecting specimens, physical and chemical characteristics of the water were recorded at the sample site. Temperature ($\pm 0.5^\circ\text{C}$), dissolved oxygen (± 1.0 ppm) and pH (± 0.1 pH) were monitored using an Horiba U-7 water checker. Conductivity ($\mu\text{s} \pm 0.3\%$) was measured using a Crison 523 conductivity meter. Alkalinity, calcium, total water hardness and carbonate hardness values were obtained *in situ* with Merck Aquamerck kits. The influence of the measured water physical parameters on gravidity was explored using a logistic regression analysis.

RESULTS

Population Dynamics and Growth

The raw data on collected, dissected and gravid specimens, and the number and size of the incubated phases for each gravid animal from each size class and month are in Araujo (1995). Appendix 1 summarizes this information.

Monthly histograms of the different size classes (Fig. 3) showed that there was only one reproductive period per year. Juveniles 1–2 mm long only appeared in May, when the next size class (2–3 mm) reaches its highest annual frequency. This suggests that birth occurs between April and May. Between June and July, there was notable mortality in the oldest classes (Fig. 3). The largest individuals (10–11 mm) represent a minimum ratio in June and August, and were not present the rest of the year. There is hardly any growth in winter. In fact, the Bhattacharya (1967) method identified only two cohorts that coexisted from late spring to late summer (Table 1). Recruitment occurred in May, and no members of the parental generation appeared in August, indicating that the life span of each generation is about 15 months. Values in Table 1 show that animals were born proportionally very big, and that their growth was fast, reaching a mean size between 3.3 and 4.5 mm in summer when the reproductive period began. This growth continued until December delaying until February, when it started again but more slowly. Animals were largest at the time of juvenile release, and stabilized at around 8 mm until the disappearance of the adults.

The growth curve of this population was tested using a Ford-Walford plot (Table 2). The corresponding regression analysis (Fig. 4) gave the formula: $L(t + 1) = 1.499 + 0.818L(t)$, which approaches the 45° line as expected if growth fits the von Bertalanffy formula. Therefore, the value of the asymptotic length ($L_{inf} = 8.245$ mm) was employed to calculate the dependent variable of regression in the von Bertalanffy method, the data from which appear in Table 2. Values for the intercept and slope of this regression are $a = 0.183$ and $b = 0.201$, respectively. Parameters of the growth formula of von Bertalanffy are: $L_{inf} = 8.254$; $K = 0.201$; $t_0 = 0.913$ years.

Figure 5 shows the monthly frequencies of gravid animals in each size class. No gravid animals under 3–4 mm have ever been found. Therefore, the minimum size to be gravid appears to be 3–4 mm, although this size was only recorded in October (50% of collected specimens). From June (just after juvenile recruitment) to September, some gravid animals in classes up to 7 mm were found, but from October to May practically the whole adult population was gravid. The logistic regression to test the influence of the size and the period of the year on gravidity revealed a significant ($X^2_{12} = 182.66$; $p < 10^{-5}$) model

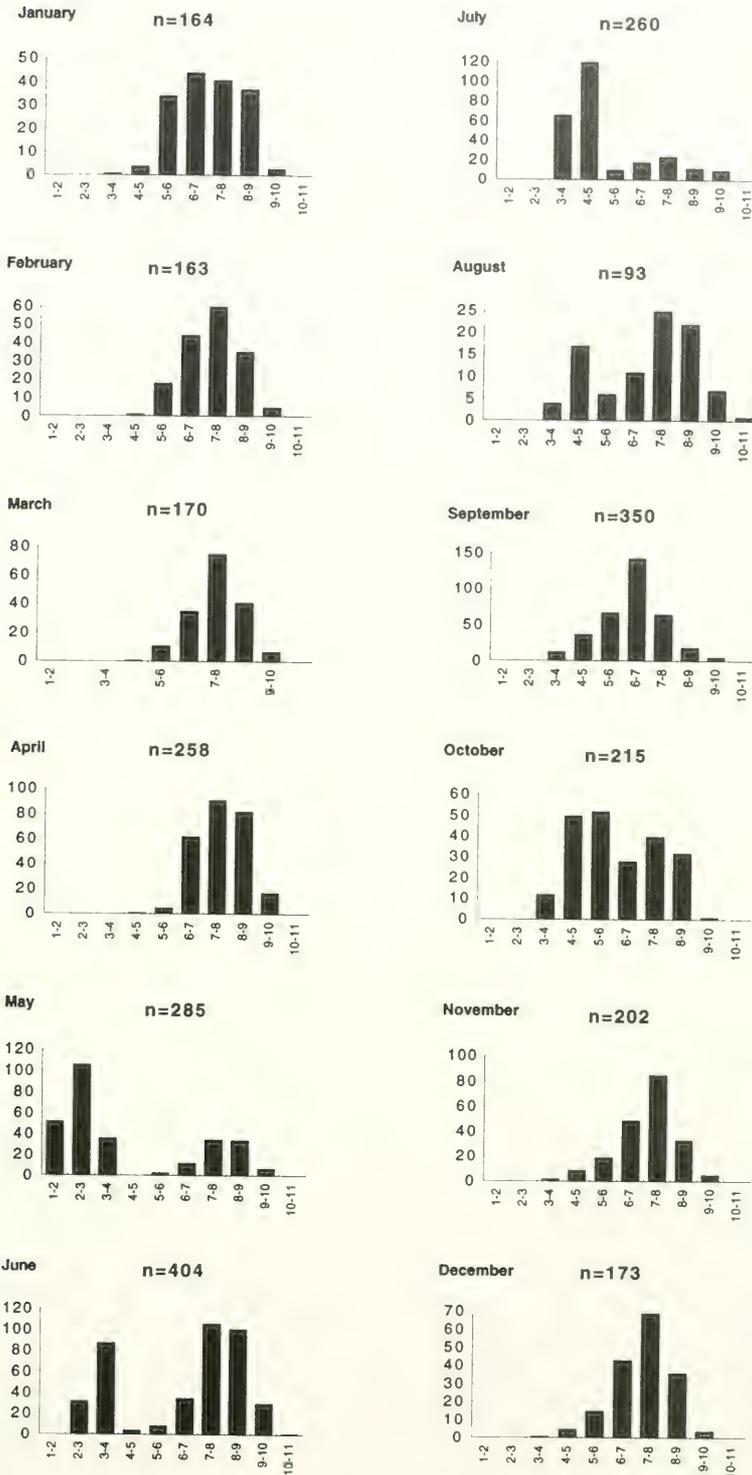


FIG. 3. Size histograms of the population structure of *P. amnicum* in the Miño River between June 1990 and May 1991.

TABLE 1. Mean length (mm) of the cohorts identified by the Bhattacharya (1967) method on the size distribution of *P. amnicum*.

Months	Cohort 1 (mm)	Cohort 2 (mm)
	\bar{x}	\bar{x}
January		7.00
February		7.27
March		7.47
April		7.66
May	2.42	7.83
June	3.27	7.90
July	4.21	7.72
August	4.57	7.92
September	5.77	
October	6.12	
November	7.11	
December	7.22	

TABLE 2. Variables used for the Ford-Walford and von Bertalanffy regression analyses. *t expressed in months.

Time (t)*	Ford-Walford Plot		von Bertalanffy Plot
	Lt	L(t + 1)	$\ln(1 - (Lt/L_{\infty}))$
1	2.42	3.27	0.3470
2	3.27	4.21	0.5045
3	4.21	4.57	0.7135
4	4.57	5.77	0.8067
5	5.77	6.12	1.2008
6	6.12	7.11	1.3527
7	7.11	7.22	1.9761
8	7.22	7.00	2.0772
9	7.00	7.27	1.8843
10	7.27	7.47	2.1267
11	7.47	7.66	2.3539
12	7.66	7.83	2.6314
13	7.83	7.90	2.9685
14	7.90	7.73	3.1489
15	7.73	7.92	2.7568
16	7.92		

that correctly classified 87.2% of individuals. The model accuracy was higher for "gravids" (90.6%) than "non-gravids" (72.3%). The seasonal variation observed in Figure 5 is significant (Wald = 52.33; df = 11; $p < 10^{-5}$), time being the main factor influencing gravidity in this species as can be deduced from the partial correlation ($r_{\text{par}} = 0.302$). The effect of individual size on gravidity, although weaker than that of seasonal variation (Wald = 10.42; df = 1; $p = 0.001$; $r_{\text{par}} = 0.159$), is positive, and the probability of a clam being gravid is higher among the biggest specimens throughout the year. The effect of size on gravidity does not

change during the year (interaction between month and size: Wald = 1.914; df = 11; $p > 0.95$).

The repeated measures ANOVA test gave no significant differences either in number ($F_{1,263} = 0.009$, $p = 0.922$) or size ($F_{1,263} = 0.182$, $p = 0.67$) of incubated phases between the two gills. Thus, the corresponding values for the two gills of each individual were added for analysis.

The ANCOVA test showed that both, the month ($F_{(8,254)} = 10.27$; $p < 10^{-5}$) and the mother size ($F_{(1,254)} = 324.50$; $p < 10^{-5}$) highly influence the number of incubated embryos (Table 3, Fig. 6). However, in this case the effect of time is not homogeneous within all age classes, because the interaction between these two factors is highly significant ($F_{(8,246)} = 10.686$; $p < 10^{-5}$). From December to May, we found a progressive decrease in the total number of embryos (larvae), especially in the smaller classes. It is minimum in July and August, and thereafter the number of embryos increased following the oocyte fertilization period, which occurs from June to mid-autumn (Araujo & Ramos, 1999). Figure 7 illustrates how the seasonal variation is lower among the smaller gravid specimens. The influence of mother size is positive ($r = 0.749$). In other words, in the same month, as a whole, larger clams contained more embryos (or larvae) than smaller clams. To show this temporal variation, the standardized regression coefficients were calculated for all the months. (The period June-August was excluded because of the lack of enough gravid specimens.) Their values were positive and similar for all the months except May when negative values (Table 4, Fig. 8) were recorded.

Regarding the size of the incubated phases (Table 5, Fig. 9), the ANCOVA analysis results, although weaker than the ones regarding number of embryos, suggest that both month ($F_{(8,255)} = 173.45$; $p < 10^{-5}$) and mother size ($F_{(1,255)} = 89.83$; $p < 10^{-5}$; $r = 0.51$) have an effect. The effect of the latter is not homogeneous, as it changes monthly ($F_{(8,247)} = 1.99$; $p = 0.05$): in a given month, larger classes incubated larger embryos (or larvae) than smaller ones. The monthly variation of this regression can be observed in Figure 10. The progressive increase after August was proportional to each size class, with a maximum in April-May just before birth. In the last month of incubation, larval growth was highest.

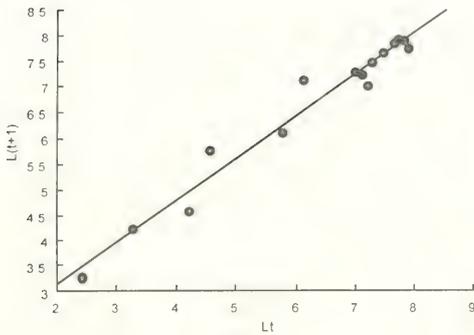


FIG. 4. Ford-Walford plot of *P. amnicum* from the Miño River in which the mean length of each age grouping (L_t) has been plotted against the succeeding age grouping (L_{t+1}).

Influence of Abiotic Factors

Monthly physico-chemical water values are shown in Table 6. There were significant seasonal variations in conductivity that may be due to tidal influences, total hardness, alkalinity, calcium values, and water temperature. The latter increased progressively from April–May (15–20°C) to July (27°C). Conductivity values are low, as occurs in rivers on granitic soils poor in soluble salts. This parameter remained constant between 60 and 70 mScm^{-1} at 25°C during the year, increasing in June to reach 225 mScm^{-1} in August, and decreasing to 112 mScm^{-1} in September. A summer increase also occurs in total hardness and alkalinity. Calcium values change in summer, reaching a maximum in September.

The influence of the physical water parameters on the reproductive cycle of *P. amnicum* was analyzed using logistic regression (dissolved oxygen was excluded, because of the lack of measurements for two months). The stepwise procedure (backward) obtained a highly significant model ($X^2_7 = 168.41$; $p = 10^{-4}$), accounting for 88.30% of the observed variability in gravidity. The accuracy was higher when explaining “gravid” (91.37%) than “non-gravid” (75%). The model identified four water variables as significant (Table 7), water temperature being the most important, as shown by the partial correlation coefficients, although calcium, pH and alkalinity were also significant.

To test to what extent the reproductive cycle of *P. amnicum* (measured by the frequency of gravid specimens during the year), is influenced by abiotic factors, a multiple regression

analysis was performed using the residuals obtained from the logistic model (gravidity = f (mother size, month)) as the dependent variable. The result of the regression analysis was not significant ($r = 0.028$; $F_{(7, 334)} = 0.039$; $p = 0.99$), which means that none of the tested water parameters were directly influencing the reproduction of the species after removing the effect of mother size and month of the year, the previous significances being a secondary effect of the seasonal variation of these parameters and their influence on other intrinsic biotic factors.

DISCUSSION

Reproductive Strategies

In the Spanish study population, *P. amnicum* had only one annual reproductive cycle with births and juvenile recruitment in April–May, when water temperature ranged between 15°C and 20°C. Danneel & Hinz’s (1976) findings for a German population were similar, although the new generation appeared in May–June, a little later than the Spanish one. The apparent absence of gravid animals in August in the German population, and in June and July in the English one (Bass, 1979) was probably a consequence of the method used, as larval stages prior to shell formation were ignored. With very few *P. amnicum* specimens from two different localities in Germany, Meier-Brook (1970) cited a total of eight gravid individuals containing embryos between 0.25 and 0.4 mm long in September, and concluded that the reproductive period was synchronous and began in autumn; also data for six individuals collected at the beginning of spring in England agree with this, “they had big embryos between 0.5 and 1.” Similar data were reported by Thiel (1928) in Germany and Odhner (1929) in Sweden, whereas in a Finnish lake juveniles were born later on in summer (July) (Holopainen, 1979). Table 8 provides a comparative summary of the data on the five studied populations of *P. amnicum*.

The species in Europe seems to be very conservative in reproductive cycle with synchrony among populations: it begins in summer, the newborn appearing in spring, with some differences depending on the country. Such variation seems to follow a latitudinal cline, which might be related to water temperature. One cycle follows as soon as the former has finished. Bass (1979) reported that in

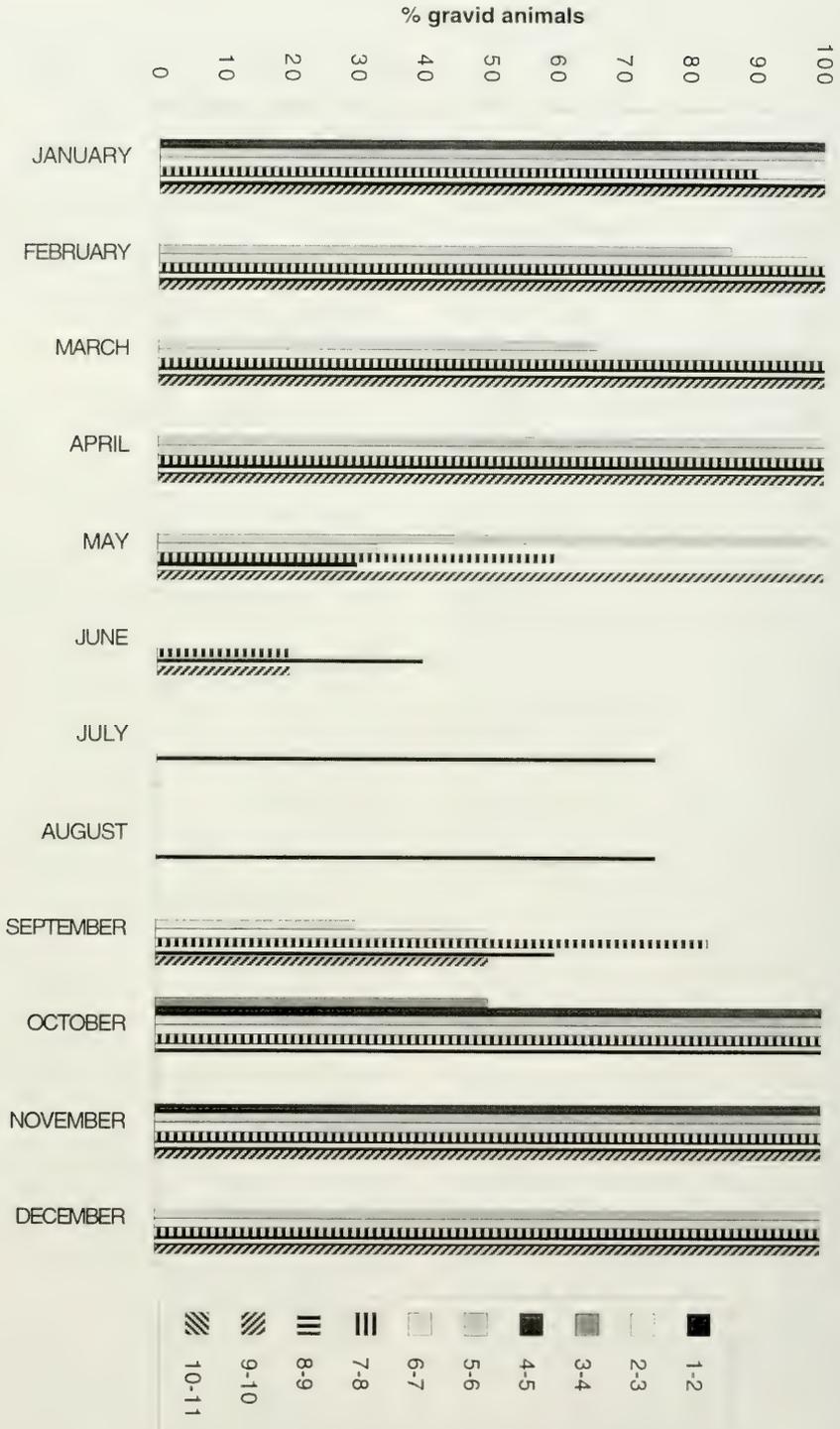


FIG. 5. Monthly variation, by size class, in the frequencies of gravid animals in relation to the number of animals dissected.

TABLE 3. Mean number and standard deviation of the embryos (larvae) occurring in the gravid animals.

CLASS (mm)	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
1-2	—	—	—	—	—	—
2-3	—	—	—	—	—	—
3-4	—	—	—	—	—	—
4-5	7.00	—	—	—	—	—
5-6	7.9 ± 2.28	10.5 ± 3.94	3.67 ± 3.21	11.5 ± 0.71	11.00	—
6-7	16 ± 4.92	16.2 ± 5.29	15.5 ± 4.95	15.5 ± 5.27	19 ± 7.07	—
7-8	34.33 ± 11.18	36.1 ± 14.43	30.3 ± 12.41	29.3 ± 9.43	16.17 ± 16.13	10.00
8-9	44.1 ± 10.94	48.3 ± 14.69	44.6 ± 15.85	43.1 ± 9.37	11.67 ± 5.03	6 ± 4.24
9-10	73.00	56 ± 1.41	63 ± 5.66	42.2 ± 11.61	5.5 ± 6.36	11.00
10-11	—	—	—	—	—	—
TOTAL	26.07 ± 18.09	31.08 ± 19	29.74 ± 18.11	30.08 ± 14.56	13.71 ± 11.4	8.25 ± 3.59

CLASS (mm)	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
1-2	—	—	—	—	—	—	—
2-3	—	—	—	—	—	—	—
3-4	—	—	—	6.5 ± 7.78	—	—	6.5 ± 7.78
4-5	—	—	13.00	13.33 ± 2.07	15 ± 2.83	—	13 ± 2.87
5-6	—	—	12 ± 4.63	18.5 ± 5.95	15.33 ± 2.52	14.5 ± 3.69	11.89 ± 5.6
6-7	—	—	18.33 ± 6.11	28.83 ± 6.97	28.33 ± 4.84	23.11 ± 9.95	19.33 ± 7.73
7-8	—	—	25.6 ± 2.89	31.83 ± 6.49	37.5 ± 6.56	42.17 ± 6.62	31.45 ± 12.33
8-9	3 ± 2	2.33 ± 1.53	25.67 ± 4.93	35.67 ± 7.5	41.17 ± 11.65	43.33 ± 9.65	36.97 ± 17.61
9-10	—	—	42.00	—	40.00	44.33 ± 16.62	42.17 ± 20.36
10-11	—	—	—	—	—	—	—
TOTAL	3 ± 2	2.33 ± 1.53	21.94 ± 8.64	24.44 ± 11.06	31.58 ± 11.92	32.57 ± 14.71	—

England a small proportion of the largest adults initiated a second reproductive cycle, but died before the brood was fully developed. None of the European populations show a decline in fertility in relation to age, as occurs in the related genus *Sphaerium* (Meier-Brook, 1970).

A very close similarity can also be found between the reproductive strategies of *P. amnicum* and the vicariant North American species *P. dubium*. Both species are of similar size, share the same incubation and birth months, and, in both cases, the mean number and size of larvae increased with the size of the parents, although the number of incubated larvae was considerably higher in the Spanish population. The strategy of the Canadian population of *P. amnicum* (Vincent et al., 1981) also fits perfectly with the one of *P. dubium*, with only two exceptions: *P. dubium* attains its sexual maturity in its first year of life, and its number of larvae is considerably higher, suggesting that these two populations might belong to the same taxon.

Meier-Brook (1970) suggested that, with the exception of *P. amnicum* for which no data were available, sexual maturity in populations of *Pisidium* occurs when individuals reach 50 or 60% of maximum size. According to this rule, as the maximum size of the Spanish

population ranges from 10-11 mm, it follows that sexual maturation would occur in a size class near 5-6 mm, which is higher than the 4-5 mm that we found to be the minimum needed to be gravid (with the exception of two gravid specimens of 3-4 mm collected in October). However, it applies perfectly to the maximum theoretical length (8.25 mm) estimated by the von Bertalanffy method. More surprising are the proportions reported by Daneel & Hinz (1976) (Table 8). Thus, although most of the available data (Table 8) seem to follow Meier-Brook's rule, in some populations maturity is reached before specimens are 40-50% of maximum size. Then, if any rule were applicable to *P. amnicum*, we would expect a positive correlation between minimum gravid length and maximum adult size throughout the species range (Table 8), which was not found.

The main feature of the Spanish population of *P. amnicum* seems to be the high number of larvae incubated during the months immediately before birth. The difference with all the other previous data may be explained by differences related to temperature and/or latitude. In warmer climates, the same species becomes bigger, incubates a greater number of larvae (maximum of 73 in Spain and 12 in Finland), and the larvae are also bigger. Such

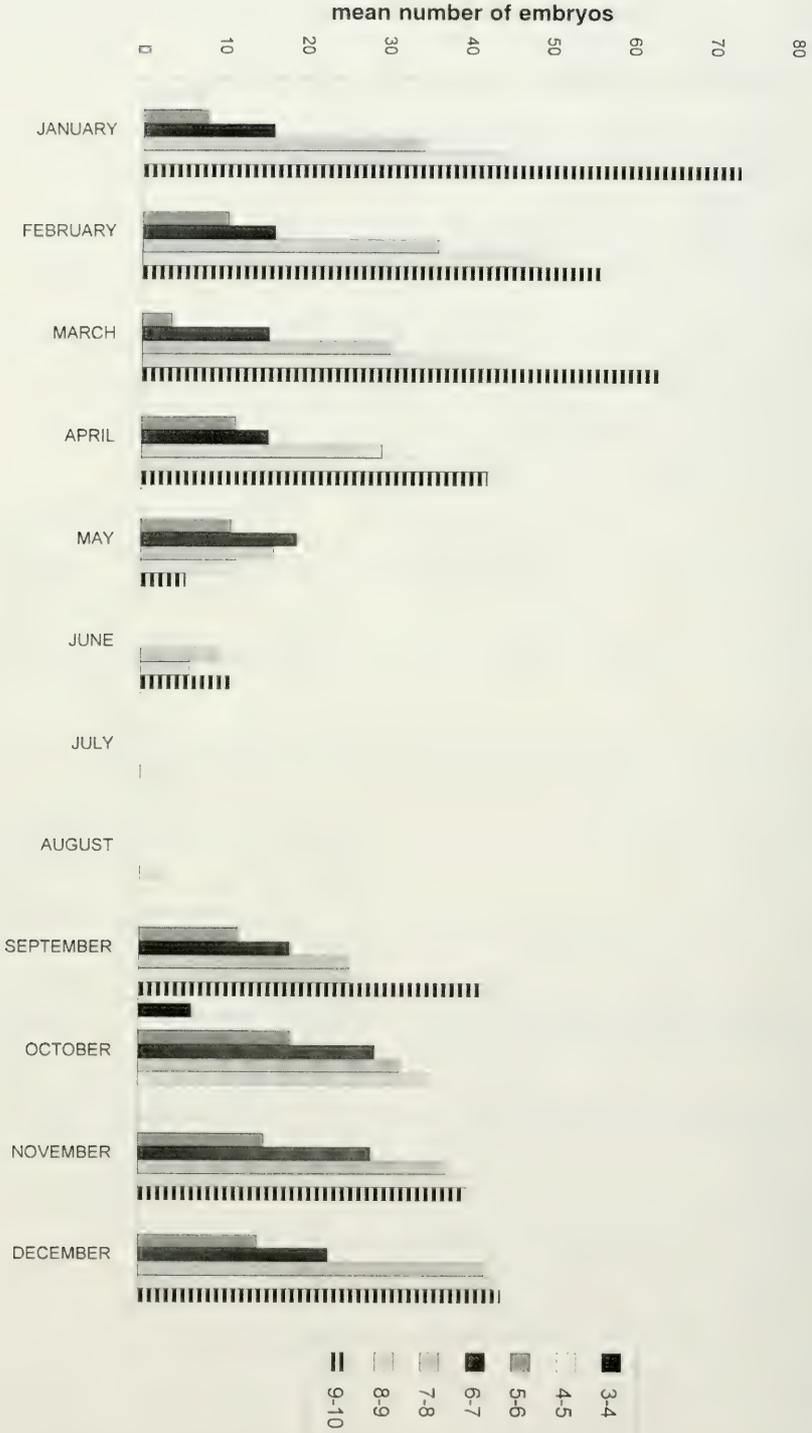


FIG. 6. Monthly variation, by size class, in the mean number of embryos (larvae) occurring in gravid animals.

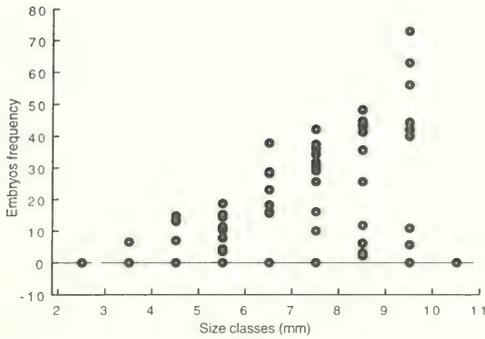


FIG. 7. Variation in the mean number of embryos (larvae) in each size class with parental size.

TABLE 4. Monthly standardized regression coefficients of the variation in the number of incubated embryos in relation with the mother size.

Month	BETA	St. Err. of BETA
January	0.898	0.070
February	0.837	0.091
March	0.842	0.094
April	0.800	0.101
May	-0.325	0.272
September	0.824	0.157
October	0.846	0.097
November	0.794	0.129
December	0.733	0.133

north-south clinal reproductive behaviour was apparently not found in preliminary studies with the related North American species *P. idahoense* Roper, in which the northern populations were smaller but had more progeny (Heard, 1965). On the other hand, as the life span of *P. amnicum* in Spain appears to be about 15 months, our results do not confirm Bass's idea (1979) of an extended life span or a successful second brood in southern areas.

The scarce data on the Finnish population, one of the northermost in the species range, indicate a reduction in fertility in relation to the central European populations. This reduction may be accompanied by a long life span, allowing iteroparity, as reported by Holopainen & Hanski (1986) and Vincent et al., (1981) in Finland and Canada, respectively. Given that the Spanish population broods the maximum number of larvae in the species range, and that the maximum embryo length is similar to all other reported populations, we may state that in *P. amnicum* there is no trade-off between litter size and embryo size as was pro-

posed by Holopainen & Hanski (1986) for the genus *Pisidium*. However, the characteristics of the Spanish population as a whole reflect its breeding success. The species seems to be so well adapted to local conditions in this range margin that it is possible to speculate that such attributes might allow the species to expand its range if the opportunity presented itself. In fact, the Goian (Fig. 2) colony, which experienced a drastic reduction after colonization by *Corbicula fluminea* (Müller, 1774) (Araujo et al., 1993), has re-established itself, sharing its habitat with the latter species as we have observed recently (unpublished data).

Study of the phenomenon that Meier-Brook (1977) called intramarsupial suppression of fetal development has deserved special attention. This author cited 50% of the brood dying before birth in *P. obtusale* and *P. liljeborgii*, whereas it is very variable in *P. amnicum* (0–54%) according to Danneel & Hinz (1976). Our results show a progressive increase in embryos from June to December, which means that settlement of eggs or zygotes may occur over several months. On the other hand, we observed that from December, and in several size classes, the embryo number may decrease, suggesting that not all the initial embryos complete their development. Nevertheless, this phenomenon does not seem to be very important in the Spanish population. The idea suggested by Araujo & Ramos (1997) that ova fertilization occurs in the gills and not in the spermatiduct as was proposed (Okada, 1935; Odhner, 1929; Meier-Brook, 1970), may provide a new perspective, suggesting that the so called "embryos" present in the gill during the first months of the reproductive period may be eggs not yet impregnated. This may explain the differences found by other authors when comparing the initial and final number of larval stages in the gills.

Although there is the same high mortality following the birth of juveniles, the corresponding ratio of the size classes over 6 mm in the Spanish population is higher than in the others during the year.

Influence of Environmental Factors

None of the water measured parameters in itself seems to directly influence the reproductive cycle of the Spanish population of *P. amnicum* as measured by specimen gravidity, although seasonal variation in water temperature, calcium, pH, and alkalinity might be im-

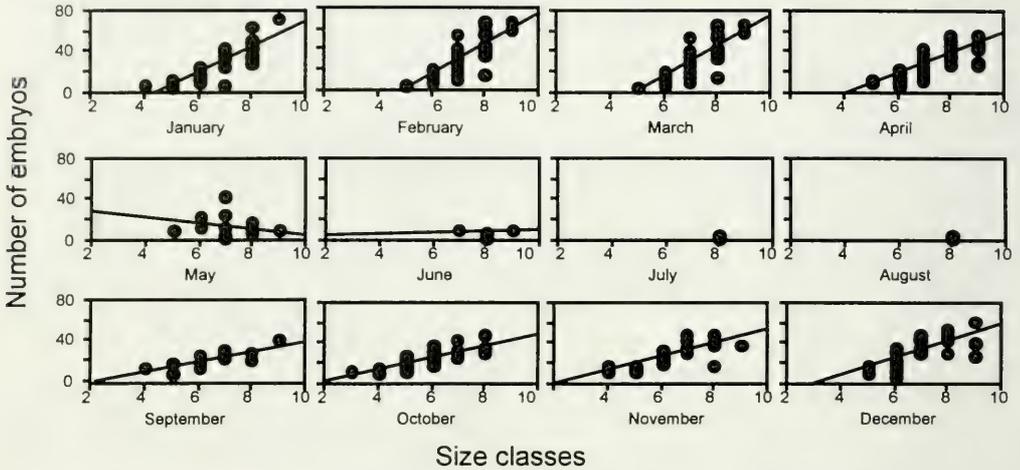


FIG. 8. Monthly variation in the relation between the mean number of embryos and parental size.

TABLE 5. Mean length (mm) and standard deviation of the embryos (larvae) occurring in the gravid animals

CLASS (mm)	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
1-2	—	—	—	—	—	—
2-3	—	—	—	—	—	—
3-4	—	—	—	—	—	—
4-5	0.5	—	—	—	—	—
5-6	0.52 ± 0.12	0.55 ± 0.1	0.45 ± 0.39	0.76 ± 0.04	1.3	—
6-7	0.66 ± 0.19	0.73 ± 0.88	0.84 ± 0.09	0.88 ± 0.16	1.37 ± 0.19	—
7-8	0.73 ± 0.14	0.87 ± 0.11	0.92 ± 0.09	1.23 ± 0.25	1.61 ± 0.4	0.08
8-9	0.79 ± 0.17	0.9 ± 0.1	0.94 ± 0.13	1.2 ± 0.22	2.02 ± 0.08	0.09 ± 0.04
9-10	0.85	1 ± 0.14	1.09 ± 0.13	1.08 ± 0.15	2.26 ± 0.09	0.1
10-11	—	—	—	—	—	—
TOTAL	0.67 ± 0.17	0.8 ± 0.16	0.87 ± 0.2	1.08 ± 0.25	1.77 ± 0.4	0.09 ± 0.02

CLASS (mm)	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
1-2	—	—	—	—	—	—	—
2-3	—	—	—	—	—	—	—
3-4	—	—	—	0.08 ± 0	—	—	0.08 ± 0
4-5	—	—	0.08	0.12 ± 0.04	0.13 ± 0.07	—	0.15 ± 0.13
5-6	—	—	0.09 ± 0.01	0.18 ± 0.10	0.25 ± 0.09	0.42 ± 0.07	0.46 ± 0.24
6-7	—	—	0.12 ± 0.02	0.22 ± 0.02	0.37 ± 0.07	0.42 ± 0.14	0.62 ± 0.29
7-8	—	—	0.09 ± 0.01	0.22 ± 0.04	0.34 ± 0.03	0.55 ± 0.15	0.75 ± 0.47
8-9	0.08 ± 0.01	0.08 ± 0.02	0.14 ± 0.12	0.25 ± 0.03	0.34 ± 0.05	0.6 ± 0.08	0.75 ± 0.47
9-10	—	—	0.18	—	0.35	0.45 ± 0.15	0.84 ± 0.54
10-11	—	—	—	—	—	—	—
TOTAL	0.08 ± 0.01	0.08 ± 0.02	0.11 ± 0.05	0.19 ± 0.07	0.32 ± 0.09	0.49 ± 0.14	—

portant for their influence on other species intrinsic factors (i.e., mother size, previous episodes of reproduction). Moreover, since *P. amnicum* incubates its larvae within a ctenidial marsupium, larvae release and subsequent growth could be induced, both directly and indirectly, by these factors.

Temperature increases progressively over April-May, when births occur, to July, when fertilization begins. The 27°C maximum is more or less maintained in August and September,

and it is followed by a sudden decrease of about 9°C in October, precisely when the period of maximum population growth ends. It could be argued that temperature has so important relation with birth that larvae big enough to be born in April did not because temperature was below 15°C. A relationship between temperature and growth in freshwater bivalves has also been described in the Asiatic clam *Corbicula fluminea* (Morton, 1977; Eng, 1979; Joy, 1985; Ituarte, 1985).



FIG. 9. Monthly variation, by size class, in mean embryos (larvae) size in gravid animals.

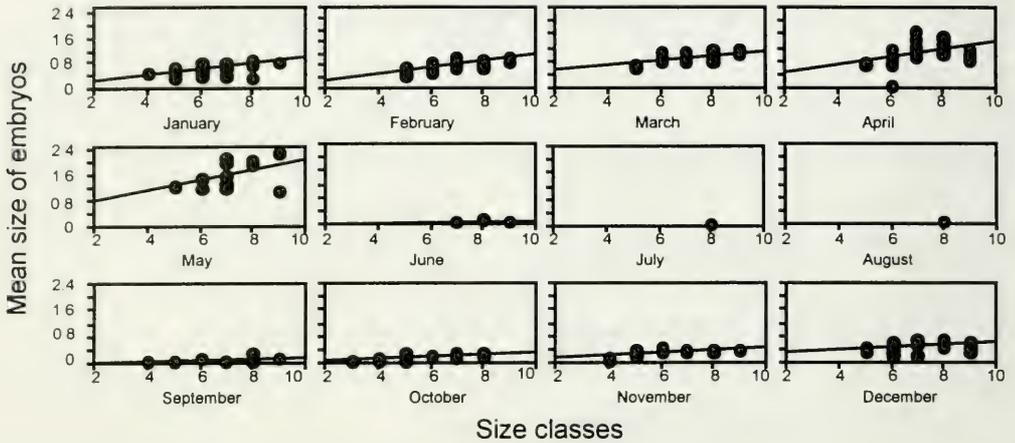


FIG. 10. Monthly variation in the relation between mean embryo size and parental size.

TABLE 6. Monthly and mean values of the chemical and physical properties of the water at the sample site

Month	Temperature (°C)	pH	Dissolved oxygen (ppm)	Conductivity ($\mu\text{s cm}^{-1}$)	Calcium (mg/l)	Alkalinity (mmol/l)	Total hardness (°dh)	Carbonate hardness (°dh)
January	8.13	6.33	12.23	53.00	8.00	0.50	1.20	1.00
February	9.17	7.13	—	60.00	9.00	0.50	1.75	1.40
March	10.80	6.00	14.77	60.00	10.00	0.35	1.30	1.40
April	13.97	6.07	13.97	68.00	11.00	0.50	1.30	1.40
May	18.83	5.97	9.77	78.00	7.00	0.60	1.50	1.70
June	22.73	5.87	11.23	84.80	8.00	0.40	1.20	1.50
July	26.97	8.17	8.43	149.00	7.00	0.45	1.60	1.50
August	26.17	7.33	—	225.00	13.00	0.80	2.00	1.50
September	26.27	7.37	9.93	112.00	20.00	0.80	1.90	1.70
October	17.67	8.27	8.43	92.50	10.00	0.75	1.55	1.50
November	14.80	7.23	9.77	82.00	10.00	0.65	1.85	1.60
December	11.63	7.20	9.93	76.00	13.00	0.50	1.70	1.40
Mean	17.26	6.91	10.85	95.03	10.50	0.57	1.57	1.47
sd	6.62	0.81	2.07	46.53	3.45	0.15	0.27	0.17

TABLE 7. Water parameters selected by the logistic regression analysis as the most important influencing the gravidity of *P. amnicum*

Variable	Wald	df	sig.	r
Alkalinity	3.97	1	0.05	-0.077
Calcium	16.44	1	0.001	0.209
pH	10.27	1	0.001	0.158
Temperature	53.25	1	10^{-5}	-0.40

Calcium, alkalinity and pH increased in summer suggesting that a relation with the higher growth speed in these months may also exist. Our results show a certain correspondence between the increase in the values of the abovementioned parameters and the disappearance of the adults.

Finally, Hornbach & Cox (1987) suggested that in *Pisidium casertanum* (Poli) there exists

a positive relation between high calcium and alkalinity values and adult shell length, and probably also embryo number and size. If we compare the values of these two parameters for the Spanish *P. amnicum* (alkalinity: mean = 0.57 mmol/l; calcium: mean = 10.50 mg/l) with those of the Finnish population (alkalinity: 0.17–0.19 mmol/l; calcium = 6.1 mg/l) (Holopainen, 1979), the only available ones, we could explain the biggest population parameters found in Spain (Table 6) as a function of the relatively "high" calcium and alkalinity values.

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TABLE 8. Reproductive and growth features of several populations of *P. amnicum*. (1) Several specimens live longer but do not breed. (2) Recorded two months before birth in adults bigger than 4 mm. (3) Not all months recorded. (4) Recorded in the month immediately after birth. (5) Recorded in the first year of life. (*) Values dependant on parents' size and date.

	Germany Danneell & Hinz (1976)	England Bass (1979)	Finland Holopainen (1979)	Canada Vincent et al. (1981)	Spain This study
Maximum adult length (mm)	8.9	8.5	8.8	9	10–11
Life span (months)	12	12(1)	—	36	15
Birth period	May–June	May	July	June	April–May
Minimum gravid length (mm)	2–3	4	4.3	4	3–4
% of gravid animals (2)	80–100%	100%	—	42–62	100%
Maximum number of embryos/larvae*	34	37	12	18	73
Maximum length of larvae (mm)*	2	1.4	0.6(3)	—	2.4
Lateralization	no	—	—	—	no
% of new born in relation to whole population (4)	< 50%	< 50%	—	62%	< 50%
Premature births	yes	probably	—	—	yes
Annual mean growth of adults (mm)	3	4	—	2–3 (5)	5–6
Winter interruption of larval growth	yes	no	—	—	no

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APPENDIX 1. Summarized information on the specimens collected, dissected and gravid, and of the total mean number and mean size of the incubated embryos (or larvae). Frequency of gravid animals at 1%

month	class	collected	freq. collected %	dis- sected	gravid	freq. gravid	total of embryos	mean number of embryos	mean length of embryos
January	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	1	0.61	0	0	0	0	0	0
	4-5	4	2.44	1	1	1	7	7	0.5
	5-6	34	20.73	10	10	1	79	7.9	0.52
	6-7	44	26.83	10	10	1	160	16	0.66
	7-8	41	25.00	10	9	0.9	309	34.33	0.73
	8-9	37	22.56	10	10	1	441	44.1	0.79
	9-10	3	1.83	1	1	1	73	73	0.85
	10-11	0	0	0	0	0	0	0	0
Total January		164	100	42	41	0.98	1069	26.07	0.67
February	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0
	4-5	1	0.61	0	0	0	0	0	0
	5-6	18	11.04	7	6	0.86	63	10.5	0.55
	6-7	44	26.99	10	10	1	162	16.2	0.73
	7-8	60	36.81	10	10	1	361	36.1	0.87
	8-9	35	21.47	10	10	1	483	48.3	0.9
	9-10	5	3.07	2	2	1	112	56	1
	10-11	0	0	0	0	0	0	0	0
Total February		163	100	39	38	0.97	1181	31.08	0.8
March	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0
	4-5	1	0.59	0	0	0	0	0	0
	5-6	11	6.47	3	2	0.66	11	3.67	0.45
	6-7	35	20.59	10	10	1	155	15.5	0.84
	7-8	75	44.12	10	10	1	303	30.3	0.92
	8-9	41	24.12	10	10	1	446	44.6	0.94
	9-10	7	4.12	2	2	1	126	63	1.09
	10-11	0	0	0	0	0	0	0	0
Total March		170	100	35	34	0.97	1041	29.74	0.87
April	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	0	0	0	0	0	0	0	0
	4-5	1	0.39	0	0	0	0	0	0
	5-6	5	1.94	2	2	1	23	11.5	0.76
	6-7	62	24.03	10	10	1	155	15.5	0.88
	7-8	91	35.27	10	10	1	293	29.3	1.23
	8-9	82	31.78	10	10	1	431	43.1	1.2
	9-10	17	6.59	5	5	1	211	42.2	1.08
	10-11	0	0	0	0	0	0	0	0
Total April		258	100	37	37	1	1113	30.08	1.08
May	1-2	52	18.25	10	0	0	0	0	0
	2-3	106	37.19	11	0	0	0	0	0
	3-4	36	12.63	10	0	0	0	0	0
	4-5	0	0	0	0	0	0	0	0
	5-6	3	1.05	1	1	1	11	11	1.3
	6-7	12	4.21	3	1	0.33	38	38	2.73
	7-8	35	12.28	10	6	0.6	97	16.17	1.61
	8-9	34	11.93	10	3	0.3	35	11.67	2.02
	9-10	7	2.46	2	2	1	11	5.5	1.72
	10-11	0	0	0	0	0	0	0	0
Total May		285	100	57	13	0.23	192	14.77	1.78

(continued)

APPENDIX 1. (Continued)

month	class	collected	freq. collected %	dis- sected	gravid	freq. gravid	total of embryos	mean number of embryos	mean length of embryos
June	1-2	0	0	0	0	0	0	0	0
	2-3	32	7.92	0	0	0	0	0	0
	3-4	87	21.53	0	0	0	0	0	0
	4-5	4	0.99	0	0	0	0	0	0
	5-6	8	1.98	2	0	0	0	0	0
	6-7	35	8.66	5	0	0	0	0	0
	7-8	106	26.24	5	1	0.2	10	10	0.06
	8-9	101	25	5	2	0.4	12	6	0.07
	9-10	30	7.43	5	1	0.2	11	11	0.08
	10-11	1	0.25	0	0	0	0	0	0
Total June		404	100	22	4	0.18	33	8.25	0.07
July	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	66	25.38	4	0	0	0	0	0
	4-5	120	46.15	4	0	0	0	0	0
	5-6	10	3.85	0	0	0	0	0	0
	6-7	18	6.92	3	0	0	0	0	0
	7-8	24	9.23	5	0	0	0	0	0
	8-9	12	4.62	4	3	0.75	9	3	0.06
	9-10	10	3.85	0	0	0	0	0	0
	10-11m	0	0	0	0	0	0	0	0
Total July		260	100	20	3	0.15	9	3	0.06
August	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	4	4.3	0	0	0	0	0	0
	4-5	17	18.28	5	0	0	0	0	0
	5-6	6	6.45	1	0	0	0	0	0
	6-7	11	11.83	5	0	0	0	0	0
	7-8	25	26.88	4	0	0	0	0	0
	8-9	22	23.66	4	3	0.75	7	2.33	0.08
	9-10	7	7.53	2	0	0	0	0	0
	10-11	1	1.08	0	0	0	0	0	0
Total August		93	100	21	3	0.14	7	2.33	0.08
September	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	13	3.71	2	0	0	0	0	0
	4-5	37	10.57	8	1	0.125	13	13	0.08
	5-6	67	19.14	10	3	0.3	26	4.33	0.03
	6-7	143	40.86	6	3	0.5	55	18.33	0.12
	7-8	65	18.57	6	5	0.83	128	25.6	0.09
	8-9	19	5.43	5	3	0.6	77	25.67	0.14
	9-10	6	1.71	2	1	0.5	42	42	0.18
	10-11	0	0	0	0	0	0	0	0
Total September		350	100	39	16	0.41	341	17.95	0.09
October	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	12	5.58	4	2	0.5	13	6.5	0.06
	4-5	50	23.26	6	6	1	80	13.33	0.12
	5-6	52	24.19	6	6	1	111	18.5	0.18
	6-7	28	13.02	6	6	1	173	28.83	0.22
	7-8	40	18.6	6	6	1	191	31.83	0.22
	8-9	32	14.88	6	6	1	214	35.67	0.25
	9-10	1	0.47	0	0	0	0	0	0
	10-11	0	0	0	0	0	0	0	0
Total October		215	100	34	32	0.94	782	24.44	0.19

APPENDIX 1. (Continued)

month	class	collected	freq. collected %	dis- sected	gravid	freq. gravid	total of embryos	mean num- ber of embryos	mean length of embryos
November	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	2	0.99	0	0	0	0	0	0
	4-5	9	4.46	2	2	1	30	15	0.13
	5-6	19	9.41	3	3	1	46	15.33	0.25
	6-7	49	24.26	6	6	1	170	28.33	0.37
	7-8	85	42.08	6	6	1	225	37.5	0.34
	8-9	33	16.34	6	6	1	247	41.17	0.34
	9-10	5	2.48	1	1	1	40	40	0.35
	10-11	0	0	0	0	0	0	0	0
Total November		202	100	24	24	1	758	31.58	0.32
December	1-2	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0
	3-4	1	0.58	0	0	0	0	0	0
	4-5	5	2.89	0	0	0	0	0	0
	5-6	15	8.67	4	4	1	58	14.5	0.42
	6-7	43	24.86	9	9	1	208	23.11	0.42
	7-8	69	39.88	6	6	1	253	42.17	0.55
	8-9	36	20.81	6	6	1	260	43.33	0.6
	9-10	4	2.31	3	3	1	133	44.33	0.45
	10-11	0	0	0	0	0	0	0	0
Total December		173	100	28	28	1	912	32.57	0.49
TOTAL		2737		398	276	0.69	7438	26.95	0.66

RELATIONSHIPS BETWEEN LENGTH OF PREY/PREDATOR FOR THE MOST
IMPORTANT PREY OF THE CUTTLFISH *SEPIA OFFICINALIS* L.
(MOLLUSCA: CEPHALOPODA)

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ABSTRACT

As is typical of cephalopods, the cuttlefish *Sepia officinalis* L. is an opportunist predator. This study shows that *Sepia* selects the length of its prey taking into account its own size. In the natural environment, the most important prey are fishes and crabs. For each group, we have linked the prey remains (found regularly in the stomach contents of the cuttlefish) to total prey length. Prey remains consist of the merus of pereopods 2 to 5 for crabs and otoliths for fishes. These pieces are not attacked by enzymes during their transit through the digestive tract.

Fishes become prey when they measure between 25–80% of the DML (Dorsal Mantle Length) of the cephalopod. The cuttlefish attack crabs when they measure between 20–40% of their own DML. Cuttlefish attack crabs by jumping or pouncing without using their tentacles.

INTRODUCTION

Throughout its geographical distribution, the diet of the cuttlefish *Sepia officinalis* L. consists of fishes and crustaceans (Brachyura and Macrura): in the English Channel (Richard, 1971), in the Bay of Biscay (Pinczon du Sel, 1996), on the Spanish coast (Castro & Guerra, 1989), on the Italian coast (Scalera Liaci & Piscitelli, 1982), and on the Tunisian coast (Najai & Ktari, 1979). In the Ria de Vigo, Guerra (1985) showed that the composition of the diet of *Sepia officinalis* changes with growth (Amphipoda and Caridea for individuals with a DML less than 45 mm, and Porcellanidae, Brachyura, and fishes when the DML is above 55 mm). Castro & Guerra (1990) remarked that in the diet of *Sepia officinalis* the proportion of crustaceans decreases whereas that of fishes increases with cuttlefish growth. However, the decrease of crustaceans in general did not modify the importance of Brachyura for larger cuttlefish.

Only a few studies have been carried out to determine the relationships between the length of the prey and the length of the cuttlefish. Moreover, these studies have been carried out only in the laboratory (Boulet, 1964; Duval et al., 1984).

Fishes and Brachyura were identified respectively by the otoliths and principally by the pereopods or walking legs. We next linked these to the size of the prey. Thus, we can

begin to elucidate the relationship between length or size of the predator to its prey.

MATERIALS AND METHODS

All cuttlefish examined for this study were collected by 2 trawls (10 × 3 × 0.6 m with 20 mm mesh size and 3.5 × 1.2 × 0.4 m with 5 mm mesh size to capture young) in the Bay of Biscay from June 1990 to July 1992, thus taking into account all the stages between hatching and death. Only two *Sepia* were under 10 cm, 19 between 10–14 cm, 12 between 15–19 cm, 15 between 20–24 cm, and 12 *Sepia* greater than 25 cm. The prey items was identified under binocular microscope. The stomach contents were examined in all 60 cuttlefish.

Crabs parts were present in 28 and fishes in 32 of the cuttlefish studied. For fishes and crabs, when the weight of the stomach contents is significant there is only one type of prey present in the stomach (Pinczon du Sel, 1996). In five cuttlefish, there were two prey items in the stomach (two fishes or fishes with Brachyura not identified).

Carcinus maenas L. (Crustacea: Brachyura)

In the Bay of Biscay, the most important Brachyura taken by *Sepia officinalis* is *Carcinus maenas* L. (Pinczon du Sel, 1996). To ap-

preciate the size range of the captured crabs, a total of 100 crabs were measured and weighed. References to size are based on the maximum length and width of the carapace and maximum width of the merus of pereiopods 2 to 5 (Fig. 1). This portion of the walking legs was often found in the stomach contents. In a laboratory study, cuttlefish eat only 52.5% of the crabs and do not consume the legs (Pinczon du Sel, 1996). All the measurements were made to the nearest 0.1 mm, and weights to the nearest 0.1 g.

The merus of the walking legs identified in the stomach contents were measured. Then, the width of the crab was estimated from the allometric relation as well as the surface area of the cephalothorax. When several merus were present in the same gut content, the pairs were reconstituted by minimizing the variations. The size used to calculate crab length was the mean width of each pair. Next, the width of the cephalothorax was linked to the size of the merus (Fig. 2) and to the surface area of the cephalothorax (surface area was calculated by length times width) (Fig. 3).

Duval et al. (1984) studied the method used by cuttlefish to capture crabs in relation to the surface area of the cephalothorax. The comparison index is $R = \text{weight of the cuttlefish (g)} / \text{surface of the cephalothorax of the crab (mm}^2\text{)}$. Duval et al. (1984) noted that when R was greater than 1 (i.e., the crab was "small"), the crab was captured using the tentacles. However, when R was less than 1 (i.e., the crab was large), capture was done by jumping or pouncing without using the tentacles (data not submitted).

Fishes

A diversity of fishes constitute the second most important prey for cuttlefish. The remains of fish prey are identified in stomach contents by the presence of otoliths, bones of the inner ear, shape. Moreover, as is typical of bones, there is an allometric relationship between the length of the otolith and the total length (Lt: from head to caudal fork) and weight of the fish.

This study was carried out on seven species of fishes, which represented the most important elements found in the stomach contents (Table 1, Fig. 4). We compare otoliths found in stomach contents with a collection of otoliths made for several fish species of the Bay of Biscay.

Before using the allometric relationship be-

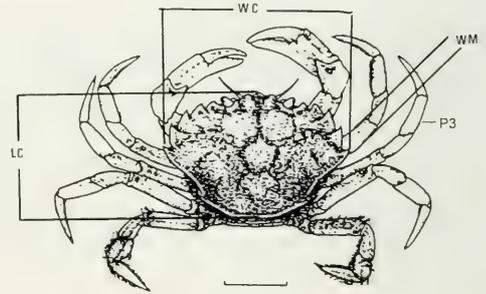


FIG. 1. Measurements made on the crab *Carcinus maenas* L. (scale bar = 2 cm). LC, length of the cephalothorax; WC, width of the cephalothorax; WM, width of the merus of P3 (pereiopods 3) (drawing from Christiansen, 1969)

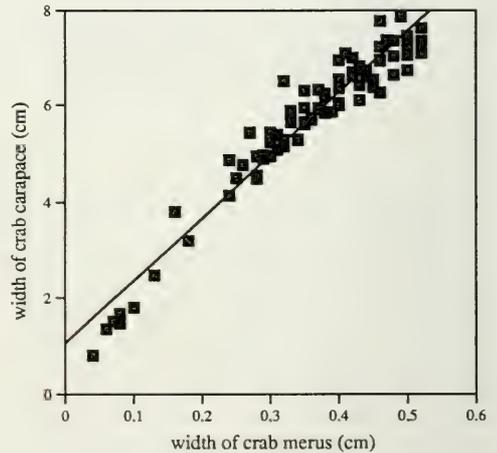


FIG. 2. *Carcinus maenas* L. Allometric relationship between width of the merus of pereiopods 2 to 5 and width of the cephalothorax. $Y = 13.082x + 1.040$; $r = 0.965$

tween length of otolith and length of fish, we had to be sure that the otoliths were not eroded by digestive enzymes during their passage through the cuttlefish digestive tract. As a control, fishes were weighed, measured and the length of the otolith estimated and fed to cuttlefish. When otoliths were recovered in the faeces, the two lengths could be compared.

All otoliths found in the cuttlefish stomach content were identified and measured to the nearest 0.01 mm. When several otoliths were present in the same stomach, they were all measured and the pairs were reconstituted by minimizing the variations. Then, the allometric

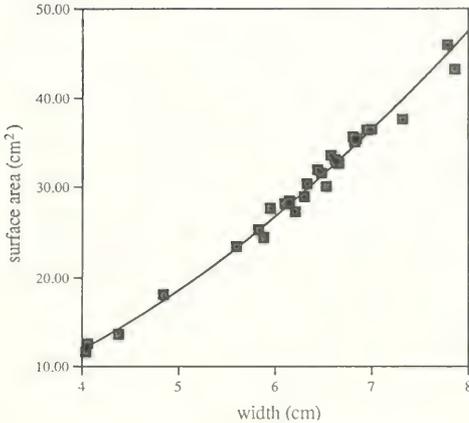


FIG. 3. *Carcinus maenas* L. Allometric relationship between width of the cephalothorax and its surface area. $Y = 0.739 \times 2.003$; $r = 0.995$

relations allowed us to estimate the length and weight of the fishes caught by *Sepia*.

RESULTS

Of the 60 cuttlefish examined, 28 contained crabs parts in the stomach. The cuttlefish that contained crab pereopods ranged in size 11–29 cm DML and 200–2200 g in total body net weight.

The ratios of prey to predator sizes (width of the cephalothorax of prey to DML of predator = WC/DML) ranged from 20% about 35% of the predator DML (Fig. 5). Thus, the lengths of captured crabs increase as the length of the predator increases. Therefore, there was a slight trend for crab size to decline as cuttlefish size increases. The size range of wild crabs was between 30–80 mm in carapace width. As the cuttlefish grow, if they do not have enough large crabs, they eat smaller ones. The ratios of weights range are between 0.1–4.0% of the weight of the cuttlefish, with a greater inter-individual variation. Some adult cuttlefish ($w = 1.875$ kg) eat small crabs ($w = 38.04$ g) and vice versa (Fig. 6).

The ratio R (weight of cuttlefish to surface area of crab cephalothorax) is given in the Table 2. The majority of the ratios are less than 1 and the crabs are considered to be "large".

The size range of cuttlefish with otoliths in their gut was between 4 cm to 29 cm, with a weight between 18 g to 2025 g. The results of our study on the digestion of otoliths indicates that they are not attacked by the cuttlefishes'

digestive enzymes (Table 3). Thus, we can use the allometric relationship between length of otolith and length of fish to estimate the size of the captured fish.

The majority of the size ratios (length of fish to DML of cuttlefish) are between 25–80% (Fig. 7) in Morbihan Bay (breeding area) as well as in the Morbraz (growth area). These are both areas of the Bay of Biscay. Only three cuttlefish had otoliths in their stomach contents, indicating fishes with length ratios greater than 80% of the DML. Moreover, we can see that among these three cases, two show a ratio greater than 100% (116 and 120%). These three fish belong to the same species with a greatly elongate shape, the eel *Anguilla anguilla* L.

The majority of cuttlefish (73%) eat fish with an estimated weight of less than 15 g (Fig. 8). *Sepia* seems to be more selective of fishes than crabs. Anguillidae, Gadidae and Labridae eaten were greater than 15 g, Gobiidae and Atherinidae less than 15 g.

DISCUSSION

Although the cuttlefish, *Sepia officinalis* is opportunist in its choice of prey-species (Najai & Ktari, 1979), it seems to be more selective when it comes to prey size. Size ratios for fishes ranges from 25–80% of the DML of the predator and between 20–40% for the crabs. *Sepia officinalis* L. can also seize its prey with its arms by jumping on it (Wilson, 1946; Messenger, 1968).

The prey's capacity to defend or to escape capture increases with prey size; larger prey are also more difficult for the cuttlefish to attack and capture successfully. In *Sepia elegans*, there was a relationship between tentacle club length and body size. Individual cuttlefish equipped with comparatively longer clubs can capture larger prey (Bello, 1991).

Boulet (1964) observed that the ratio between the size of the crab and of the cuttlefish may inhibit predatory behaviour of the cephalopod; if the crab is too big the cuttlefish will not attempt to capture it.

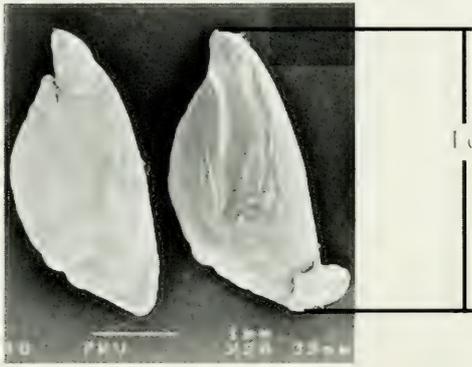
A study on the energy expended in prey capture and the energy gained from ingestion and assimilation could perhaps explain the larger size limit. In the case of *Octopus vulgaris*, the stimulus for predation is controlled by the repletion ratio of the crop (number of full stomachs of the octopus to number of total number of stomach contents examined (Young, 1964;

TABLE 1. Allometric relationship between otolith length and the length and weight of several species of fish prey.

Family	Species	L(mm) = f(Lo(mm))	r	n	References
Anguillidae	<i>Anguilla anguilla</i> L.	$L = 186.73Lo - 85.35$	0.94	25	Monaix, 1992
Gadidae	<i>Trisopterus luscus</i> L.	$L = 31.877Lo - 89.924$	0.92	58	Pinczon du Sel, 1996
Atherinidae	<i>Atherina presbyter</i> Cuvier	$L = 31.91Lo - 0.35$	0.99	30	La Mao, 1985
Callionymidae	<i>Callionymus lyra</i> L.	$L = 87.323Lo - 72.783$	0.88	28	Pinczon du Sel, 1996
Gobiidae	<i>Gobius</i> sp./ <i>Pomatochistus</i> sp.	$L = 29.461Lo - 9.238$	0.99	32	Pinczon du Sel, 1996

Lo = length of the otolith (see also Fig. 4)

CALLIONYMIDAE'S OTOLITH



ATHERINIDAE'S OTOLITH

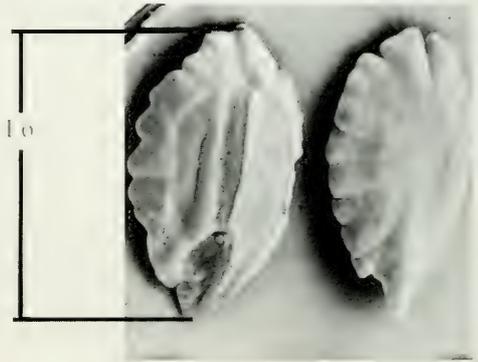


FIG. 4. Measurements made on fish otoliths. Lo, length of otolith.

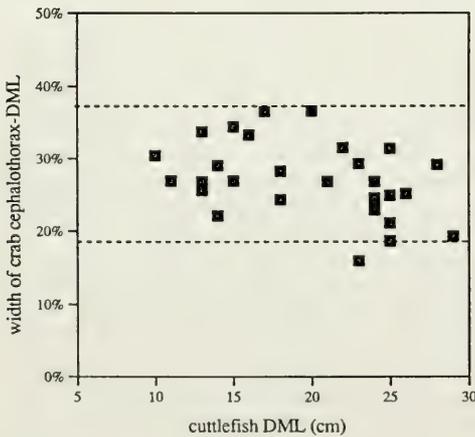


FIG. 5. Ratio of the "width of the cephalothorax of the captured crab ÷ DML of the cuttlefish" plotted against cuttlefish (predator) DML.

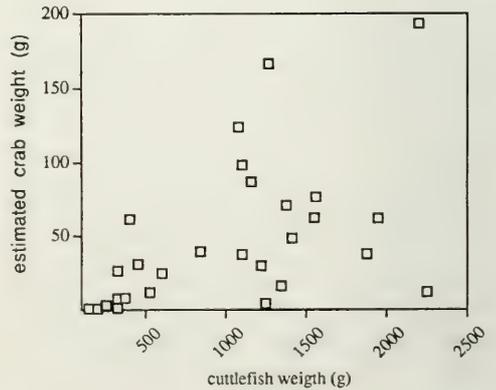


FIG. 6. Relationship between cuttlefish weight and estimated crab weight.

TABLE 2. Estimates of the surface area of the cephalothorax of crabs captured by cuttlefish in their natural environment and the calculation of the ratio R.

<i>Sepia officinalis</i>		<i>Carcinus maenas</i>			Ratio R
DML (cm)	Weight (g)	Width of the carapace (mm)	Carapace surface area (mm ²)	Crab weight (g)	
11	200	29.8	632.9	1.74	0.32
13	250	33.3	796.3	3.26	0.31
14	372	40.6	1178.2	8.86	0.32
15	325	51.6	1888.3	27.11	0.17
17	400	62.1	2716.6	61.33	0.15
18	525	43.8	1364.3	12.66	0.38
20	1078	73.2	3758.5	123.84	0.29
21	840	56.2	2232.5	39.65	0.38
22	1100	69.3	3371.8	98.13	0.33
23	1246	36.6	957.7	5.27	1.30
24	1100	55.0	2181.6	37.64	0.50
24	1413	58.8	2441.9	48.44	0.58
25	1220	52.9	1983.7	30.35	0.62
25	1265	78.6	4320.4	166.43	0.29
26	1560	65.3	3005.8	76.52	0.52
10	145	30.4	666.3	2.01	0.22
13	225	43.8	1364.3	12.66	0.16
13	254	34.9	871.9	4.16	0.29
14	325	30.9	689.1	2.20	0.47
15	325	40.4	1163.3	8.59	0.28
16	450	53.3	2012.8	31.37	0.22
18	600	50.8	1832.1	25.30	0.33
23	1157	67.3	3186.2	86.84	0.36
24	1375	64.2	2900.1	70.77	0.47
25	1344	46.4	1529.6	16.62	0.88
25	1550	62.3	2739.3	62.45	0.57
28	2200	81.4	3409.6	193.20	0.64
29	1875	55.7	2191.7	38.04	0.86

R = weight of the cuttlefish (g), surface of the cephalothorax (mm²) (Duval et al., 1984).

Nixon, 1965, 1966). Mather (1980) obtained the same results with a second species of octopus, *Octopus joubinii*.

There is another hypothesis that must also be taken into account in the attack and capture of larger prey. This relates to the efficiency of the toxic secretions from the posterior salivary glands of *Sepia* on larger fishes or crabs. The injection of a toxic saliva provokes the rapid paralysis of the crab (Chichery & Chichery, 1992).

For each type of prey (crabs and fishes), the length ratios remain constant in relation to the DML of the predator. The anatomical characteristics of the digestive tract constrains the ability of the cuttlefish to break down prey tissue into particles that can pass through the oesophagus where it penetrates the central nervous system. Guerra et al. (1988) showed that the antero-posterior length of the buccal mass increases in size as the oesophagus grows, until the cuttlefish reaches a DML of 150 mm.

This implies that the size of ingested particles is limited by these two parameters. In other words, the larger the prey is, the more the buccal mass—the beaks and radula—must work to reduce the prey before ingestion take place.

Fishes appear to be preferentially captured by the tentacle strategy, whereas crabs are captured by both strategies (Messenger, 1968). The tentacle strategy was often used for smaller crabs (Chichery & Chichery, 1991).

Messenger (1968) demonstrated in a laboratory study that crabs can be attacked by two methods—a jump or tentacular strike. Duval et al. (1984) indicated that the attack behaviour is dependent on the ratio R (weight of the cuttlefish/surface area of the crabs' cephalothorax). Estimates based on stomach contents show that the majority of crabs are captured by the jump method. Control of the two first parts of the attack (attention and positioning) is a visually controlled loop system (Messenger, 1968). The prey and its movements

TABLE 3. Comparison of estimated otolith lengths after digestion of the fish prey by the cuttlefish (n = 14)

Teleostan family	Species	Total length of the fish (mm)	Otolith length estimated (mm)	Otolith length following digestion (mm)
Gadidae	<i>Trisopterus luscus</i>	200	9.29	8.70
Gadidae	<i>Trisopterus luscus</i>	180	8.47	8.60*
Gadidae	<i>Trisopterus luscus</i>	215	9.57	9.80*
Gadidae	<i>Trisopterus luscus</i>	230	10.04	10.60*
Gadidae	<i>Trisopterus luscus</i>	94	5.77	5.30
Callionymidae	<i>Callionymus lyra</i>	205	3.18	3.55*
Callionymidae	<i>Callionymus lyra</i>	230	3.47	3.41
Callionymidae	<i>Callionymus lyra</i>	185	2.95	3.02*
Callionymidae	<i>Callionymus lyra</i>	255	3.75	3.55
Callionymidae	<i>Callionymus lyra</i>	275	3.98	3.58
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	91	3.40	3.55*
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	73	2.79	2.78
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	103	3.81	3.71
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	54	2.15	2.14
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	89	3.33	3.30
Gobiidae	<i>Gobius</i> sp. or <i>Pomatoschistus</i> sp.	102	3.78	3.30
Mean ± sd			4.98 ± 2.72	4.96 ± 2.77

*Some data on otolith length following digestion were greater than these of estimated otolith length. The latter came from the equations given in Table 1. There were also variations.

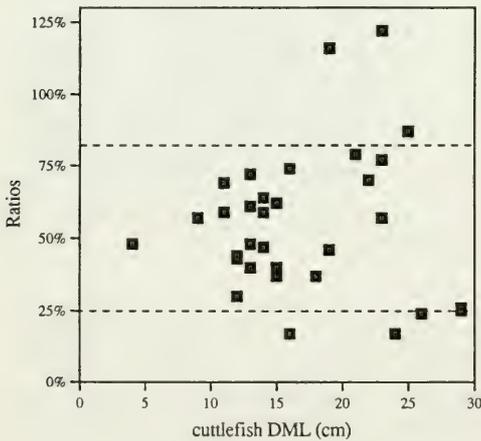


FIG. 7. Ratio of the "length of the captured fish ÷ DML of the cuttlefish" plotted against DML cuttlefish (predator) DML.

are identified by the cuttlefish before beginning an attack. The possibility of identification of the prey in terms of type and size, possible with an accurate spatial location, increases the rate of attack success. The tentacle strategy is the best adapted for prey that possess a rapid escape response (Duval et al., 1984; Chichery & Chichery, 1992).

Hurley (1976) noted that the young squid *Loligo opalescens* showed an absence of selectivity for prey length. But this author also noticed that, although success of the hunt de-

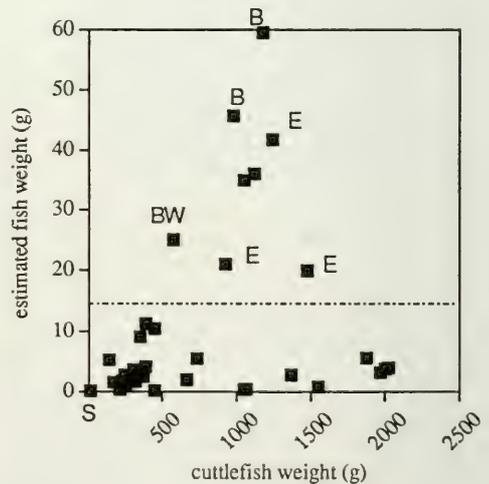


FIG. 8. Relationship between cuttlefish weight and estimated fish weight.

E: eels G: gobies
B: bibs S: sand smelts
BW: ballan wrasses

pended on prey size, Other factors have to be taken into account to explain the variations in behaviour—the prey species hunted, and the age, experience, and motivation of the predator.

Otoliths are routinely used to identify fishes and to estimate their sizes. Digestive enzymes do not attack these hard structures.

Jobling & Breiby (1986) noticed the same result with the squid *Todarodes sagittatus*. They estimated that the pH (5.2 to 6.3) of the digestive tract of oceanic squid was not acidic enough to dissolve these structures.

The only fish that is attacked even if the length ratio is unfavourable to the predator is the eel. Our observations in the laboratory revealed that the tentacular method undergoes some modifications. In these cases, the head of the eel is not targeted first. The first bite is made on the spinal column. Subsequently, manipulation to bring the head of the fish to the mouth can be done because risks of flight have been minimized.

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THE ACCUMULATION OF TAXONOMIC KNOWLEDGE: THE HISTORY OF SPECIES DESCRIPTIONS OF SOME PREDATORY GASTROPODS

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ABSTRACT

In order to assess how perceived temporal and spatial patterns of distribution of species depend on taxonomic knowledge, I compiled dates of description of all 558 known gastropod species with shells having a labral tooth. Peaks in the number of described species were reached in the intervals of 1820–1859 and 1980–1999 for Recent species, and 1920–1939 for fossil taxa. Distributional patterns were already evident by 1859, when about half of the living and 13% of the fossil species had been described; but geographical and temporal ranges are very sensitive to accumulated taxonomic knowledge, and have changed substantially as more species were described. An asymptote in the number of species has not yet been approached either in these gastropods or in molluscs and other organisms generally. This fact underscores the importance of continuing support for taxonomic research.

Key words: taxonomy, gastropods, labral tooth, history.

INTRODUCTION

For some years I have been studying the functional morphology, taxonomy, phylogeny, and distribution in time and space of predatory gastropods with a labral tooth, a sharp, ventrally and sometimes anteriorly directed protrusion of the abapical or medial sector of the shell's outer lip. In all studies of this kind, questions arise about the completeness of taxonomic data, and about the robustness of the patterns that those data reveal. One approach to tackling these questions is to document the history of description of species since the beginnings of formal zoological taxonomy in 1758. If the number of species described per time interval falls off, we may infer that an asymptotic number is being approached, which would mean either that we are close to discovering all existing or knowable species, or that taxonomic interest is waning. This latter possibility is potentially a matter of concern, because it affects directly the amount and quality of fundamental data on which most studies of ecology, evolution, biogeography, stratigraphy, and many other fields depend.

Here I report the results of my compilation of the times of description of all fossil and living gastropod species with a labral tooth known to me. Although these gastropods represent a tiny and potentially unrepresentative

fraction of gastropods as a whole, to say nothing of living things generally, they reveal patterns of description that are both surprising and informative.

METHODS

I have assembled a list of all fossil and living gastropod species with a labral tooth. A species was included if I observed a labral tooth on specimens, or if authors describing or discussing the species in question clearly indicated the presence of a labral tooth. In a large number of cases, the original describers were unaware of, or failed to record, the presence or characteristics of the labral tooth. My own examination of material in museum collections therefore revealed many cases that would not have come to light from a search of the literature alone.

For each species, I used the earliest published name, even if that name is not the currently accepted one because of homonymy. Names that on the basis of my studies or taxonomic revisions by other authors appear to be synonyms were not included.

I grouped dates of publication into 20-year time intervals. For each interval, I compiled separately the number of living and fossil species.

RESULTS AND DISCUSSION

My current tally shows that 558 species (219 Recent and 339 fossil) have a labral tooth. These species range in age from Late Cretaceous (Campanian) to Recent, and belong to ten families. These include the tonnoidean Ranellidae, and the neogastropod families Muricidae, Buccinidae, Melongenidae, Nassariidae, Fascioliariidae, Pseudolividae, Turbinellidae, Lividae, and Marginellidae.

The temporal pattern of description of Recent species differs from that of fossil ones (Fig. 1). For Recent species, there was a broad peak from 1820 to 1859, and a very conspicuous maximum in the present 20-year interval, 1980–1999. The first half of the 20th century (1900–1959) represents a broad trough in the number of described Recent species. The fossil data reveal a general rise in the number of described species per interval to a peak in the years 1920–1939, followed by more than half a century of stability at a high level (about 30 species per 20-year interval) of taxonomic activity.

A striking result of this study is that the most recent time interval (1980–1999) has contributed a sizable fraction of our accumulated taxonomic knowledge. Of the 219 Recent species, 48 (21.9%) have been described during this interval. Among the 339 fossil species, the corresponding number is 34 (9.4%). For Recent species, taxonomic knowledge has doubled since 1860; for fossil species, the number has doubled since 1920.

As Sepkoski et al. (1981) and Sepkoski (1993) have pointed out in their studies of the number of families and genera of marine fossil invertebrates, increases in taxonomic knowledge do not necessarily require revision in our perception of temporal and spatial patterns of distribution of species. A general increase in the number of taxa over geological time, and the existence of a latitudinal increase in diversity from the poles to the equator, are robust patterns that were discernible by 1859, the date of publication of Charles Darwin's *Origin of Species*. Similarly, the broad outlines of distribution of gastropods with a labral tooth were already apparent by this date when, according to my data, about half the living species with a labral tooth and 13% of the presently known fossil taxa had been named. By the middle of the 19th century, observers able to examine specimens could already have concluded that the inci-

dence of labral teeth is higher among living predatory gastropods on the Pacific than on the Atlantic side of the Americas, and that the incidence in Europe declined from the Miocene to the Recent.

Other details of distribution, especially first appearances and unusual geographical records, are very sensitive to accumulated knowledge. For example, only one of 30 Cretaceous gastropods with a labral tooth – *Buccinopsis parryi* Conrad, 1857 – was described before 1859, and this species was based on material so poorly preserved that no contemporary scholar could have inferred the presence of a labral tooth. Muricids with a labral tooth from the Oligocene, the earliest epoch from which this structure is known in this family, were not described until 1918. In that year, Clark described *Thais packi*, but its labral tooth was not recognized until 1993 (Amano et al., 1993). A second Oligocene muricid with a labral tooth was described by Vokes (1963), but its age was originally thought to be early Miocene. Until about 1970, therefore, taxonomists might legitimately have concluded that muricids with a labral tooth did not originate until early Miocene time.

The continuing high rate of discovery of species with a labral tooth mirrors a general pattern for molluscs (Bouchet, 1997) and for organisms generally (Winston & Metzger, 1998). This is surprising in view of the fact that most labral-tooth-bearing gastropods are relatively large (greater than 2 cm in height) and thus more easily collected than are micromolluscs.

The fossil data in Figure 1 are likely to be less representative for the description of fossil taxa in general than are the data for Recent species. Large parts of the world remained essentially unexplored paleontologically until the present century. This is especially true for older rocks and for small fossils. Despite this, the vigor of descriptive paleontological activity in the latest 20-year interval as indicated in Figure 1 is noteworthy.

The early peak (1820–1859) in taxonomic description of Recent gastropods with a labral tooth probably reflects the collective efforts of major scientific expeditions throughout the world. Many of the common shallow-water taxa became known during this time. The 1980–1999 maximum, preceded by a rise in taxonomic activity that began in the 1960s, likely represents the efforts of divers and of expanded trawling and dredging operations. It

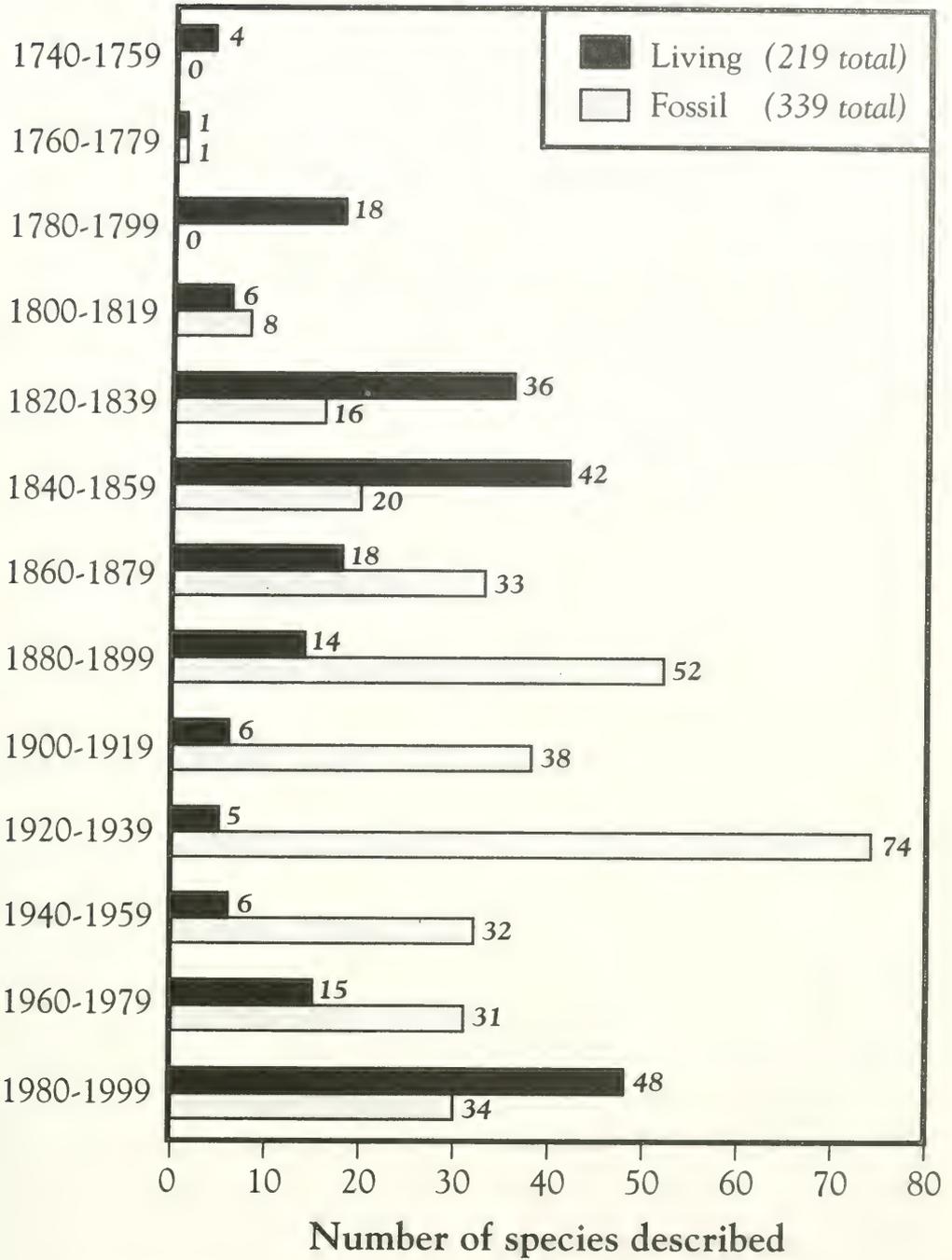


FIG. 1. Number of gastropod species with a labral tooth described per 20-year interval, 1740–present.

also demonstrates unequivocally that interest in molluscan taxonomy is as strong as ever.

The pattern of description of fossil species probably reflects exploratory activities by geologists and paleontologists working in the oil industry or for other commercial interests. It is noteworthy that, whereas the number of described Recent species reached a broad low in the decades during and between the great world wars, the fossil data show no such apparent effect of worldwide conflict. There is no dramatic upsurge in the number of described fossil species in the most recent time interval as there is for Recent species, but neither is there the decline that might be expected if taxonomic expertise and the number of working taxonomists were declining.

In the future, the rate of species description is likely to dwindle for either or both of two reasons. The first, and most worrying, is the long feared reduction in funding for, and interest in, taxonomy. This problem may be mitigated somewhat, at least for shell-bearing molluscs, by the increased participation of highly knowledgeable amateurs. According to my data, amateurs account for about one-quarter of the authors of labral-tooth-bearing gastropod species since 1980. The second reason is that fewer species remain to be discovered. Potentially counteracting this trend is the finding that taxa previously interpreted as representing a single species in fact comprise two or more genetically distinct species. In any case, the limited data in Figure 1 give no indication that either taxonomic activity or the rate of discovery of species is declining or reaching asymptotic values.

The fact that taxonomic data are fundamental to all branches of comparative and historical biology and that large numbers of taxa remain to be described provides a strong argument for continued support of what many observers disparage as mundane, descriptive science. Phylogenetic and ecological studies are only as good as their underlying data and assumptions. Continued research and publication on taxonomy should remain an impor-

tant component of the study of the history and distribution of living things.

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THE ROLE OF SUBSTRATUM STABILITY IN DETERMINING ZEBRA MUSSEL LOAD ON UNIONIDS

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ABSTRACT

Data reported herein do not support the existence of preference for or attraction to unionids by settling or migrating zebra mussels compared to alternative hard substrata. Despite claims and inferences in the *Dreissena* literature suggesting that unionids are preferred substrata, higher *Dreissena* loads on unionids compared to alternative hard substrata can be explained by mechanisms other than preference. Our data indicate that substratum conditions are often critical in determining the relative zebra mussel loads that accrue on unionids. On stable and relatively hard lake/river bottoms, zebra mussel loads on unionids tend to be similar to those on other hard substrata. However, on bottoms mostly composed of very soft or unstable substrata, discreet hard objects become silted-over and/or buried, hence sub-optimal for zebra mussels. Under such conditions, unionids develop a higher load of zebra mussels due to their ability to maintain position in relation to the sediment/water interface. We conclude that high *Dreissena* loads on unionids relative to other substrata are not a matter of preference for or attraction to the unionids, but are the outcome of differential survival/emigration of the *Dreissena* due to unstable or changing bottom conditions.

Key words: *Dreissena*, zebra mussel, substratum, preference, unionids, stability.

INTRODUCTION

Among the many ecological changes attributed to the zebra mussel invasion of North American lakes, none is more readily apparent than the virtual elimination of entire unionid communities (Schloesser & Kovalak, 1991; Schloesser & Nalepa, 1994; Nalepa, 1994). This consequence is especially unfortunate due to the already precarious status of the majority of unionid taxa (Williams et al., 1993; Stein & Flack, 1997).

The common impression from examination of unionids in lakes recently invaded by zebra mussels is that they are highly suitable as substrata for attachment. This often gives the appearance that unionids are being singled out by zebra mussels by some mechanism of preference (Lewandowski, 1976; Mackie, 1993; Ricciardi et al., 1995, 1996). One of the difficulties in using the word preference in the context of settling zebra mussels is that it specifies active choice, a choice based on the ability to discriminate among alternatives. The underlying mechanism would of necessity involve an ability to detect positive or negative stimuli from amongst alternatives (e.g., different kinds of substrata) and to choose based

on the perception of those qualities. With the example of settling larvae in marine ecosystems, preference is typically based on detection of chemical or textural cues originating from the biofilm or from components of the biofouling community (Morse, 1991; Rodriguez et al., 1993). However, it is possible for a different mechanism, not involving preference, to result in clumped or aggregated distribution of a species. In such cases, larvae settling on both suitable and non-suitable substrata would experience post-settlement differential mortality with higher mortality among the latter group. The resulting greater aggregation on suitable substrata might give the impression of preference, but in the absence of active choice the resulting distribution would simply be the outcome of differential mortality on dissimilar substrata.

Although it is often taken for granted that zebra mussels "prefer" hard substrata, they have been reported living on soft substrata and can achieve surprisingly high densities (Hunter & Bailey, 1992; Dermott & Munawar, 1993; Coakley et al., 1997). Despite these reports, the highest densities are on hard substrata, and it is likely that they can colonize soft substrata only from an initial seed object

and if the bottom is undisturbed by current. Zebra mussels are clearly epifaunal and the morphological adaptations for this mode of existence are obvious: presence of a byssus and heteromyarian shell morphology (Morton & Yonge, 1964; Yonge & Campbell, 1968).

One form of preference that has been documented for zebra mussels is settlement on or near conspecifics. This was first suggested by Lewandowski (1976) and supported by the field studies of Wainman et al. (1996), Chase & Bailey (1996), and Toczyłowski & Hunter (1997). Although none of these studies experimentally addressed the release of chemical cues by established adults, the results of Wainman et al. (1996) suggest that it is more likely that conspecific shell surface chemistry was the basis for veliger preference.

Some of the recent literature largely reinforces the perception of unionid preference by *D. polymorpha*, without specifically identifying the mechanism by which preferred substrata are recognized (Ricciardi et al., 1995, 1996). A previous study (Toczyłowski & Hunter, 1997) found no evidence to support zebra mussel attraction to unionids based on field studies of *Dreissena* settling on unionid and non-unionid test surfaces. The present work extends and refines those findings and examines the effects of substratum stability on differential mortality of zebra mussels attached to stones as compared to unionids.

We hypothesize that zebra mussels do not actively choose unionids over other hard substrata. Higher *Dreissena* density on unionids in relation to other hard substrata, where it occurs, is the outcome of differential mortality resulting from bottom instability.

EXPERIMENTAL DESIGN AND METHODS

Site Descriptions

All of the field sites were in the upper Clinton River or in Loon Lake, a lake connected to the Clinton River located in Oakland County in southeastern Michigan (Fig. 1). The upper mainstem of the Clinton River between Loon Lake and Dawson's Millpond Outlet (DMO) is mostly less than 10 m in width, with discharge rates averaging 1.42 and 1.75 m³/s for the 1995 and 1996 calendar years respectively (Blumer et al., 1997). The range in 1996 was 0.28 – 15.01 m³/s. The dominant unionid species in this region of the river, *Ptychobranchnus fasciolaris* (23%), *Elliptio dilatata* (21%), and *Strophitus undulatus* (15%), are

indicative of a hydrological tendency toward a stable river as opposed to an event river (Hunter et al., 1997; DiMaio & Corkum, 1995). The water is relatively clear with the silt load sufficiently low that one can see the bottom in most places. Mean seston load for 1995 from 12 monthly measurements was 8.7 mg/L dry mass (range = 0.7 – 37.4 mg/L) and values for 1996 were similar (Hunter et al., 1997). In this stretch of the river, the watershed is mostly residential with a few small towns and no heavy industry. The river bottom is mostly hard consisting of cobble and/or sand with soft silt elsewhere.

Loon Lake is a mesotrophic lake with an area of 0.95 km² and maximum depth of 21 m. The bottom is mostly soft mud except near shore where it is mostly cobble and sand except for a few marshy areas. Macrophyte growth along the shore is moderate.

Natural Density of Zebra Mussels on Unionids and Stones

To determine the field density of zebra mussels on unionids and stones, samples of natural substrata were collected at DMO on 16 October 1996 (Fig. 1). Unionids and stones were from an area where the water was about 0.5 m deep and the bottom was partially cobble and partially sand. Bottom sediments in this area were subject to considerable movement during periods of high water flow; consequently hard objects, such as stones, were alternately buried in sand or exposed as cobble. At this site, the most abundant three unionids were *Elliptio dilatata*, *Lampsilis siliquoidea*, and *Ptychobranchnus fasciolaris*.

The density measured in this instance, refers to number or biomass of zebra mussels per unit of unionid or stone surface area, not per unit total bottom area. Although the latter meaning is the standard one, our modified density (= suitable substrate density) allows a more direct comparison between different types of discreet hard surfaces. Ten unionids along with ten stones of approximately the same exposed surface area as the unionids were selected. Both the unionids and the stones were carefully transported to the lab where all attached zebra mussels were removed with a scalpel under a dissecting microscope to ensure that small individuals were included. After removal, the zebra mussels were counted and their length was measured using digital calipers. For each of the two surface types, area above the sedi-

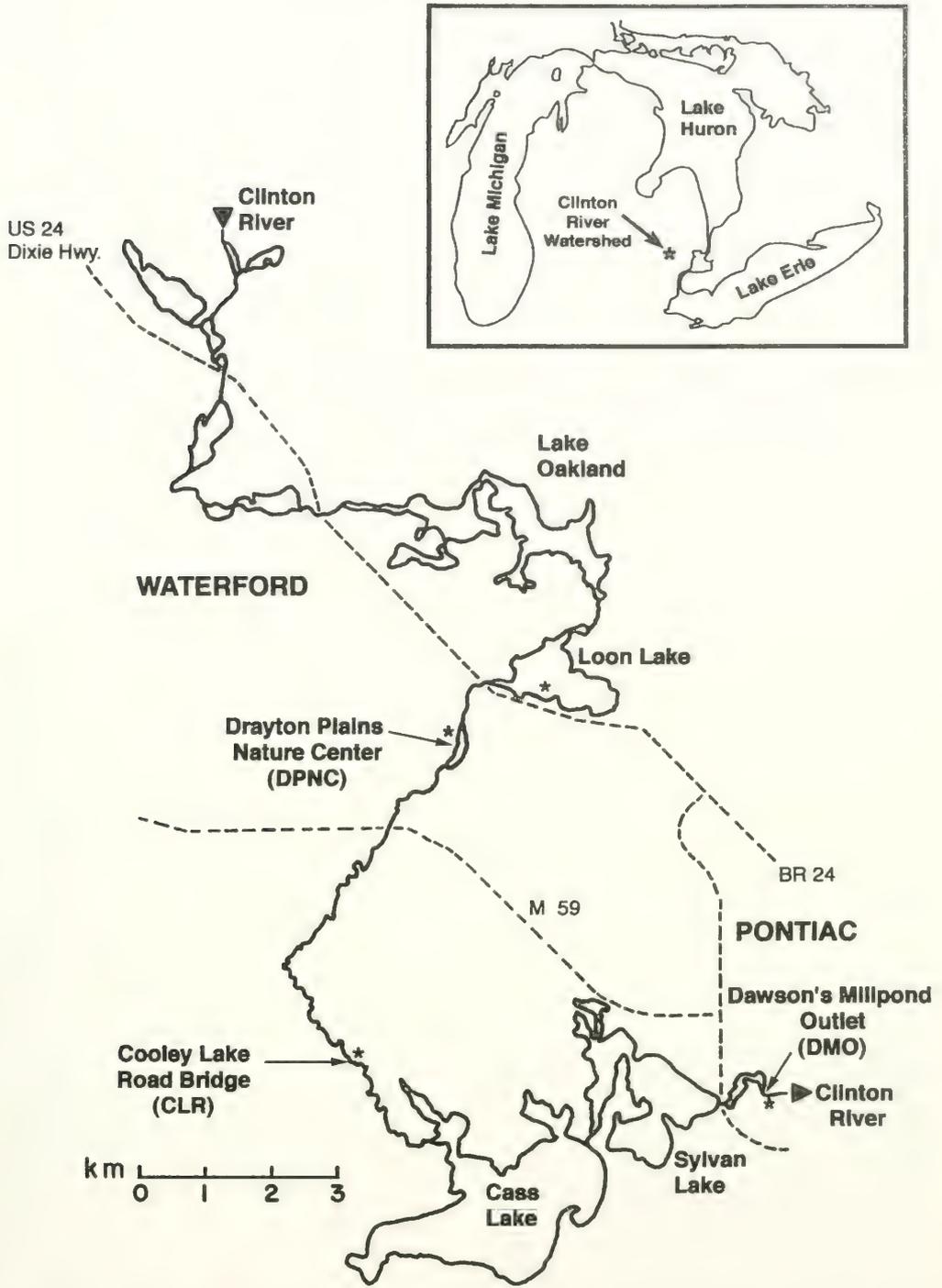


FIG. 1. Map of the upper mainstem of the Clinton River in southeastern Michigan with the sample and study sites indicated.

ment/water interface was obtained using a foil method. This method is essentially the same as that used in Mackie (1993). Because buried areas on the test surfaces became darkened, this made the sediment/water interface clearly visible, so the area exposed to zebra mussel settling was easy to see. The method involved removing attached zebra mussels, covering the exposed area with aluminum foil, removing the foil, and trimming and flattening it. After placing the foil on metric graph paper, the outline was traced and the surface area counted. Data from this study were compared using an unpaired t test to determine if there were significant differences between zebra mussel density on stones and unionids. Mean values given in the text are accompanied by standard error.

Settling and Migration Preference Study

An experimental test of preference utilized test quadrats placed in Loon Lake and in the Clinton River at Drayton Plains Nature Center (DPNC; asterisks mark these locations in Fig. 1). Quadrats at Loon Lake were placed in water of 1.2 – 1.4 m deep and ~10 m from shore, where the bottom sediment was soft and silty, lacking in cobble or stones. The DPNC quadrats were placed at a depth of about 1.0 m at the center of a slow-flowing stretch of the river where the bottom was also mostly silt. Quadrats were circular and enclosed an area of about 0.75 m². Each quadrat was an open-topped enclosure using plastic garden edging as a boundary, with about 3 cm above and about 9 cm below the sediment, to facilitate recovery of unionids later in the summer. Each quadrat contained four replicates of each of the following test surfaces; unionids with no attached zebra mussels (= control), unionids with marked zebra mussels, control stones, and stones with marked zebra mussels. Although they differed slightly in size, the surface area of each object was measured at the end of the experiment and the density of attached zebra mussels expressed per unit area. Average total exposed unionid surface area was 31 cm² and that of stones was 43 cm².

The species composition of test unionids at Loon Lake was *Pyganodon grandis*, *Lampsilis siliquoidea*, and *Elliptio dilatata* in a ratio of 4:3:1 and at a density of 8/quadrat. Unionids placed in Clinton River quadrats were collected from the river nearby and included *Strophitus undulatus*, *Ptychobranchus fasciolaris*, and *Pyganodon grandis* in a ratio of ap-

proximately 4:2:1, also at a density of 8/quadrat.

The unionids and stones with zebra mussels each had about 5 - 30 zebra mussels at the outset of the experiment. Each of these zebra mussels was marked with a dot of enamel model paint; those on unionids were marked with red paint and those on stones were marked with blue. All unionids and stones had an identification number painted on them at the start of the experiment. By marking the zebra mussels attached to the test surfaces at the beginning (= residents), it was possible to identify non-residents that immigrated onto the test surfaces during the experiment.

Test surfaces were put into Loon Lake on 6 June 1996 and remained *in situ* for 103 days. At the Clinton River site they were put in on 10 June 1996 and were *in situ* for 99 days. This period coincided with the major period of the veliger presence and spat settling for Loon Lake. After completion of the experiment, all attached zebra mussels were removed and counted. Area of the test surfaces above the sediment/water interface was measured using the foil method. For unionids with attached zebra mussels, the surface area of the original resident zebra mussels was included in the total surface area. However, for the density and biomass data, the original attached zebra mussels were not included.

The mean of four replicates of each of the four test surfaces was statistically analyzed using two-way ANOVA, with treatment and site as the factors. To evaluate the relationship between number of attached zebra mussels at the start and number of immigrating zebra mussels that attached during the study, a regression ANOVA was used that calculated an F-statistic using sum of squares and mean square. This indicated how important the independent variable was in explaining the behavior of the dependent variable. Emigration data was expressed as % decrease of resident mussels for plotting, but two-factor ANOVA was done on arcsin transformed data ($p' = \arcsin[\sqrt{p}]$), the results of which are reported in Table 1B.

Zebra Mussel Survival and Migration on Unstable Substrata

To determine survival and migration of zebra mussels on unionids and stones located on shifting river bottom, we performed a field experiment in the Clinton River at the CLR site (Cooley Lake Road Bridge, Fig. 1).

Flow rate at this site is moderately fast for the upper Clinton River, and the bottom is relatively unstable. At a given point in time, most of the bottom is sand with a few areas of cobble, the boundaries of which change over the year. Depressions in the sand are common here, and their size and location also shift. The test surfaces were located on the upstream slopes of such depressions. Test surfaces consisting of unionids and stones of approximately the same size were collected and given an identification mark. The test unionids were collected on site and included *Villosa iris*, *Elliptio dilatata*, *Pyganodon grandis*, *Strophitus undulatus*, *Lampsilis siliquoidea*, and *Ptychobranchus fasciolaris* in ratios of 20:3:3:2:2:1. Unionids were allocated to replicates in approximate proportion to their natural abundance. Zebra mussels on both test surfaces were counted and marked at the start of the experiment, so that any unmarked zebra mussels found at the end of the experiment could be recognized as immigrants. To ensure recovery at the conclusion of the experiment, unionids were tethered to a stake in the river bottom using nylon string attached to one valve with cyanoacrylate glue. Stones were not tethered, and were simply placed on the sand bottom. Ten unionids and ten stones were placed around each of the three replicate stakes on 23 June 1997 and remained *in situ* for 78 days, after which they were retrieved and brought to the laboratory. In the lab, the number of resident and non-resident zebra mussels was recorded for each test surface. Surface area was not measured due to the inability to determine the exact amount of the test surface that was above the sediment-water interface (i.e., the sediment was not chemically reducing at this site). The data were analyzed with an unpaired t test to detect significant differences using mean number of zebra mussels per test surface at each stake, hence $N = 6$ (i.e., 3 replicates \times 2 treatments/replicate). When the total number of zebra mussels (residents + immigrants) was expressed as a % change on unionids and stones, the decimal equivalents were arcsin transformed before using the t test; $p' = \arcsin(\sqrt{p})$.

RESULTS

Natural Density of Zebra Mussels on Unionids and Stones

Unionids at DMO had a zebra mussel density of $1.40/\text{cm}^2$, which was significantly

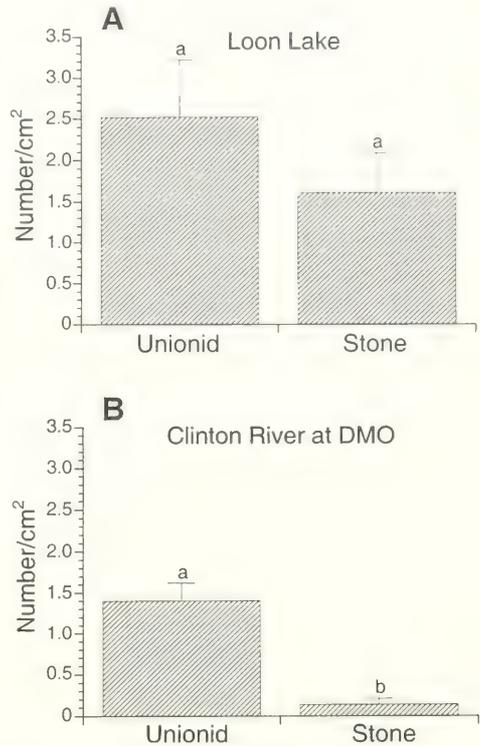


FIG. 2. Natural density of zebra mussels (mean \pm SE) on unionids and on stones from (A) Loon Lake and (B) Clinton River at DMO (Dawson's Millpond Outlet). Histograms with the same letter are not significantly different from each other.

greater than the zebra mussel density observed on stones, $0.14/\text{cm}^2$ (Fig. 2B; $t_{(18)} = 5.332$; $P = 0.0001$). There was also a significant difference in wet biomass; unionids had $600.3 \text{ mg zebra mussels}/\text{cm}^2$, stones had $78.0 \text{ mg}/\text{cm}^2$ ($t_{(18)} = 4.568$; $P = 0.0002$). Hence, the river results did not agree with those from the Lake. DMO is a river site that is frequently exposed to high current due to the proximity of a dam used to maintain nearby lake levels. As a result, it is a high-energy location with an unstable bottom. The results we obtained at this site contrasted with those obtained from Loon Lake a year earlier (Toczyłowski & Hunter, 1997).

Settling Preference Experiment

All test surfaces in Loon Lake, whether stones, unionids, with or without resident zebra mussels, attracted a significantly greater density of unmarked (= immigrant)

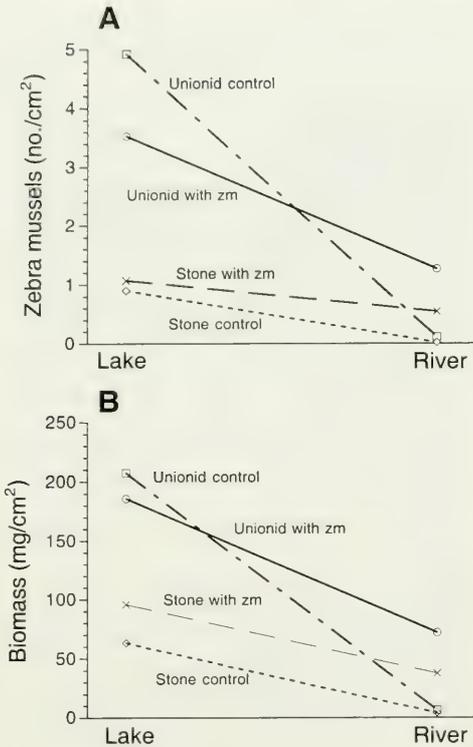


FIG. 3. Interaction plots for the settling preference study. (A) Numerical density of zebra mussels in Loon Lake and Clinton River treatments. (B) Biomass density of zebra mussels in Loon Lake and Clinton River treatments. The four treatments used at each site are identified on the plots.

zebra mussels/cm² than those in the Clinton River (Fig. 3A). The lake test surfaces averaged five times as many newly recruited zebra mussels as their river counterparts. As used here, immigrants denotes both spat zebra mussels that have recently settled out of the plankton directly onto the test surface, as well as later stage individuals that settled elsewhere and subsequently relocated onto the test surfaces. This latter group consists of young-of-the-year juveniles as well as adults that settled in previous years. No attempt was made to separate these groups in this data set; spat and later stages were lumped as "immigrants". This higher rate of accumulation of zebra mussels on lake compared to river substrata is simply the result of higher settling rates due to higher veliger density in the lake (Hunter et al., 1997; unpublished data for 1996).

There were also significant differences in immigrant zebra mussel density among the test surface types (Table 1A). Unionids in the lake had about a 4× higher density of immigrant zebra mussels than did the stones. However, there was no such difference at the river site; that is, both unionids and stones appeared to attract similar numbers of immigrants. It also appeared to be of little importance whether the test surface had resident zebra mussels or not. That is, immigration of zebra mussels was not enhanced by the presence of previously settled (resident) conspecifics. There was also a significant interaction between the main effects (Table 1A). Hence, zebra mussel density was responsive to surface type at the lake site, but not at the river site.

The biomass data showed a similar pattern of significance as was seen in the numerical density data above (Fig. 3B). There were strong site effects; unionids had higher zebra mussel loads than did stones, and presence of resident zebra mussels had little effect (two-factor ANOVA $F_{(1, 112)} = 29.6$; $P < 0.0001$). Unionid test surfaces had a significantly greater biomass of immigrants (approximately five times greater) than the river unionid surfaces ($F_{(3, 112)} = 7.3$; $P = 0.0002$). There was also a significant interaction between main effects ($F_{(3, 112)} = 6.5$; $P = 0.0005$). Density differences between lake and river are great for unionids but small for stones.

In order to evaluate the effects of emigration of resident zebra mussels and whether it differed by test surface type, the percent decrease of marked individuals was calculated. There was a significant difference in percent decrease by site and surface type (Table 1B). Loon Lake had a larger percent decrease of resident zebra mussels on both unionid and stone test surfaces compared to those in the river (Fig. 4). Stone surfaces had a significantly greater overall percent decrease in resident mussels regardless of site (Table 1 B). However, in contrast to number and biomass data, the interaction of the two factors was not significant.

The number of resident zebra mussels at the start of the study was not a good predictor of the number of mussels that subsequently immigrated to that surface at the lake site; however, the river site showed the opposite result. At Loon Lake, neither unionids ($F_{(31)} = 0.391$; $P = 0.537$) nor stones ($F_{(22)} = 2.105$; $P = 0.162$) attracted more immigrants if the number of resident mussels at the start was

TABLE 1. Analysis of variance tables based on settling preference data. (A) Results of a three-factor ANOVA on number of immigrant zebra mussels per cm². (B) Results of a two-factor ANOVA on percent decrease (emigration) of resident zebra mussels from test settling surfaces.

A. Immigration: no./cm ²			
Source of variation	Degrees of freedom	Mean square	F
Main effects			
site	1	122.6	29.2**
surface	1	90.4	21.6**
res/non-res	1	0.3	0.8 ns
Two-way interaction			
site x surface	1	54.3	12.9*
surf x res/non-res	1	1.5	0.3 ns
res/non-res x site	1	14.1	3.4 ns
Three-way interaction			
site x surface x res/non-res	1	8.1	1.9 ns
Error	104	4.2	
Total	111	6.8	

*P = 0.0005; **P < 0.0001

B. Emigration: % decrease of residents			
Source of variation	Degrees of freedom	Mean square	F
Main effects			
site	1	1.758	14.99*
surface type	1	1.160	9.89**
Two-Way interaction			
site x surface type	1	0.243	2.07 ns
Error	56	0.117	
Total	59	3.278	

*P = 0.0003; **P = 0.003; ns = P > 0.05

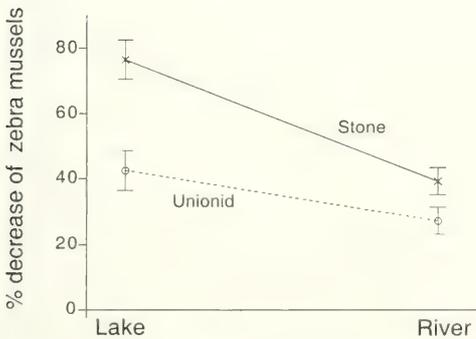


FIG. 4. Emigration of zebra mussels as % decrease (mean ± SE) of residents from stones and unionids by the end of the settling preference study. See Table 1B for statistical analysis.

greater (Fig. 5A). When unionids and stones at the river site were analyzed, there was a significant correlation between the two variables (unionids, $F_{(28)} = 528.6$; $P = 0.0001$; stones, $F_{(28)} = 210.1$; $P = 0.0001$; Fig. 5B). In other words, at the river site, having more res-

ident mussels at the start significantly increased the number of zebra mussels that recruited to the test surfaces. However, at the lake site, where recruitment was about an order of magnitude higher, there was no such relationship. The mean length of recruited zebra mussels did not differ between surface type ($t_{(6)} = -2.205$; $P = 0.696$).

Zebra Mussel Survival and Migration on Unionids and Stones on Unstable Substrata

Test unionids and stones at the start of this experiment had virtually identical mean numbers of attached zebra mussels by object: unionids, 6.48 ± 0.435 zebra mussels, and stones, 6.45 ± 0.449 zebra mussels ($t_{(52)} = 0.041$; $P = 0.967$). After 78 days on the unstable river bottom at CLR, unionids had a significantly higher number of zebra mussels ($t_{(4)} = 4.855$; $P = 0.008$), averaging 8.4 ± 0.91 zebra mussels, whereas stones averaged 1.9 ± 0.50 zebra mussels (Fig. 6A). This gain of zebra mussels by unionids occurred for two

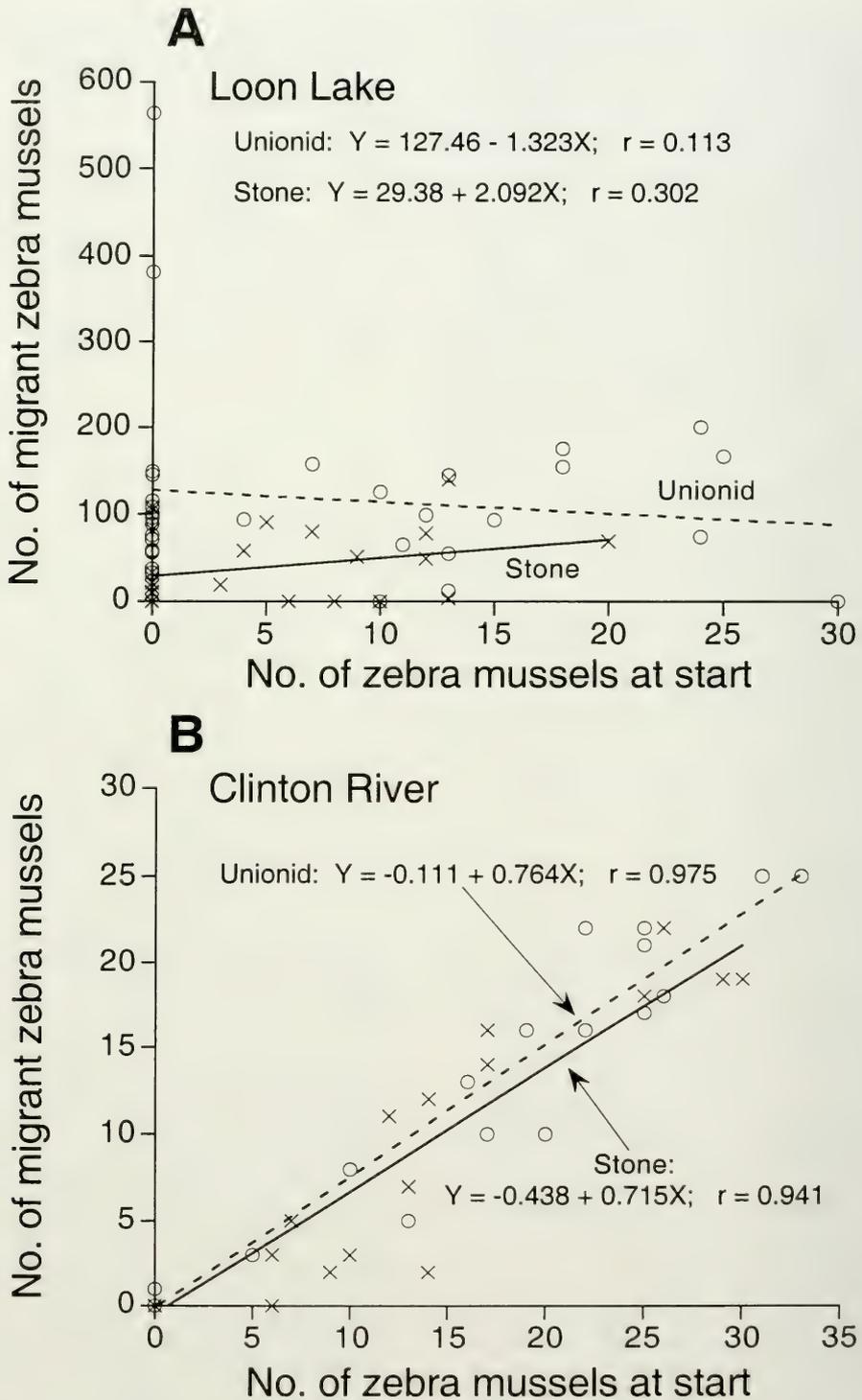


FIG. 5. Scatter plot of the number of zebra mussels immigrating to unionids and to stones at Loon Lake (A) and the Clinton River (B) in relation to the number of resident zebra mussels on these test objects at the start of the settling preference study. Lines are fitted by least squares regression.

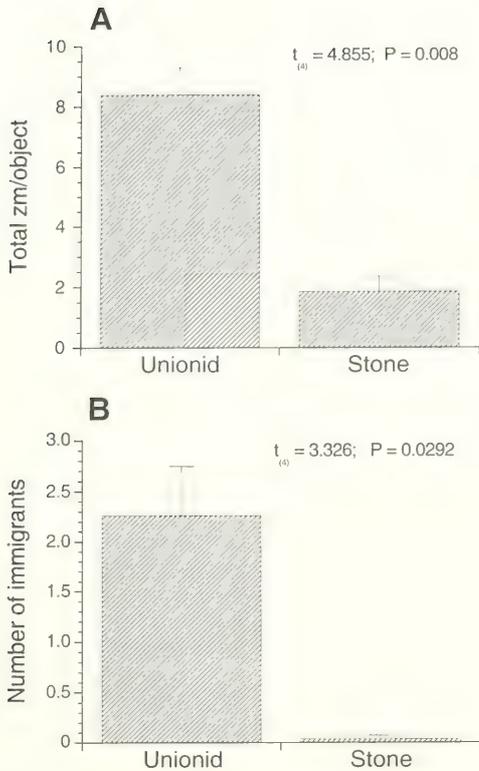


FIG. 6. (A) Total number of zebra mussels (mean \pm SE) on unionid and stone test objects at the end of the experiment at CLR (Cooley Lake Road; unstable substratum site). (B) Number of immigrant zebra mussels (mean \pm SE) that were present on test objects at the end of the experiment.

reasons. First, resident zebra mussels on unionids (those marked at the start) showed little tendency to emigrate, so that the mean number of residents on unionids at the end of the experiment was 6.1 compared to 6.5 at the start. In contrast, the number of resident zebra mussels on stones at the end of the experiment averaged 1.8, a mean decrease of 4.7 zebra mussels on each stone. Second, there was greater immigration of zebra mussels to unionids than there was to stones, so that at the end of the experiment, the unionids averaged 2.3 ± 0.48 immigrants per unionid compared to 0.04 ± 0.037 immigrants for stones (Fig. 6B). These values were significantly different: $t_{(4)} = 3.326, P = 0.029$.

It is not known if the decrease in resident zebra mussels was due to mortality or emigration. Evidence for the former came from

field observations that many of the stones were entirely buried, and these often had empty shells of resident zebra mussels still attached. These differences between unionids and stones in terms of survival and behavior of attached zebra mussels led to large percentage changes by the end of the experiment (Fig. 7). Average percent change in zebra mussels attached to unionids was both positive and significantly higher ($24.5 \pm 8.66\%$) than that for stones, the latter decreasing by $73.1 \pm 7.36\%$ ($t_{(4)} = 5.14; P = 0.007$).

DISCUSSION

Our data show that unionids in both lake and river conditions are not preferentially settled on or colonized by zebra mussels compared to other hard substrata. We found no evidence to suggest that unionids are more attractive to either migrating zebra mussels or to settling veligers than are alternative hard substrata nearby. This finding agrees with Toczyłowski & Hunter (1997), who examined a variety of substrata, including natural (live unionids, unionid shells, wood) and artificial surfaces (tiles, plastic mesh) and reached the same conclusion. The density of zebra mussels on unionids is approximately the same as it is on other natural hard substrata located in the same area, the outcome that would be predicted if there were no preference. These data were obtained for lake and river bottoms that are relatively firm and in which inanimate hard substrata do not become buried.

However, under different bottom conditions, we obtained different results. When the bottom is soft enough for hard substrata to become buried, or if the bottom sediment is unstable due to a strong current, as in a river, then any hard surfaces that are buried or silted-over become sub-optimal for zebra mussels. In these conditions, unionids maintain their position with respect to the sediment-water interface, and the zebra mussels attached to the posterior end are carried above the sediment. Zebra mussels on stones or other kinds of inanimate substrata become buried and either die or are forced to emigrate. Therefore, under conditions of unstable substrata, greater loads of zebra mussels will develop on unionids than on inanimate hard substrata due to differential survival of the immigrant zebra mussels.

This study provides one observation and

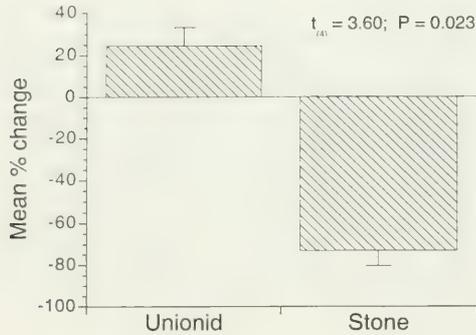


FIG. 7. Percent change (mean \pm SE) in the total number of zebra mussels (residents + immigrants) on unionids and on stones at CLR (unstable substratum site) over the course of the experiment.

two experiments that support our hypothesis. The observation is from measurements of natural zebra mussel density on unionids and on stones from a river site with an unstable bottom (DMO). Test stones and unionids from this site were located on patchy sand and cobble where sand movement alternately buried and exposed the cobble, rendering the stones less suitable for zebra mussels. The result was that unionids carried significantly higher zebra mussel loads per unit area of exposed surface than did stones from the same site. This result differs from that reported in an identical study in Loon Lake in 1995 (Toczyłowski & Hunter, 1997).

We believe the different outcomes of these two studies can be explained by different substratum stability. The lake work was done on a predominantly hard substratum (cobble), on which test stones had little tendency to sink and maintained their position relative to the sediment/water interface. Unionids, which were similarly positioned, had loads that were basically the same as those on the stones. At the river site, with its unstable bottom, zebra mussels located on stones were buried and died, whereas those on unionids survived by remaining above the sediment.

Our hypothesis was further tested by experiments conducted at both lake and river sites. Pre-marked test substrata placed on an unstable bottom in the Clinton River at CLR confirmed that zebra mussels previously attached to unionids were not only more likely to remain attached over the summer than their counterparts attached to stones, but were also more likely to be joined by immigrants.

On stones, not only were there negligible numbers of immigrants, but previously attached zebra mussels died or emigrated due to burial under bottom sediments.

A further test was provided by experiments on a soft bottom site in Loon Lake and a similar site in the Clinton River (DPNC). Under such conditions, we would predict that unionids should accumulate a higher zebra mussel load than stones, due to siltation of the stone surfaces that occurred during the course of the experiment. Our data supported the hypothesis in both in lake and river conditions; however, the results were more pronounced under lake conditions despite the similarity of substratum type. We believe this was due to the presence of low current at the river site. Current may have reduced silt accumulation on upper stone surfaces and prevented reduced O_2 levels from occurring at the sediment/water interface. These improved bottom conditions would reduce the negative effects to which zebra mussels on the river bottom were exposed, reducing mortality. These same subtle lake/river bottom differences would also explain our finding that emigration/mortality of previously marked resident zebra mussels from lake test surfaces was higher than that from river test surfaces.

More recently, studies by Ricciardi et al. (1995, 1996) have purported to demonstrate that unionids are preferred substrata, and consequently zebra mussels reach greater densities on unionids than on the bottom in general. A model presented in Ricciardi et al. (1995) in support of preference was tested using data from sites that were mostly soft substrata. Under these conditions, the preference by *Dreissena* for unionids is simply a choice between hard and soft substrata. This model has tested the obvious: that zebra mussels reach higher density on hard, compared to soft substrata. The control that was not done would have examined zebra mussel load at the same sites on stones, bricks, or other hard substrata of comparable size and surface texture. Although their model is useful in extrapolating from general zebra mussel density to zebra mussel loads on unionids, it does not provide any useful insight into the real issue of preference: do zebra mussels select unionids over other natural hard surfaces?

In a further examination of the impact of *Dreissena* on unionids, Ricciardi et al. (1996) offered more evidence in support of preference by showing that there was higher zebra

mussel density on unionids (in the mud bottom of a canal) than on the concrete walls of the canal. The problem with these data is that *Dreissena* density on the wall is based on the total wall area, whereas the density of *Dreissena* on unionids is based only on the area of unionid shell, not on total area of the bottom (unionids plus mud). Using the terminology of Bailey et al. (1995), what Ricciardi et al. (1996) have done is to express zebra mussel density on unionids in terms of number per unit unionid surface (BMI/RSA) and zebra mussel density on the concrete wall in terms of number per unit wall area (BMI/BA). It is not surprising that the former density is greater. Because unionid shells are discreet optimal surfaces for zebra mussel settling and survival and are dispersed in a habitat that is generally sub-optimal, the result is intense crowding. In contrast, the entire wall is suitable substratum.

We have shown the absence of preference where the substratum is relatively firm and an appearance of preference where the substratum is soft and/or unstable. In this latter case, it is likely that higher zebra mussel densities on unionids are simply the outcome of the ability of unionids to actively maintain their posterior shell surface in an exposed position on the bottom. Unionids are a relatively stable surface due to their responsiveness to changing bottom conditions. Therefore, the development of higher *Dreissena* densities on unionids is not an outcome of preference involving substratum choice using sensory perception and subsequent taxes or directed movement. Instead, our data indicate that these density differences have arisen from lower mortality and emigration rates on a responsive live surface compared to that on unresponsive, inanimate hard surfaces.

Although it is well known that unionids move both horizontally and vertically (extent of protrusion from the sediment), the conditions stimulating these responses and the adaptive value of specific movements are largely unclear and have received scant attention in most species (Balfour & Smock, 1995; Amyot & Downing, 1997). Vertical movement involving shifts from epibenthic to endobenthic position are known to occur in some species. The best studied species is *Elliptio complanata*, which showed seasonal movement resulting in most of the population becoming endobenthic in winter and epibenthic in summer (Amyot & Downing, 1997). In terms of the present study, should any of the species that were test

unionids become endobenthic in winter it could be a means of eliminating or reducing their load of zebra mussels. The authors have observed unionids in the field that are almost entirely buried, with only the siphons visible. Yet adjacent to the siphons are a few zebra mussels attached to the extreme posterior margins of the unionid shell, where they have avoided burial but remain at the sediment/water interface. Because they are epifaunal, zebra mussels are likely to be less tolerant of burial than unionids, hence might be partially or entirely eliminated over winter when much of the unionid community is endobenthic (unpublished observations). Although as yet we have no overwinter survival data for zebra mussels attached to unionids, we do have evidence from zebra mussel loads in fall and the following spring that indicate there is no significant reduction of loads over winter (Hunter et al., 1997). It is entirely possible that once a significant load of zebra mussels has accumulated, it may act as a kind of stop, limiting the ability of the unionid to bury itself in the substratum. If this occurs, it would prevent freshwater mussels that vertically migrate from becoming endobenthic.

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GANULA GADIRANA N. SP., A NEW HYGROMIIDAE FROM SOUTHERN SPAIN
(PULMONATA: HELICOIDEA)

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ABSTRACT

Ganula gadirana Muñoz, Almodóvar & Arrébola, is proposed as a new species from the southernmost corner in the Iberian Peninsula. This species, sometimes erroneously recorded as *Helix* (= *Ganula*) *lanuginosa* Boissy, 1835, is characterized by a globose conical-depressed shell, with ovate aperture, small umbilicus, and periostracum with long, persistent hairs; it has a right omatophore retractor between the penis and the vagina, a penial nerve arising from the right cerebral ganglion, ring-shaped glandular area on the distal penial wall, a fenestrate and elongate penial papilla, and a circumvaginal tuft of long, annulated digitiform glands. A dart-sac complex is on one side of the vagina and is formed by an outer dart-bearing stylophore and inner apically bilobed dartless stylophore; each open separately into a canaliculate deep groove that divides two large pleats, distally detached from the vaginal wall. Comparisons with *Ichnusotricha* and *Ganula* suggest that the new species may be closely related to *G. lanuginosa* (Boissy, 1835). The bilobate distal portion of the inner stylophore, the larger number of tufts in the digitiform glands, the fenestrate penial papilla, the penial glandular area, and the radular formula to distinguish *G. gadirana* from *G. lanuginosa*.

Key words: *Ganula gadirana*, Gastropoda, Stylommatophora, Hygromiidae, Spain.

INTRODUCTION

Many new generic taxa have been recently described by the Giusti-Manganelli's team for the western Mediterranean area, most of them being monotypical: *Helicotricha carusoi* from the Aeolian Islands (Giusti et al., 1992), *Cilliellopsis oglasae* from Montecristo Island (Giusti & Manganelli, 1990), *Schileykiella* for *Helix parlatoris* Bivona, 1839, and *Helix reinae* Pfeiffer, 1856, from Sicily (Manganelli et al., 1989), *Ichnusotricha berninii* and *Nienhuisiella antonellae* from Sardinia (Giusti & Manganelli, 1987), and *Tyrreniella josephi* from Sardinia/Capraia Islands (Giusti & Manganelli, 1989). The number of new morphological patterns is striking, and more new generic taxa can be expected.

Ganula lanuginosa, according to bibliographical records, seems to be distributed in four areas: eastern Balearic Islands (type locality of *Helix lanuginosa* Boissy, 1835) Sardinia (Giusti & Manganelli, 1987), southern Spain (Servain, 1880), and northwestern Africa (Terver, 1839; Bourguignat, 1864; Letourneux & Bourguignat, 1887). This species could be of great zoogeographical value if its presence in these areas is anatomically confirmed (Giusti & Manganelli, 1984), although

Balearic records could be the result of an introduction from northwestern Africa during the Middle Ages (Gasull, 1963).

Nevertheless, the anatomical confirmation has been carried out only for the Balearic Islands (Hesse, 1931; Gittenberger, 1968) and Sardinia (Giusti & Manganelli, 1987). Searching to confirm its presence in southern Spain, where it was repeatedly cited (Servain, 1880; Sacchi, 1956, 1957; Sacchi & Nos, 1958; Gasull, 1963), was unsuccessful. In nearly identical shells, we have found snails with genitalia similar to those in *Ganula lanuginosa*, but differing in the configuration of the dart-sac complex and in a larger number of tufts in the digitiform glands. These characters support introduction of a new species from southern Spain: *Ganula gadirana*.

MATERIALS AND METHODS

Living specimens were drowned in tap-water and preserved in 70% ethanol. Removed bodies were dissected and studied by optical stereomicroscopes (Zeiss and Nikon). Genital system and other structures were drawn using a camera lucida (Zeiss and Nikon). Nerve rings were removed from the

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connective tissue with a pointed watchmaker forceps prior to drawings.

Serial microscopical sections were coloured with Mallory's stain and photographed with a Zeiss Stemi SV6 photostereomicroscope. Radulae were removed from buccal bulbs by hot digestion in KOH and then washed in pure ethanol. Some radulae were mounted on metallic blocks with electron-conductive glue, coated with gold, and photographed in a JEOL 820 SEM, and others were stained by a modified hemalumpicroindigocarmine method and observed using a Zeiss stereomicroscope. Shells were photographed with the same SEM or with a Pentax P30N camera.

Shell parameters were measured in adult specimens using a Zeiss stereomicroscope with millimetric lens. Features of the central nervous system were described according to the character states proposed by Tillier (1989: 40–42).

The nomenclature for the genital system (that used by other authors in brackets) is the following: outer stylophore (= dart sac or lower stylophore), inner dartless stylophore (= accessory sac or upper stylophore), penial papilla (= verge), digitiform glands (= mucous glands).

SYSTEMATIC DESCRIPTION

Ganula gadirana Muñoz, Almodóvar & Arrébola, n. sp.

Helix lanuginosa, Servain, 1880: 51 (*non* Boissy) ["Environs d'Algesiras"].

Fruticicola lanuginosa, Sacchi, 1956: 17 (*non* Boissy) [only: "nelle regioni tra Malaga e Algesiras"].

Fruticicola lanuginosa, Sacchi, 1957: 19 (*non* Boissy) [only: "presso Malaga e presso Algesiras"].

Fruticola lanuginosa, Sacchi & Nos, 1958: 93 (*non* Boissy) [only: "region d'Algesiras"].

Shell (Figs. 1, 2). Shell medium sized, globose-depressed, conical-convex above, inflated below, uniform light brown translucent (Fig. 1A–B). Spire low conical, consisting of 5–5 1/2 convex, regularly increasing whorls separated by deep sutures. Apex small, protruding apex; protoconch with 1 1/4–1 1/2 whorls, striated, with hair scars (Fig. 2A). Last whorl large, one and one half times broader than the penultimate whorl, rounded at periphery, and variably descending near aperture. Umbilicus very small (0.6–0.8 mm), deep, partly covered by the reflected margin

of peristome. Aperture oblique, oval or nearly circular, without internal lip, but with a whitish band (reddish in external view). Peristome interrupted straight, smooth, not thickened, with very separated, non-convergent marginal edges; columellar edge gently curved, widened at origin, very reflected over umbilicus. External teleoconch surface with long (0.4 mm), erect, persistent hairs in transverse rows 0.4 mm far apart, with a density of 14–17 hairs/mm² (Fig. 2B); microsculpture of teleoconch formed by fine, small crests among hairs; hair scars curved.

Dimensions (*n* = 16): Shell diameter, 11.0–14.0 mm (holotype, 13.0 mm); shell height, 8.0–10.9 mm (holotype, 9.8 mm); aperture maximum diameter, 6.8–8.2 mm (holotype, 7.9 mm).

Foot (Fig. 2C). Sole with a tripartite appearance (also visible in preserved specimens): central zone light, lateral zones darker.

Lung roof (Fig. 3A) with an irregular pattern of small, blackish, irregular spots, which are larger over kidney borders.

Kidney (Fig. 3B) with numerous, long, raised internal pleats, more numerous on ureteric side; primary ureter broad, mostly situated on the kidney; secondary ureter, closed at first, but open 1 mm from the ureteric angle (in front of heart ventricle) to form a broad ureteric groove.

Heart oval, somewhat longer than half length of kidney. Slender primary pulmonary vein with inconspicuous secondary veins.

Mantle collar (Fig. 3C). Left lateral lobe, thin, small, with lobed upper border and straight lower border. Right lateral lobe long, thick, with an upper corner forming an anal lobe. Both left and right dorsal lobes with free marginal borders. Subpneumostomal lobe separating anal and pneumostomal orifices.

Central nervous system (Fig. 3D–F). Cerebral commissure shorter (CC3) and right cerebropedal connective somewhat longer (CPD2) than right cerebral ganglion width; both right and left cerebropedal connectives of similar length (CPR2), somewhat longer than cerebropedal connectives; both right and left pleural ganglia closer to pedal ganglia than cerebral ganglia (PLD1, PLG1); visceral ganglion in median plane of pedal ganglia (VG2); right parietal ganglion in contact with both visceral and right pleural ganglia (PAD2), apparently fused with the latter; left parietal ganglion only in contact with visceral ganglion (PAG3), both ganglia apparently fused (FG3).

Genital system (Figs. 4A–B, 5A–E, 6A–E).

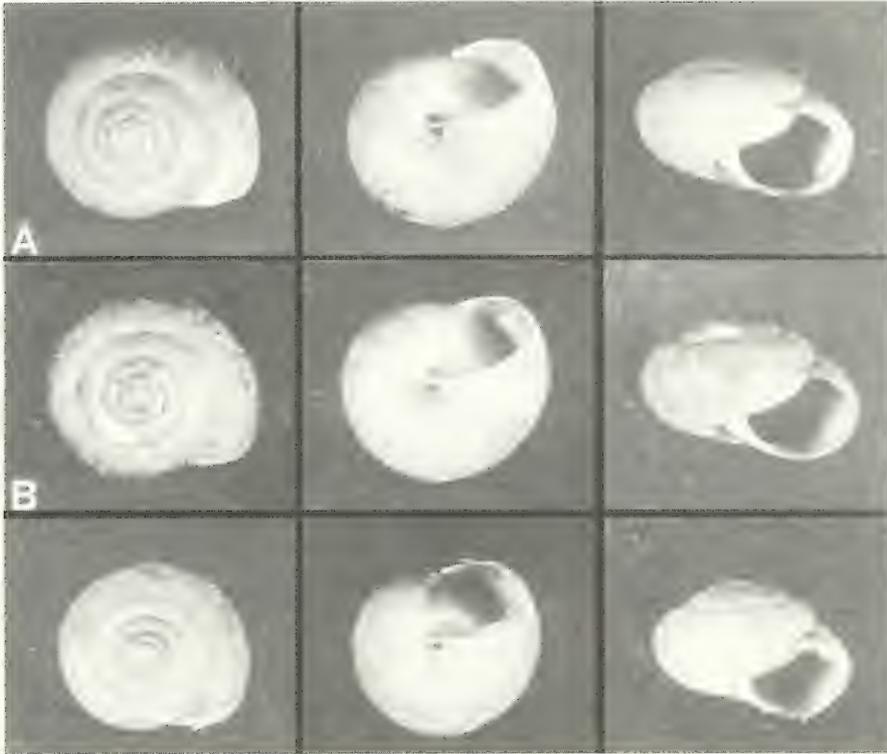


FIG. 1. Shells of *Ganula gadirana*, n. sp., from the type locality. A. Holotype; B. Two paratypes (upper collected on 30 October 1992, and lower on 14 May 1992).

Multilobate gonad and hermaphrodite duct without special features (not figured). Albumen gland relatively short. FPSC (Fig. 5A), with both seminal receptacle and fertilization pouch simple, sac-like, the former shorter, slender. Ovispermiduct (Fig. 4A), wide, circumvolute, with prostatic and uterine parts well differentiated. Vas deferens slender.

Penial complex (Figs. 4A–B, 5D) consisting of flagellum, epiphallus (i.e., from end of vas deferens to attachment point of penial retractor muscle), and penis (i.e., from point of attachment of penial retractor muscle to genital atrium); penial retractor muscle attached to penial complex proximal to point in which base of penial papilla is contained; a strong muscular band (Fig. 4B) extends from outer penial wall of proximal penis to wall of genital atrium, bending the penial complex; flagellum short, 1/3 of epiphallus length, conical in shape, with thick walls; epiphallus cylindrical, somewhat longer than penis, its inner wall pleated; penis with distal yellowish, ring-shaped, glandular area (Fig. 5C–D); penial

papilla long, cylindrical (Fig. 5B), with three fenestrations through which wide distal lacunae inside penial papilla walls communicate with penial cavity; a nearly isolated central canal traverses the penial papilla, its walls fixed by radial septa to walls of penial papilla.

Female part (Fig. 4A–B) consisting of a short free oviduct (half length of bursa copulatrix duct); bursa copulatrix large, shoe-shaped, with short pedunculus; vagina short, with digitiform glands and dart-sac complex, the latter located on one side. Digitiform glands (Fig. 5E) composed of many (14–20), long, unbranched or basally branched tubes disposed all around vagina; tubes with an annulate appearance, the inner secretory epithelium forming small ridges (Fig. 7A, G). Dart-sac complex (Figs. 6A–B, D–G, 7A–F) short, broad, composed of two stylophores; outer stylophore basally surrounded by groove of vaginal wall (Fig. 6A–B); inner stylophore broader, dartless, with thick walls and narrow inner cavity, extended into two apical

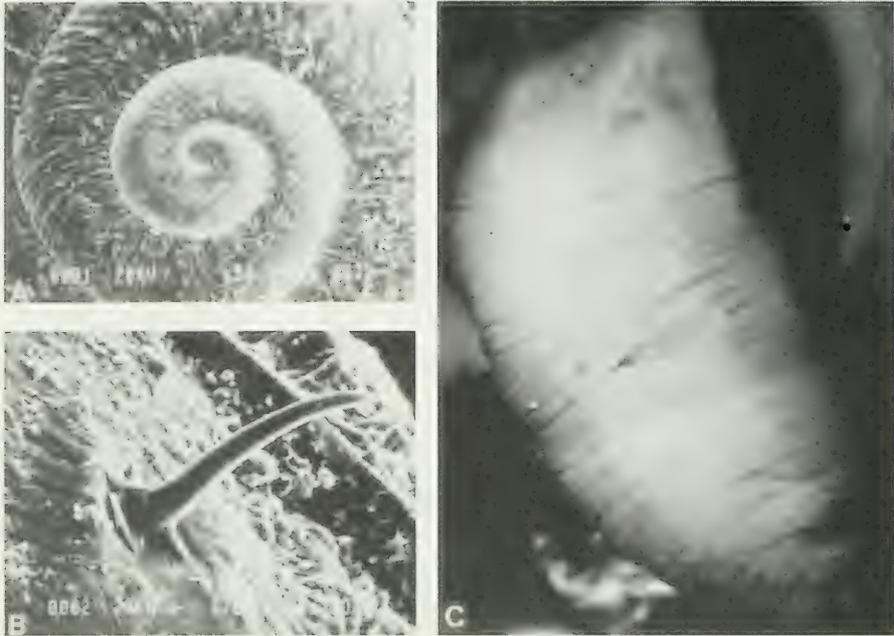


FIG. 2. Shell (SEM micrographs) and pedal sole of *Ganula gadirana*, n. sp. A. Protoconch of a paratype from Cortijo de Ahojiz; B. Microsculpture and one hair of teleoconch (same locality); C. Ventral view of pedal sole showing the tripartite sole (from type locality).

lobes (Fig. 6D, 7A). Inner stylophore opening into vagina through a wide orifice, far from outer stylophore aperture (Fig. 6B, 7C). Dart (Fig. 6C) smooth, straight, circular in section, but with flattened, keeled tip, elongate, extending out of outer stylophore. Inner vagina (Fig. 6B, F–G, 7C–F) with two thick, large pleats, which distally fuse to form a tongue-like structure, U-shaped in traverse section, its tip detached from vaginal walls; outer stylophore and inner stylophore open far apart inside groove of tongue-like structure.

Juvenile specimens (Fig. 6E–G) with dart-sac complex placed at half of vagina length, with outer stylophore shorter than bilobed inner stylophore and tongue-like structure present inside vagina.

Jaw (Fig. 3H) odontognathous, with 24 ribs, central larger.

Radula (Fig. 8) consisting of many rows of teeth each with a formula of $35-37 + C + 35-37$, with the lateral/marginal transition towards the 15–16th tooth. Central tooth with wide basal plate, pointed vertices and body with large mesocone and two small ectocones nearly fused to base of mesocone. Lateral teeth with wide basal plates and body with endocone, large, pointed mesocone and short

ectocone; both mesocone and ectocone progressively slender towards radular margin, mesocones with little lateral protuberance and pointed ectocones split into two points.

Other anatomical characters. Right ommatophore retractor running between penis and vagina. Penial nerve apparently arising from right cerebral ganglion.

Type Locality

Arroyo de la Cabañuela, Puerto de Bolonia, Tarifa (Cádiz, Spain, UTM: 30STE552990).

Type Material

Holotype (shell and dissected specimen) – 30 October 1992, A. Almodóvar, B. Muñoz and P. Refoyo leg. Museo Nacional de Ciencias Naturales, Madrid, Spain.

Paratypes:

– Type locality, 15 paratypes: 11 specimens (10 dissected), 30 October 1992, A. Almodóvar, B. Muñoz and P. Refoyo leg., 1 shell, 9 May 1994, A. Almodóvar leg.; 3

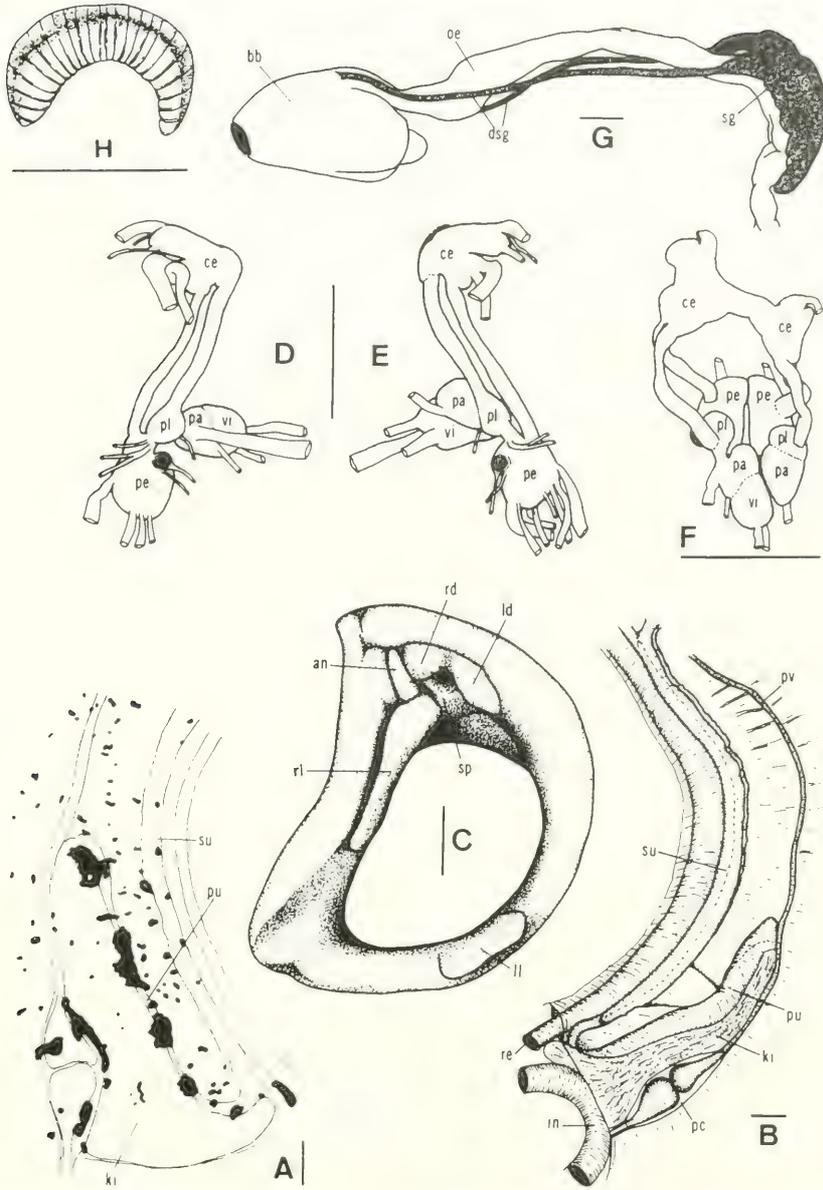


FIG. 3. Some body parts of *Ganula gadirana*, n. sp., from Los Barrios, 16 May 1993 (A–G) and the type locality (H). A. Pigmentary patches on the pulmonary cover (external view); B. Kidney, ureters and pericardium; C. Mantle collar; D–E. Left and right views of central nervous system; F. Posterodorsal view of central nervous system from another specimen; G. Position of salivary glands in respect to buccal bulb; H. Jaw. Abbreviations: an, anal lobe; bb, buccal bulb; ce, cerebral ganglion; dsg, ducts of salivary glands; in, intestine; ki, kidney; ld, left dorsal lobe; ll, left lateral; oe, oesophagus; pa, parietal ganglion; pc, pericardium; pe, pedal ganglion; pl, pleural ganglion; pu, primary ureter; pv, pulmonary vein; rd, right dorsal lobe; re, rectum; rl, right lateral lobe; sg, salivary glands; sp, supneumostomal lobe; su, secondary ureter; vi, visceral ganglion. Scale bar, 1mm.

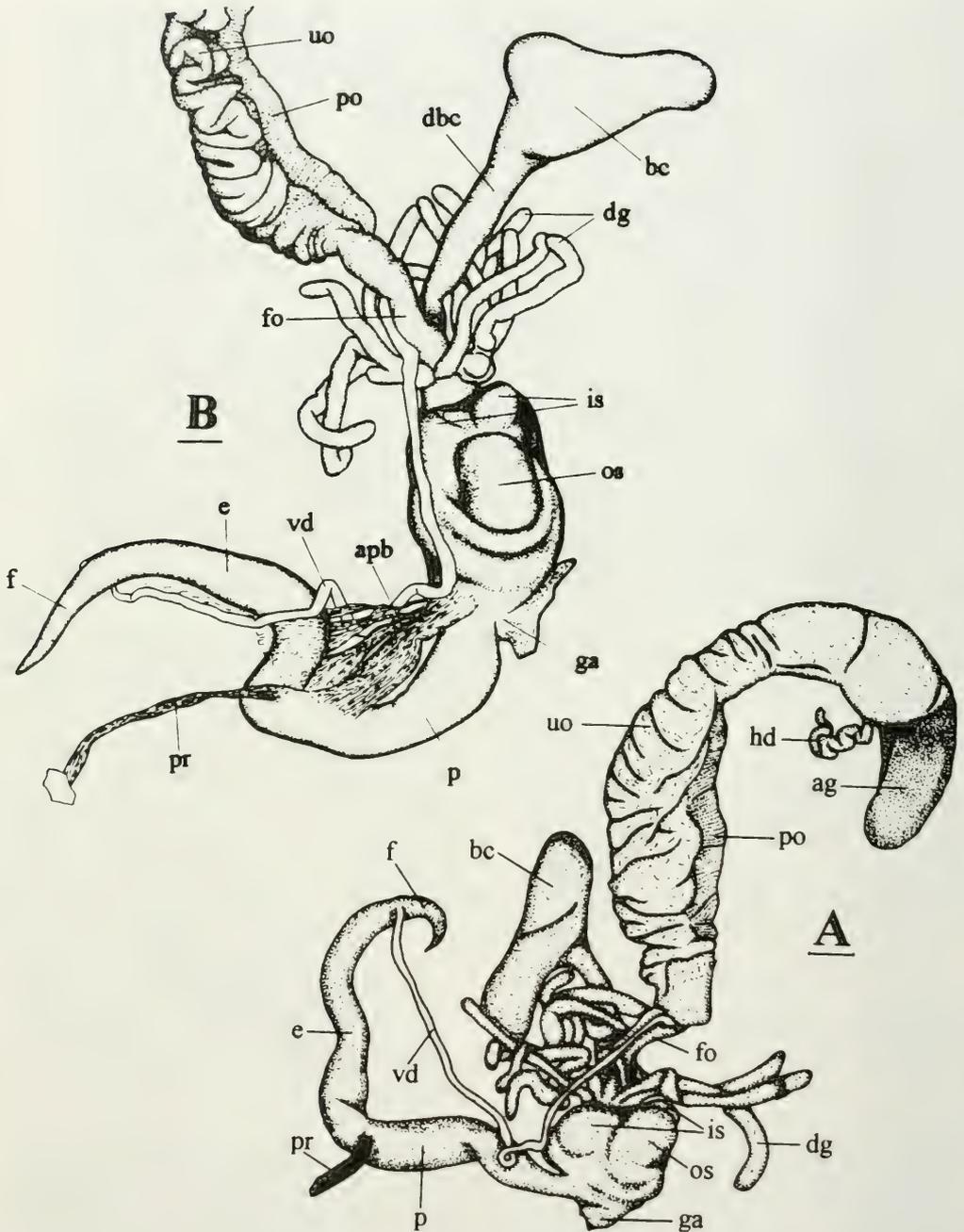


FIG. 4. Genital system of *Ganula gadirana*, n. sp. A. Specimen from type locality (gonad excluded); B. Specimen from Los Barrios (distal ducts), 3 November 1991. Abbreviations: ag, albumen gland; apb, atrio-penial muscular band; is, inner stylophore; bc, bursa copulatrix; dbc, duct of bursa copulatrix; dg, digitiform glands; os, outer stylophore; dsc, dart sac complex; e, epiphallus; f, flagellum; fo, free oviduct; ga, genital atrium; hd, hermaphrodite duct; p, penis; po, prostatic part of ovispermiduct; pr, penial retractor muscle; uo, uterine part of ovispermiduct; v, vagina; vd, vas deferens. Scale bar, 1 mm.

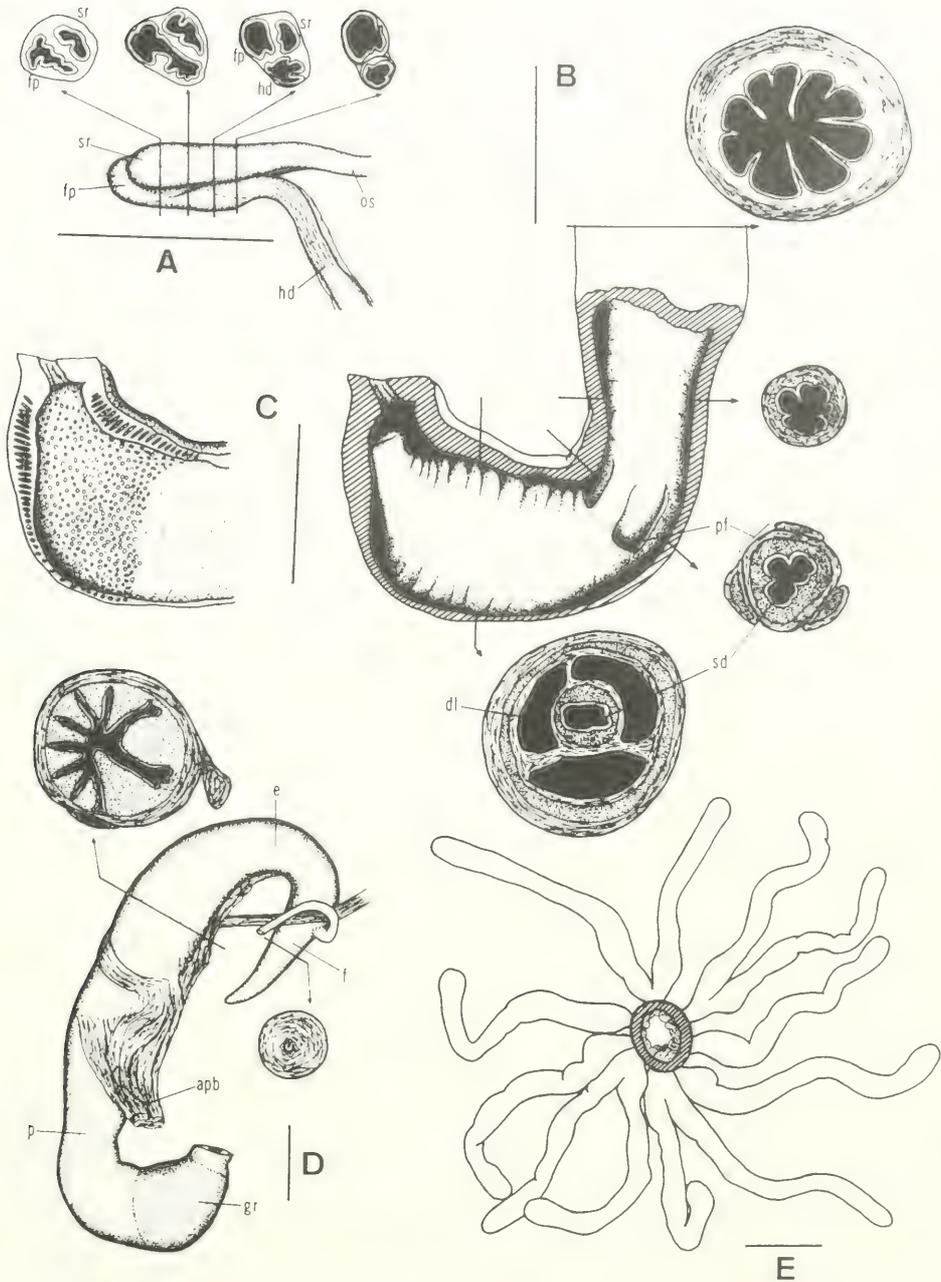


FIG. 5. Some genital parts of one specimen of *Ganula gadirana*, n. sp., from Los Barrios, 16 May 1993. A. Fertilization pouch and seminal receptacle complex (external view) and four transversal sections; B. Penial papilla within penis and transversal sections at different levels (1.33 times enlarged); C. Glandular area on distal part of penis (internal view); D. Penial complex (contracted) and transversal sections (1.5 times enlarged) of epiphallus and flagellum; E. Shape and disposition of digitiform glands on vagina. Abbreviations: apb, atrio-penial muscular band; dl, distal lacuna; e, epiphallus; f, flagellum; fp, fertilization pouch; gr, glandular ring; hd, hermaphrodite duct; os, ovispermiduct; p, penis; pf, papillar fenestration; sd, seminal duct; sr, seminal receptacle. Scale bar, 1 mm.

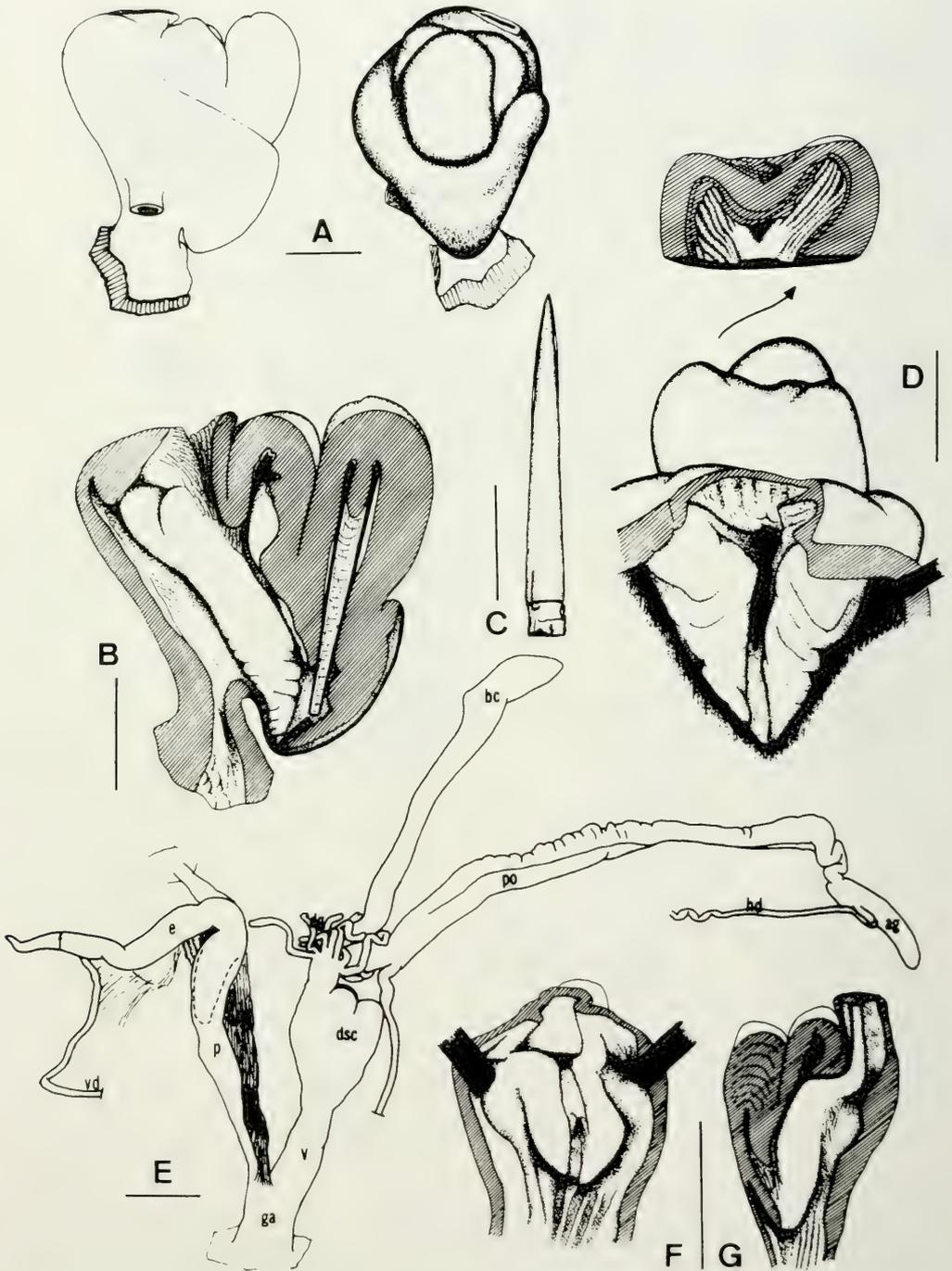


FIG. 6. Some genital parts of *Ganula gadirana*, n. sp. A. External views of dart-sac complex in a contracted specimen from Los Barrios, 16 May 1993; B. Same dart-sac complex in longitudinal section; C. Dart of a specimen from type locality; D. Inner vaginal view of dart-sac complex and cavity of bilobed inner stylophore from a specimen from Los Barrios, 5 February 1990; E. Genital system (gonad excluded) of a juvenile specimen from Los Barrios, 16 May 1993. Note position of dart-sac complex on vagina and bilobation of inner stylophore; F. Inner vaginal view; G. Longitudinal section of dart-sac complex of same juvenile specimen. Abbreviations as in Fig. 4. Scale bar, 1mm.

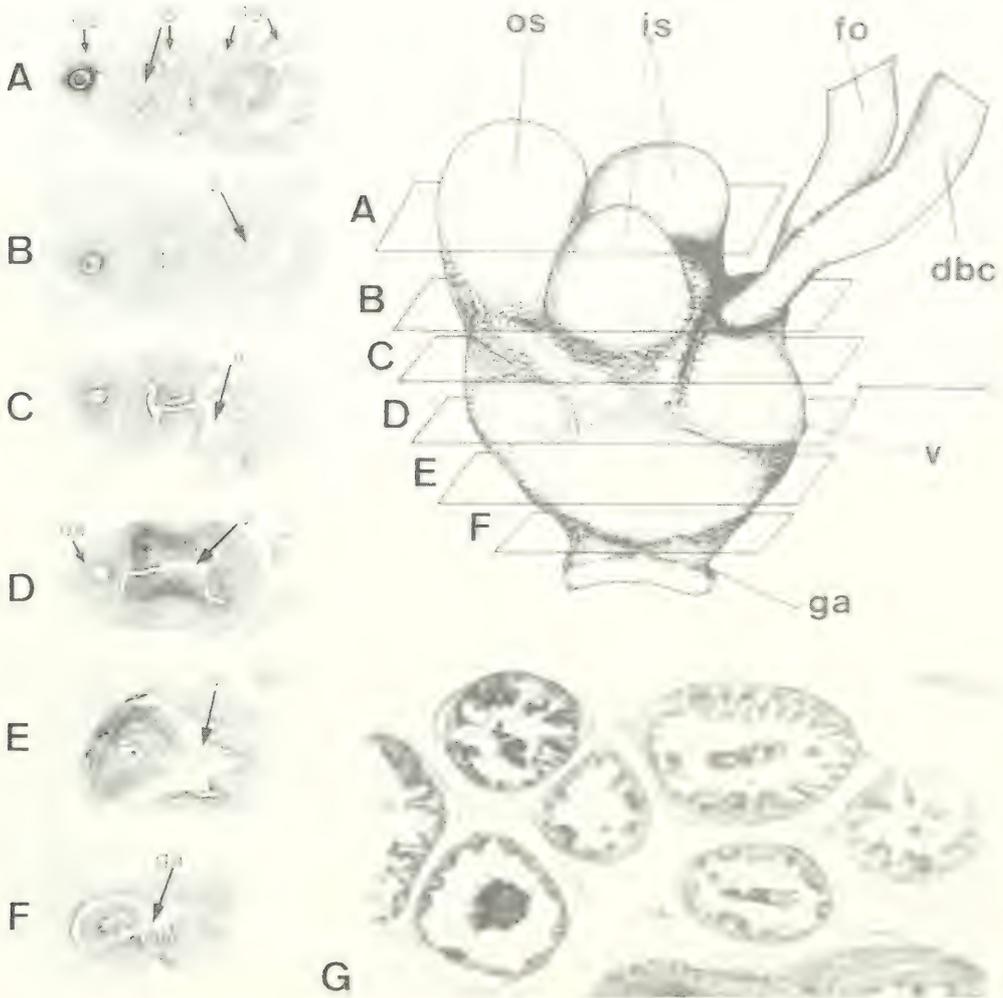


FIG. 7. Outline of dart-sac complex (digitiform glands not figured) and transversal sections at different levels. A. Bilobed cavity of inner stylophore and inner ridges of digitiform glands; B. Monoluminar cavity of inner stylophore and opening of a digitiform gland inside vagina; C. Opening of inner stylophore inside groove formed by vaginal pleats; D. Groove between inner and outer stylophores forming a functional channel; E. Opening of outer stylophore inside groove and fused pleats partially detached from vaginal wall; F. Opening of outer stylophore inside groove and fused pleats partially detached from vaginal wall; G. Enlarged transversal section of digitiform glands showing inner ridges and secreted mucus.

specimens (dissected), 14 May 1994, A. Almodóvar leg.

—Cortijo de Ahojiz, between km 90–91 of C-440 road to Los Barrios (Cádiz, UTM: 30STF7010), 16 paratypes: 3 specimens (one dissected), 5 February 1990; 6 specimens (2 dissected), 18 May 1991; 1 specimen (dissected), 24 March 1991; 6 specimens (3 dissected), 3 November 1991. All, J. Arrébola leg.

—‘Campo de Gibraltar’ sawmill, Los Barrios

(Cádiz, UTM: 30STF7507), 30 paratypes: 21 specimens (mostly juveniles, 5 dissected) and 9 shells, 16 May 1993, E. Unamuno and J. C. Ruiz leg.

—Algeciras, near a shelter (Cádiz, UTM: 30STE7394), 1 paratype (damaged shell), 16 May 1993, E. Unamuno and J. C. Ruiz leg.

—2 km towards Punta Paloma from N-340 road, Tarifa (Cádiz, UTM: 30STE5694), 10 paratypes (3 dissected), 6 December 1993, J. Arrébola leg.

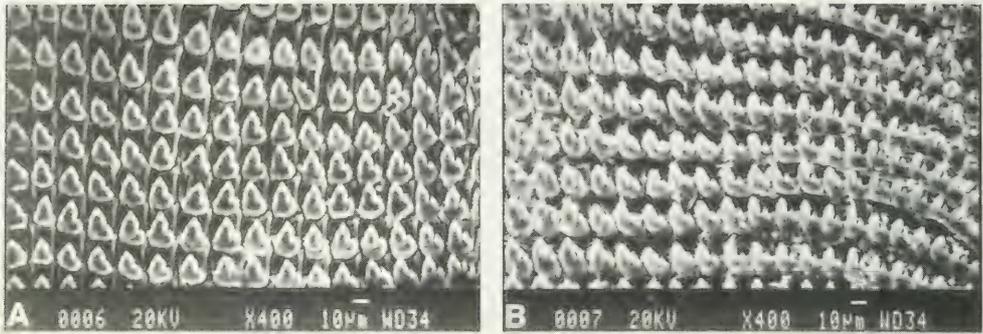


FIG. 8. SEM micrographs of the radula of *Ganula gadirana*, n. sp. A. Central tooth and lateral teeth; B. Last lateral and marginal teeth.

Derivatio Nominis

From the name of the Fenician colony Gadir, which gave origin to Cádiz, in the southernmost province of Spain, where the described snails were collected.

Ecology

Ganula gadirana has been found under stones, half buried in the ground or on herbaceous vegetation associated to mediterranean bushes (*Chamaerops humilis* and *Nerium oleander*) and close to periodically river flows. The associated malacological fauna is composed by *Rumina decollata* (Linnaeus, 1758), *Ferussacia follicula* (Gmelin, 1791), *Helix aspersa* Müller, 1774, *Cochlicella acuta* (Müller, 1774), *Cochlicella barbara* (Linnaeus, 1758), *Gasullliella simplicula* (Morelet, 1845), *Caracollina lenticula* (Michaud, 1831), *Xerotracha apicina* (Lamarck, 1822), *Cerneuella virgata* (Da Costa, 1778), *Theba pisana* (Müller, 1774), *Otala lactea* (Müller, 1774), *Candidula gigaxii* (Pfeiffer, 1848), *Oestophora barbula* (Rossmässler, 1838), and *Oestophora tarnieri* (Morelet, 1854). Specimens from Los Barrios, collected on 16 July 1993, were intensively parasitized by small nematodes, which were found in all growth stages inside the pulmonary cavity.

Geographical Distribution (Fig. 9)

All known localities are in the province of Cádiz in the southernmost corner of Spain, concentrated on a small region around the northern side of the Gibraltar Strait. Because the previously recorded presence of *Ganula lanuginosa* has not been confirmed for that region after many searches (see Introduction),

all records from southern Spain referred to this species have been assigned to *Ganula gadirana*.

There are older conchological records for *Helix lanuginosa* from northern Africa – from Morocco (Hidalgo, 1909: "Muluya") to northwestern Algeria (Terver, 1839; Bourguignat, 1864), but with one record from Tunisia (Letourneux & Bourguignat, 1887). Other two nominal species are considered to be related to *G. lanuginosa* – *Helix flava* Terver, 1839, and *Helix roseotincta* Forbes, 1838 (Clessin, 1881; Richardson, 1980). *Helix flava* from "Gourayah," near Bougie (Terver, 1839), and *H. roseotincta* was cited from northeastern Algeria (Bourguignat, 1864) to northern Tunisia (Letourneux & Bourguignat, 1887) (Bourguignat, 1864, considered the two species as synonymous). Taking into account that *Ganula gadirana* has been an overlooked species identified as *H. lanuginosa*, there is a possibility that some of the African records belong to *Ganula gadirana*. Further studies must be carried out to test this. Nevertheless, Giusti & Manganelli (1987) stated that *H. flava* is an Algerian species clearly distinct from *G. lanuginosa* and also from *G. gadirana* (Giusti, pers. com.).

DISCUSSION

The new species has similar features to *Ichnusotricha berninii* Giusti & Manganelli, 1987, and *Ganula lanuginosa* (Boissy, 1835). *Ichnusotricha berninii* has a similar shell shape, but the sutures are more superficial, the aperture is descendent, the umbilicus is nearly closed, and the periostracal hairs are very short. Anatomically, it differs from the new species, because *I. berninii* has a long,

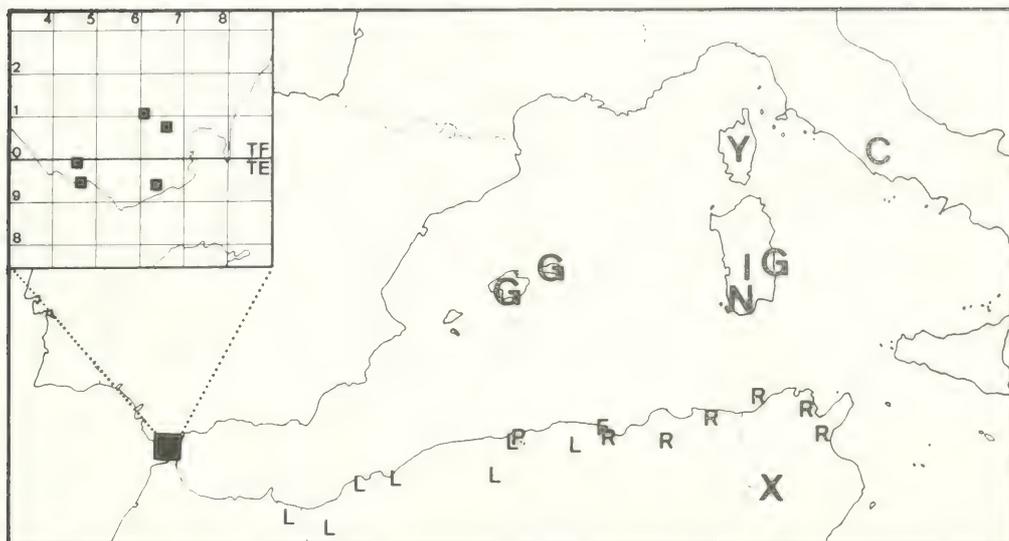


FIG. 9. Geographical distribution of *Ganula gadirana* n. sp. (square and upper left inset) and other endemic Hygromiinae from the western Mediterranean area (larger letters): C, *Ceruellopsis ghisottii* Manganelli & Giusti, 1987; G, *Ganula lanuginosa* (Boissy, 1835); I, *Ichnusotricha berninii* Giusti & Manganelli, 1987; N, *Nienhuisiella antonellae* Giusti & Manganelli, 1987; Y, *Cyrtoscheba corsica* (Shuttleworth, 1843); X, *Xeroplana lacosteana* (Bourguignat, 1864). Smaller letters from northern Africa show old conchological records for *Helix flava* Terver, 1839 (F), *Helix lanuginosa* Boissy, 1835 (L), and *Helix roseotincta* Forbes, 1838 (R).

non-fenestrated penial papilla; small, narrow dart-sac complex placed far apart; shorter digitiform glands; and long cylindrical vagina, with two long, slender pleats fused distally to form an apical tap or dart gun (Giusti & Manganelli, 1987).

Ganula lanuginosa has a nearly identical shell, although it is convex-depressed above, with more superficial sutures and less convex whorls, shorter (length, 0.3 mm; in shells from Mallorca), less spaced hairs (25–30/mm²), wider umbilicus (1/10 of shell diameter), and descending aperture. Anatomically, *G. lanuginosa* and *G. gadirana* are very different, because *G. lanuginosa* has four bifurcated digitiform glands, standard inner stylophore, and non-fenestrated penial papilla (Gittenberger, 1968; Giusti & Manganelli, 1987). *Ganula gadirana* has 14–20 digitiform glands, and the tubes have an annulate appearance, apically bilobed inner stylophore, fenestrate penial papilla, and penial glandular area.

On the other hand, in the pedal sole *G. gadirana* appears tripartite, and the left parietal ganglion is in contact with the visceral ganglion; these features are unknown in *G. lanuginosa*, but are present in a few species of the family Hygromiidae. The tripartite appearance of the pedal sole appears frequently

among helicoids (Schileyko, 1978), and is illustrated in a drawing of *Leucozonella rubens*, although we do not know other similar cases. The genera of Hygromiidae studied by Tillier (1989) have a more anterior position of the left parietal ganglion, being in contact with both visceral and left pleural ganglions.

Ganula lanuginosa has radular formula of 32 + C + 32 (Giusti & Manganelli, 1987), whereas *G. gadirana* has a formula of 35–37 + C + 35–37; both species have a similar central tooth, but *G. lanuginosa* has lateral teeth with an apex formed by a wide, robust, pointed mesocone and a short, sharp, robust ectocone, whereas the new species has lateral teeth with a large endocone, pointed mesocone, and short ectocone. Two points on the mesocone apex of the extreme marginal teeth appear occasionally in *G. lanuginosa*, whereas there is a little lateral protuberance in *G. gadirana*.

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COMPARATIVE KARYOLOGICAL STUDY OF CUPPED OYSTER SPECIES

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ABSTRACT

Chromosomes of six cupped oyster species were studied using karyometric analysis after conventional Giemsa staining, and silver staining. Karyotypes of *Crassostrea angulata* (nine metacentric and one submetacentric chromosome pairs), *C. sikamea* (nine metacentric and one submetacentric chromosome pairs), *C. virginica* (eight metacentric and two submetacentric chromosome pairs), *C. ariakensis* (eight metacentric and two submetacentric chromosome pairs), *C. gasar* (six metacentric and four submetacentric chromosome pairs), and *Saccostrea commercialis* (eight metacentric and two submetacentric chromosome pairs) are distinguishable by the number and position of the submetacentric chromosome pair and by the location of nucleolus organizer regions. Comparative karyological analysis of these six cupped oysters and of *C. gigas* was made using a Principal Component Analysis and a Hierarchical Clustering Analysis. *Crassostrea gasar* appears isolated from the other oyster species. Then, two clusters are separated. The first one groups *C. gigas*, *C. angulata* and *C. sikamea*, in which *C. gigas* is pleiomorphic. The second one consists of *C. ariakensis*, *C. virginica* and *S. commercialis*. Results are discussed with regards to oyster species relationships based on other genetic characters and to hybridization possibilities.

Key words: cupped oyster, chromosome, karyotype, NORs, cytotaxonomy, Bivalvia.

INTRODUCTION

Chromosomes of Ostreidae have been studied in 22 species (Nakamura, 1985; Vituri et al., 1985; Ieyama, 1990). Cupped oyster species of the genera *Crassostrea* and *Saccostrea* show a common diploid chromosome number of $2n = 20$, and their karyotypes include only metacentric and submetacentric chromosomes (Table 1). Interspecific differences consist of the occurrence and differing proportions of these morphological types, identified either by observation or after chromosome measurements. Karyotype differences may be seen within a species (e.g., *C. rhizophorae*; Table 1) which could be due either to intraspecific polymorphism or to the different techniques used.

Oyster species might have become differentiated through pericentric inversions of centric shifts. However, cytotaxonomic comparison needs to be based on karyological analysis carried out by the same technique and the same worker. For example, the concentration and time of incubation in the colchicine and in the hypotonic treatment, resulting in differential condensation or elonga-

tion of chromosomes (Sharma & Sharma, 1980), vary from one author to another. Karyometric analysis brings a more quantitative method to assess chromosome morphology, but still depends on the condensation or elongation of chromosomes.

Banding techniques have been found to be very useful for the identification of individual chromosomes and also of particular regions of chromosomes. Few studies have looked at banding patterns in the chromosomes of oysters (Rodriguez-Romero et al., 1979c; Insua & Thiriot-Quévrevex, 1991; Li & Havenhand, 1997). Fluorescence *in situ* hybridization has been tested in *Crassostrea gigas* (Clabby et al., 1996; Guo & Allen, 1997). Selective staining of the nucleolus organizer regions (NORs) has been shown to have potential as a cytotaxonomic tool (e.g., Amemiya & Gold, 1990). Patterns of specific NORs have been described in five species of oysters (Thiriot-Quévrevex & Insua, 1992; Insua & Thiriot-Quévrevex, 1993; Ladron de Guevara et al., 1994). Identification of structural chromosome features is useful in hybrid breeding programs and in oyster stock conservation.

In the present study, karyotypes and NORs

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TABLE 1. Chromosome data in cupped oysters.

Species	2n	karyotype	Origin	Authors
<i>Crassostrea</i>				
<i>C. amasa</i> (Iredale)	20		Australia	Menzel, 1968
<i>C. angulata</i> (Lamarck)	20		Portugal	Menzel, 1968
	20	10m*	France (Barfleur)	Thiriôt-Quiévreux, 1984
<i>C. belcheria</i> (Sowerby)	20	10 m-sm	Japan	Ieyama & Inaba, 1974
<i>C. corteziensis</i> (Hertlein)	20	7m-3sm*	Mexico	Rodríguez-Romero et al, 1979a
<i>C. gigas</i> (Thunberg)	20	8m-2sm	USA	Ahmed & Sparks, 1967
	20	10m*	France (Barfleur)	Thiriôt-Quiévreux, 1984
<i>C. glomerata</i> (Gould)	20		West Pakistan	Ahmed, 1973
<i>C. gryphoides</i> (Scholteim)	20		West Pakistan	Ahmed, 1973
<i>C. iredalei</i> (Faustino)	20		Philippines	Menzel, 1968
<i>C. rhizophorae</i> (Goulding)	20	5m-5sm*	Mexico	Rodríguez-Romero et al., 1979b
	20	8m-2sm*	Venezuela	Marquez, 1992
<i>C. "rivularis</i> (Gould)"	20		West Pakistan	Ahmed, 1973
Syn. <i>C. ariakensis</i> (Fujita)	20	10m-sm	Japan	Ieyama, 1975
<i>C. sikamea</i> (Amemiya)	20		West Pakistan	Ahmed, 1973
(Kumamoto variety of <i>C. gigas</i>)				
<i>C. virginica</i> (Gmelin)	20	6m-4sm*	East coast USA	Longwell et al., 1967
	20	6m-4sm*	Mexico	Rodríguez-Romero et al., 1978
	20	6m-4sm*	Venezuela	Marquez, 1992
<i>Saccostrea</i>				
<i>S. commercialis</i> (Iredale & Roughley)	20		Australia	Menzel, 1968
<i>S. cucullata</i> (Born)	20	10m*	India	Goswami, 1992
<i>S. echinata</i> (Quoy & Gaimard)	20	10m-sm	Japan	Ieyama & Inaba, 1974
<i>S. mordax</i> (Gould)	20	10m-sm	Japan	Ieyama & Inaba, 1974

2n: diploid chromosome number

*: after chromosome measurements

m: metacentric; sm: submetacentric

were studied in six species of cupped oysters: *Crassostrea angulata*, *C. sikamea*, *C. virginica*, *C. ariakensis*, *C. gasar*, and *Saccostrea commercialis*. These species originating from different areas were imported and reared in common quarantine facilities. Comparative karyological analysis was made with reference to *C. gigas* (Thiriôt-Quiévreux, 1984, and unpublished data, 1997).

MATERIALS AND METHODS

Species Studied

Five cupped oyster species of the genus *Crassostrea* and one of the genus *Saccostrea* were studied, none native to Europe. *Crassostrea gigas* and *C. angulata* have been introduced into the natural environment for decades (Grizel & Héral, 1991) or centuries (Boudry et al., 1998) respectively. The other species were recently imported into France as part of a genetic resources research program. They have been strictly confined to the quar-

antine facilities of the IFREMER hatchery in La Tremblade, Charente-Maritime, France, according to international recommendations. All the oysters studied were reared in the same environmental conditions for at least three months before sampling. The *C. angulata* oysters studied originate from the Rio Sado estuary, Setubal, Portugal. The taxonomic status of these oysters was assessed using mitochondrial DNA markers as described in Boudry et al. (1998). *Crassostrea sikamea* were imported from Bodega Marine Laboratory, University of California, USA. Their taxonomic status was confirmed using mitochondrial DNA markers as described in Banks et al. (1993). *Crassostrea virginica* were imported from a wild stock located in Shippagan, New Brunswick, Canada. *Crassostrea ariakensis* ("*C. rivularis*," *auctt.*) were imported from the Shellfish Research Laboratory, Rutgers State University, New Jersey, USA. This species was introduced from Japan into the Northwest waters of the USA, and its aquaculture potential has been recently reviewed by Langdon & Robinson

(1996). Mangrove oysters, *C. gasar*, were imported from a wild stock located in Kafountine, Casamance, Senegal. *Saccostrea commercialis* were collected from the wild at Port Stephens, New South Wales, Australia.

Because of the low number of animals available, only two animals from each species (except three of *C. sikamea*) were used for this study.

Chromosome Preparations

Oysters were incubated for 7 h with 0.005% colchicine in sea water. The gills were then dissected out and treated for 30 min in 0.9% sodium citrate in distilled water. The material was then fixed in a freshly prepared solution of absolute ethanol and acetic acid (3:1), with three changes of 20 min duration each. Slide preparation was made using an air-drying technique (Thiriot-Quévieux & Ayraud, 1982). For conventional karyotypes, slides were stained directly with Giemsa (4%, pH 6.8) for 10 min. Photographs of suitable mitotic metaphases were taken with a Zeiss III photomicroscope, and after karyotyping, chromosome measurements of ten metaphases in each species were made with a digitizer table (Summa Sketch II) interfaced with a Macintosh. Data analysis was performed with an Excel macro program. Terminology relating to centromere position follows that of Levan et al. (1964). NORs were silver-stained directly on unstained slides using the technique of Howell & Black (1980), modified by Gold & Ellison (1982).

Statistical Analysis

In order to evaluate the relationships between the six species studied here and *C. gigas*, a principal component analysis (PCA) was carried out. The data set is a matrix of 70 objects, that is, ten metaphases in seven species described by the centromeric index values of ten chromosome pairs. Means of centromeric index values for each species were considered as supplementary objects and were projected in the PCA space. The position (i.e., component score) of the ten metaphases around this mean point gives information of the scattering of each species. Their correlations give a criterion of their explanation by the PCA axes considered. As a second step, a hierarchical clustering analysis (HCA) was performed between the species described by their component scores on the first four axes of the PCA, using the Ward ag-

glomeration method (Ward, 1963). This clustering offers the possibility of representing the distances between species by a dendrogram. PCA and HCA were computed with the SPAD software (CESIA) (Lebart et al., 1995).

RESULTS

The results obtained for each species are summarised in Table 2.

Crassostrea angulata

The karyotype (Fig. 1A, Table 3) consists of nine metacentric and one submetacentric (no. 8) chromosome pairs. Ag-NORs were found terminally on the metacentric pair 10. The two homologous chromosomes showed heteromorphism involving apparent NOR activity. The most frequent case (69%) was one silver-stained NOR chromosome (Fig. 2A).

Crassostrea sikamea

The karyotype (Fig. 1B, Table 3) shows nine metacentric and one submetacentric (no. 6) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs nos. 9 and 10 (Fig. 2B). A variable number of one to three Ag-NORs was observed. 54% of the silver stained metaphases only showed NORs on pair 10. The most frequent case (61%) was one silver-stained NOR chromosome in pair 10.

Crassostrea virginica

The karyotype (Fig. 1C, Table 3) has eight metacentric and two submetacentric (nos. 4 and 8) chromosome pairs. Ag-NORs were found terminally on the short arms of metacentric pairs nos. 1 and 5 (Fig. 2C). A variable number of one to three Ag-NORs was observed. The most frequent case (52%) was one silver-stained NOR chromosome in pair 1 and in pair 5.

Crassostrea ariakensis

The karyotype (Fig. 1D, Table 3) consists of eight metacentric and two submetacentric (nos. 4 and 8) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs 9 and 10. A variable number of one to three Ag-NORs was observed (Fig. 2D). 68% of the silver stained metaphases showed Ag-NORs only on pair 10.

Crassostrea gasar

The karyotype (Fig. 1E, Table 3) includes six metacentric and four submetacentric (nos.

TABLE 2. Summary of karyological data of the six cupped oysters studied.

Species	No. metaphases studied		No. karyotypes studied		2n	Chromosome type (no. chromo. pairs)		No. of (haploid) NOR-chromosomes
	Giemsa	NOR	Giemsa	NOR		m	sm	
<i>C. angulata</i>	42	31	13	7	20	9	1	1 (pair 10)
<i>C. sikamea</i>	32	62	15	9	20	9	1	2 (pairs 9 and 10)
<i>C. virginica</i>	29	57	10	8	20	8	2	2 (pairs 1 and 5)
<i>C. ariakensis</i>	30	46	17	12	20	8	2	2 (pairs 9 and 10)
<i>C. gasar</i>	33	55	13	8	20	6	4	1 (pair 2)
<i>S. commercialis</i>	34	35	13	7	20	8	2	2 (pairs 9 and 10)

*after chromosome measurements of 10 metaphases
m: metacentric; sm: submetacentric

2, 8, 9, and 10) chromosome pairs. Ag-NORs were found terminally on the short arms of two homologous chromosomes of the metacentric pair 2 (Fig. 2E). Heteromorphism involving NOR-size occurred in 49% of the metaphases examined.

Saccostrea commercialis

The karyotype (Fig. 1F, Table 3) shows eight metacentric and two submetacentric (nos. 4 and 7) chromosome pairs. Ag-NORs were found terminally on the metacentric pairs 9 and 10. A variable number of one to three NORs were observed. 77% of the silver stained metaphases showed Ag-NORs only on pair 10 (Fig. 2F).

Comparative Karyological Analysis

Figure 3 shows ideograms constructed from relative length and centromeric index values (Table 3) of the six oyster species studied here and of *Crassostrea gigas*. Chromosome measurements of this later species were taken from ten metaphases of animals collected at La Tremblade in 1997. Mean values of relative length and centromeric index are similar to those found in *C. gigas* from Barleur (Thiriot-Quévroux, 1984). *Crassostrea gasar* is distinguishable from the other species first, due to the occurrence of four submetacentric chromosome pairs. *Crassostrea angulata* and *C. sikamea* showed only one submetacentric chromosome pair, whereas *C. virginica*, *C. ariakensis* and *S. commercialis* have two submetacentric chromosome pairs. *Crassostrea angulata* and *C. sikamea* may be differentiated by the different positions of the submetacentric chromosome pair and by the Ag-NORs which appear on pair 10 and on pairs 9 and 10 respectively. *Crassostrea virginica* and *C. ariak-*

ensis share a similar karyotype, but Ag-NORs are observed in different locations (pairs 1 and 5, and pairs 9 and 10, respectively). *Saccostrea commercialis* is close to *C. ariakensis*. Their karyotypes differ by the position of the second submetacentric pair and by the frequencies of Ag-NORs observed on pair 10. *Crassostrea gigas* has the most symmetrical karyotype, with only metacentric chromosome pairs.

Principal component analysis of the data set of 70 objects (ten metaphases for seven species described by centromeric index values of ten chromosome pairs) gives percentages of variance for the first five axes of 31.74, 20.06, 12.61, 11.17 and 7.77 respectively. The variance decreases progressively from 5th axis. We have thus only considered the information provided by the first four axes as relevant. The 1/2 plan (Fig. 4) explains 51.80% of the variance. It shows the separated position of *C. gasar* (correlation with 1/2 plan of 0.98) without continuity with the other species. The six other species overlap along a continuum. *Crassostrea gigas* (correlation of 0.87) is the most distant from this continuum. Then, *C. ariakensis* and *C. virginica* are very close and overlap a part of *S. commercialis* (correlations of 0.57, 0.43 and 0.52, respectively). *Crassostrea sikamea* (correlation of 0.38) shows a larger scattering. *Crassostrea angulata* is unexplained by this plan, as shown by its correlation of 0.01. The 3/4 plan explained less of the total variance: 23.78%. There is a trend of separation between *C. virginica*, *C. ariakensis* and *S. commercialis* (correlations of 0.40, 0.17 and 0.55 respectively). *Crassostrea angulata* and *C. sikamea* remain together (correlations of 0.48 and 0.55 respectively). Figure 5 shows the dendrogram of a Hierarchical Clustering Analysis made using the information from the



FIG. 1. Giemsa-stained karyotypes of six cupped oysters. A: *Crassostrea angulata*, B: *Crassostrea sikamea*. C: *Crassostrea virginica*, D: *Crassostrea ariakensis*, E: *Crassostrea gasar*, F: *Saccostrea commercialis*. Arrows show submetacentric chromosome pairs. Scale bar = 5 μ m.

TABLE 3. Chromosome measurements and classification in ten cells of six cupped oyster species.

Chromosome pair No.	Relative length		Arm ratio		Centromeric index		Chromosome Type
	Mean	SD	Mean	SD	Mean	SD	
<i>C. angulata</i>							
1	12.81	0.91	0.79	0.08	43.79	2.50	m
2	11.34	0.41	0.84	0.09	45.22	2.45	m
3	10.75	0.41	0.83	0.09	45.02	2.65	m
4	10.33	0.63	0.64	0.09	38.46	2.89	m
5	10.12	0.74	0.82	0.06	44.79	1.86	m
6	9.82	0.60	0.62	0.07	38.01	2.56	m
7	9.53	0.82	0.88	0.07	46.47	1.94	m
8	9.25	0.68	0.59	0.07	36.84	3.01	sm
9	8.98	0.66	0.68	0.12	40.07	3.83	m
10	7.08	0.67	0.75	0.10	42.36	3.44	m
<i>C. sikamea</i>							
1	12.40	1.17	0.85	0.17	45.24	4.35	m
2	11.28	0.59	0.77	0.10	43.08	2.99	m
3	11.20	0.64	0.87	0.12	46.03	3.38	m
4	10.39	0.93	0.86	0.12	45.92	3.14	m
5	10.38	0.78	0.85	0.13	45.41	3.89	m
6	9.87	0.89	0.53	0.13	34.20	5.27	sm
7	9.50	0.76	0.79	0.13	43.72	4.36	m
8	9.27	1.19	0.79	0.08	43.52	2.48	m
9	8.61	0.89	0.82	0.20	44.28	6.39	m
10	7.10	1.05	0.95	0.18	47.75	4.98	m
<i>C. virginica</i>							
1	12.71	0.67	0.88	0.08	46.46	2.51	m
2	11.50	0.56	0.87	0.11	46.27	3.03	m
3	10.89	0.90	0.79	0.11	43.61	3.62	m
4	10.57	0.71	0.46	0.08	30.97	3.49	sm
5	10.33	0.47	0.82	0.08	44.63	2.34	m
6	9.62	0.61	0.74	0.09	42.18	3.19	m
7	9.38	0.92	0.79	0.16	43.52	5.43	m
8	9.16	0.69	0.43	0.10	29.34	4.57	sm
9	8.64	0.51	0.73	0.11	41.81	3.78	m
10	7.19	0.54	0.86	0.12	45.63	3.49	m
<i>C. ariakensis</i>							
1	12.10	0.63	0.82	0.07	44.65	2.09	m
2	11.34	0.43	0.77	0.10	42.98	3.47	m
3	10.67	0.46	0.78	0.07	43.71	2.10	m
4	10.54	0.59	0.51	0.05	33.77	2.32	sm
5	10.21	0.79	0.81	0.09	44.44	2.55	m
6	9.91	0.39	0.81	0.12	44.30	3.78	m
7	9.64	0.57	0.74	0.07	42.12	2.03	m
8	9.44	0.78	0.53	0.08	34.25	3.08	sm
9	9.09	0.90	0.79	0.17	43.41	5.00	m
10	7.07	0.43	0.76	0.12	42.64	3.86	m
<i>C. gasar</i>							
1	11.36	0.63	0.79	0.10	43.64	3.11	m
2	11.19	0.74	0.38	0.05	27.52	2.34	sm
3	11.04	0.41	0.85	0.08	45.62	2.47	m
4	10.62	0.76	0.62	0.07	37.80	2.31	m-sm
5	10.54	0.51	0.90	0.09	46.95	2.45	m
6	9.97	0.43	0.86	0.08	45.77	2.33	m
7	9.64	0.54	0.84	0.12	45.21	3.54	m
8	9.54	0.64	0.45	0.07	30.78	3.43	sm
9	8.82	0.60	0.41	0.06	28.65	3.01	sm
10	7.28	0.56	0.39	0.08	27.89	3.78	sm

TABLE 3. (Continued)

Chromosome pair No.	Relative length		Arm ratio		Centromeric index		Chromosome Type
	Mean	SD	Mean	SD	Mean	SD	
<i>S. commercialis</i>							
1	13.41	1.07	0.81	0.08	44.41	2.45	m
2	12.39	1.04	0.78	0.06	43.58	1.77	m
3	10.84	0.56	0.81	0.09	44.50	2.68	m
4	10.36	0.70	0.44	0.05	30.48	2.29	sm
5	9.89	0.74	0.80	0.08	44.07	2.58	m
6	9.82	0.66	0.81	0.11	44.32	3.48	m
7	9.13	0.98	0.48	0.07	32.24	2.98	sm
8	9.11	0.63	0.78	0.11	43.42	3.56	m
9	8.44	0.59	0.81	0.10	44.34	3.01	m
10	6.60	0.63	0.79	0.12	43.19	3.76	m

m: metacentric; sm: submetacentric

first four axes of the PCA. *Crassostrea gasar* appears clearly separated from the other species. Then, two clusters are differentiated, one with the grouping of *C. virginica*, *C. ariakensis* and *S. commercialis*, and the other with two close species *C. angulata*, *C. sikamea* and *C. gigas* at a higher distance.

DISCUSSION

Our chromosome study of these six cupped oyster species confirms the diploid chromosome number of $2n = 20$ found up to now in all cupped oysters examined (Table 1).

The karyotype of *C. angulata* differs from that described in the animals reared at Barfleur (Thiriot-Quévieux, 1984). This difference could be due to the origin of animals. Samples in this study came from the Bay of Setubal, Portugal, and are considered as pure *C. angulata* (Boudry et al., 1998). The origin of samples in the Barfleur study is unknown. The karyotype of *C. virginica*, showing two submetacentric chromosome pairs, differs from those with four submetacentric chromosome pairs described by Longwell et al. (1967), Rodriguez-Romero et al. (1978), and Marquez (1992). However, the position of these submetacentric chromosome pairs is different between these authors. Genetic discontinuity has been observed in this American oyster along the Atlantic coast and the Gulf of Mexico (Buroker, 1983; Reeb & Avise, 1990; Hare & Avise, 1996). The origin of our animals is close to those studied by Longwell et al. (1967). Therefore, the karyological variation observed could be due either to the effect of acclimation or to differences in the technique (e.g., different concentrations of colchicine:

0.02% in the Longwell et al. 1967 study and 0.005% in this study). Karyotypes made by the same scientist, with the same techniques carried out within a short period of time give a more valid comparison than karyotypes made by different authors.

Karyotypes of *C. sikamea*, *C. ariakensis*, *C. gasar* and *S. commercialis* are first described here.

Our observations on Ag-NORs are original in the six species studied. In *C. virginica*, Longwell & Stiles (1996) suggested that NOR sites could be located on the secondary constriction observed on the longest metacentric chromosome pair. Our results confirm the location of Ag-NORs on this pair 1, but another Ag-NOR was observed on pair 5. Heteromorphism involving apparent NOR activity and NOR-size is a common phenomenon in bivalves (Thiriot-Quévieux & Insua, 1992; Insua et al., 1994; Martinez-Exposito et al., 1994). However, the number of Ag-NORs, their chromosomal location and their position within karyotypes are considered as species-specific characters (Sumner, 1990). In this study, the majority of species showed Ag-NORs on pair 9 or pairs 9 and 10, in a frequency that varies according to the species considered. The position of NORs was different in *C. virginica* and *C. gasar*. Ag-NORs allowed the separation of *C. angulata* and *C. sikamea*, and of *C. virginica* and *C. ariakensis* which have similar karyotypes.

Comparative karyological analysis (Figs. 3–5) highlights the isolation of *C. gasar*. Then two clusters are separated. The first cluster consists of *C. gigas*, *C. angulata* and *C. sikamea*, in which *C. gigas*, with the most symmetrical karyotype, could be considered as plesiomorphic. *Crassostrea gigas* and *C. an-*



FIG. 2. Silver-stained karyotypes of six cupped oysters. A: *Crassostrea angulata*, B: *Crassostrea sikamea*, C: *Crassostrea virginica*, D: *Crassostrea ariakensis*, E: *Crassostrea gasar*, F: *Saccostrea commercialis*. Arrows show Ag-NORs. Scale bar = 5 μ m.

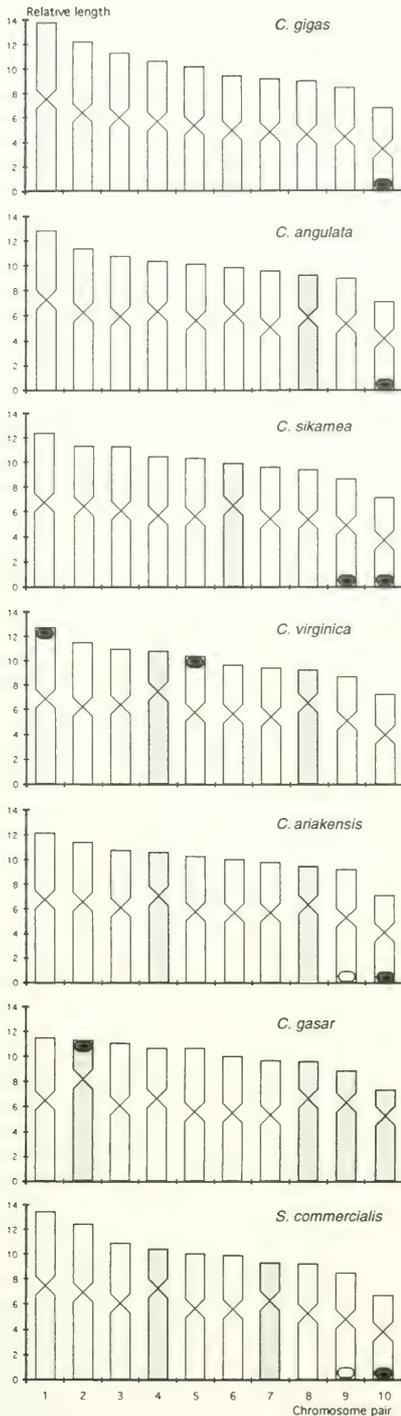


FIG. 3. Ideograms of seven cupped oysters constructed from relative length and centromeric index values. White chromosome: metacentric, grey chromosome: submetacentric. Circles indicate Ag-NORs, dark circles the most frequent case.

gulata are often considered as a same species (Menzel, 1974), as are *C. gigas* and *C. sikamea*, of which the latter has sometimes been considered as the "Kumamoto variety" (Ahmed, 1973). Recent molecular genetic studies have displayed differences between *C. gigas* and *C. sikamea* (Banks et al., 1994), and between *C. gigas* and *C. angulata* (Boudry et al., 1998; Ó Foighil et al., 1998). Our karyological study confirms these genetic differences. The second cluster put together *C. ariakensis*, *C. virginica* and *S. commercialis*. Molecular phylogenetics of cupped oysters (Littlewood, 1994) distinguished two lineages: (1) *C. gigas*, *C. belcheri*, "*C. rivularis*" (= *C. ariakensis*) and (2) *C. virginica*, *C. rhizophorae* and *S. commercialis*. Ó Foighil et al. (1995) using mitochondrial 16S ribosomal gene sequences confirm a genetic divergence between *C. virginica* and two Asian congeners *C. gigas* and *C. ariakensis*. Ladron de Guevara et al. (1996) suggested that *C. virginica* showed the most primitive karyological features when compared with *C. rhizophorae* and *C. corteziensis*. Our study is in agreement with the relationship between *C. virginica* and *S. commercialis*, but does not agree on the position of *C. ariakensis*. Multidisciplinary approaches would help in understanding evolutionary relationships of oyster taxa.

Interspecific hybridizations have been produced in cupped oyster species (Gaffney & Allen, 1993, provide a review). *Crassostrea gigas* is known to hybridize with *C. angulata*, *C. sikamea* and, rather less successfully, with *C. ariakensis*. These observations are in agreement with our study. Looking at karyological features, *C. ariakensis* and *S. commercialis* would be good candidates for hybridization, although this cross has apparently never been tried. Ag-NORs observed in *C. virginica* isolate this species from the others. This could explain inviability of hybrids of *C. gigas* and *C. ariakensis* with *C. virginica* (Allen & Gaffney, 1993). In the future, it will be of great interest to study the mitotic and meiotic chromosomes of interspecific hybrids such as *C. gigas* × *C. sikamea* or *C. gigas* × *C. ariakensis* and their backcross offspring.

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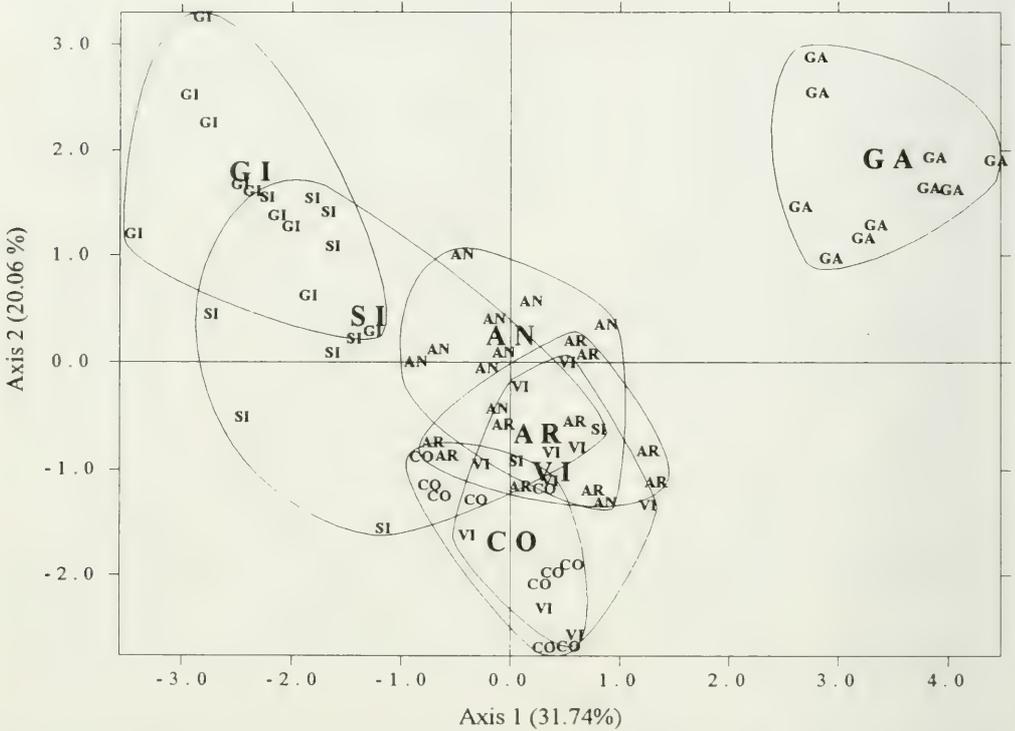


FIG. 4. 1/2 plan determined by Principal Component Analysis of chromosome data. Small characters represent active objects, large characters indicate the mean for each species. A line is drawn around each species to show the dispersion within species. AN: *Crassostrea angulata*. CO: *Saccostrea commercialis*. GA: *Crassostrea gasar*, GI: *Crassostrea gigas*, AR: *Crassostrea ariakensis*, SI: *Crassostrea sikamea*, VI: *Crassostrea virginica*.

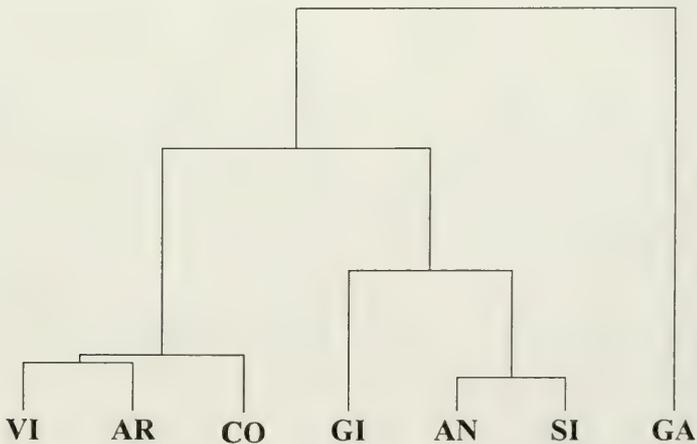


FIG. 5. Hierarchical Clustering Analysis showing the distances between the seven species from the first four axes of the PCA. AN: *Crassostrea angulata*. CO: *Saccostrea commercialis*. GA: *Crassostrea gasar*. GI: *Crassostrea gigas*, AR: *Crassostrea ariakensis*, SI: *Crassostrea sikamea*, VI: *Crassostrea virginica*.

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ULTRASTRUCTURAL AND CYTOCHEMICAL STUDY OF THE KIDNEY AND NEPHRIDIAL GLAND CELLS OF THE MARINE PROSOBRANCH MOLLUSC *NUCELLA LAPILLUS* (L.) IN RELATION TO FUNCTION

Vasilis K. Dimitriadis¹ & Elizabeth B. Andrews²

ABSTRACT

There are three epithelial cell types over the dorsal wall folds of kidney of *Nucella lapillus* (L.). The most numerous, excretory cells, are unciliated columnar cells, characterised by the presence of a variety of large membrane-bound cytoplasmic vacuoles, some containing granular material that gives a positive reaction for periodate-reactive carbohydrates. The second type, resorptive cells, are usually cone-shaped ciliated cells, with a well-developed endocytotic canal system and numerous vesicles in their apical cytoplasm. The apical cytoplasm contains large clusters of periodate-reactive granules, possibly glycogen and occasional large vacuoles containing some granular material. The third type, "small mucous cells," are carbohydrate-containing cells with many small mucous granules, which react positively for sulphated and carboxylated carbohydrates showing a reticulate positive reaction.

In the "secondary" folds, which are interspersed with the primary ones, the epithelium is cuboidal and in some places almost squamous, and most of the cells display some cilia, well-recognizable microvilli, many pinocytotic vesicles, phagosomes and elements of the canal system on their apices. Many mitochondria and deposits of glycogen are also observed.

The nephridial gland epithelium is composed of ciliated resorptive cells and "small mucous cells," very similar to those in the dorsal wall folds. The significance of the intracellular presence of carbohydrates in relation to the ability of the tissues to absorb and store sugars is discussed.

INTRODUCTION

The questions addressed by Fretter & Graham (1962) regarding the complex kidney of the carnivorous mesogastropods and neogastropods are still not adequately answered. A series of papers by Delhaye (1974–1976) gives a comparative systematic, primarily histological, survey of a range of archaeogastropods and mesogastropods, while other investigations deal with the excretory system of neogastropods, archaeogastropods, prosobranches or molluscs in general (Little & Andrews, 1977; Andrews, 1981; 1985; Taylor & Andrews, 1987; Andrews, 1988). However, there is little information on kidney of neogastropods at the ultrastructural level.

Andrews (1981) describes the cell types composing the kidney epithelium of the carnivorous neogastropod *Nucella lapillus* (L) and other monotocardians, such as *Littorina*, *Viviparus*, *Assimineia*, *Turritella* and *Lunatia*. According to this study, three main types of epithelial cell have been identified in the two

regions of the kidney folds and nephridial gland of *Nucella lapillus*, only one, the ciliated resorptive cells, being common to both and possibly implicated in reabsorption of organic solutes on the basis of well-developed apical microvilli with coated vesicles at their bases. The vacuolated excretory cells appear to have prominent microvilli, although coated vesicles are rarely observed.

Data for *Littorina* indicate that the nephridial gland is the major resorptive site for glucose (Andrews & Taylor, 1990), which is consistent with ultrastructural observations showing that the dorsal wall is also involved in uptake of solutes. The latter is believed to be responsible for the resorption of residual glucose and for nitrogenous excretion (Taylor & Andrews, 1987; Andrews & Taylor, 1990). Thus, one of the goals of the present study is the investigation of the intracellular presence of carbohydrates in the kidney and nephridial gland epithelium of *Nucella lapillus* and the comparison of the results with those obtained from other gastropods.

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In the present study, the ultrastructure of the kidney and nephridial gland cells of *Nucella lapillus* is considered and compared with analogous ones of other carnivorous or herbivorous molluscs. In addition, cytochemical tests for carbohydrate presence and acid phosphatase activity were applied.

MATERIALS AND METHODS

Specimens of *Nucella lapillus* from the University Marine Station, Millport, Scotland, were kept in an aquarium with circulating artificial sea water at 10°C. Some of the specimens were fixed for electron microscopy and histochemistry on arrival and others were used within a few days.

Electron Microscopy

Samples were fixed in 3% glutaraldehyde in Sorensen's phosphate buffer, pH 7.2 adjusted to 1100 mOsm with 14% sucrose. Material was embedded in TAAB resin, and thin sections were stained in 1% aqueous uranyl acetate and Reynolds' lead citrate.

Cytochemistry

For carbohydrate cytochemistry, finely minced pieces of tissues were incubated overnight after fixation in high iron diamine (HID) (Spicer et al., 1978; Sannes et al., 1979) using a medium containing 36 mg N,N-dimethyl-m-phenylenediamine, 6 mg of the para isomer and 0.45 ml ferric chloride 40%, in 15 ml d water. Other pieces were incubated in low iron diamine (LID) (Takagi et al., 1982), using a medium containing 27 mg N,N-dimethyl-m-phenylenediamine, 4.5 mg of the para isomer, and 0.45 ml ferric chloride 40% in 45 ml d water. After incubation, the tissues were post-fixed with 2% aqueous osmium tetroxide and embedded in Spurr's resin. Thin sections of these specimens were stained with the thiocarbonyl-silver proteinate (TCH-SP) sequence, using a medium containing 2% thiocarbonylhydrazine in 20% acetic acid, to reveal periodate-reactive substances. Control tissues were incubated in 1M MgCl₂ in place of LID or HID. Specimens without osmium tetroxide treatment were used for the postembedding periodate-thiocarbonylhydrazine-silver proteinate (PA-TCH-SP) method (Thiéry, 1967). Control sections were stained without the periodate treatment.

For acid phosphatase demonstration, a modification (Lewis & Knight, 1992) of the method proposed by Barka & Anderson (1962) was applied. Glutaraldehyde was used as a fixative, and the tissues were incubated in a medium containing 0.2M tris/maleate as a buffer and 0.1 M β-glycerophosphate as substrate at 37° for 15–30 min. Control sections were taken through an identical sequence except that the substrate is omitted from the incubation medium or had 0.01 M sodium fluoride added.

RESULTS

Kidney

The dorsal wall of kidney of *Nucella lapillus* (L.) (Fig. 1) consists of two sets of folds, "primary" folds, which receive blood from a branch of the afferent renal vein running over the ventral wall of the kidney sac, and smaller "secondary" folds, which receive blood from a branch running over its dorsal wall (Fretter & Graham, 1962).

Three different cell types comprise the epithelium of the "primary" folds. The most common, excretory cells (Fig. 2), are columnar cells, which present a variety of excretory vacuoles in their cytoplasm (Figs. 4, 8).

The vacuolated excretory cells have prominent microvilli (Figs. 4, 8), which are lost in later stages when apical blebs are formed. The most conspicuous organelles of these cells are a series of large excretory vacuoles containing in certain cases a concentration of finely fibrous excretory material (Fig. 4). Due to the presence of the large vacuoles in the apical and mid cytoplasmic region, the ovoid nuclei are usually displaced in their base (Fig. 4). The fibrous material of the excretory vacuoles in most cases gives a positive reaction for periodate-reactive carbohydrates (Fig. 8). In certain cases, this material reacts positively for acid phosphatase activity (Fig. 11). Other clusters of fibrous and/or granular periodate-reactive material is also present in the cytoplasm of these cells (Fig. 8). Small vesicles are seen in the process of fusing with excretory vacuoles of varying size and may appear as evaginations of the vacuolar membrane.

The rough endoplasmic reticulum of the excretory cells is usually restricted to a narrow zone of apical cytoplasm, often associated with clusters of glycogen. The Golgi complexes lie close to the nuclei and are small,

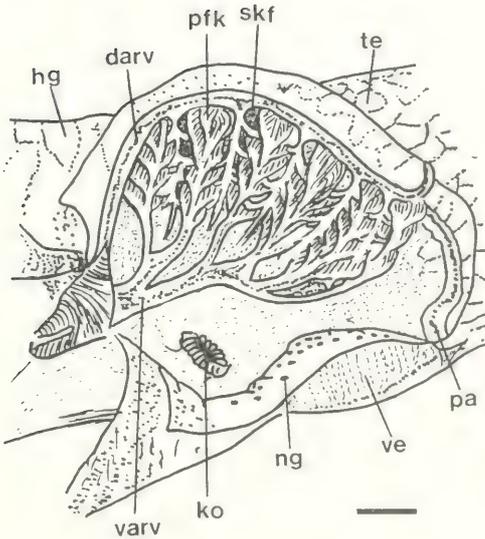


FIG. 1. Drawing of the kidney sac of *Nucella lapillus*, opened to show double series of folds on the wall, based on the original in Fretter & Graham (1962). darv, dorsal branch of afferent renal vein; hg, hypobranchial gland; ko, kidney opening; ng, nephridial gland; pa, posterior aorta; pfk, primary kidney folds; skf, secondary kidney folds; te, testis; varv, ventral branch of afferent renal vein; ve, ventricle. Bar = 1 mm.

conspicuous and rarely observed, whereas the mitochondria are positioned parallel with the infoldings of the basal plasma membrane. In the basal cytoplasm, the excretory cells display long slender basal processes, which invaginate the basal lamina and extend into underlying blood spaces (Fig. 9). Near the basal membrane, in the narrow cytoplasmic spaces between the adjacent infoldings and very close to the blood spaces clusters of periodate-reactive granules, mostly glycogen are present (Fig. 9).

The second cell type in the "primary" dorsal folds, the ciliated resorptive cells (Fig. 5), bear apical microvilli and cilia. Usually, they appear to be cone-shaped, with their distended apices bulging into the lumen in umbrella-like fashion over the neighbouring cells (Fig. 5).

The dense cytoplasm of the resorptive cells possesses an endocytotic canal system and many small vesicles (Fig. 10), some near the apical membrane being coated. Pinocytosis occurs at the base of the microvilli and the pinocytotic vesicles are coated (Fig. 10). Pinocytotic vesicles are coated on the cytoplasmic face by closely spaced, bead-like particles. The apical membrane itself bears a fine gly-

cocalyx and microvilli 0.7–1.2 μm long are interspersed amongst groups of long cilia 4.5–5 μm long.

In the apical cytoplasm there are some lysosomes, usually in the form of residual bodies and occasional large vacuoles, some of them containing a little granular material. Also in the apical cytoplasm are prominent large clusters of periodate-reactive particles, mostly glycogen (Fig. 10). Similar particles are also present throughout the cytoplasm.

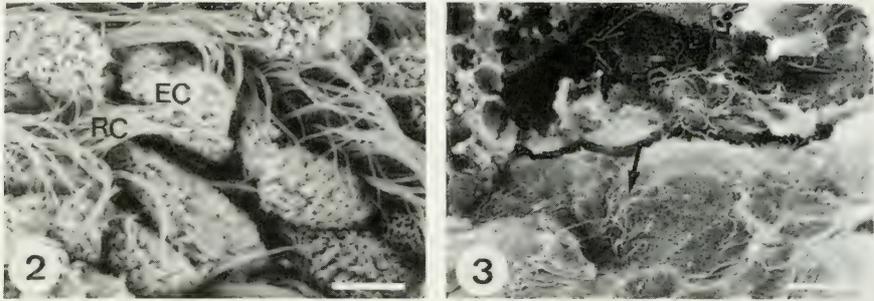
The third cell type, the "small mucous cell" (Fig. 6), is very rare and consists of small cells with small periodate-reactive secretory granules, or cells having discharged their secretion. The granules of these cells reacted positively for sulphated and carboxylated carbohydrates showing a reticulate positive reaction (not shown).

The "secondary" folds of dorsal wall of kidney of *N. lapillus* are present in troughs between "primary" folds. In the "secondary" folds the epithelium is cuboidal and in some places almost squamous (Fig. 7), while the presence of fewer cilia comparing to those of the primary kidney folds makes the recognition of the cell limits under the scanning microscope obscure, comparing to the "primary folds" (Figs. 2, 3). In the apical portion, the cells show many pinocytotic vesicles, phagosomes and elements of the canal system. Many mitochondria, lysosomes and deposits of periodate-reactive particles are also observed. In the "secondary" folds, the presence of the "small mucous cells" is less obvious than in the "primary" ones.

Nephridial Gland

The nephridial gland of *N. lapillus* receives post branchial blood from the auricle. In the epithelium of this gland, the same type of ciliated resorptive cell covers the surface and the more superficial parts of the tubules (Figs. 12, 13). Unlike the resorptive cells of the dorsal wall folds, cone-shaped forms in the nephridial gland are very rare, the cells consisting mostly of cuboidal form. The resorptive cells show apical microvilli and cilia and a well-developed endocytotic canal system with small coated vesicles. In the apical portion, the presence of small, empty vacuoles is usually apparent (Fig. 13). Periodate-reactive particles, mostly glycogen, are also located in clusters usually in the apical cytoplasm (Fig. 14).

Dense bodies frequently lie in the apical portion of the cells and usually exhibit an elec-



FIGS. 2 & 3. Scanning electron micrographs of the apical surface of "primary" (Fig. 2) and "secondary" folds (Fig. 3) of the kidney dorsal wall. In the "secondary" folds, the decreased presence of cilia (arrow), compared to the "primary" folds, makes the recognition of the cell limits not well recognizable. EC, excretory cell; RC, resorptive cell. Fig. 2 Bar = 2.5 μ m, Fig. 3 Bar = 8 μ m.

tron-dense matrix. The material of most dense bodies gives a positive reaction for periodate-reactive carbohydrates, as well as for sulphated (Fig. 15) and carboxylated carbohydrates. In certain cases, membranous remnants of the dense bodies display a positive acid phosphatase reaction (not shown).

Like the dorsal wall epithelium of the kidney, in the nephridial gland there is a second cell type, the "small mucous cells" consisting of small cells with small periodate-reactive secretory granules giving also a reticulate positive reaction for sulphated and carboxylated carbohydrates (not shown).

Control sections of all cytochemical techniques constantly lacked reaction product.

DISCUSSION

The kidney of *N. lapillus* is histologically similar to that of other monotocardians prosobranchs (Andrews, 1981) in that the epithelium is composed of distinct vacuolated excretory and ciliated resorptive cells. The morphological and functional parameters of the excretory and ciliated cells of *Nucella lapillus* documented by the results of the present study, such as the structure of their excretory vacuoles, canal system compartments, and basal processes, are very similar to those of the herbivorous mesogastropod *Littorina littorea* (Andrews, 1981; Taylor & Andrews, 1987; Andrews, 1988), albeit that *Nucella* is carnivorous. A differentiation in the density of the excretory vacuoles contents was reported by Andrews (1981) between *Nucella* and herbivores species, being denser in *Nucella* than in the other species, as well as a differentiation in the colour of the lysosomes of the cili-

ated cells, being greenish in the herbivores species studied.

The vacuolated excretory cells of the dorsal wall folds of the kidney of *Nucella*, like the corresponding cells in *Littorina* (Andrews, 1988), contain excretory vacuoles with finely fibrous excretory material, unlike those of archaeogastropods and amphibious and terrestrial species, which contain layered concentrations. It is generally accepted that the excretory vacuoles are formed and increase in size after fusion of smaller vesicles and finally are ultimately shed in blebs of apical cytoplasm that are nipped off from the cell membrane (Andrews, 1981, 1985). Delhaye (1976) demonstrated pinocytosis at the bases of the excretory cells of *Monodonta* by which the cells abstracted ferritin injected into the blood. A second indication for this function is the clearly well-recognisable long slender processes that invaginate the basal lamina of the excretory cells of *Nucella*, and as in *Littorina* (Andrews, 1988) and *Monodonta* (Andrews, 1985), permeate the underlying blood spaces, which suggest transport of molecules across the lamina.

The ciliated resorptive cells comprising the epithelium of *N. lapillus* display many similarities to those of the ciliated resorptive cells of the dorsal wall folds. The ciliated cells in both epithelia were regarded as cells of the same cell type (Andrews, 1981; Andrews & Taylor, 1990). The results of the present study show that the ciliated cells in the kidney folds differ from those in the nephridial gland in certain features, for example their cone shape, where they are interspersed with excretory cells. However, other differences, such as the number of their apical vesicles and lysosomes, should be attributed to the different phases of

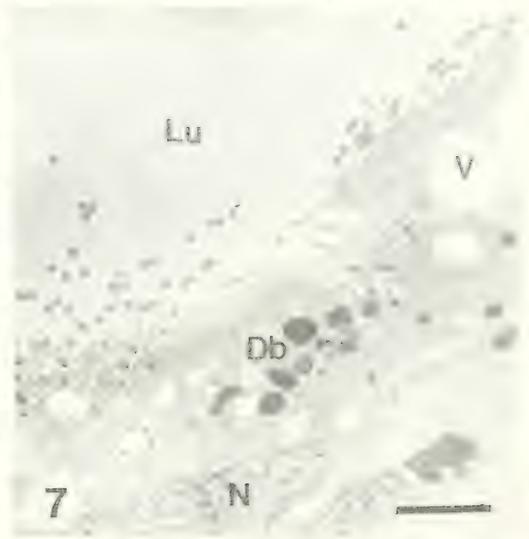


FIG. 4. Apical portion of excretory cells in a "primary" fold of dorsal wall of kidney showing large excretory vacuoles (EV). Lu, lumen; N, nucleus. Bar = 4 μ m.

FIG. 5. The apex of a ciliated resorptive cell displaying microvilli and cilia, as well as apical vesicles (asterisk), is protruded into the lumen. Bar = 3.5 μ m.

FIG. 6. A "small mucous cell" (asterisk) is positioned in the apical portion of the epithelium, possibly just before the secretion of the cell product. EV, excretory vacuole; Lu, lumen; N, nucleus. Bar = 3 μ m.

FIG. 7. In a "secondary" fold of the kidney dorsal wall, there is a transition in the form of the cells from columnar-cuboidal towards cuboidal-squamous. Db, dense body; Lu, lumen; N, nucleus; V, apical vesicle. Bar = 5 μ m.

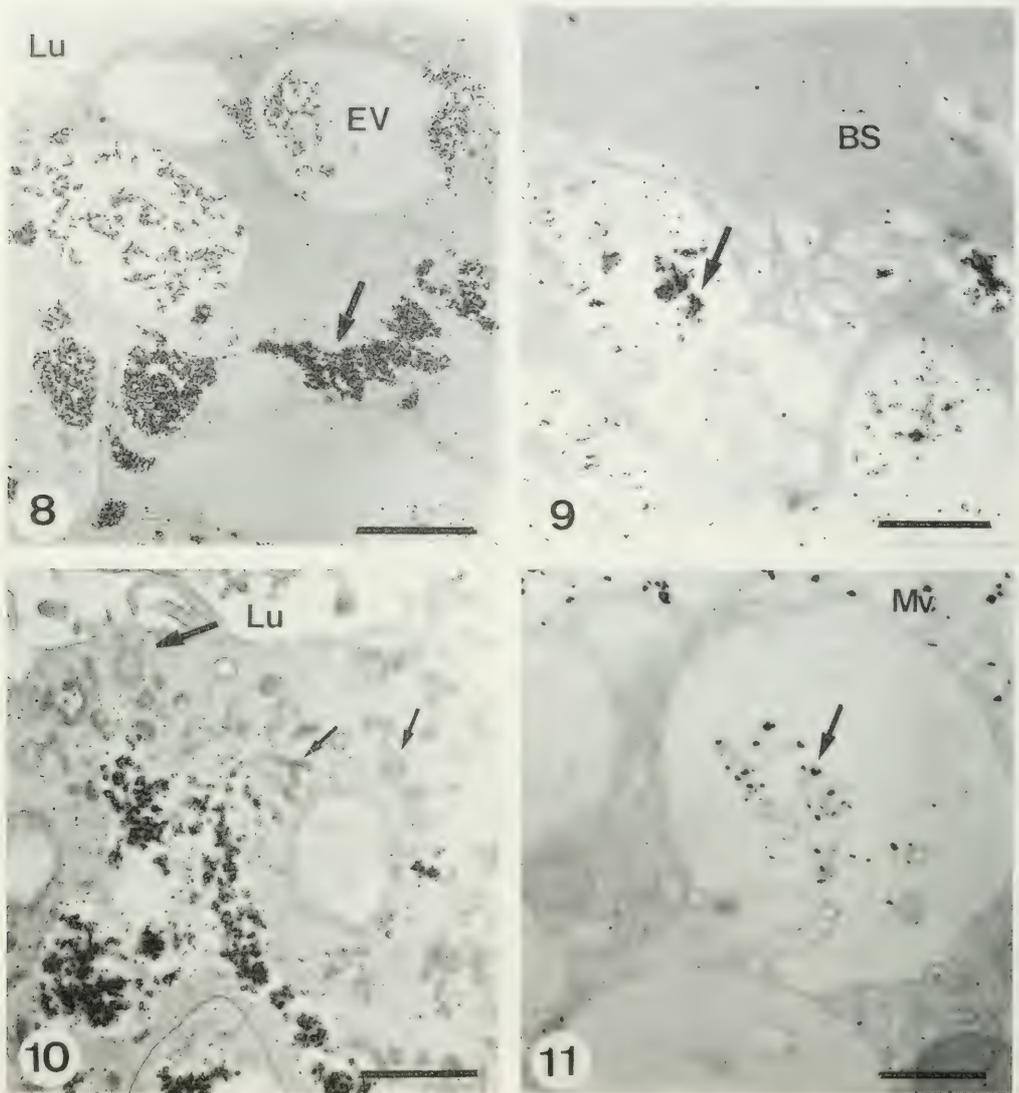


FIG. 8. Cytochemistry for periodate-reactive carbohydrates. Excretory cells show periodate-reactive fibrous material in large excretory vacuoles (EV). Other "primary" fold cells show large clusters of other periodate-reactive material in their cytoplasm (arrow). Lu, lumen. Bar = 3 μ m.

FIG. 9. Cytochemistry for periodate-reactive carbohydrates. Basal cytoplasm of excretory cell. The basal membrane infoldings form long slender cytoplasmic processes, which permeate the blood spaces (BS) and contain periodate-reactive carbohydrates (arrow). Bar = 0.6 μ m.

FIG. 10. Cytochemistry for periodate-reactive carbohydrate. Apical cytoplasm of a ciliated resorptive cell in the kidney of *Nucella lapillus*. Clusters of periodate-reactive particles, mostly glycogen, are located in the cytoplasm. Note the coated vesicle (arrow) and the well-stained vesicles and tubules of the canal system (small arrows). Bar = 0.8 μ m.

FIG. 11. Cytochemistry for acid phosphatase. Materials in an excretory vacuole of an excretory cell react positively for enzyme presence (arrow). Mv, microvilli. Bar = 1 μ m.

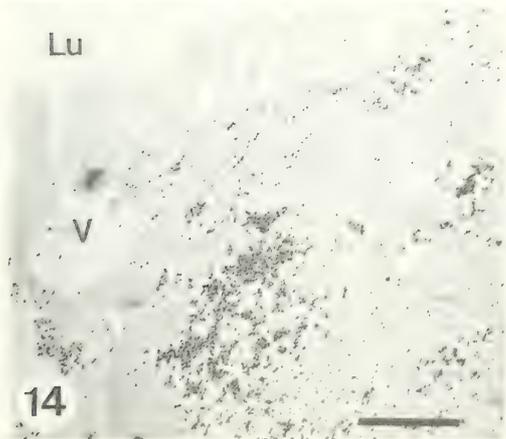


FIG. 12. Ciliated resorptive cells and a "small mucous cell" (asterisk) in the nephridial gland of *Nucella lapillus*. Db, dense body; Lu, lumen. Bar = 3.5 μ m.

FIG. 13. Ciliated resorptive cells in the nephridial gland of *Nucella lapillus* display many mitochondria, some lysosomes and many apical small vesicles (arrow). Ci, cilium; Db, dense body; Mv, microvilli. Bar = 2 μ m.

FIG. 14. Carbohydrate cytochemistry. The cytoplasm of a ciliated resorptive cell shows abundance of peroxide-reactive particles, mostly glycogen. Lu, lumen; V, apical vesicle. Bar = 1.5 μ m.

FIG. 15. In the nephridial gland, a dense body (Db) in the apical region of a ciliated resorptive cell react positively for sulphated carbohydrates. Lu, lumen. Bar = 4 μ m.

cell activity rather, than to differences between cell types.

The apical canal system observed in the resorptive cells of both kidney dorsal wall and nephridial gland cells of *Nucella lapillus* is also observed in the pericardial and kidney

cells of *Scrobicularia plana* (Andrews & Jennings, 1993) and the left kidney of archaeogastropods (Andrews, 1985), as well as in the digestive gland of *Nucella* (Dimitriadis & Andrews, submitted for publication), *Lasaea* (McQuiston, 1969), *Cardium* (Owen, 1970),

Mytilus (Owen, 1972), and *Rissoa* (Wigham, 1976), where it was regarded as structure related to the delivery of nutrients to the endocytotic system. In the light of the recent data, the elements of the canal system should be regarded as endosomes, that is, the cell compartments, where the endocytosed material enters the lysosomal pathway (Alberts et al., 1994).

In the excretory cells of *N. lapillus*, periodate-reactive fibrous material is located inside the excretory vacuoles, while in certain cases this intravacuolar material reacts positively for acid phosphatase. Carbohydrates, among other substances, have been identified in the excretory vacuoles in the right kidney of diotocardians (Delhaye, 1976). The presence of a significant amount of carbohydrates and hydrolases inside the excretory vacuoles probably indicates that the role of these vacuoles should be regarded as multiple and that they are implicated in more functions than the transport and release of the excretory material.

In the kidney dorsal wall of *N. lapillus*, clusters of periodate-reactive particles, mostly glycogen, are present in the cytoplasm of the resorptive cells. It is well known that carbohydrates are the major source of energy in gastropods molluscs (Livingston & De Zwaan, 1983) and that carbohydrates are largely stored as glycogen, especially in connective tissue. The excretory system of prosobranch gastropods, which forms urine by a filtration mechanism, has the capacity to resorb valuable organic solutes, such as glucose, from the primary urine, limiting markedly the amount that is lost to the exterior (Andrews & Taylor, 1990). Glucose resorption has been demonstrated in the abalone *Haliotis rufescens* (Harrison, 1962) and the mesogastropod *Littorina littorea* (Taylor & Andrews, 1987), while glucose influx to both dorsal wall and nephridial gland of the kidney appeared to reflect net inward transport by a saturable Na⁺-dependent carrier mechanism believed to be responsible for reabsorption in vivo (Taylor & Andrews, 1987). On the other hand, in the herbivorous mesogastropod *Littorina littorea*, the nephridial gland cells were regarded as sites implicated in resorption of organic solutes on the basis of well-developed apical microvilli with coated vesicles at their bases (Andrews & Taylor, 1990). Also in *Littorina*, Delhaye (1974b) demonstrated the capacity of the nephridial gland cells to remove indian ink, iron saccharide and ferritin from

the blood, like the resorptive cells of the dorsal wall, while their ability to accumulate [³H] glucose has been shown in vitro (Taylor & Andrews, 1990). In *N. lapillus*, where the morphological features of the nephridial gland cells are very similar to those in *Littorina*, an equivalent absorptive function of the cells is very possible. Thus, the presence of large amounts of periodate-reactive particles in the resorptive cells of kidney folds and nephridial gland is a strong indication that these cells absorb carbohydrates and store, at least a part of them, in their cytoplasm.

The "small mucous cells" observed in the examined tissues of *N. lapillus* exist also in the digestive gland of the same gastropod, where they also reacted positively for complex carbohydrates (Dimitriadis and Andrews, submitted for publication). Probably this cell type is related to the mucoid cells described in the nephridial gland of other monotocardian prosobranch gastropods (Andrews, 1981), as well as in the kidneys of freshwater mesogastropods (Andrews, 1988), where they were specialised as cells related to the resorptive mechanism of the epithelium. The secretion product of these mucoid cells is not stained by alcian blue and is probably a mucoprotein or neutral mucopolysaccharide.

In conclusion, by using light and electron microscopic observations in combination with cytochemical characterization, the present study provides information about the structure and function of the kidney and nephridial gland cells. Additional information, especially by the use of histochemistry in cryosections and immunocytochemistry, is necessary for the better understanding of the fine structure and physiology of the studied interesting cell types.

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ENERGETICS OF THE RED SLUG *ARION RUFUS* (GASTROPODA) AND OF THE GASTROPOD COMMUNITY IN A BEECH FOREST ON LIMESTONE

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ABSTRACT

The energy budget of the red slug *Arion rufus* (L.) in a mull beech forest on limestone in Lower Saxony, Germany, was calculated. Somatic growth, reproduction, secretion of mucus, respiration, excretion of faecal materials, and assimilation efficiency were measured experimentally. The consumption was calculated from the excretion of faecal materials, using the assimilation efficiency.

Mean biomass of the population of *A. rufus* in the forest studied was $0.2 \text{ g d wt m}^{-2}$. The average density of slugs that grew to maturity and reproduced was one slug per 3 m^2 . The annual consumption of the population of *A. rufus* in the beech forest studied was equivalent to 361.9 kJ m^{-2} , the investment into somatic growth to 52.5 kJ m^{-2} , the reproduction to 3.6 kJ m^{-2} , the respiration to 27.6 kJ m^{-2} , and the excretion of faecal materials to 79.7 kJ m^{-2} . About 105.3 kJ m^{-2} were invested into the secretion of mucus, which was 65% of the production. The considerable investment of gastropods in locomotion probably limits the range of conditions under which populations can survive. Ecosystem characteristics and slug qualities that allow the high slug biomass in the beech forest ecosystem were discussed.

The population of *A. rufus* and the whole gastropod community consumed 0.15% and 2.4% of the annual leaf litter input, 3.3% and 26% of the annual production of green plant material, and 0.24% and 1.7% of the annual net primary production (NPP), respectively. The direct contribution of the gastropod community to decomposition processes therefore was small. The consumption of green plant material by the gastropod community was similar to that annually ingested by phytophages (Curculionidae and lepidopteran larvae) in the forest studied.

Key words: *Arion rufus*, gastropod community, energy budget, mucus, beech forest.

INTRODUCTION

In the present investigation, we calculate the energy budget of the red slug *Arion rufus* (L.) in order to understand life history strategies of this slug species in the forest ecosystem. We are especially interested in the role of mucus in the energy flux of *A. rufus*. In previous studies, the energy budget of *A. rufus* and *A. ater* in beech forests was calculated (Stern, 1969; Jensen, 1975), however the authors did not quantify the secretion of mucus. Since mucus is a major component of gastropod production (Denny, 1980; Davies et al., 1990), the omission of the mucus term caused a considerable underestimation of production.

Arion rufus feeds on dead organic material, living plants, fungi and carcasses and therefore contributes directly to decomposition processes (Jennings & Barkham, 1976; 1979a). In the present investigation, special

attention was given to the consumption data of the population of *A. rufus*, because with these data the impact of *A. rufus* on its food and the direct contribution to decomposition processes could be evaluated. Furthermore, we extrapolated the consumption data of *A. rufus* to the gastropod community in order to assess the role of gastropods in the forest ecosystem. Schaefer (1990) constructed the energy budget of the heterotrophic subsystem of the forest investigated in the present study. He found that the contribution of the gastropod community to the energy and material turnover was low. However, the calculations were made without data on *A. rufus*, because biomass data of *A. rufus* were not available. Presumably, this omission resulted in a drastic underestimation of the role of the gastropod community in nutrient cycling in the forest ecosystem studied. *Arion rufus* has a lifespan of little more than a year (Laviolette, 1950;

Smith, 1966), and since the body weight of mature specimens is high, the population of *A. rufus* builds up a high biomass during a short time period. Therefore, we assume that the energy flow through the population of *A. rufus* is high and that *A. rufus* makes a significant direct contribution to plant decomposition processes.

As a result of the high production of biomass of the population of *A. rufus* in the forest ecosystem studied, we hypothesize that this slug species turns high amounts of living plants and dead organic material into faecal material, which is then colonised by a rich community of microfauna (Theenhaus & Scheu, 1996a) and mesofauna (Theenhaus, 1997). Furthermore, it is assumed that in the forest ecosystem studied the population of *A. rufus* secretes high amounts of slug mucus. Since slug mucus contains high nutrient concentrations, *A. rufus* directly modulates the availability of resources for microorganisms through the secretion of mucus (Theenhaus & Scheu, 1996b). We assume that the creation of habitats for other species through the excretion of faecal material and the secretion of slug mucus is of quantitative importance in the forest ecosystem studied. *Arion rufus* might therefore be called an ecosystem engineer (Lawton, 1994).

MATERIALS AND METHODS

Study Site

The Göttinger Wald beech forest is situated on a plateau of Muschelkalk at about 420 m above sea level in southern Lower Saxony, Germany. The forest, which is approximately 110–125 years old, has a uniform canopy layer, which consists almost exclusively of beech trees (*Fagus sylvatica*). A shrub layer is not developed. The herb layer is dominated by *Allium ursinum* and *Mercurialis perennis*. In the years 1995 and 1996, in which the experiments were conducted, mean annual temperature in the litter layer was 9.0°C and 7.1°C, respectively, and mean annual precipitation was 700 mm and 567 mm, respectively.

Energy Budget of *A. rufus*

In the present study, we first calculated the energy budget of a "model specimen" of *A. rufus*, which is an animal that grows to matu-

rity, deposits one egg clutch, and then dies. Based on this model specimen, the energy budget of the population of *A. rufus* in the forest studied was estimated. The energy budget was calculated using the equation given by Petruszewicz (1967), which was modified to include a mucus term (e.g., Edwards & Welsh, 1982; Davies et al., 1990): $C = P_g + P_r + P_m + R + F$, where C = consumption, P_g = somatic growth, P_r = reproduction, P_m = secretion of mucus, R = respiration, and F = faecal materials.

Defined-area traps similar to those of Ferguson et al. (1989) were constructed in cooperation with C. Döring to estimate slug populations in the beech forest. The traps consisted of a square PVC frame (1 m² area, 30 cm high). This frame was placed onto the forest soil to enclose slugs, which were supposed to be caught during the following trapping period. A PVC lid could be screwed tightly on a metal rim, which was attached to the inner edge of the frame. Twelve round holes in the lid, which were sealed with gauze (meshsize 1 mm) created a light regime (twilight) inside the traps being favourable for slugs. To prevent slug movements in or out of the traps, outside the traps soil was piled up (20 cm high) and pressed tightly to the frame. A glass jar (diameter 5 cm, 11 cm high; Barber, 1931) was imbedded into the soil inside the traps and filled with beer (3 cm high), which served as baiting agent. During periods of drought, the ground inside the traps was moistened regularly. In the years 1995 and 1996, the population of *A. rufus* was estimated using 12 traps, which were arranged in four groups (distance 10 m) of three traps each (distance 3–5 m; block-design; Sokal & Rohlf, 1995). Every two weeks, the glass jars were emptied and refilled with fresh beer. Trapped slugs were identified, and their ash-free dry weight was determined. When no slugs were captured in the traps for four weeks, it was assumed that all slugs inside the traps were caught, and therefore the trapping period was finished. Traps were rearranged, and a new trapping period started.

To quantify the production of eggs, in autumn 1994 and 1995 mature specimens of *A. rufus* were collected in the beech forest and transferred into the laboratory. Each egg clutch was divided into three portions and incubated at 5, 10 and 20°C, respectively. The time between egg deposition and hatching of juveniles was measured.

The CO₂ production of slugs was measured

titrimetrically (1N KOH as a CO₂ absorbent) at 5, 10 and 15°C for 24 h (day/night cycle of 12/12 h). Measurements were done in experimental chambers, which consisted of perspex tubes (diameter 6 cm, 15 cm high). Slugs were acclimated at the respective temperature for 14 days prior to measurements.

To quantify the excretion of faecal materials, specimens of *A. rufus* were collected in the beech forest in summer following periods of drought, in summer following wet periods, and in autumn (three parallel measurements). Still in the forest, each slug was placed into a separate round, perspex vessel (12 cm diameter, 9 cm height), which contained food (leaves of *Allium ursinum*, *Mercurialis perennis*, and *Sambucus nigra*). The vessels were then transported into a temperature controlled room, in which temperature and day/night cycle were close to those of the respective time of year. The fresh weight of each slug and the dry weight of faecal materials, which were excreted during the incubation by each slug, were determined 24 h after the collection of slugs.

To quantify the secretion of mucus, specimens of *A. rufus* (fresh weight between 0.3 and 15.6 g) were incubated in pre-weighed perspex vessels (see above) at defined temperature and humidity conditions (5, 10, 15 and 20°C in combination with 100% RH and 10°C in combination with 75% and 55% RH, respectively) for 2 h. The fresh weight of mucus, which was secreted during the time of incubation, was determined by reweighing the vessels after the incubation and calculating the difference in weight. Slugs were acclimated at the respective experimental conditions (temperature, RH) for 12 h prior to use.

The assimilation efficiency of juvenile *A. rufus* (fresh weight between 0.8 g and 2.2 g) feeding on leaves of *M. perennis* and *A. ursinum* and of mature *A. rufus* (fresh weight between 7 g and 15 g) feeding on leaves of *M. perennis* and *Sambucus nigra* was determined gravimetrically. Before measurement, slugs were fed with one of the respective herbs for three days. Then the slugs were transferred to perspex vessels (see above), which contained 7 g f wt of one of the respective herbs of known water content, five juvenile slugs or one mature slug per vessel (10°C, day/night cycle of 14/10; juvenile slugs: six parallel measurements; mature slugs: 15 parallel measurements). After three days of incubation, the dry weight of faecal materials, which was excreted during the incubation, and the dry

weight of herb materials were determined. The dry weight of herbs, which were eaten by slugs during the three days of incubation, was calculated as the difference before and after feeding. Carbon content of faecal materials and herbs was determined using an elemental analyser (Carlo Erba Co., Milan). In order to determine the change in weight of herbs without the influence of slugs, nine vessels without slugs but with herbs (three vessels with *M. perennis*, *A. ursinum* and *S. nigra*, respectively) were incubated for three days. The assimilation efficiency of slugs was calculated with the formula:

$$a = \frac{d \text{ wt food} \times cc \text{ food} - d \text{ wt faecal materials}}{d \text{ wt food} \times cc \text{ food} - < cc \text{ faecal materials}} \times 100.$$

where a = assimilation efficiency, cc = carbon content.

The conversion of data into calorific values was accomplished with conversion factors from the literature: 1 g d wt tissue of *A. rufus* and of egg mass was equated with 20.1 kJ (Jensen, 1975; Bless, 1978); 1 g d wt mucus was equated with 18.8 kJ (Calow, 1974; Richardson, 1975); 1 l CO₂ was equated with 21.8 kJ. This conversion factor applies to animals with a respiratory quotient of 0.95 (Southwood, 1991), which was found for the slug *Ariolimax columbianus* by Denny (1980). One g d wt faecal materials was equated with 21.4 kJ, since 1 g carbon corresponds to 46 kJ (Humphreys, 1979), and the mean carbon content of slug faecal materials is 46.5%. One g d wt green plant material was equated with 18.8 kJ (Schaefer, 1990, 1991).

RESULTS

In both years (1995 and 1996), traps were moved three times (four catching periods in each year; Table 1). To get a conservative estimate of the mean yearly biomass of the population of *A. rufus* in the forest studied, we assumed that during the time when no trapping occurred the dry weight of *A. rufus* was zero. As a result, the mean dry weight of the population of *A. rufus* was 0.2 g m⁻² (0.15 and 0.25 g d wt m⁻² in 1995 and 1996, respectively).

Mean fresh weight of *A. rufus* after the deposition of eggs was 9.4 g and 8.4 g in the year 1994 and 1995, respectively (Table 2). Juvenile slugs hatched 134.0 (n = 39, SD = 8.7) and

TABLE 1. Ash-free d wt [mg] of specimens of *Arion rufus* caught with 12 defined-area traps in the Göttinger Wald in the years 1995 and 1996.

Catching period	Ash-free d wt per specimen	Mean	(SD)
23.03.–19.05.95	14, 25, 214, 227, 235, 241, 316, 475, 491, 827, 2095	*306	(228)
20.05.–28.06.95	66, 91, 94, 155, 742, 975, 1030, 1083	529	(438)
29.06.–11.08.95	960	960	(0)
12.08.–05.10.95	407, 1267, 1762	1145	(559)
07.05.–02.07.96	137, 300, 412, 581, 790, 1466, 1690	768	(550)
03.07.–12.08.96	231, 939, 1100, 1990	1065	(626)
13.08.–10.10.96	812, 1280, 1560, 1650, 1916, 1920, 2009	1562	(396)
11.10.–03.12.96	1, 2, 6, 17	7	(6)

*without the mature specimen of 2095 mg

TABLE 2. Mean fresh weight (f wt) of *Arion rufus* and egg clutches after the deposition of eggs in the year 1994 and 1995, number of slugs and egg clutches examined (n), standard deviation (SD), minimum and maximum values and mean number of eggs per clutch.

	year	f wt (g)	n	SD	minimum	maximum	number of eggs per clutch
<i>A. rufus</i>	1994	9.40	11	3.7	4.6	17.7	
	1995	8.40	32	2.8	4.5	19.3	
egg clutch	1994	2.57	11	1.2	1.31	5.13	58
	1995	2.26	32	1.0	0.68	5.94	63

TABLE 3. Regression lines between dry weight of specimens of *Arion rufus* and CO₂ production at 5, 10 and 15°C. R: CO₂ production [ml CO₂ h⁻¹]; W: d wt of *A. rufus* [g]; n: number of slugs examined; inter.: intercept; exp.: exponent.

Temperature	Regression equation	n	R ²	p inter.	SD inter.	SD exp.
5°C	R = 0.17 × W ^{1.06}	14	0.59	< 0.001	0.041	0.246
10°C	R = 0.36 × W ^{0.92}	15	0.99	< 0.001	0.019	0.026
15°C	R = 0.49 × W ^{1.07}	15	0.89	< 0.001	0.016	0.101

TABLE 4. Regression lines between the dry weight of specimens of *Arion rufus* and the excretion in summer following dry and wet periods and in autumn (three parallel measurements in each season). F: d wt cast materials (mg 24 h⁻¹); W: d wt *A. rufus* (g); n: number of slugs collected.

	Regression equation	range of n	range of R ²	P (Slope)
Summer, following dry periods	F = 10.6 × W ^{0.71}	52–73	0.47–0.65	< 0.001
Summer, following wet periods	F = 29.2 × W ^{0.94}	68–79	0.68–0.79	< 0.001
Autumn	F = 19.4 × W ^{0.98}	58–77	0.36–0.64	< 0.001

66.8 days following the deposition of eggs (n = 69, SD = 6.1) at 5°C and 10°C, respectively. At 20°C slugs did not hatch.

The CO₂ production of *A. rufus* increased with increasing temperature. The relationship between the dry weight of specimens and the respiration rate was almost linear (Table 3).

There was a positive correlation between the log dry weight of *A. rufus* and the log ex-

cretion of faecal materials (Table 4). In summer following periods of drought, the faecal weight excreted per unit weight of slug decreased with increasing weight of slugs. In summer following wet periods, and in autumn, the exponent of the regression equations ranged between 0.88 and 1.00 indicating an almost linear relationship between the excretion and slug weight.

The weight of mucus secreted increased with the weight of the slug following the equation $S = a \times W^b$, where $S = d$ wt mucus, $W = d$ wt *A. rufus*, $0 < b < 1$ (Fig. 1).

The assimilation efficiency of juvenile slugs was 78.7% ($n = 6$; SD = 8.1) and 88.5% ($n = 6$; SD = 5.6) when feeding on *M. perennis* and *A. ursinum*, respectively. The assimilation efficiency of mature slugs was lower than that of juvenile slugs, being 73.1% ($n = 15$; SD = 5.9) and 71.8% ($n = 15$; SD = 7.2) when feeding on *M. perennis* and *S. nigra*, respectively.

ENERGY BUDGETS

The Energy Budget of *Arion rufus*

Monthly weight classes of *A. rufus* were calculated using literature data (Künkel, 1916; Abeloos, 1944; Laviolette, 1950; Frömring, 1954; Smith, 1966) and present data. As a result, the model specimen deposits one egg clutch at the beginning of September (Table 5). Mean temperature in the litter layer in September and October is 10°C, and therefore it is assumed that the juveniles hatch two months following the deposition of egg clutches (beginning of November). The model specimen weighs 9 g in July, deposits one egg clutch in September, and then dies. Based on these data, the energy budget of the model specimen was calculated as follows: mean dry weight of slugs, which deposited egg clutches in the laboratory was 1.35 g (27.2 kJ). In the laboratory, slugs never deposited more than one egg clutch, and therefore it was assumed that the model specimen deposits only one egg clutch. Average d wt of egg clutches that were deposited in the laboratory 1994 and 1995 was 0.54 g (10.9 kJ). To calculate the amount of mucus secreted, it was assumed that during a period of 24 h slugs are active for 6 h (Newell, 1971) and that during slug activity relative humidity is 100%. Therefore, the model specimen secretes 4.58 g d wt mucus (85.9 kJ) during its life (Table 5). Considering the monthly average temperature in the litter layer, the model specimen respire 2.0 l CO₂ (43.5 kJ) during its life (Table 5). The regression line between dry weight of *A. rufus* and weight of faecal materials excreted was similar in summer following wet periods and in autumn (Table 2). Therefore, data of both seasons were pooled, resulting in the regression equation $F = 19.3 \times W^{0.96}$. For June, July and August the excretion

of faecal materials was calculated from the regression equation of data from summer following periods of drought, which is $F = 10.6 \times W^{0.71}$. As a result, during its life the model specimen excretes 3.2 g d wt faecal materials (68.5 kJ; Table 5). The consumption rate of the model specimen was calculated from the excretion of faecal materials using the assimilation efficiency of 75%. This value is closer to that of mature slugs (72%) than to that of juveniles (84%), because the contribution of the model specimen to total consumption rate is higher in the mature than in the juvenile stage. As a result, the model specimen consumes 5.96 g carbon during its life, which is equivalent to 13.2 g dry plant material and 248.7 kJ.

Summing up, the energy budget of the model specimen in the Göttinger Wald is:

$$248.7 \approx 27.2 + 10.9 + 85.9 + 43.5 + 68.5 \text{ [kJ]} \\ (C \approx P_g + P_r + P_m + R + F)$$

The Energy Budget of the Population of *Arion rufus*

Using data of Table 1, the number of mature *A. rufus* per m² was determined. Slugs with a dry weight of 0.75 g and more were regarded as mature, and only those catching periods were considered in which slugs deposit eggs (August-October). As a result, the number of mature specimens per 12 m² was two and seven in the year 1995 and 1996, respectively, the average number being one mature slug per 3 m². Based on this abundance, the energy budget of the population of *A. rufus* was calculated. It is assumed that each mature slug lays one egg clutch in autumn, which consists of 61 eggs. In November, 61 juveniles hatch, of which one specimen survives until July. In September, this specimen deposits an egg clutch and then dies. Based on these data, the monthly abundance of *A. rufus* in the Göttinger Wald was calculated using a survivorship curve. Survivorship curves of slug populations in forest ecosystems are not known, but those of laboratory cultures (Szabó & Szabó, 1929) and of slug populations on permanent pasture (South, 1989) revealed a more or less constant mortality rate throughout life. Therefore, a survivorship curve with a constant mortality rate was used, which is $N_t = N_0 e^{-rt}$, where N_0 = number of juveniles hatched, N_t = number of slugs at time t , r = death rate, and t = time (Slobodkin, 1962). Using the abundance data, monthly secretion of mucus, respiration and excretion

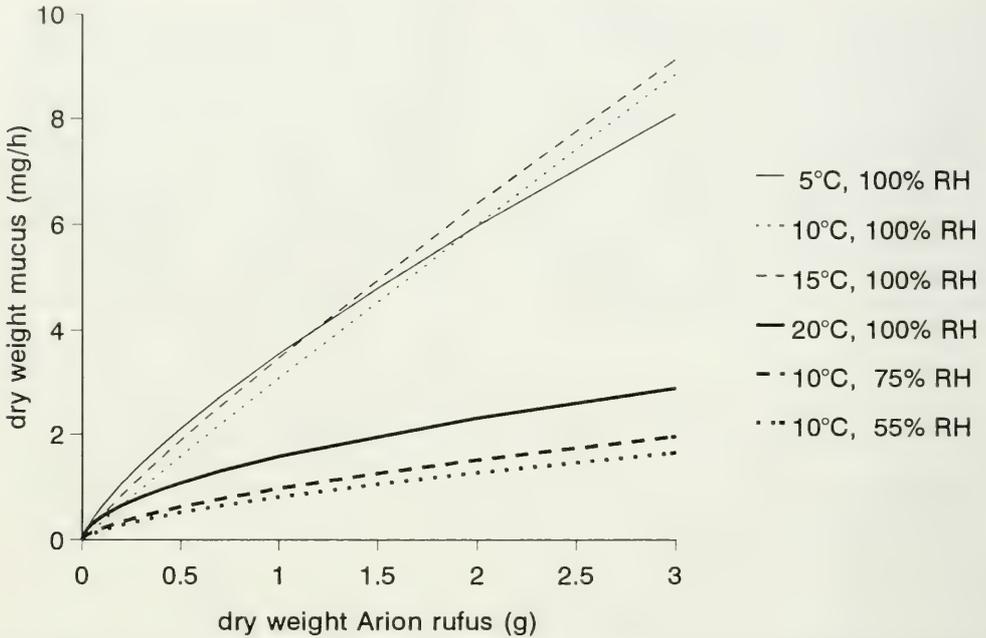


FIG. 1. Weight of mucus secreted by *A. rufus* at different temperatures and relative humidities as a function of animal dry weight. Regression equations are for 5°C and 100% RH: $S = 3.55 \times W^{0.75}$ ($n = 49, R^2 = 0.81, p(\text{slope}) < 0.001$), for 10°C and 100% RH: $S = 3.08 \times W^{0.96}$ ($n = 53, R^2 = 0.83, p(\text{slope}) < 0.001$), for 15°C and 100% RH: $S = 3.47 \times W^{0.88}$ ($n = 45, R^2 = 0.52, p(\text{slope}) < 0.001$), for 20°C and 100% RH: $1.58 \times W^{0.55}$ ($n = 34, R^2 = 0.54, p(\text{slope}) < 0.001$), for 10°C and 75% RH: $S = 0.97 \times W^{0.64}$ ($n = 60, R^2 = 0.79, p(\text{slope}) < 0.001$), and for 10°C and 55% RH: $S = 0.81 \times W^{0.65}$ ($n = 54, R^2 = 0.66, p(\text{slope}) < 0.001$), where RH = relative humidity, $S = \text{d wt mucus [mg h}^{-1}\text{]}$, $W = \text{d wt } A. rufus \text{ [g]}$, $n = \text{number of slugs examined}$.

TABLE 5. Mean monthly temperature in the litter layer and relative humidity (RH) 2 m above ground (Göttinger Wald, mean data of the years 1991–1995). Mean monthly fresh weight (F wt) and dry weight (D wt) of the model specimen of *Arion rufus*. Monthly secretion of mucus (Mucus), respiration and excretion of faecal materials (F) of the model specimen.

Month	Temp [°C]	Temp. [°C] rounded off	RH [%]	F wt [g]	D wt [g]	Mucus [mg d wt]	Respiration [ml CO ₂]	F [mg d wt]
September	11.2	10	85			egg stage		
October	8.2	10	89			egg stage		
November	4.4	5	92	0.05	0.01	20	0.7	7
December	2.0	5	92	0.05	0.01	21	0.7	7
January	1.0	5	87	1	0.15	160	17.1	97
February	0.3	5	85	1	0.15	144	15.5	87
March	2.7	5	78	3	0.46	368	55.8	284
April	5.7	5	72	3	0.46	356	54.0	275
May	9.1	10	71	6	0.92	528	247.7	552
June	11.4	10	81	6	0.92	511	239.8	300
July	14.3	15	78	9	1.38	857	514.8	412
August	14.7	15	75	9	1.38	857	514.8	412
September	11.2	10	85	9	1.38	756	348.5	789
Sum						4578	2009.4	3222
Sum [kJ]						85.9	43.5	68.5

TABLE 6. Number of *Arion rufus*, monthly secretion of mucus, respiration and excretion per 3 m².

Month	<i>A. rufus</i> [Number]	Mucus [mg d wt]	Respiration [ml CO ₂]	Faecal Material [mg d wt]
September			----- egg stage -----	
October			----- egg stage -----	
November	61	1220	43	427
December	36.5	767	26	256
January	21.9	3504	374	2124
February	13.1	1886	203	1140
March	7.8	2870	435	2215
April	4.7	1673	254	1293
May	2.8	1478	694	1546
June	1.7	869	408	510
July	1	857	515	412
August	1	857	515	412
September	1	756	349	789
Sum		16737	3816	11124
Sum [kJ]		315.8	82.8	239.2

was calculated (Table 6). The consumption rate of the population was calculated using an assimilation efficiency of 80%. As a result, the annual consumption rate of the population is 8.7 g carbon m⁻², which is equivalent to 19.3 g dry plant material and 361.9 kJ. The annual investment of the population of *A. rufus* into the somatic growth was calculated as sum of dry weight of slugs, that die each month, which is 2.6 g d wt m⁻² (52.5 kJ).

Summing up, the energy budget of the population of *A. rufus* in the Göttinger Wald is:

$$361.9 \approx 52.5 + 3.6 + 105.3 + 27.6 + 79.7$$

$$[\text{kJ m}^{-2} \text{ yr}^{-1}]$$

$$(C \approx P_g + P_r + P_m + R + F)$$

DISCUSSION

Slug populations are difficult to estimate, because, particularly during periods of drought, slugs hide underground, where they can stay for up to three months (Künkel, 1916). For the determination of slugs in the field, Ferguson et al. (1989) constructed defined-area traps, which consisted of an iron ring covering 0.1 m². A wet sacking was placed into the traps to encourage the slugs to remain above ground. In the present investigation, defined-area traps similar to those of Ferguson et al. (1989) were constructed. These traps cover a larger area (1 m²) than those of Ferguson et al. (1989), and a glass jar with beer is imbedded into the soil inside the traps, because beer is supposed to be a good baiting medium for slugs. However, during periods of drought, only low number of

slugs were caught by the traps, presumably because the slugs hid underground. Obviously, during dry seasons the slug number is underestimated by the defined-area trap method. Furthermore, the defined-area traps are not suitable for the determination of the age distribution of slug populations, because in the present investigation the number of juvenile slugs was much lower than one would expect from the number of adult specimens (Table 1). Presumably juvenile slugs are not baited by the beer as efficiently as mature slugs. As a consequence of these shortcomings of traps, in the present investigation the energy budget of the population of *A. rufus* was calculated using data on the abundance of mature slugs only, and the monthly population structure of *A. rufus* derived from a survivorship curve. However, the abundance of mature specimens was calculated from the average value of two abundance values only, which were strongly dissimilar (two and seven mature *A. rufus* per 12 m² in the year 1995 and 1996, respectively). Furthermore, reproduction does occur at various times of the year and therefore our model is too simple. Finally, it was assumed that the mortality rate of *A. rufus* remains constant throughout life. This assumption was based on observations on other slug species made in the laboratory and on permanent pasture (Szabó & Szabó, 1929; South, 1989). However, respective data on *A. rufus* in the forest ecosystem are not available, and therefore the survivorship curve used in the present investigation is just a broad estimate. Therefore, when evaluating the energy budget of the population of *A. rufus* it should be kept in mind that this budget

is based on a broad estimate of slug abundance.

Mean biomass of the population of *A. rufus* was $0.2 \text{ g d wt m}^{-2}$. Corsmann (1990) determined the biomass of *A. rufus* in the forest investigated in the present study by hand sorting and found a very similar biomass ($0.19 \text{ g d wt m}^{-2}$). This accordance shows that the defined-area trap of the present study is a suitable tool for the determination of slug biomass in forest ecosystems. Biomass data of *A. ater* in different beech forests are comparable to data of *A. rufus* found in the present investigation. Jensen (1975) estimated $0.27 \text{ g d wt m}^{-2}$ in a forest in Denmark, and Jennings & Barkham (1976) calculated $1.5 \text{ g fresh weight m}^{-2}$ in Great Britain, which is equivalent to a dry weight of 0.2 g m^{-2} . The similar biomass of *A. rufus* and *A. ater* in different beech forests suggests that slug biomass is limited by the same factor in different forests. Jennings & Barkham (1979b) suggested that on windy nights and during drought or sub-zero temperatures, intraspecific competition for food may occur, because slugs remain low on the ground or beneath the litter layer where green food is unavailable. However, another limiting factor of slug biomass may be the number of hiding places in the forest soil. Hiding places are essential for the survival during drought or harsh weather conditions. In different forest ecosystems, the number of hiding places might be similar, and therefore the regulation of slug density through the number of hiding places would result in a similar slug biomass in different beech forests. This hypothesis is supported by the fact, that slugs usually keep the same hiding place for a long period of time ("homing"; Frömring, 1954; Cook, 1979).

McNeill & Lawton (1970) calculated a regression line between annual production and respiration of short-lived poikilotherms being $\log R = 1.17 \log P + 0.14 [\text{kcal}]$. The insertion of the production of *A. rufus* ($P = P_g + P_r + P_m$) into this equation results in a respiration value which is 60 times of that actually calculated in the present investigation. However, in their calculations, McNeill & Lawton (1970) explicitly omitted data on animals characterized by high mucus secretion, and when P_m was excluded from our calculations the respiration value of *A. rufus* would be one fourth of that expected by McNeill and Lawton's equation. The very high net population production efficiency may be a consequence of life history characteristics of *A. rufus*. This slug species

builds up a very high biomass within just one year, reproduces and then dies. Therefore, no standing crop of older individuals has to be maintained. However, in the present investigation the respiration of *A. rufus* was extrapolated from laboratory measurements at three temperatures to the field. This extrapolation of course is too simple, because in the field slugs are exposed to a complex environment with fluctuating biotic and abiotic conditions. In a further experiment, the respiration rate of *A. rufus* should be measured in the field.

In the present investigation, the secretion of mucus constitutes an important component of the energy budget; however, the determination of this parameter implies some inaccuracies. First, handling of slugs in the laboratory might have stimulated mucus production during incubation, causing an artificial high value of P_m . However, preceding experiments showed that the amount of mucus secreted during the first hour of incubation in the laboratory was not significantly different from that secreted during the second and third hour. Second, the secretion of mucus during copulation of slugs was not considered in the present calculation. Since copulation is very expensive in mucus and since slugs may copulate several times, the amount of mucus lost due to copulation might cause a significant increase in P_m . Third, when determining the energy content of mucus of *A. rufus* in the present investigation, the mean of calorific values of the mucus of two snail species were used (23.9 and $13.9 \text{ kJ g}^{-1} \text{ dw mucus}$ for *Lymnaea stagnalis* and *Cepaea nemoralis*, respectively). Obviously, such calorific values vary widely interspecifically, and had another value been used the outcome of the energy budget would have been different. Fourth, it was assumed that during a period of 24 h slugs are active for 6 h. However, during cold seasons, slug activity may be very low, resulting in low mucus secretion. If between November and April (months of an average temperature of 5°C ; Table 5) zero mucus secretion is assumed, the term P_m of the energy budget of the population of *A. rufus* would decrease by 35%.

The exponent b in the relationship between the weight of mucus secreted by *A. rufus* and animal dry weight was about 1 at favourable (100% RH, $5\text{--}15^\circ\text{C}$), and about $2/3$ at unfavourable conditions (20°C or low RH, Fig. 1). This dichotomy may reflect the different shapes of slugs during moving, being more or less flat at favourable and more or less spher-

ical (rather elliptic) at unfavourable abiotic conditions. In general, doubling in weight of a flat body results in doubling of the surface ($b = 1$), whereas the surface of a spherical body doubles when its weight increases by a factor of 3 ($b = 2/3$).

Denny (1980) stated that gastropod crawling is the most costly form of locomotion in the animal kingdom. Actually, in the present investigation, the secretion of mucus of the model specimen (85.9 kJ) comprises 69% of total production and therefore is an important component of the energy budget. In many calculations of the energy flow through molluscs, the secretion of mucus is ignored (Paine, 1965; Stern, 1969; Hughes, 1970; Mason, 1971; Jensen, 1975; Jennings & Barkham, 1976; Streit, 1976; Andreassen, 1981; Phillipson & Abel, 1983; Workman, 1983). The secretion of mucus is considered in the calculations of Carefoot (1967), Paine (1971), Kofoed (1975), Richardson (1975), Otto (1976), Edwards & Welsh (1982), Horn (1986), Davies et al. (1990), and Santini et al. (1995). Calow (1974) calculated for freshwater gastropods that 13–23% of the assimilated energy is lost via mucus. The respective value for *Cepaea nemoralis* is 12% (Richardson, 1975), for *Ilyanassa obsoleta* 80% (Edwards & Welsh, 1982), and for *Patella vulgata* 52% (Davies et al., 1990). In the present investigation, the model specimen of *A. rufus* invested 51% of the assimilated energy into the secretion of mucus. Most obviously, the considerable investment of gastropods in locomotion limits the range of conditions under which populations can survive. In the light of this high investment of energy into mucus, the question arises as to which ecosystem characteristics and slug qualities allow the relative high slug biomass in the beech forest ecosystem? First, the dense herb layer of the beech forest provides plenty of food for population growth, at least during favourable weather conditions. Second, since slugs are omnivorous, their food intake is independent of single food items, and therefore food limitation becomes rare. Third, the high assimilation efficiency of gastropods allows an exceptionally high efficiency in food utilization. These factors might contribute to the high biomass of gastropods in the beech forest ecosystem and make up for the high cost of gastropod crawling.

In this and the following section, data obtained for *A. rufus* are used to draw broad conclusions about the energetics of the gastropod community in the forest ecosystem

studied. For example, it is assumed that the biomass/excretion ratio of all gastropod species in the forest ecosystem studied is identical to that of *A. rufus*. This of course is a broad generalization, because there may be marked differences in the ecological efficiencies of different pulmonates (Lamotte & Stern, 1987). Therefore, the calculated values of excretion and consumption of the gastropod community should be regarded as a broad estimate. The production of faecal materials of the population of *A. rufus* in the forest ecosystem studied is equivalent to $1.7 \text{ g carbon m}^{-2} \text{ yr}^{-1}$, which is $3.7 \text{ g d wt faecal materials m}^{-2} \text{ yr}^{-1}$. The biomass of *A. rufus* makes up 19% of total biomass of gastropods in the forest studied (Corsmann, 1990). Assuming that the biomass/excretion ratio of all gastropod species in the forest studied is identical to that of *A. rufus*, the gastropod community deposits $19 \text{ g m}^{-2} \text{ yr}^{-1}$ as faeces. In comparison, the excretion of faecal materials of lumbricids (more than $10 \text{ kg m}^{-2} \text{ yr}^{-1}$; Scheu, 1987) is much higher, which shows that the relative contribution of the gastropod community to the bioturbation in the forest soil is negligible.

The population of *A. rufus* consumes $19.3 \text{ g d wt food m}^{-2} \text{ yr}^{-1}$ in the forest studied. The input of canopy litter fall in this forest is $331 \text{ g d wt m}^{-2} \text{ yr}^{-1}$ (leaves and bud scales; Schaefer, 1990). About 2.6% of food material of *A. rufus* is leaf litter (Corsmann, 1990), and therefore the population of *A. rufus* has an annual consumption of $0.50 \text{ g d wt leaf litter m}^{-2}$, which represents 0.15% of the leaf litter input per year. Corsmann (1990) determined the biomass of the most abundant gastropod species of the beech forest studied. Furthermore, the author examined the food material in the gut of these gastropod species and determined the relative amount of certain food material (e.g., leaf litter, green plant material, fungi) in their gut. Considering these data and assuming that the biomass/excretion ratio of all gastropod species in the forest studied is identical to that of *A. rufus*, the gastropod community consumes $8.1 \text{ g d wt leaf litter m}^{-2} \text{ yr}^{-1}$, which is 2.4% of the annual leaf litter input. This value compares with those obtained by Phillipson (1983) and Phillipson & Abel (1983) in a beech forest. The authors calculated that the gastropod community consumes 1.8–2.9% of leaf litter input per year. Much higher values were calculated by Jennings & Barkham (1976, 1979a). These authors found that the population of *A. rufus* consumes 1.54% and that the slug commu-

nity consumes 8.4% of the annual leaf litter input of a beech forest, and therefore makes a significant direct contribution to plant decomposition processes. In contrast, data of the present investigation suggest that the direct contribution of the gastropod community to decomposition processes is low.

Mean above- and below-ground net primary production (NPP) of the herb layer in the forest studied is $100 \text{ g d wt m}^{-2} \text{ yr}^{-1}$ (Schaefer, 1990). Green herb material makes up 17% of the food material of *A. rufus* (Corsmann, 1990), and therefore the population of *A. rufus* consumes $3.3 \text{ g d wt green herb material m}^{-2} \text{ yr}^{-1}$, which represent 3.3% of the annual production of green plant material. The gastropod community has a consumption of $26.1 \text{ g d wt green herb material m}^{-2} \text{ yr}^{-1}$, which represent 26% of the annual production of green plant material. This phytomass compares well with that which is annually ingested by phytophages (Curculionidae and lepidopteran larvae) in the forest studied (Schaefer, 1990), and therefore the relative consumption of green plant material by the gastropod community is high.

The NPP of higher plants in the forest studied is $2078 \text{ g d wt m}^{-2} \text{ year}^{-1}$ (Schaefer, 1990), and the population of *A. rufus* consumes 0.24% of the annual NPP. The gastropod community consumes $34.5 \text{ g d wt higher plants m}^{-2} \text{ year}^{-1}$, which is 1.7% of the NPP of higher plants. Obviously, the gastropod community consumes a very small part of the phytomass in the forest ecosystem and therefore most probably the feeding pressure of gastropods on plants is low. This is partly due to the fact that gastropods are omnivorous and that therefore the strength of predation diffuses to different trophic unities (Polis, 1994). It is stated by many authors (e.g., Hairston et al., 1960; Fretwell, 1987; Oksanen et al., 1981) that herbivores of forest ecosystems consume only a small part of the phytomass, because their biomass is controlled by predators (top-down control). The low feeding pressure of gastropods on higher plants, however, most probably is not caused by top-down forces, because gastropod biomass is limited by abiotic factors (e.g., drought; Jennings & Barkham, 1979b).

The energy budget of the present investigation is based on a series of assumptions, which are strongly subjective. For example, the population structure of *A. rufus* was calculated using data on the abundance of mature

slugs and on the mean number of eggs per clutch and a survivorship curve. "Mature slugs" were defined as specimens with a dry weight of 0.75 g and more, and had another weight been taken the abundance of mature specimens would have been different. The survivorship curve used is a broad estimate taken from the literature, and it therefore represents another shortcoming of the present investigation. Furthermore, the conversion of data into calorific values was accomplished with conversion factors from the literature, and therefore the calorific energy budget should be evaluated with caution. In addition, the monthly amount of respiration, secretion of mucus and excretion of faecal materials of *A. rufus* was calculated using mean monthly temperature and air humidity values of the forest under study. Of course, the abiotic characteristics of the forest ecosystem are incompletely simulated by this approach. However, the energy budgets of populations in general embody many assumptions about population structure and generally lack true seasonal changes in budget terms. The energy budget of the present investigation should be regarded as a broad estimate.

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SPECIES OF *MACOMA* (BIVALVIA: TELLINIDAE) FROM THE PACIFIC COAST OF RUSSIA, PREVIOUSLY DESCRIBED AS *ABRINA* (BIVALVIA: SEMELIDAE)

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ABSTRACT

Abrina cuneipyga Scarlato, 1981; *A. sachalinica* Scarlato, 1981; *A. shiashkotanika* Scarlato, 1981; and *A. tatarica* Scarlato, 1981, described from the Pacific coast of Russia belong instead in the genus *Macoma* Leach, 1819. For one species, a new combination is suggested, *Macoma cuneipyga* (Scarlato, 1981). *Abrina sachalinica*, *A. tatarica* and *A. shiashkotanika* are synonyms of *Macoma loveni* (Jensen, 1905), *Macoma calcarea* (Gmelin, 1791), and *Macoma sp.*, respectively. The main morphological characteristic on the basis of which these species were previously included in *Abrina* Habe, 1952, was the presence of an internal ligament in an oblique resilifer posterior to the cardinal teeth. Studies of the common northwestern Pacific *M. loveni*, *Macoma balthica* (Linné, 1758), *Macoma crassula* (Deshayes, 1855), *Macoma lama* Bartsch, 1921, *Macoma incongrua* (Martens, 1865), and *M. calcarea* (Gmelin, 1791) show the presence of a similar morphological characteristic in young specimens. In *Macoma*, this characteristic is reduced as the molluscs grow, but it is preserved in adult *Abrina*. The results of a comparative analysis of species of genera *Abrina* and *Macoma*, and expanded descriptions of *M. cuneipyga*, *M. loveni*, and *Macoma sp.*, are presented.

Key words: *Macoma*, *Abrina*, northwestern Pacific, systematics, morphology, distribution.

INTRODUCTION

Scarlato (1981), in his monograph on fauna and distribution of bivalve molluscs of the northwestern Pacific, gave a description with very schematic pictures of the type specimens of four new species of the genus *Abrina* Habe, 1952 (Fam. Semelidae Stoliczka, 1870): *Abrina cuneipyga* Scarlato, 1981; *A. sachalinica* Scarlato, 1981; *A. shiashkotanika* Scarlato, 1981; and *A. tatarica* Scarlato, 1981. Previously, representatives of this genus were not mentioned in lists of the Russian molluscan fauna and were found only in subtropical latitudes of the western Pacific (Gould, 1861; Kuroda, 1951; Habe, 1952, 1958, 1977, 1981).

A study of the type specimens of *Abrina* species from the Pacific coast of Russia, as well as material of *Macoma* species from the Arctic seas and the northwestern Pacific, and of *Abrina* species from Japan, have shown that the species described by Scarlato (1981) should instead be included in the genus *Macoma* Leach, 1819 (Fam. Tellinidae Blainville, 1814).

For one of the four species described by Scarlato (1981) as *Abrina*, a new combination is suggested, *Macoma cuneipyga* (Scarlato,

1981). *Abrina sachalinica*, *A. tatarica*, and *A. shiashkotanika* are considered to be synonyms of *M. loveni*, *M. calcarea* and *Macoma sp.*, respectively. The goal of this paper is to present the results of a comparative analysis of species of genera *Abrina* and *Macoma* and to give an expanded description of *M. cuneipyga*, *M. loveni* and *Macoma sp.*, supplemented by new data on their shell morphology, ecology, and geographical distribution.

MATERIALS AND METHODS

In this study we have used the material collected by expeditions of the following research institutes:

PRIFO expeditions on the shelf zone of the northern Sea of Okhotsk (R/V "8-452", 1977; R/V "8-461", 1979), eastern coast of Sakhalin Island, Sea of Okhotsk (R/V "8-452", 1977), western coast of Kamchatka, Sea of Okhotsk (R/V "Professor Levanidov", 1996), eastern coast of Kamchatka, Pacific Ocean (R/V "Mys Tikhyy", 1985), and Bering Sea (R/V "Mys Tikhyy", 1984). A joint IMB-PRIFO expedition on the shelf and bathyal zones of the Kuril Islands (R/V "Tikhookeansky", 1987).

A joint FERHI-SakhRIFO expedition under

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the supervision of ENL Company on the shelf zone of the northeastern Sakhalin Island, Sea of Okhotsk, in the vicinity of an oil-drilling site (R/V "Pavel Gardienko", 1997).

Expeditions of IMB on the shelf zone of the Tatar Strait, Sea of Japan (R/V "Atna", 1974; R/V "Beril", 1977) and Peter the Great Bay, Sea of Japan (R/V "Ametist", 1983). A joint IMB-PIBOC expedition on the shelf zone of the Sea of Japan (R/V "Akademik Oparin", 1995).

The material from the western Kamchatka, Sea of Okhotsk, southeastern Kamchatka, Pacific Ocean, and the Bering Sea was fixed and stored in 4% formaldehyde in PRIFO. All the other material was fixed in 70% ethanol and stored dry in IMB.

We have also used collections of the following taxa: *M. balthica* from the Bering Sea (MIMB); *M. calcarea*, *M. crassula* and *M. incongrua* from the Sea of Japan (MIMB); *M. lama* from the Sea of Okhotsk (MIMB); *M. loveni* from the Arctic seas, the Sea of Japan, the Sea of Okhotsk, and the North Atlantic (USNM, ZIN); *Abrina lunella* (Gould, 1861) and *A. kinoshitai* (Kuroda & Habe, 1958) from the coastal waters of Japan (Drs. T. Kurozumi and E. Tsuchida, NHMI, NSMT); *A. cuneipyga*, *A. sachalinica*, *A. shiashkotanika*, and *A. tatarica* from the Pacific seas of Russia (ZIN). *Macoma* from the Arctic and Pacific seas of Russia were fixed and stored in 70% ethanol. All other material was stored dry.

For the collection material stored in ZIN, inventory numbers are given for holotypes; for other specimens, their catalogue numbers are given.

Shell Measurements

For measurements, we have chosen those parameters most often used in diagnosis, description, and comparative analysis of species of the genus *Macoma* (Dunnill & Ellis, 1969; Coan, 1971; Scarlato, 1981; Kamenev, 1989, 1990; Kafanov et al., 1997).

Figure 1 shows the position of our shell morphology measurements. Shell length (L), height (H), width of each valve (W) (not shown), anterior end length (A), maximal distance from posterior shell margin to top of pallial sinus (L1), and minimal distance from top of pallial sinus to anterior adductor muscle scar (L2) were measured for each valve. The ratios of these parameters to shell length (H/L, W/L, A/L, L1/L, L2/L, respectively) were determined. Shell measurements were made

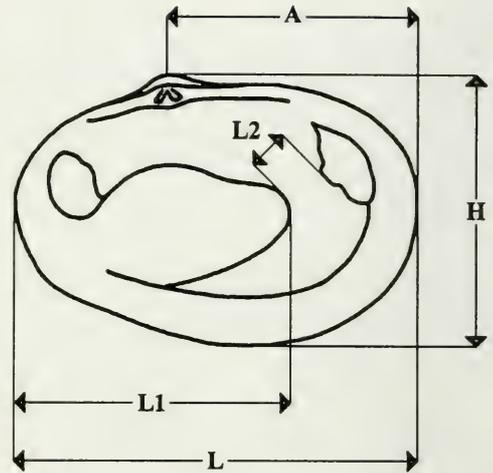


FIG. 1. Placement of shell measurements: L—shell length; H—height; A—anterior end length; L1—maximal distance from posterior shell margin to top of pallial sinus; L2—minimal distance from top of pallial sinus to anterior adductor muscle scar.

using a calipers and an ocular micrometer with an accuracy of 0.1 mm.

We made measurements of:

- (1) 51 specimens and 2 left valves of *M. loveni* from Spitzbergen (USNM 108789, 2 spec.); Laptev Sea (ZIN 106, 3 spec.); Gulf of St. Lawrence, North Atlantic (USNM 95638, 1 spec., 1 left valve); Gulf of Maine, North Atlantic (USNM 159769, 1 spec.); Bering Sea (our material, 1 spec.); Kronotsky Bay, eastern Kamchatka, Pacific Ocean (our material, 4 spec.); Sea of Okhotsk (ZIN 9853, holotype of *A. sachalinica*; ZIN 57, 2 spec.; USNM 204814, 1 spec.; our material, 5 spec.); Kuril Islands (our material, 19 spec.); Sea of Japan (ZIN 65, 3 spec.; ZIN 67, 1 spec.; USNM 2048015, 2 spec.; our material 5 spec., 1 left valve).
- (2) 22 specimens, 2 right, 7 left valves of *M. cuneipyga* from Kuril Islands (ZIN 9800, holotype of *A. cuneipyga*; ZIN 10, 1 left valve; our material, 18 spec., 2 right, 6 left valves); Sea of Okhotsk (our material, 3 spec.).
- (3) 3 specimens of *Macoma* sp. from Shiashkotan Island, Middle Kuril Islands (ZIN 9867, holotype of *A. shiashkotanika*; 2 spec. (ZIN 1) from the type locality).
- (4) 3 specimens and one right valve of *M. calcarea* from the Sea of Japan (ZIN 9900, holotype of *A. tatarica*; *A. tatar-*

- ica* (ZIN 5), 1 spec.); Sea of Okhotsk (*A. tatarica* (ZIN 6), 1 spec., 1 left valve).
- (5) 2 specimens, 5 right, 2 left valves of *A. lunella* from Sagami Bay, Japan (NSMT 45451).
 - (6) 1 specimen, 1 right, 7 left valves of *A. kinoshitai* from Esu-zaki, Wakayama Prefecture, Japan (NSMT 51909, 1 spec.); Funakashi Bay, Iwate Prefecture, Japan (material of Drs. T. Kurozumi and E. Tsuchida (NHMI, no number); 1 left, 1 right valves), Otsuchi Bay, Iwate Prefecture, Japan (material of Drs. T. Kurozumi and E. Tsuchida (NHMI, no number), 6 left valves).

Statistics

Statistical analysis of the material used a package of statistical programs for Windows STADIA 6.0 (Kulaichev, 1996) and STATGRAPHICS.

The calculated indices (H/L; W/L; A/L; L1/L and L2/L) are less susceptible to change compared to other measured parameters. A comparison by pairs of different parameters of the samples using parametric and nonparametric tests was conducted using these indices. All data was tested with a Kolmogorov test for their fit to a normal distribution. For the left valves of *M. loveni* and *M. cuneipyga*, the original data on L2/L and the \log_{10} of H/L corresponded to the norm; for the right valves of these species, original data on H/L, A/L and L2/L also corresponded to the norm. For this reason, we used the Student (T) parametric test for a comparison by pairs of different valves of *M. loveni* and *M. cuneipyga* for these indices.

The distribution of the original data by other indices was different from the norm. Fifteen transformations were used in an attempt to bring the obtained data to the norm; however, none were successful. Therefore, in the comparative analyses of the samples of other indices (W/L, A/L, and L1/L for left valves and W/L and A/L for right valves), we used the nonparametric criterion of Kolmogorov-Smirnov (two sample test).

We used a discriminant analysis to test the validity of the hypothesis of division of all studied specimens into two groups (*M. loveni* and *M. cuneipyga*).

In linear and stepwise multiple discriminant analyses of the samples of *M. loveni* and *M. cuneipyga*, we used original data on the parameters H, L, W, L1, L2. Data on these

parameters corresponded to the norm; 51 specimens of *M. loveni* and 22 specimens of *M. cuneipyga* were used for the analysis.

Throughout this study, statistical significance was defined as $P < 0.05$.

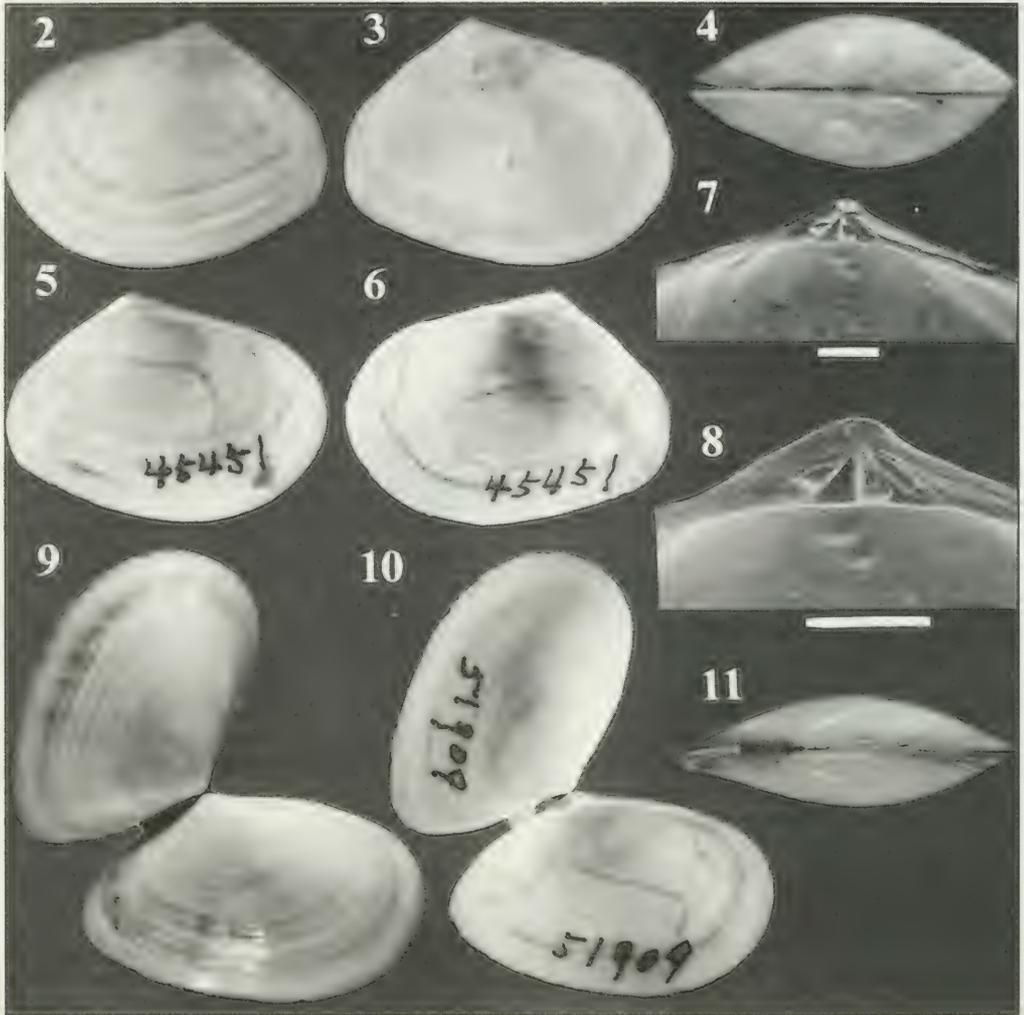
The following abbreviations are used in the paper: ENL—commercial company “Exon Neftegas Limited”; FERHI—Far East Research Hydrometeorological Institute, Vladivostok; IMB—Institute of Marine Biology, Russian Academy of Sciences, Vladivostok; MIMB—Museum of the Institute of Marine Biology, Vladivostok; NHMI—Natural History Museum and Institute, Chiba; NSMT—National Science Museum, Tokyo; PIBOC—Pacific Institute of Bio-organic Chemistry, Russian Academy of Sciences, Vladivostok; PRIFO—Pacific Research Institute of Fisheries and Oceanography, Vladivostok; SakhRIFO—Sakhalin Research Institute of Fisheries and Oceanography, Yuzhno-Sakhalinsk; USNM—United States National Museum of Natural History, Smithsonian Institute, Washington, D. C.; ZIN—Zoological Institute, Russian Academy of Sciences, St. Petersburg.

COMPARATIVE ANALYSIS

In Tables 1 and 2 the results of a morphometric analysis of the common Japanese spec. *Abrina lunella* and *A. kinoshitai* are presented (Figs. 2–11). On the basis of the results of these studies, as well as descriptions, pictures and photos of *Abrina* (Gould, 1861; Kuroda, 1951; Habe, 1952, 1958, 1961, 1964, 1977, 1981; Ito, 1967; Kuroda et al., 1971; Ito et al., 1986; Ito, 1989; Tsuchida & Kurozumi, 1995) and *Macoma* species (Golikov & Scarlato, 1967; Keen, 1969; Coan, 1971; Bernard, 1979; Lubinsky, 1980; Scarlato, 1981), Table 3 has been constructed giving the main diagnostic characteristics of *Abrina* and *Macoma*.

Table 3 shows that the genera *Abrina* and *Macoma* are similar in most morphological characteristics. The main distinguishing characteristic of *Abrina* is the presence of a well-developed internal ligament in a resilifer, a narrow groove posterior to the cardinal teeth. In *Macoma*, an internal ligament is absent.

The results of studies different age groups of the well-identified and widely distributed northwestern Pacific *M. loveni*, *M. balthica*, *M. calcarea*, *M. crassula*, *M. incongrua* and *M. lama* have shown that young individuals of these species have a rather large, well-devel-



FIGS. 2–11. Shells of *Abrina* species. 2–8. *Abrina lunella* (Gould, 1861) (NSMT 45451), Sagami Bay, Japan. 2–6: Largest specimen, shell length 16.1 mm. 7, 8: Hinge of the right valve, valve length 13.0 mm (bar = 1 mm). 9–11. *Abrina kinoshitai* (Kuroda & Habe, 1958) (NSMT 51909), Esu-zaki, Wakayama Prefecture, Japan, shell length 14.8 mm.

oped internal ligament (Figs. 12–21). In its form, position, and size it is similar to the internal ligament of the genus *Abrina*. It is situated in an oblique resilifer posterior to the cardinal teeth and almost reaches the ventral edge of the hinge plate (Figs. 12, 13). With age, its relative size is reduced (Figs. 14, 21), and in adult specimens of *M. crassula*, *M. balthica*, *M. calcarea*, *M. incongrua* and *M. lama*, it disappears. In adult specimens of *M. loveni*, it can just be noticed on the hinge plate ventral to the beaks.

Also, the form of the resilifer changes with age. In young specimens of *M. crassula*, *M.*

balthica, *M. calcarea*, *M. incongrua*, and *M. lama* (shell length, <5–6 mm) and *M. loveni* (< 10 mm), the form and size of the resilifer relative to the width of the hinge plate (Figs. 15, 16, 18, 20) is similar to those of adult *Abrina lunella* and *A. kinoshitai*. In larger specimens (> 8–10 mm), the resilifer is much shorter than the width of the hinge plate and ovate (Fig. 19). With the exception of *M. loveni*, in adult specimens of all investigated species it is absent. In adult specimens of *M. loveni*, it is preserved in the form of ovate pit on hinge plate ventral to the beaks posterior to the cardinal teeth (Fig. 17).

TABLE 1. *Abrina lunella* (Gould, 1861). Shell measurements (mm), indices and summary statistics of all characteristics (NSMT 45451): L — shell length; H — height; W — width; A — anterior end length; L1 — maximal distance from posterior margin to the top of pallial sinus; L2 — minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates shell measurements and indices for the left valve, denominator — for the right valve. NM — not measured.

Statistics	L	H	W	A	L1	L2	H/L	W/L	A/L	L1/L	L2/L
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	7.8	6.0	1.7	4.8	6.2	0.8	0.77	0.22	0.62	0.79	0.10
	10.8	7.7	2.1	6.6	7.2	1.5	0.71	0.19	0.61	0.67	0.14
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	11.1	7.9	2.4	7.0	7.6	1.8	0.71	0.22	0.63	0.68	0.16
	11.4	8.0	2.1	7.2	8.0	1.3	0.70	0.18	0.63	0.70	0.11
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	11.9	8.2	2.5	7.6	7.7	2.3	0.69	0.21	0.64	0.65	0.19
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	13.0	10.0	3.0	8.0	8.3	2.3	0.69	0.21	0.64	0.65	0.19
	14.1	10.2	2.8	9.1	9.5	2.4	0.72	0.20	0.65	0.67	0.17
	14.0	10.3	3.2	9.1	9.0	3.0	0.74	0.23	0.65	0.64	0.21
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	15.0	10.5	3.3	9.0	10.0	2.5	0.70	0.22	0.60	0.67	0.17
	16.1	12.5	3.5	9.8	10.7	3.0	0.78	0.22	0.61	0.66	0.19
	16.1	12.5	4.0	9.8	11.4	2.4	0.78	0.25	0.61	0.71	0.15
Mean	13.10	9.60	2.63	8.18	8.85	2.05	0.728	0.198	0.625	0.675	0.153
	12.70	9.34	2.87	7.90	8.60	2.17	0.737	0.226	0.624	0.683	0.166
SD	2.46	2.23	0.67	1.52	1.56	0.79	0.036	0.017	0.019	0.017	0.035
	2.76	2.13	0.74	1.67	1.71	0.70	0.037	0.013	0.017	0.053	0.035
SE	1.23	1.12	0.34	0.76	0.78	0.40	0.018	0.009	0.010	0.009	0.018
	1.05	0.81	0.28	0.63	0.65	0.26	0.010	0.005	0.006	0.020	0.013
Min	10.8	7.7	2.1	6.6	7.2	1.3	0.70	0.18	0.61	0.66	0.11
	7.8	6.0	1.7	4.8	6.2	0.8	0.69	0.21	0.60	0.64	0.10
Max	16.1	12.5	3.5	9.8	10.7	3.0	0.78	0.22	0.65	0.70	0.19
	16.1	12.5	4.0	9.8	11.4	3.0	0.78	0.25	0.65	0.79	0.21

Thus, a well-developed internal ligament lodged in oblique resilifer in representatives of the genus *Macoma* is a juvenile characteristic that is preserved in *Abrina* during its entire life.

A study of the type material of *Abrina* species from the seas of Russia has shown that the main character for their description as new species of *Abrina* is the presence of an internal ligament posterior to the cardinal teeth (Fig. 22) and having different degrees of development. It is most distinct in *A. shiashkotanika* and *A. cuneipyga*. However, the type specimens of all the species of *Abrina* described by Scarlato (1981) have a less developed internal ligament compared with that of *Abrina* species inhabiting the coastal waters of Japan. The size of internal ligament of the holotypes of these species corresponds to their shell size and species, similar to the species of *Macoma* studied. In other taxonomic characteristics, they are also identical to the genus *Macoma*. All the new species described by Scarlato (1981) are of a relatively

small size. Therefore, we consider that Scarlato (1981) described new species of *Abrina* on the basis of a study of young specimens of *Macoma* that had internal ligaments. We consider that all species of *Abrina* described from the Pacific coast of Russia should be included in the genus *Macoma*.

SYSTEMATICS

Family Tellinidae Blainville, 1814
Genus *Macoma* Leach, 1819

Type species (by monotype): *Macoma tenera* Leach, 1819; = *Tellina calcarea* Gmelin, 1791

Diagnosis

Shell small to large in size (20 to 100 mm), medium in thickness to heavy, moderately inflated, ovate-triangular, rounded-triangular, ovate or round, white, chalky, smooth with

TABLE 2. *Abrina kinoshitai* (Kuroda & Habe, 1958). Shell measurements (mm), indexes and summary statistics of all characteristics: L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates shell measurements and indices for the left valve, denominator—for the right valve. NM—not measured.

Statistics	L	H	W	A	L1	L2	H/L	W/L	A/L	L1/L	L2/L
Otsuchi Bay (39°22'N, 142°00'E), Iwate Prefecture, Japan, depth 75 m, sandy mud											
	7.5	4.7	1.1	4.8	5.3	0.6	0.63	0.15	0.64	0.71	0.08
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	8.3	5.1	1.3	5.2	5.8	0.9	0.61	0.16	0.63	0.70	0.11
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	8.5	5.3	1.3	5.4	6.2	0.9	0.62	0.15	0.64	0.73	0.11
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	9.5	5.9	1.5	5.9	7.0	1.0	0.62	0.16	0.62	0.74	0.11
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	10.0	6.3	1.6	6.5	7.2	1.1	0.63	0.16	0.65	0.72	0.11
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	12.8	8.0	2.2	8.3	9.3	1.6	0.63	0.17	0.65	0.73	0.13
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
Funakochi Bay (39°23'N, 142°00'E), Iwate Prefecture, Japan, depth 70 m, mud and sand											
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	12.0	7.7	1.9	8.0	8.1	2.1	0.64	0.16	0.67	0.68	0.18
	12.7	8.0	2.2	8.3	9.3	1.5	0.63	0.17	0.65	0.73	0.12
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
Esu-Zaki, Wakayama Prefecture, Japan (NSMT 51909)											
	14.8	9.3	2.4	9.8	11.1	2.1	0.63	0.16	0.66	0.75	0.14
	14.8	9.3	2.4	9.8	10.2	3.0	0.63	0.16	0.66	0.69	0.20
Mean	10.51	6.58	1.70	6.78	7.65	1.21	0.625	0.160	0.643	0.726	0.114
	13.40	8.50	2.15	8.90	9.15	2.55	0.635	0.160	0.665	0.685	0.190
SD	2.61	1.66	0.50	1.81	2.04	0.49	0.008	0.008	0.013	0.016	0.018
	1.98	1.13	0.35	1.27	1.49	0.64	0.007	0	0.007	0.007	0.014
SE	0.92	0.59	0.18	0.64	0.72	0.17	0.003	0.003	0.005	0.006	0.006
	1.40	0.80	0.25	0.90	1.05	0.45	0.005	0	0.005	0.005	0.010
Min	7.5	4.7	1.1	4.8	5.3	0.6	0.61	0.15	0.62	0.70	0.08
	12.0	7.7	1.9	8.0	8.1	2.1	0.63	0.16	0.67	0.69	0.2
Max	14.8	9.3	2.4	9.8	11.1	2.1	0.63	0.17	0.66	0.75	0.14
	14.8	9.3	2.4	9.8	10.2	3.0	0.64	0.16	0.67	0.69	0.20

TABLE 3. Diagnostic characteristics of the genera *Abrina* and *Macoma*.

	<i>Abrina</i> Habe, 1952. Type species: <i>Abrina kanamarui</i> Kuroda, 1951; = <i>Macoma lunella</i> Gould, 1861	<i>Macoma</i> Leach, 1819. Type species: <i>Macoma tenera</i> Leach, 1819; = <i>Tellina calcarea</i> Gmelin, 1791
Shell form and proportions	Ovate or triangular-ovate, moderately inflated; anterior end longer than posterior; posterior end with radial ridge along posterodorsal margin, twisted to the right	Ovate to subtrigonal, moderately inflated; equivalve or with left valve somewhat larger; equilateral to longer anteriorly; posterior end with radial ridge along posterodorsal margin, twisted to the right
Shell surface	Smooth, with faint growth checkmarks	Smooth, with faint growth checkmarks
Periostracum	Thin, colorless, silky or shiny	Thin, dark to colorless, silky or shiny
Hinge	Weak, two cardinal teeth in each valve, lateral teeth absent	Weak, two cardinal teeth in each valve, lateral teeth absent
Ligament	Both external and internal: external—seated on a nymph not projecting above dorsal margin; internal—lodged in oblique groove behind cardinal teeth	External, seated on a nymph not projecting above dorsal margin
Pallial sinuses	Long, often of different length and form in right and left valves, partly confluent with pallial line	Long, often of different length and form in right and left valves, partly confluent with pallial line



FIGS. 12–21. Hinge of *Macoma* species. 12–17. *Macoma loveni* (Jensen, 1905). 12: Cardinal teeth and internal ligament of right valve (ZIN 106), Laptev Sea ($76^{\circ}38'N$, $118^{\circ}20'E$), 46 m, valve length 7.1 mm (bar = 1 mm). 13: Cardinal teeth and internal ligament of left valve, Iturup Island ($44^{\circ}23'4''N$, $147^{\circ}37'8''E$), 230 m, valve length 8.7 mm (bar = 1 mm). 14: Cardinal teeth, external and internal ligament of right valve (ZIN 65), western coast of Sakhalin Island, Tatar Strait, Sea of Japan, 84–86 m, valve length 13.4 mm (bar = 1 mm). 15: Cardinal teeth and resilifer of right valve, Iturup Island ($44^{\circ}23'4''N$, $147^{\circ}37'8''E$), 230 m, valve length 8.6 mm (bar = 100 μ m). 16: Cardinal teeth and resilifer of right valve (USNM 159769), Gulf of Maine, North Atlantic, valve length 5.0 mm (bar = 100 μ m). 17: Cardinal teeth and ligament pit of right valve, Shikotan Island ($43^{\circ}45'N$, $146^{\circ}55'E$), 100 m, valve length 15.6 mm (bar = 1 mm). 18, 19. *Macoma balthica* (Linné, 1758) (MIMB 12/14742), Signalny Island, Olyutorskiy Bay, Bering Sea, intertidal zone. 18: Cardinal teeth and resilifer of left valve, valve length 4.5 mm (bar = 100 μ m). 19: Cardinal teeth and ligament pit of right valve, valve length 8.2 mm (bar = 100 μ m). 20. *Macoma calcarea* (Gmelin, 1791), cardinal teeth and resilifer of right valve, Peter the Great Bay, Sea of Japan, 16 m, valve length 4.2 mm (bar = 1 mm). 21. *Macoma nipponica* (Tokunaga, 1906), cardinal teeth and ligament of right valve, Peter the Great Bay, Sea of Japan, 12 m, length 6.3 mm (bar = 100 μ m).

TABLE 4. *Macoma cuneipyga* (Scarlato, 1981). Summary statistics of the shell measurements (mm) and indexes: L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates the summary statistics for the left valve, denominator—for the right valve.

Characteristics	Mean	SD	SE	Min	Max	n
L	<u>11.29</u>	<u>5.52</u>	<u>1.03</u>	<u>3.1</u>	<u>20.0</u>	<u>29</u>
	10.43	5.66	1.16	3.1	19.7	24
H	<u>7.93</u>	<u>3.75</u>	<u>0.70</u>	<u>2.4</u>	<u>14.0</u>	<u>29</u>
	7.50	3.96	0.81	2.4	14.0	24
W	<u>2.28</u>	<u>1.13</u>	<u>0.21</u>	<u>0.6</u>	<u>4.1</u>	<u>29</u>
	2.05	1.09	0.22	0.6	3.8	24
A	<u>8.29</u>	<u>4.19</u>	<u>0.78</u>	<u>1.8</u>	<u>15.3</u>	<u>29</u>
	7.73	4.35	0.89	1.8	15.3	24
L1	<u>7.22</u>	<u>3.41</u>	<u>0.63</u>	<u>2.2</u>	<u>12.9</u>	<u>29</u>
	5.80	3.07	0.63	1.9	11.0	24
L2	<u>1.70</u>	<u>0.94</u>	<u>0.17</u>	<u>0.4</u>	<u>3.5</u>	<u>29</u>
	2.50	1.54	0.31	0.7	5.5	24
H/L	<u>0.714</u>	<u>0.038</u>	<u>0.007</u>	<u>0.65</u>	<u>0.80</u>	<u>29</u>
	0.730	0.031	0.006	0.69	0.80	24
W/L	<u>0.201</u>	<u>0.015</u>	<u>0.003</u>	<u>0.17</u>	<u>0.24</u>	<u>29</u>
	0.197	0.013	0.003	0.17	0.22	24
A/L	<u>0.721</u>	<u>0.010</u>	<u>0.056</u>	<u>0.58</u>	<u>0.82</u>	<u>29</u>
	0.723	0.013	0.061	0.58	0.82	24
L1/L	<u>0.653</u>	<u>0.040</u>	<u>0.007</u>	<u>0.59</u>	<u>0.75</u>	<u>29</u>
	0.565	0.037	0.008	0.46	0.65	24
L2/L	<u>0.146</u>	<u>0.027</u>	<u>0.005</u>	<u>0.09</u>	<u>0.19</u>	<u>29</u>
	0.233	0.043	0.009	0.18	0.30	24

faint growth lines, equivalve or with left valve somewhat larger, equilateral to longer anteriorly. Posterior end with a radial ridge along posterodorsal margin, twisted to the right, usually slightly gaping. Periostracum thin, dark or colorless, silky or shiny. Bevelled esutcheon present in some species. Hinge weak, two cardinal teeth in each valve, lateral teeth absent. Ligament external, seated on a nymph that does not project above dorsal margin. In young specimens, ligament both external and internal; internal ligament small, lodged in lanceolate resilifer posterior to cardinal teeth. Pallial sinus long, often of different length and form in each valve, partly confluent with pallial line.

Macoma cuneipyga (Scarlato, 1981)
(Figs. 22–37, Table 4)

Abrina cuneipyga Scarlato, 1981: 371–372, figs. 192, 193; Kabanov, 1991: 79.

Type material and Locality

Holotype (ZIN 9800), Onekotan Island, Middle Kuril Islands, 150 m, silty sand, bottom water temperature of 0.7°C, Coll. N. S. Spirina, 18-VII-1954 (R/V "Lebed").

Other Material Examined

1 lot (ZIN 6) from Green Island (1 spec.); 1 lot from Shikotan Island (5 spec.); 5 lots from Kunashir Island (5 spec.); 5 lots from Iturup Island (ZIN 2, 1 spec.; 4 lots of our material, 7 spec.); 3 lots from Onekotan Island (ZIN 10, 1 spec.; 2 lots of our material, 4 spec.); 1 lot (ZIN 11) from Makanrushi Island (1 spec.); 2 lots from Fourth Kuril Strait (5 spec.); 1 lot from Paramushir Island (1 spec.); 3 lots from the western coast of Kamchatka, Sea of Okhotsk (3 spec.); 1 lot from northeastern coast of Sakhalin Island, Sea of Okhotsk (1 spec.). Total of 35 specimens.

Description

(Expanded from that of Scarlato, 1981)—Exterior: Shell small (to 20.0 mm in length, west coast of Kamchatka, Sea of Okhotsk), ovate, of moderate height (H/L = 0.65–0.80), inequilateral, moderately inflated (W/L of left valve 0.17–0.24; W/L of right valve 0.17–0.22), thin, white under periostracum, smooth with faint growth lines, inequivalve (left valve slightly longer, more inflated, less twisted to right than right valve); posterior end distinctly twisted, slightly gaping. Periostracum non-

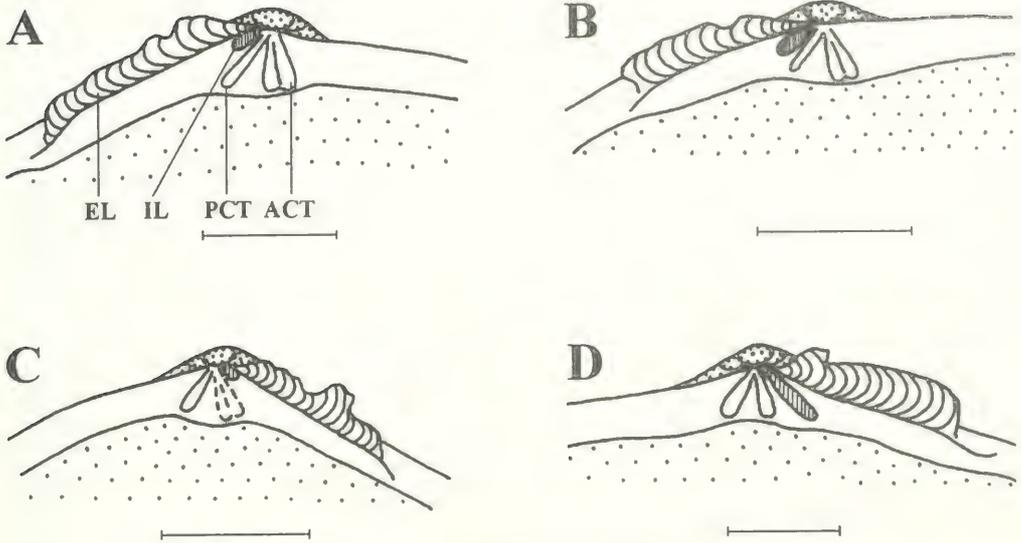
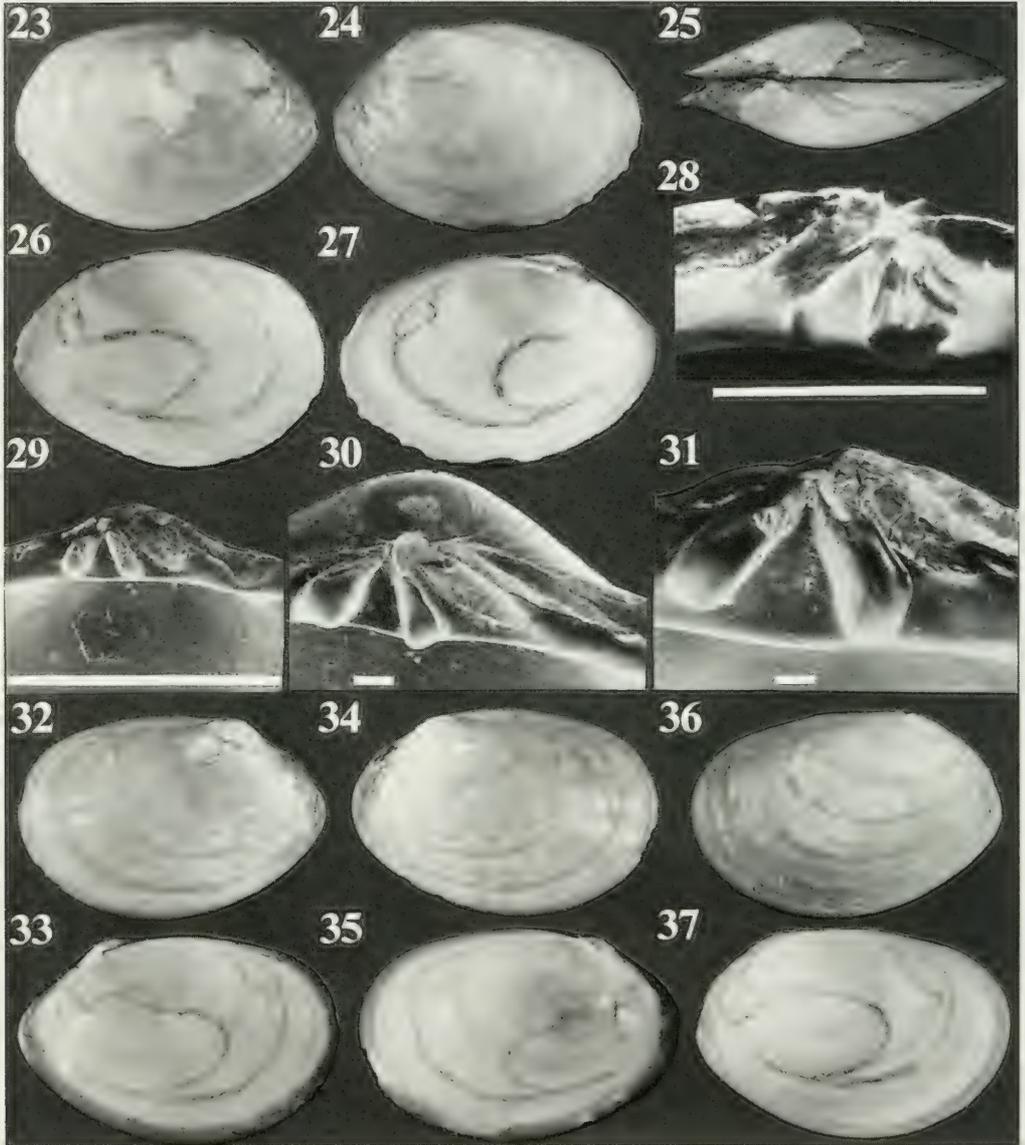


FIG. 22. The hinge plate of holotypes of *Abrina* species from Pacific seas of Russia aided by camera lucida: A — *Abrina sachalinica* (left valve, length 15.0 mm); B — *Abrina cuneipyga* (left valve, 13.0 mm); C — *Abrina tatarica* (right valve, posterior cardinal tooth is broken, 9.4 mm); D — *Abrina shiashkotanika* (right valve, 11.8 mm). ACT: anterior cardinal tooth; EL: external ligament; IL: internal ligament; PCT: posterior cardinal tooth. Bar = 1 mm.

polished, thin, adherent, sometimes peeling off near beaks, gray, yellowish or pink-brown, thrown into numerous small wrinkles, more conspicuous at shell margins. Beaks small, somewhat projecting above dorsal margin, rounded, not opisthogyrate, well posterior to midline ($A/L = 0.58-0.82$). Anterior end obtusely rounded, expanded vertically. Posterior end pointed, angular, with a faint radial ridge extending from posterior portion of beaks to transition of posterior margin to ventral margin. Anterodorsal margin near beaks slightly convex, forming small smooth obtuse angle, extending almost horizontally, smoothly transitioning to rounded anterior margin. Ventral margin slightly curved. Posterodorsal margin very short, straight, steeply extending ventrally, forming a hardly noticeable angle at transition to posterior margin, better expressed in left valve. Posterior margin straight, abruptly transitioning to ventral margin, forming slightly smoothed acute angle in left valve, more distinctly rounded in right valve. External ligament short ($1/2-2/3$ of posterodorsal margin length).

Interior: Hinge plate very narrow, not arcuate in area of cardinal teeth. Hinge weak, with two cardinal teeth in each valve. In left valve, anterior tooth wide, deeply bifid, long, reach-

ing edge of hinge plate; posterior tooth narrow, lamellate, shorter, not reaching edge of hinge plate. In right valve, anterior and posterior teeth of almost same length and width (anterior slightly longer), reaching edge of hinge plate; posterior tooth sometimes slightly bifid. Internal ligament in adult specimens (> 15 mm) weakly developed, short ($< 1/2$ of hinge plate width), lodged in a small pit on hinge plate under beaks posterior to cardinal teeth; internal ligament in young specimens (< 8 mm) well developed, almost reaching edge of hinge plate, lodged in lanceolate resilifer, which extends obliquely posterior to beaks. Anterior adductor muscle scar large, ovate, angular, vertically extended; posterior adductor scar large, rounded, shorter and wider than anterior scar. Pallial sinuses distinct, of different length and shape in each valve. In left valve, pallial sinus long, with wide rounded top, reaching past midline ($L1/L = 0.59-0.75$); ventral sinus branch confluent with pallial line for more than $1/2$ of its length. In right valve, pallial sinus shorter ($L1/L = 0.46-0.65$) with a narrow, rounded top; ventral sinus branch confluent with pallial line for more than $1/2$ of its length. Shell polished, within sometimes with faint radial striae especially noticeable along ventral margin.



FIGS. 23–37. *Macoma cuneipyga* (Scarlato, 1981). 23–27: Holotype of *Abrina cuneipyga* (ZIN 9800), Onekotan Island, 150 m, shell length 13.0 mm. 28: Cardinal teeth and resilifer of left valve of specimen from the type locality (ZIN 10), length 13.5 mm (bar = 1 mm). 29: Cardinal teeth and ligament of right valve, South Kuril Strait (44°12'N, 146°32'E), 103 m, valve length 3.6 mm (bar = 1 mm). 30: Cardinal teeth and resilifer of right valve, Iturup Island (44°55'1"N, 148°02'4"E), 197 m, valve length 6.6 mm (bar = 100 μ m). 31: Cardinal teeth and ligament of right valve, Iturup Island (44°55'1"N, 148°02'4"E), 197 m, valve length 11.1 mm (bar = 100 μ m). 32–35: Fourth Kuril Strait, 200 m, left valve length 17.7 mm, right valve length 17.5 mm. 36, 37: Kunashirsky Strait, Kunashir Island (43°58'5"N, 145°27'2"E), 200 m, left valve length 15.0 mm.

Variability

Shell shape and proportions change little with age. In young specimens (< 8 mm) in con-

trast to adults, the shell is more rounded, higher (H/L = 0.73–0.80) and angular; the anterodorsal margin is horizontal, then bends (forming a smooth obtuse angle) and is

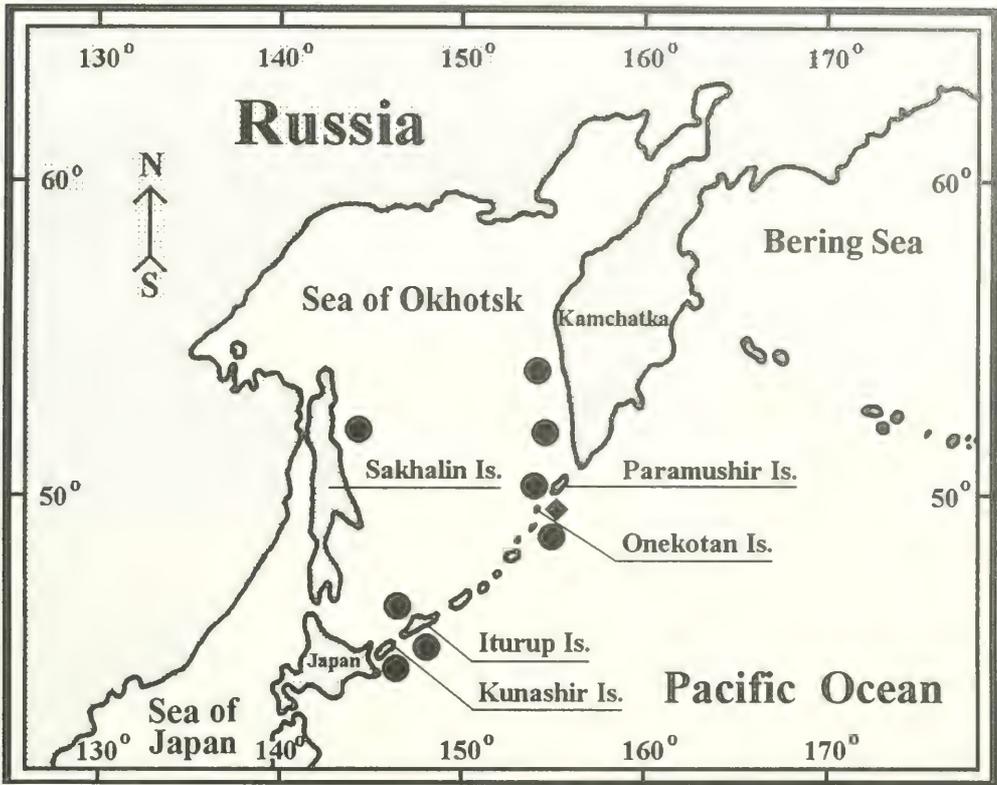


FIG. 38. Distribution of *Macoma cuneipyga* (◆—type locality).

steeply turned ventrally; the posterodorsal margin at the transition to posterior margin forms a distinct smooth angle; the posterior margin at the transition to ventral margin forms a pointed acute angle; the beaks are placed less posteriorly and only slightly rounded; the ridge on the posterior end of the shell is better expressed; in left valve, the anterior tooth is non-bifid and only slightly sulcate; the internal ligament is very well developed, reaching edge of hinge plate and lodged in a lanceolate resilifer; the pallial sinus in left valve is longer and reaches closer to the anterior adductor muscle scar ($L1/L = 0.65-0.75$; $L2/L = 0.11-0.16$). In adult specimens, the degree of confluence of the pallial sinus with pallial line varies. In left valve, ventral sinus branch confluent for $1/4$ to $1/2$ of its length with pallial line; in right valve, sometimes completely confluent.

Distribution and habitat (Figure 38)

Green Island ($43^{\circ}19'3''N$, $146^{\circ}23'7''E$); Shikotan Island ($43^{\circ}39'N$, $147^{\circ}08'E$); South Kuril

Strait, Kunashir Island (from $43^{\circ}48'1''N$, $147^{\circ}30'5''E$ to $44^{\circ}12'N$, $146^{\circ}32'E$); Blizky Island, Kunashirsky Strait, Kunashir Island ($43^{\circ}58'5''N$, $145^{\circ}27'2''E$); Rok Bay, Iturup Island ($44^{\circ}00'N$, $147^{\circ}44'E$); Kasatka Bay, Iturup Island ($44^{\circ}52'7''N$, $147^{\circ}46.1'E$); Iturup Island, coast of the Sea of Okhotsk ($45^{\circ}21'76''N$, $148^{\circ}18'7'E$); Drakon Cape, Iturup Island ($44^{\circ}55'N$, $148^{\circ}02'4''E$); Onkotan Island ($49^{\circ}16'N$, $155^{\circ}38'7''E$); Makanrushi Island, Fourth Kuril Strait (from $49^{\circ}32'3''N$, $155^{\circ}47'2''E$ to $49^{\circ}46'6''N$, $155^{\circ}46'E$); Dym Island, Paramushir Island ($49^{\circ}36'1''N$, $156^{\circ}06'E$); west coast of Kamchatka, Sea of Okhotsk ($52^{\circ}45'N$, $155^{\circ}42'E$; $54^{\circ}00'N$, $155^{\circ}30'E$); northeastern coast of Sakhalin Island, Sea of Okhotsk ($43^{\circ}45'15''N$, $143^{\circ}45'15''E$).

This species was registered off the South Kuril Islands at a depth from 53 m (South Kuril Strait ($43^{\circ}51'3''N$, $146^{\circ}05'5''E$)) to 300 m (Rok Bay, Iturup Island, and Kunashir Island) on sand and silty sand, sometimes with some admixture of gravel and small stones; near the North Kuril Islands from 150 to 590 m on sand with a admixture of gravel, small stones and

TABLE 5. *Macoma loveni* (Jensen, 1905). Summary statistics of the shell measurements (mm) and indexes: L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates the summary statistics for the left valve, denominator—for the right valve.

Characteristics	Mean	SD	SE	Min	Max	n
L	15.56	5.95	0.82	3.9	32.5	53
	15.36	5.86	0.81	3.9	32.0	52
H	10.90	4.04	0.56	3.0	23.0	53
	10.88	3.97	0.56	3.0	23.0	51
W	3.12	1.30	0.18	0.8	7.0	53
	2.89	1.12	0.16	0.8	6.1	51
A	10.52	3.96	0.54	2.4	21.5	53
	10.48	3.90	0.55	2.4	21.5	51
L1	9.76	3.47	0.48	2.8	18.0	53
	8.11	3.00	0.42	2.2	15.6	52
L2	2.24	1.06	0.15	0.4	5.0	53
	3.77	1.60	0.22	1.0	7.0	52
H/L	0.705	0.024	0.003	0.65	0.77	53
	0.706	0.025	0.003	0.66	0.77	51
W/L	0.199	0.016	0.002	0.17	0.23	53
	0.187	0.016	0.002	0.15	0.22	51
A/L	0.675	0.035	0.005	0.62	0.80	53
	0.667	0.073	0.010	0.22	0.80	51
L1/L	0.639	0.047	0.006	0.55	0.88	53
	0.530	0.034	0.005	0.46	0.61	52
L2/L	0.139	0.025	0.003	0.08	0.20	53
	0.243	0.031	0.004	0.18	0.32	52

debris; near the western coast of Kamchatka at a depth of 50–53 m, on silty sand at a bottom water temperature of 0.83° to 1.42°C. Near the Kuril Islands, it was observed at a bottom water temperature of 0.7–5.4°C (Scarlato, 1981).

Comparison

M. cuneipyga is distinguished from all other species of *Macoma* by its short, pointed posterior end. In shape and proportions, this species is closest to *M. loveni*, from which it is distinguished in having a very narrow, pointed gaping posterior end, beaks less projecting above the dorsal margin and less posteriorly placed, and a narrower hinge plate that is not arcuate in the area of the cardinal teeth.

Remarks

Scarlato (1981) described this species from of 22 specimens (10 samples) (Scarlato by mistake mentioned 12 samples: specimens in ZIN 1 and 3, 7 and 9 were taken from 2 samples, not from 4), of which the holotype is the largest (shell length, 13.0 mm). We have studied all of this material. In addition to the holo-

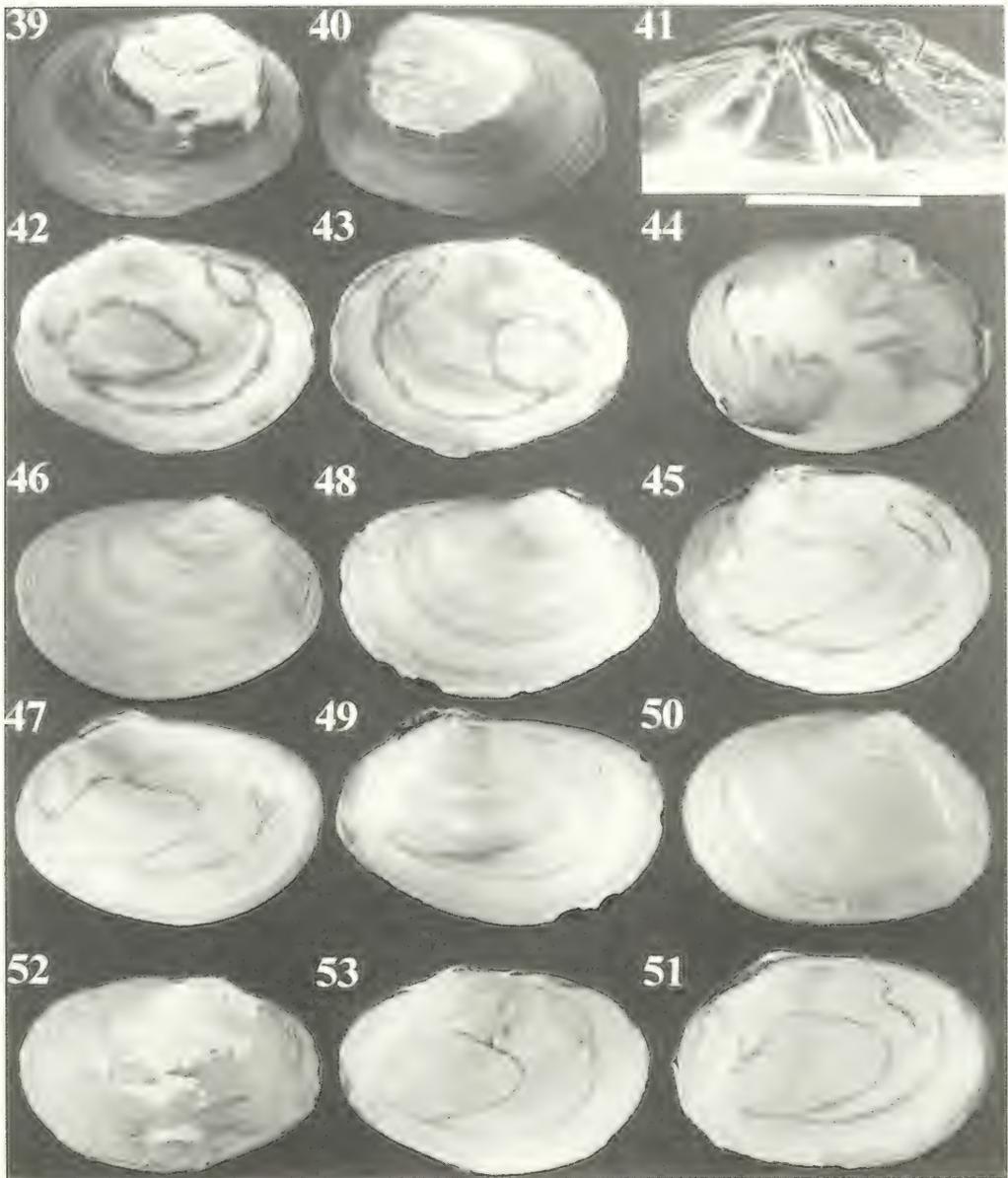
type, only 4 specimens (samples 2, 6, 10 and 11) could be surely identified as *M. cuneipyga*. All others are young specimens of the genus *Macoma* that are not easily identified and are different from the holotype.

In our material, we have found 31 specimens of different ages. The studies show that the typical characteristics of this species are not much prone to age or individual variation. A well-developed internal ligament is a juvenile characteristic. We consider that *A. cuneipyga* is a separate species of *Macoma*, for which a new combination of *M. cuneipyga* is suggested.

Macoma loveni (Jensen, 1905)
(Figs. 12–17, 22, 39–51, Table 5)

Tellina (Macoma) loveni Jensen, 1905: 45, pl. 1, figs. 5a–h (Steenstrup MS) (cited from Coan, 1971); Coan, 1971: 31–32, fig. 19, pl. 8, figs. 42, 43, synonymy.

Macoma loveni (Jensen, 1905), Coan, 1971: 31–32, fig. 19, pl. 8, figs. 42, 43, synonymy; Bernard, 1979: 49, fig. 81; Lubinsky, 1980: 42, pl. 9, figs. 2, 5, 8, 11; Scarlato, 1981: 362–363, fig. 362, synonymy; Bernard, 1983: 45; Romejko & Kamenev, 1985: 94; Baxter,



FIGS. 39–53. *Macoma loveni* (Jensen, 1905). 39–43: Holotype of *Abrina sachalinica* (ZIN 9853). Terpeniya Bay, Sakhalin Island, Sea of Okhotsk (48°22'N, 145°17'E), 220 m, shell length 15.0 mm. 44, 45: ZIN 106, Laptev Sea (76°38'N, 118°20'E), 46 m, left valve length 11.2 mm. 46, 47: Yury Island (43°11'N, 146°14'E), 300 m, left valve length 22.0 mm. 48, 49: Paramushir Island (49°45'9"N, 155°58'5"E), 135 m, left valve length 17.5 mm. 50, 51: Northern Sea of Okhotsk (53°34'N, 139°34'E), 130 m, left valve length 13.3 mm. 52, 53: Northern Sea of Okhotsk (58°16'5"N, 142°54'3"E), 134 m, left valve length 26.1 mm.

1987: 25; Kafanov, 1991: 72; Feder et al., 1994: 160; Kamenev, 1995: 6.

Abrina sachalinica Scarlato, 1981: 372, fig. 194; Kafanov, 1991: 79.

Material Examined

Abrina sachalinica—holotype (ZIN 9853) and 3 specimens of this species from the type locality, Terpeniya Bay, Sakhalin Island, Sea of Okhotsk (48°22'N, 145°17'E), 220 m, silty sand, Coll. L. G. Nazvich, 30-VI-1951 (R/V "Vityaz"); 1 lot (ZIN 4) from Hokkaido Island, southern Sea of Okhotsk (1 spec.); 1 lot (ZIN 5) from west coast of Kamchatka, Sea of Okhotsk (1 spec.); *M. loveni* — 1 lot (USNM 108789) from Spitzbergen (2 spec.); 1 lot (ZIN 94) from Barents Sea (3 spec.); 1 lot (ZIN 101) from Franz-Joseph Land, Barents Sea (4 spec.); 3 lots (ZIN 107) from Novaya Zemlya, Kara Sea (17 spec.); 1 lot (ZIN 106) from the Laptev Sea (4 spec.); 1 lot (ZIN 98) from the East Siberian Sea (7 spec.); 2 lots from Vrangeli Island, Chukchi Sea (2 spec.); 1 lot (USNM 95638) from Murray Bay, Gulf of St. Lawrence, Atlantic Ocean (2 spec.); 1 lot (USNM 159769) from Gulf of Maine, Atlantic Ocean (1 spec.); 1 lot (ZIN 57) from Terpeniya Bay, Sakhalin Island, Sea of Okhotsk (2 spec.); 3 lots from Shikotan Island (ZIN 82, 1 spec.; 2 lots of our material, 3 spec.); 1 lot (ZIN 67) from Tatar Strait, Sea of Japan (1 spec.); 1 lot (ZIN 65) from the western coast of Sakhalin Island, Tatar Strait, Sea of Japan (5 spec.); 1 lot (USNH 204814) from Aniva Bay (1 spec.); 1 lot (USNM 204815) from coast of Japan ("coast Yesso", 37°9'N), Sea of Japan (2 spec.); 2 lots from Peter the Great Bay, Sea of Japan (2 spec.); 1 lot from east coast of Sakhalin Island, Sea of Okhotsk (1 spec.); 3 lots from the northern Sea of Japan (5 spec.); 1 lot from Blizkiy Island, Kunashirsky Strait (3 spec.); 2 lots from Yury Island (2 spec.); 4 lots from Iturup Island (10 spec.); 1 lot from Paramushir Island (1 spec.); 1 lot from Kronotsky Bay, eastern coast of Kamchatka, Pacific Ocean (4 spec.); 1 lot from Bering Sea (1 spec.). Total of 92 specimens.

Description

(Expanded from that of Coan, 1971; Bernard, 1979; and Scarlato, 1981) — Exterior: Shell small (to 37.0 mm, Terpeniya Bay, Sakhalin Island, Sea of Okhotsk), ovate, of

moderate height ($H/L = 0.65-0.77$), inequilateral, moderately inflated (W/L of left valve 0.17–0.23; W/L of right valve 0.15–0.22), thin, white under periostracum, smooth, with faint growth lines, sometimes inequivalve (left valve sometimes slightly longer, more inflated than right valve); posterior end slightly twisted to right, without a gape. Periostracum thin, gray, grayish-olive and from light to bright brown with an iridescent sheen, dehiscent, non-polished, easily peeled off near beaks, thrown into small wrinkles, better expressed at shell margins. Beaks small, somewhat projecting above dorsal margin, rounded, not opisthograte, well posterior to midline ($A/L = 0.62-0.80$). Anterior end obtusely rounded, expanded vertically. Posterior end slightly truncate, with faint radial ridge extending from posterior portion of beaks to transition of posterior margin to ventral margin. Anterodorsal margin slightly convex, extending almost horizontally, smoothly transitioning to rounded anterior margin. Ventral margin slightly curved. Posterodorsal margin slightly concave, smoothly extending ventrally, forming a very smooth angle at transition to posterior margin. Posterior margin slightly convex, vertically extending ventrally, forming a smooth angle at transition to ventral margin. External ligament short (2/3 of posterodorsal margin length).

Interior: Hinge plate narrow, arcuate in area of cardinal teeth. Hinge weak, with two cardinal teeth in each valve. In left valve, anterior tooth wide, bifid, long, reaching edge of hinge plate; posterior tooth narrow, lamellate, shorter, not reaching edge of hinge plate. In right valve, anterior and posterior teeth of almost same length and width (posterior slightly longer), almost reaching edge of hinge plate; posterior tooth bifid. Internal ligament in adult specimens (> 15 mm) weakly developed, short (< 1/2 width of hinge plate), lodged in a small pit on hinge plate under beaks behind cardinal teeth; internal ligament in young specimens (to 5–10 mm) well developed, reaching almost to edge of hinge plate, lodged in a lanceolate resilifer, which extends obliquely posterior to beaks. Anterior adductor muscle scar large, ovate-angular, vertically extended; posterior adductor scar large, rounded, shorter and wider than anterior scar. Pallial sinus distinct, of different length and shape in each valve. In left valve, pallial sinus long, with wide, rounded top, reaching past midline ($L1/L = 0.55-0.88$), approaching anterior adductor muscle scar

($L2/L = 0.08-0.20$); ventral sinus branch confluent with pallial line for more than 1/3 of its length. In right valve, pallial sinus shorter ($L1/L = 0.46-0.61$), with narrow, rounded top stopping just short of midline; ventral sinus branch confluent with pallial line for more than 1/3 of its length. Shell interior polished, with faint radial striae especially noticeable along ventral margin.

Variability

Shell size, shape and proportions, as well as the inner shell morphology of *M. loveni* from the Arctic Ocean and the Pacific Ocean differ slightly. This species is much larger (maximal shell size: $L = 37.0$; $H = 25.5$; $W = 25.5$ mm, Terpeniya Bay, Sakhalin Island, Sea of Okhotsk; Scarlato, 1981) in the Pacific than in other parts of its distribution. In the Arctic and the North Atlantic oceans, *M. loveni* attains 20 mm (Coan, 1971; Bernard, 1979). Specimens (especially young) from the Pacific Ocean also often have a more elongate shell, with the beaks placed less posteriorly.

The shell shape of *M. loveni* from the Pacific varies from ovate to elongate-ovate. Specimens from the Bering Sea and the northern Sea of Okhotsk have a more ovate shell compared to specimens from the Kuril Islands and the Sea of Japan (Figs. 46–51). The shape of the posterior end varies from angular and distinctly truncate to almost rounded. The length and width of the posterior end also varies.

The degree of confluence of the pallial sinus with the pallial line varies in both valves. In some specimens, the ventral branch of the pallial sinus in left valve is not confluent with pallial line. Usually the ventral sinus branch in the left valve is confluent with the pallial line for 1/4–1/5 of its length. In the right valve, the ventral sinus branch is frequently confluent for 1/3 of its length.

In specimens from the Pacific Ocean with a shell length up to 10–12 mm, the resilium is large, reaching the edge of hinge plate and lodged in a lanceolate resilifer (Figs. 13, 15). In *M. loveni* from the Arctic Ocean and the North Atlantic Ocean, a similar internal ligament was observed only in specimens up to 7–8 mm (Figs. 12, 16). Moreover, in specimens from the Arctic and Atlantic oceans, the hinge plate is relatively wider and more arcuate in the area of the cardinal teeth. In some specimens from throughout the distribution,

the anterior tooth of the left valve is slightly bifid.

Distribution and Habitat in the Northwestern Pacific (Fig. 54)

In the Pacific Ocean, *M. loveni* occurs in the Sea of Japan—near Japan ($37^{\circ}9'N$), Possyet Bay, Peter the Great Bay ($42^{\circ}33'N$, $131^{\circ}22'7''E$); from Olga Bay to Syurkum Cape, near Moneron Island, near Sakhalin Island (from Kholmsk City to Kitoushi Cape ($50^{\circ}10'0''N$, $140^{\circ}57'1''E$)); in the Sea of Okhotsk—near Sakhalin Island (Aniva Bay, Terpeniya Bay, east coast) and in the northern part of the sea ($58^{\circ}16'5''N$, $142^{\circ}54'3''E$), near western Kamchatka ($53^{\circ}35'N$, $155^{\circ}11'5''E$); near the Kuril Islands—in Kunashir Strait (Blizky Island, $43^{\circ}58'5''N$, $145^{\circ}27'2''E$), near Yury Island ($43^{\circ}07'N$, $146^{\circ}12'E$; $43^{\circ}11'N$, $146^{\circ}14'E$), near Shikotan Island ($43^{\circ}29'N$, $147^{\circ}00'E$), near Iturup Island (Pacific and Sea of Okhotsk coasts), near Paramushir Island (Dym Island, $49^{\circ}45'9''N$, $155^{\circ}58'5''E$); near eastern Kamchatka in Kronotsky Bay, Pacific Ocean; in the Bering Sea ($61^{\circ}34'8''N$, $179^{\circ}59'7''E$); near Bering Island, the Commander Islands.

In the Sea of Japan, this species was obtained at a depth from 40 m (western coast of Sakhalin Island, Krasnogorsk Village ($48^{\circ}27'7''N$, $141^{\circ}56'6''E$) to 850 m (coast of Japan, USNM 204815) on sand, silty sand, sandy silt, often with admixture of gravel and shell debris at a bottom temperature of 0.4–5.7°C; in the Sea of Okhotsk from 16 m (Aniva Bay, Sakhalin Island) to 220 m (Terpeniya Bay, Sakhalin Island) on silt and silty sand with some admixture of gravel at a bottom temperature from $-1.7^{\circ}C$ ($55^{\circ}34'N$, $139^{\circ}34'E$, depth 130 m) to $3.6^{\circ}C$ (Aniva Bay, Sakhalin Island, depth 16 m); near the Kuril Islands from 75 m (Pacific coast of Iturup Island) to 1,000 m (Yury Island, $43^{\circ}07'N$, $146^{\circ}12'E$) on sand, silty sand, sandy silt and silt often with admixture of gravel at a bottom temperature from $1.3^{\circ}C$ (Shikotan Island, depth 100 m) to $6.4^{\circ}C$ (Pacific coast of Iturup Island, 75 m); in Kronotsky Bay (eastern coast of Kamchatka, Pacific Ocean)—from 134 to 150 m on silt and sandy silt with admixture of gravel and small stones at a bottom temperature of $1.0^{\circ}C$ ($53^{\circ}33'N$, $160^{\circ}09'E$, depth 150 m); near the Commander Islands at a depth of 60–80 m on sand with some admixture of gravel; in the Bering Sea at 134 m on sandy silt with some



FIG. 54. Distribution of *Macoma loveni* in the northwestern Pacific (◆—sampling site of holotype of *Abrina sachalinica*).

admixture of gravel at a bottom temperature of 2.56°C.

Comparison

In shell shape and proportions, as well as in pallial sinus size and form, this species is closest to *M. cuneipyga*. However, *M. loveni* differs in having a wide, truncate posterior end without a gape, with more projecting beaks, and a wider hinge plate that is arcuate in the area of the cardinal teeth. A comparison of these species with reference to other characteristics (Table 6) shows that the in left valves differ significantly in A/L and L1/L indices, and their right valves in all indices with the exception of L2/L. Thus, in addition to the characteristics mentioned above, the beaks of *M. loveni* compared to *M. cuneipyga* are placed less posteriorly, the pallial sinus in the left valve is more elongate, and in the right valve

is shorter proportional to shell length. Moreover, as compared with *M. loveni*, the right valve of *M. cuneipyga* is shorter and more inflated than the left valve. This is because *M. cuneipyga* is more gaping posteriorly and because the posterior end of right valve is more twisted to the right and more rounded. In *M. loveni*, the shape of posterior end of right and left valves is similar.

Discriminant analysis of all lots containing *M. loveni* and *M. cuneipyga* showed that these species differ significantly (Table 7) in a complex of parameters (group centroids for *M. cuneipyga* and *M. loveni* 1.60979 and -0.694421, respectively). Of 73 specimens analysed, 65 were accurately classified (89.04%) (1 specimen was mistakenly classified as *M. cuneipyga* and 7 as *M. loveni*). The most significant characteristics for dividing all specimens into two species were shell length (L) and shell anterior end length (A) for right

TABLE 6. Results of comparison by pairs of mean values of indices of left and right valves of *Macoma cuneipyga* and *Macoma loveni* using Student (T) and Kolmogorov-Smirnov (S) tests: L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. P—probability that the index values in *M. cuneipyga* and *M. loveni* are drawn from the same population.

Indices	Left valves			Right valves		
	Test value	P	Test	Test value	P	Test
H/L	1.10	0.272	T	3.30	0.002	T
W/L	0.259	0.162	S	0.471	0.001	S
A/L	0.581	<0.001	S	0.593	<0.001	S
L1/L	0.344	0.024	S	3.84	<0.001	T
L2/L	1.16	0.246	T	0.94	0.649	T

TABLE 7. Results of discriminant analysis for *M. cuneipyga* (22 spec.) and *M. loveni* (51 spec.): L—shell length; H—height; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus. P—probability that characteristic values in *M. cuneipyga* and *M. loveni* are drawn from the same population.

Valves	Discriminant Function	Canonical Correlation	Wilks Lambda	P	Significant characteristics	Standardized coefficient
Both	1	0.73126	0.465254	<0.001		
Right	1	0.62539	0.608883	<0.001	L	-1.20894
					A	1.612
Left	1	0.63291	0.599421	<0.001	H	4.80796
					A	-6.96378
					L1	2.562

valves, and height (H), anterior end length (A), and distance from the shell posterior margin to the top of pallial sinus (L1) for left valves.

Remarks

Scarlato (1981) described *A. sachalinica* on the basis of a study of 13 specimens (7 samples), from which the holotype is the largest (shell length, 15.0 mm). We have studied all of this material. In addition to the holotype, only 5 specimens (samples ZIN 1, 4 and 5) could be included in this species. Other specimens are young specimens (< 8 mm) of *Macoma* of uncertain identity. The studies of *M. loveni* from the Arctic Ocean and the northwestern Pacific, as well as its descriptions, photos and pictures (Golikov & Scarlato, 1967; Coan, 1971; Bernard, 1979; Lubinsky, 1980; Scarlato, 1981), show that in shell morphology the holotype of *A. sachalinica* is identical to specimens of *M. loveni*. The holotype of *A. sachalinica* has a small internal ligament that

is short in contrast to the width of the hinge plate (Fig. 41). We have found resilium of similar shape and size in the specimens of *M. loveni* of the same size as the holotype of *A. sachalinica* (Fig. 14). Therefore, we consider that *A. sachalinica* is a synonym of *M. loveni*.

Macoma calcarea (Gmelin, 1791) (Figs. 20, 22, 55–59, Table 8)

For complete synonymy, see Coan, 1971: 20–21; Scarlato, 1981: 356.

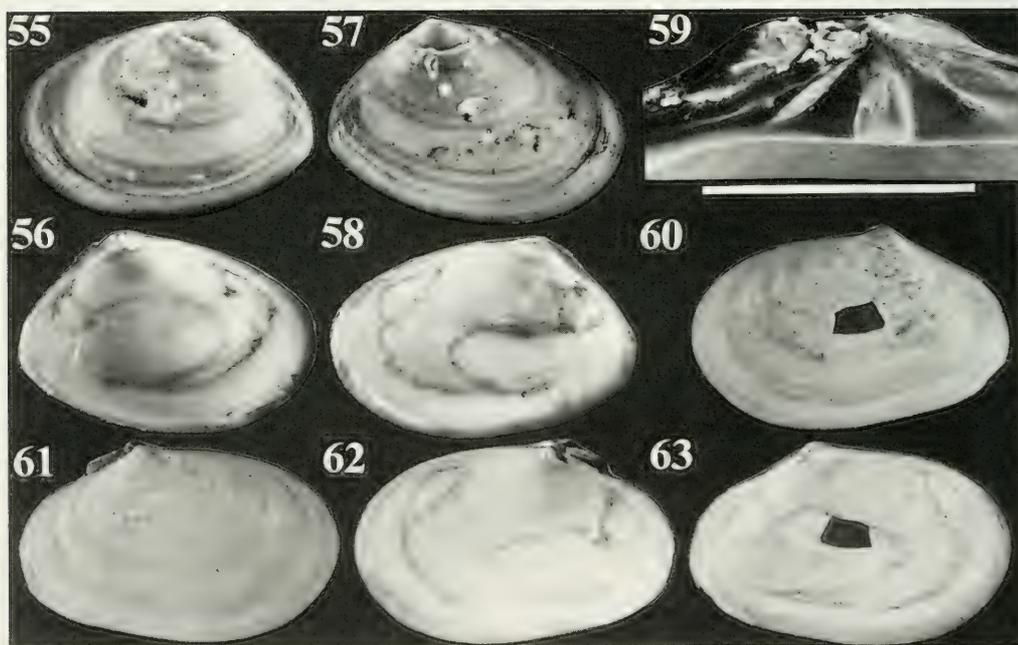
Abrina tatarica Scarlato, 1981: 373–374, fig. 197; Kafanov, 1991: 79.

Material Examined

A. tatarica—holotype (ZIN 9900), southern coast of Sakhalin Island near Kholmsk town (47°02'N, 142°00'E), Sea of Japan, 46 m, sand with some admixture of shell debris, bottom water temperature of 3.9°C, Coll. V. A. Skalkin, 8-X-1949 (R/V "Toporok"); 1 lot (ZIN

TABLE 8. *Macoma calcarea* (Gmelin, 1791). Shell measurements (mm) and indices of specimens identified as *Abrina tatarica* (ZIN 1, 5, 6): L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates shell measurements and indices for the left valve, denominator—for the right valve. NM—not measured. Holotype of *Abrina tatarica* in italics.

L	H	W	A	L1	L2	H/L	W/L	A/L	L1/L	L2/L
<i>7.0</i>	<i>4.6</i>	<i>1.0</i>	<i>4.7</i>	<i>5.1</i>	<i>0.4</i>	<i>0.66</i>	<i>0.14</i>	<i>0.67</i>	<i>0.73</i>	<i>0.057</i>
NM	NM	NM	NM	NM						
<i>9.4</i>	<i>6.2</i>	<i>1.4</i>	<i>6.2</i>	<i>6.8</i>	<i>0.7</i>	<i>0.66</i>	<i>0.15</i>	<i>0.66</i>	<i>0.72</i>	<i>0.074</i>
9.4	6.2	1.4	6.2	5.8	1.6	0.66	0.15	0.66	0.62	0.17
9.7	6.3	1.6	6.0	7.0	0.5	0.65	0.16	0.62	0.72	0.052
9.7	6.3	1.1	6.0	6.0	1.5	0.65	0.11	0.62	0.62	0.15
10.5	6.8	1.6	6.6	8.0	0.7	0.65	0.15	0.63	0.76	0.067
10.5	6.8	1.6	6.6	6.5	1.5	0.65	0.15	0.63	0.62	0.14



FIGS. 55–63. Shells of *Macoma calcarea* (Gmelin, 1791) and *Macoma* sp. 55–58. Holotype of *Abrina tatarica* (ZIN 9900), southern coast of Sakhalin Island, Sea of Japan (47°02'N, 142°00'5"E), 46 m, shell length 9.4 mm. 59. Cardinal teeth and ligament of left valve of specimen identified as *Abrina tatarica* (ZIN 6), Terpeniya Bay, Sakhalin Island, Sea of Okhotsk (49°03'1"N, 114°17'3"E), 16 m, valve length 10.5 mm (bar = 1 mm). 60–63. Holotype of *Abrina shiashkotanika* (ZIN 9867), Shiashkotan Island, 2270 m, left valve length 11.9 mm, right valve length 11.8 mm.

5) from Kievka Bay, Sea of Japan (1 spec.); 1 lot (ZIN 6) from Terpeniya Bay, Sakhalin Island, Sea of Okhotsk (1 spec., 1 left valve); *M. calcarea*—10 lots from Peter the Great Bay, Sea of Japan (64 spec.). Total of 67 specimens, 1 left valve.

Remarks

Abrina tatarica was described from a study of 16 specimens (6 samples) (Scarlato, 1981). We have studied this material and consider that in addition to the holotype only 2

TABLE 9. *Macoma* sp. Shell measurements (mm) and indices: L—shell length; H—height; W—width; A—anterior end length; L1—maximal distance from posterior margin to the top of pallial sinus; L2—minimal distance from the top of pallial sinus to anterior adductor muscle scar. Numerator indicates shell measurements and indices for the left valve, denominator—for the right valve. NM—not measured. Holotype of *Abrina shishkotanika* in italics.

L	H	W	A	L1	L2	H/L	W/L	A/L	L1/L	L2/L
9.5	6.1	1.5	5.9	5.6	1.6	0.64	0.16	0.62	0.59	0.17
9.5	6.1	1.5	5.9	4.9	2.0	0.64	0.16	0.62	0.52	0.21
<u>10.0</u>	<u>6.9</u>	<u>1.5</u>	<u>6.2</u>	<u>6.5</u>	<u>1.5</u>	<u>0.69</u>	<u>0.15</u>	<u>0.62</u>	<u>0.65</u>	<u>0.15</u>
10.0	6.8	1.5	6.2	NM	NM	0.68	0.15	0.62	NM	NM
<u>11.9</u>	<u>7.6</u>	<u>1.8</u>	<u>7.4</u>	<u>8.0</u>	<u>2.0</u>	<u>0.64</u>	<u>0.15</u>	<u>0.62</u>	<u>0.67</u>	<u>0.17</u>
11.8	7.6	1.6	7.4	6.8	3.0	0.64	0.14	0.63	0.58	0.25

specimens (samples ZIN 5 and 6) can be referred to this species. The other specimens differ significantly from holotype and are young specimens of different species of *Macoma*. Scarlato (1981) stated that the holotype is the largest specimen (shell length, 9.4 mm). However, the largest is a specimen from a sample ZIN 6 (shell length, 10.5 mm).

On the basis shell morphology, the holotype of *A. tatarica* is identical to young specimens of *M. calcarea* from Peter the Great Bay, Sea of Japan. The internal ligament of the holotype is small, very short (about 1/3 of hinge plate width) and lodged in a small pit on the hinge plate ventral to the beaks posterior to the cardinal teeth (Fig. 22). We have found resilia of similar shape and size in specimens of *M. calcarea* of the same size as the holotype of *A. tatarica*. Kafanov et al. (1997) presented the results of a morphometric analysis of a large sample (150 spec.) of specimens of different ages (L = 14.3–39.8 mm) of *M. calcarea* from the Bering Sea. A comparative analysis of these data with ours shows that the values of all the indices of *A. tatarica* are within the range of variation of similar indices (H/L = 0.657–0.773; W/L = 0.125–0.192; A/L = 0.503–0.662; L1/L = 0.566–0.801) of *M. calcarea* from the Bering Sea. Therefore, we consider that *A. tatarica* is a synonym of *M. calcarea*.

Macoma sp.

(Figs. 22, 60–63, Table 9)

Abrina shiashkotanika Scarlato, 1981: 373, figs. 195, 196; Kafanov, 1991: 79.

Type material and locality

Holotype (ZIN 9867), Shiashkotan Island, Middle Kuril Islands, 2,270 m, sand, Coll. L. G. Nazvich, 7-8-VII-1951 (R/V "Vityaz").

Other Material Examined

Two specimens (ZIN 1) from the type locality.

Description

(Expanded from that of Scarlato, 1981)—Exterior: Shell small (to 11.9 mm, holotype), elongate-ovate, of moderate height (H/L = 0.64–0.69), inequilateral, flattened (W/L of left valve 0.15–0.16; W/L of right valve 0.14–0.16), thin, smooth, with faint growth lines, inequivalve (left valve slightly longer, more inflated than right valve); posterior end distinctly twisted to right. Periostracum thin, adherent, grey, thrown into small wrinkles more conspicuous at shell margins. Beaks small, not very prominent above dorsal margin, slightly rounded, not opisthogyrate, posterior to midline (A/L = 0.62–0.63). Anterior end obtusely rounded, not expanded vertically. Posterior end slightly truncate, with faint (better expressed in right valve) radial ridge extending posteriorly from beaks to transition of posterior margin to ventral margin. Anterodorsal margin straight, extending almost horizontally parallel to ventral margin, smoothly transitioning to rounded anterior margin. Ventral margin slightly curved. Posterodorsal margin slightly concave, smoothly extending ventrally, forming a smooth angle at transition to posterior margin. Posterior margin slightly convex, almost vertical, extending ventrally and forming a strongly smooth angle at transition to ventral margin. External ligament short (approximately 1/2 of posterodorsal margin length.)

Interior: Hinge plate narrow, not arcuate in area of cardinal teeth. Hinge weak, with two cardinal teeth in each valve. In left valve, anterior tooth bifid, wide, long, reaching edge of hinge plate; posterior tooth narrow, lamellate,

shorter, not reaching edge of hinge plate. In right valve, anterior and posterior teeth of almost same length and width (posterior slightly longer); posterior tooth sometimes bifid. Internal ligament large, reaching edge of hinge plate, lodged in lanceolate resilifer, which extends obliquely posterior to beaks. Anterior adductor muscle large, ovate, extended vertically; posterior adductor scar large, rounded, shorter and wider than anterior scar. Pallial sinuses indistinct, of different length and shape in each valve. In left valve, pallial sinus long, with narrow rounded top, reaching past midline ($L1/L = 0.59-0.67$), approaching anterior adductor muscle scar ($L2/L = 0.15-0.17$); ventral sinus branch confluent for 1/2 of its length with pallial line. In right valve, pallial sinus shorter ($L1/L = 0.52-0.58$; $L2/L = 0.21-0.25$), with a narrower rounded top; ventral sinus branch half confluent with pallial line. Shell inside polished, with faint radial striae.

Distribution and Habitat

Known only from type locality.

Comparison

In shell form and proportions, this species is similar to *M. loveni* and *Macoma moesta* (De-shayes, 1855). However, it has a narrower, more elongate shell, with beaks placed less posteriorly, and a narrower posterior end compared to *M. loveni* and *M. moesta* of the same size.

Remarks

Abrina shiashkotanika was described based on the study of only three specimens (1 sample) (Scarlato, 1981), and of which the holotype is the largest (shell length, 11.9 mm). We have studied these specimens and consider them to belong to one species. In the description of this species, Scarlato (1981) mentioned that the anterior tooth of the right valve is deeply bifid. Our studies have shown that in the right valve of the holotype both teeth were non-bifid, but in the other two specimens the posterior tooth is deeply bifid. Probably, Scarlato (1981) made a mistake and described the anterior tooth of the left valve. Of the three specimens studied, only the smallest had a deeply bifid anterior tooth in the left valve.

In describing this species, Scarlato (1981) had only a small amount of material, with all specimens mainly smaller than 10 mm and in poor condition. We think that these are young specimens of a *Macoma*, for which it is difficult to make an accurate species identification. Possibly, as a result of further studies, additional material will be obtained representing all age groups of this species. It is not inconceivable that this species will be considered synonymous with a known species of *Macoma*. At present, taking into consideration the peculiarities of shell morphology and the depth from which this species was collected (2,270 m), as well as the lack of any additional material, we assume it to be a separate species of *Macoma*.

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TERRESTRIAL GASTROPOD RICHNESS OF CARBONATE CLIFF AND ASSOCIATED HABITATS IN THE GREAT LAKES REGION OF NORTH AMERICA

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ABSTRACT

The richness of terrestrial gastropod communities in 19 different habitat types in a 1,300 × 1,000 km region in the Great Lakes region of North America was analyzed using 349 0.01–0.1 ha samples. Sites supporting high-richness faunas (24 or more taxa) were limited in the study region to areas south of 45°N. Only weakly significant longitudinal gradients in richness were observed, while a significant latitudinal gradient was present. When only wooded carbonate outcrops were analyzed, a significant negative correlation between richness and latitude was present only between 44°N and 45°N. Highly significant differences in richness between habitats were also observed. Carbonate cliffs harbored the richest faunas, possessing a mean greater than 20. Approximately 25% of these sites contained 24 or more taxa, with a maximum richness of 34 being recorded. Algific talus slopes and lakeshore carbonate ledges were also found to commonly harbor faunas of 17 or more taxa. All of these sites are characterized by shaded, vertical exposures of carbonate bedrock. Only two of the habitats (old fields and open dunes) were found to never support a dozen or more co-occurring taxa. Based on these analyses, carbonate cliffs and related habitats in the Great Lakes region should be included among the most important habitats on a global scale for molluscan biodiversity.

Key words: terrestrial gastropods, community ecology, biodiversity, conservation, North America, Niagaran Escarpment, cliff ecology.

INTRODUCTION

One of the more important components of community structure is richness, or the number of co-occurring species (Peet, 1974). While Solem & Climo (1985) suggest that land snail community richness rarely exceeds 12, a number of other studies have documented much higher rates of sympatry. Tropical forest ecosystems have the highest reported richnesses at various sample grains, with up to 40 taxa being reported from individual sites in the Greater Antilles (Solem, 1984), 45 species from a 400 m² area in southwestern Cameroon (de Winter & Gittenberger, 1998), 50 species from a <4 ha site near Amboni Cave in eastern Tanzania (Emberton et al., 1997), 52 species from a 4 ha area near Manombo, Madagascar (Emberton, 1995), and 56 species from the 4.2 ha Waipipi Scenic Reserve in New Zealand (Solem et al., 1981). Communities with high land snail richness have also been reported from the temperate zone. Up to 24 species have been recorded from 0.01 ha areas in the Italian Alps (Bishop, 1980), 26 species 0.09 ha regions in British Columbia

coniferous forests (Cameron, 1986), 27 species from 9.1 ha Ekholmen Island in Sweden (Nilsson et al., 1988), 39 species from approximately 1 ha samples in SW Sweden (Waldén, 1981), and 44 species from an approximately 4 ha site on Pine Mountain in Harlan County, Kentucky (Emberton, 1995). However, such sites are uncommon enough that Tattersfield (1996) concludes, based upon his review of the international literature, that sites with 24 or more sympatric terrestrial gastropod taxa in small to moderate sample sizes (approx. <10 ha) are of global conservation importance.

Reconnaissance of a dozen eastern Wisconsin limestone and dolomite cliff sites for glacial relict snails (Nekola et al., 1996) documented three sites that possessed 24 or more co-occurring species. Previous surveys made from similar habitats in northeastern Iowa (Frest, 1982, 1987; Frest & Fay, 1981) documented at least five additional sites that also equalled or exceeded this level of sympatry. If these surveys are reflective of such sites as a whole, carbonate cliffs and associated habitats in central North America could be consid-

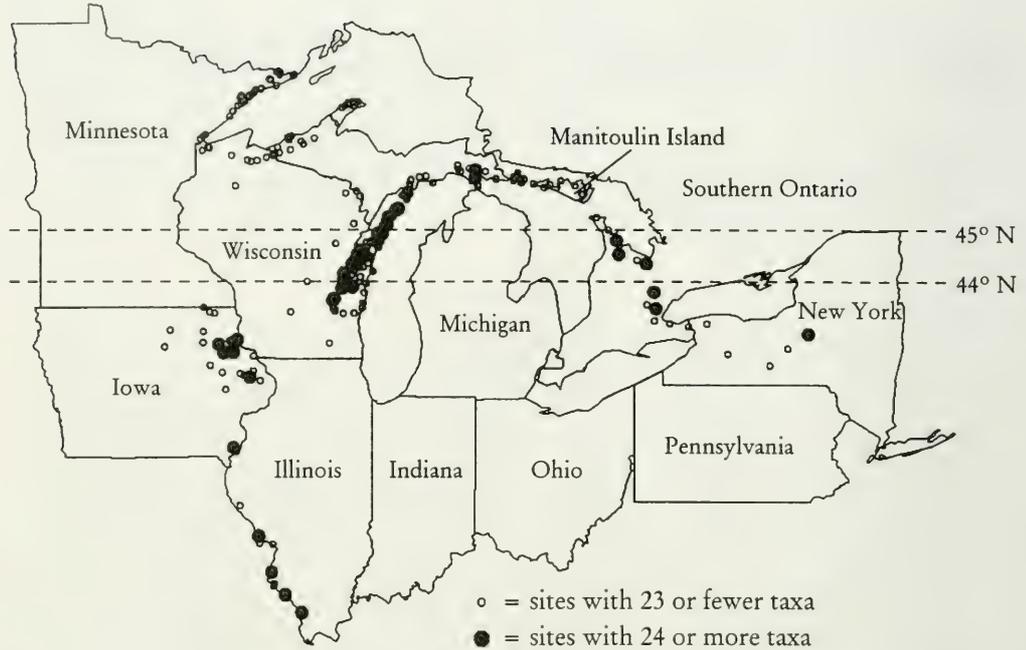


FIG. 1. Distribution of high-richness terrestrial gastropod communities within Great Lakes region.

ered among the richest global terrestrial gastropod communities, particularly at small scales. Unfortunately, given the preliminary nature of these investigations, it was not possible to determine whether these sites were simply outliers, or whether other habitats in this landscape harbored similarly high numbers of species.

The purpose of this paper is to investigate the terrestrial gastropod richness of carbonate cliffs and other habitats in a 1,300 × 1,000 km region centered on the North American Great Lakes. Through this it will be possible to better estimate the frequency of high diversity assemblages from various habitats, to identify potential geographic gradients in species richness over this extent, and to compare the richness of carbonate cliff habitats to others in this landscape.

MATERIALS AND METHODS

Study Sites

A total of 349 areas were surveyed for their terrestrial gastropod faunas (Fig. 1, Appendix I). Sites were chosen for survey if they represented typical examples of their respective

habitat and (except for anthropogenic habitats) were undisturbed. They ranged from north-central Iowa through Wisconsin, the Upper Peninsula of Michigan, and southern Ontario (including Manitoulin Island and the Bruce Peninsula), to central New York State (an extent of approximately 1,300 km), and from northeastern Minnesota and the Keweenaw Peninsula in Michigan to southern Illinois (an extent of approximately 1,000 km). The bulk of collections were made along or adjacent to the Niagaran Escarpment, a narrow zone of exposed Silurian-age carbonates extending from Rochester, New York, to West Union, Iowa. Sampling was most intensive (252 sites) from Drummond Island, Michigan, through northeastern Iowa, where an effort was made to sample along the Escarpment from all areas supporting carbonate bedrock outcrops.

Collections were made from nineteen distinct habitat types: carbonate cliffs (114 sites), igneous cliffs (72), rocky woodlands (21), lakeshore carbonate ledges (19), fens (19), algific talus slopes (16), tamarack wetlands (16), lakeshore alluvial banks (12), upland woods (8), lowland woods (8), white cedar wetlands (8), calcareous meadows (7), cobble beaches (7), alvars (6), carbonate glades

(5), igneous shorelines (4), old fields (3), shale cliffs (3), and open dunes (1).

Carbonate cliffs represent 3–30 m tall, wooded limestone or dolomite outcrops that typically support moss or fern-covered ledges. Igneous cliffs are wooded, 2–20 m tall basalt, serpentine, or granite outcrops and associated open talus slopes located on the Precambrian Shield of northern Wisconsin, the Upper Peninsula of Michigan, and northeastern Minnesota. Rocky woodlands are upland tracts with abundant bedrock or glacial-erratic boulders. Lakeshore carbonate ledges are <3 m tall, wooded limestone or dolomite outcrops that are within 3 km of the Lake Michigan or Lake Huron shore. Fens are peatland areas formed at locations of groundwater discharge that maintain higher soil moisture and a cooler soil temperatures than is otherwise found in the surrounding landscape (Nekola, 1994). Sampling was only conducted from sites in which *Sphagnum* mosses were either uncommon or lacking. Algific talus slopes are associated with mechanical karst systems harboring year-round ice reservoirs. Air and water drainage from these ice caves through loose carbonate talus has created an unique buffered microclimate where soil temperatures rarely range lower than -10°C in winter or exceed 10°C in the summer, and have a more constant soil moisture as compared to surrounding forest soils. Such sites have been shown (Frest, 1991) to support populations of the glacial relict snails *Catinella gelida* (F. C. Baker, 1927), *Discus macclintockii* (F. C. Baker, 1928), *Hendersonia occulta* (Say, 1831), and *Vertigo hubrichti* Pilsbry, 1934. Tamarack wetlands represent almost pure *Larix laricina* (DuRoi) K. Koch. stands that are open and support abundant *Alnus rugosa* (DuRoi) Spreng. and *Carex* growth. Collections were limited to areas that lacked *Sphagnum* cover. Such sites appear restricted to regions with thin soils over carbonate bedrock. Lakeshore alluvial banks represent steep wooded banks along the Lake Michigan shore that are developed into unconsolidated lacustrine material. Upland woods represent wooded tracts developed on soils lacking large rocky debris. Lowland woods represent deciduous forests found in floodplains or depressions. White cedar wetlands represent forested peatlands, dominated by *Thuja occidentalis* L., that are associated with groundwater seepage. Surficial soil chemistry can vary from acidic (where *Sphagnum* moss is abundant) to neu-

tral or alkaline (where *Sphagnum* is largely absent). Litter collections were limited to the latter class of sites. Calcareous meadows are open or very sparsely forested wet meadows found on carbonate-rich mineral (rather than organic) substrate. Cobble beaches are constantly wet shoreline grassland habitats developed on flat limestone or dolomite pavement with little or no soil development except in bedrock fracture planes. Alvares are similar to cobble beaches except that they are found in upland locations and become xeric by mid-summer. Carbonate glades are xeric grassland communities with thin soils overlying limestone, dolomite, or calcareous shales. Igneous shoreline sites occur along the Lake Superior coast in the Keewenaw Peninsula where basalts or basalt-derived conglomerate sequences are exposed. They are largely treeless, have only limited soil development, and support a number of western and arctic disjunct vascular plants. Old fields represent early successional grasslands that develop following agricultural abandonment. Shale cliffs represent wooded cliffs or banks developed into shale exposures that are often kept wet through constant groundwater seepage. Open dunes are xeric grasslands found in sandy soils along the Great Lakes shore.

Field Sampling

Documentation of the terrestrial gastropod communities from each site was accomplished by hand collection of larger shells and litter sampling for smaller taxa from representative 100–1,000 m² regions within sites. As suggested by Emberton et al. (1996), sample collection was concentrated at places of high micro-mollusc density, with a constant volume of soil litter (approximately 4 liters) being collected from each site. For woodland sites, litter collection was concentrated: (1) at places with an abundance of larger shells; (2) along the base of rocks or trees; (3) on soil covered ledges; and/or (4) at cold air vents on the cliff face or in the associated talus. For open sites, collections consisted of: (1) small blocks (approx. 125 cm³) of turf; and/or (2) loose soil and leaf litter accumulations under or adjacent to shrubs, cobbles and/or boulders.

The location of each sample was marked on USGS (or equivalent) 7.5 minute topographic maps. The latitude-longitude coordinates for each was then determined through digitization of these maps using the ATLAS DRAW software package. Conversion of loca-

tions into UTM Zone 16 coordinates was completed using ARCINFO.

Laboratory Procedures

Samples were slowly and completely dried in either a low-temperature soil oven (approx. 80–95°C) or in full sun in a greenhouse. Dried samples were then soaked in water for 3–24 hours, and subjected to careful but vigorous water disaggregation through a standard sieve series (ASTME 3/8" (9.5 mm), 10 (2.0 mm), 20 (0.85), and 40 (0.425 mm) mesh screens). Sieved sample fractions were then dried and passed again through the same sieve series. These dry, resorted fractions were then hand picked against a neutral-brown background. All shells and shell fragments were removed.

All recovered, identifiable shells were assigned to species (or subspecies) using the author's reference collection and the Hubricht Collection at the Field Museum of Natural History. From this, the total number of taxa per site was determined. All specimens have been catalogued and are housed in collections maintained at the University of Wisconsin-Green Bay.

Statistical Analyses

The frequency of high richness (24 or more taxa) sites was calculated across all habitats, and for wooded carbonate outcrops (carbonate cliffs, algific talus slopes, lakeshore carbonate ledges) only, within each of the included states or provinces (Illinois, Iowa, Minnesota, Michigan, southern Ontario, and New York). Testing for statistical differences in the ratio of high vs. normal or low richness sites was conducted via the Pearson chi-square and likelihood ratio tests. The likelihood ratio test was calculated as some of the predicted values were sparse (< 5), complicating interpretation of Pearson's chi-square statistic. The asymptotic distribution of the likelihood ratio test, however, is trustworthy when the number of observations (349 and 149, respectively) equal or exceed the number of cells (14) by a factor of ten (Zar, 1984). Based on apparent differences in the ratio of high-diversity sites between northern and southern sections of the study area, these tests were repeated following exclusion of sites from Minnesota, Michigan and Manitoulin Island.

The relationship between geographic position and richness was graphically represented

by plotting site richness vs. UTM N-S or UTM E-W coordinates for (1) all habitats, and (2) for wooded carbonate outcrop (carbonate cliff, algific slope, and carbonate lakeshore ledge) sites only. The central tendencies in these relationships were indicated through locally weighted scatterplot smoothing (Cleveland, 1979). The statistical significance of these relationships, and amount of variance in richness accounted for by geographic position, was estimated using least-squares regression. Cartesian UTM coordinates were analyzed to preclude biases originating from use of polar-coordinate latitude and longitude coordinates.

For the N-S relationships, locally weighted scatterplot smoothing indicated that the response of richness might not be constant. Tests for such differences in response were conducted by splitting the data sets into different N-S position regions, and repeating regression analyses separately for each. The p -values and r^2 for each of these models were recorded.

The central tendency in site richness among habitat types was graphically represented via a box plot with habitats being sorted along the horizontal axis from the highest to lowest means. In box plots the central line represents the median of the sample, the margins of the box represent the interquartile distances, and the fences represent 1.5 times the interquartile distances. For data having a Gaussian distribution, approximately 99.3% of the data will fall inside of the fences (Velleman & Hoaglin, 1981). Outliers falling outside of the fences are shown with asterisks. Testing for significant differences in the average richness between habitats was conducted using ANOVA.

RESULTS

Regional Patterns

Forty of 349 sites harbored 24 or more terrestrial gastropod taxa within 0.01–0.1 ha samples (Fig. 1). Seven sites (4 Iowa, 1 Illinois, 1 Ontario, 1 Wisconsin) harbored 30 or more taxa, with a maximum richness of 34 being observed from a Brown County, Wisconsin, site. Eighty-five percent of high richness sites were found on carbonate cliff habitats. The only non-carbonate cliff habitats that possessed high terrestrial gastropod richness were three algific talus slopes (all with imbedded carbonate cliffs) and single white cedar

TABLE 1. Ratio of high richness (24 or more taxa) to medium and low richness (23 or fewer taxa) sites in states and provinces within study region.

State or Province	All sites		Wooded carbonate outcrops	
	# high	# medium-low	# high	# medium-low
Illinois	4	5	4	5
Iowa	8	25	7	16
Michigan	1	74	1	18
Minnesota	0	39	0	0
New York	1	5	1	2
Ontario	5	17	5	14
Wisconsin	21	144	19	57

	All states and provinces	
	All sites	Wooded carbonate outcrops
Pearson Statistic	30.763	6.2791
<i>p</i> -value	< 0.00005	0.2800
Likelihood Ratio Statistic	34.2086	7.3618
<i>p</i> -value	< 0.00005	0.1951

Minnesota, Michigan and Manitoulin Island sites excluded	
	All sites
Pearson Statistic	12.2529
<i>p</i> -value:	0.0156
Likelihood Ratio Statistic	10.4536
<i>p</i> -value:	0.0334

wetland, tamarack wetland, and rocky woodland sites.

Approximately 11% of all sampled sites had 24 or more taxa (Table 1). The frequency of these high richness sites in the seven states or provinces varied between 0% and 44% of all sites. These differences were significant (Pearson chi-square and likelihood ratio $p < 0.00005$). It appeared possible that this difference may be attributed to the much lower frequency of high-richness sites in Minnesota, Upper Peninsula of Michigan, and Manitoulin Island. However, differences in the frequency of high-richness sites was found to remain marginally significant (Pearson chi-square $p = 0.0156$; likelihood ratio $p = 0.0334$), even after removal of the most northern regions from analysis. This marginal significance is apparently related to a lowered frequency of high-richness sites in Wisconsin.

Approximately 25% of all wooded carbonate outcrop sites harbored high richness communities. The frequency of these in the five states or provinces ranged between 5% and 44% (Table 1), and occurred over the entire extent of the sample region (Fig. 1). While carbonate cliff sites of high-richness appeared scarce in the Upper Peninsula of Michigan and Manitoulin Island, Pearson's chi-square ($p = 0.2800$) and the likelihood

ratio ($p = 0.1951$) tests demonstrated that at the state or province scale, these differences were non-significant.

Geographic Gradients

Only a marginally significant (Fig. 2; $p = 0.031$) and weak ($r^2 = 0.013$) trend was found between richness and E-W UTM position across all habitats. This relationship was found to not be significant when only wooded carbonate outcrops were analyzed (Fig. 3; $p = 0.106$). The relationship between richness and N-S UTM location, however, was found to be stronger and more significant both for all habitats ($p < 0.0005$; $r^2 = 0.188$) as well as for wooded carbonate outcrop sites only ($p = 0.003$; $r^2 = 0.059$), with northerly sites possessing lower richness than southerly sites.

The shape of the locally weighted scatterplot smoothing lines for the N-S relationships, in conjunction with additional regression analyses, demonstrate that this pattern is not constant over the study region. Across all habitats, only a weak ($r^2 = 0.063$) but statistically significant ($p = 0.001$) relationship was observed south of 5,000 km while north of this position this same relationship was more significant ($p < 0.0005$) and over 4½ times stronger ($r^2 = 0.289$; Fig. 2). When only

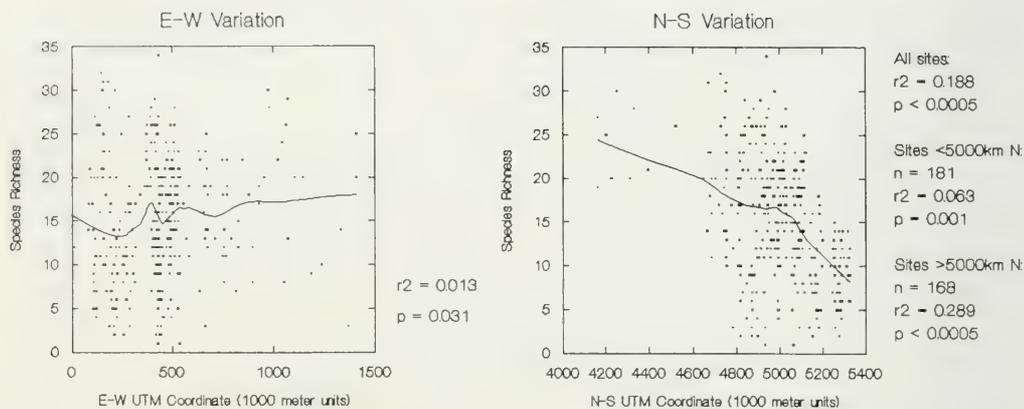


FIG. 2. Relationship of terrestrial gastropod richness to E-W and N-S UTM location across all 19 habitat types. A locally weighted scatterplot smoothing line has been fitted to each relationship.

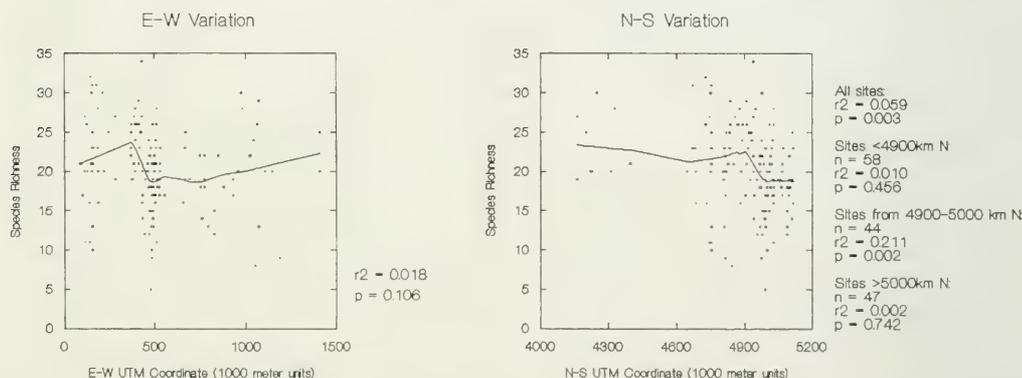


FIG. 3. Relationship of terrestrial gastropod richness to E-W and N-S UTM location for wooded carbonate outcrop sites (carbonate cliffs, algific talus slopes, and lakeshore carbonate ledges). A locally weighted scatterplot smoothing line has been fitted to each relationship.

wooded carbonate outcrops were considered, no relationship was apparent south of 4900 km (roughly 44° N; $p = 0.456$) and north of 5,000 km (approx. 45° N; $p = 0.742$). However, a significant ($p = 0.002$) and moderately strong ($r^2 = 0.221$) relationship was apparent between 4,900 and 5,000 km (Fig. 3).

Habitat Patterns

Comparison of site richness values demonstrate striking differences among the 19 sampled habitat types (Fig. 4). Carbonate cliffs were the richest habitats sampled, possessing a mean score approaching 21. Algific talus slopes and lakeshore carbonate ledges followed, having mean richness scores exceeding 17. Both carbonate cliffs and algific slopes had upper data fences that exceeded 30 species per site. Rocky woodlands, carbonate

glades, calcareous meadows, white cedar wetlands and fens had mean richness scores ranging from 15.3 to 13.9. Igneous shorelines, tamarack wetlands, lakeshore alluvial banks, lowland woods and cobble beaches had mean richness scores ranging from 12 to 10.6. Igneous cliffs, alvars, shale cliffs, upland woods, old fields, and open dunes all had mean richness scores of less than 10. ANOVA showed these differences to be highly significant ($p < 0.0005$), with almost 50% of observed variance in richness being accounted for by habitat type.

DISCUSSION

Regional Species Richness Patterns

Although Solem & Climo (1985) stated that land snail community richness rarely exceeds

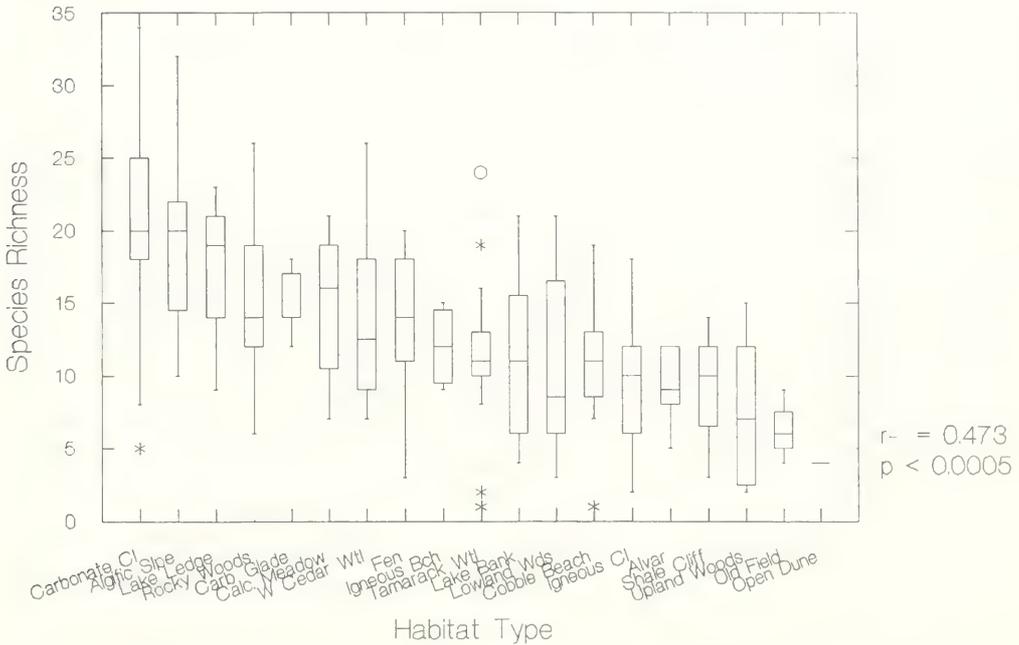


FIG. 4. Box-plot diagrams of terrestrial gastropod richness across 19 habitat types. Habitats are sorted along the horizontal axis from highest to lowest mean scores.

12 taxa, fully 232 of the sites inventoried (66% of the total) equalled or exceeded this level. Sites with 12 or more taxa were found in 17 of the 19 sampled habitats. Only old field and open dune habitats never equalled or exceeded this richness level. It is not clear whether Solem & Climo were unnecessarily pessimistic about terrestrial gastropod community richness, or if the Niagaran Escarpment in the Great Lakes region possesses uniquely rich community assemblages. While it seems likely that the former is true, it should be mentioned that sites with a dozen or more co-occurring terrestrial gastropod taxa may be less frequent in other landscapes. For example, in this study only 34% of northern Wisconsin, western Upper Peninsula and northeastern Minnesota sites had 12 or more co-occurring taxa. Burch (1956) reported maximum mean richness of nine taxa per site in the eastern piedmont and coastal plain of Virginia. Clarke et al. (1968) found no more than nine co-occurring taxa in New Brunswick forests. In their survey of 189 sites (many with carbonate substrates) in the Black Hills of South Dakota, Frest & Johannes (1993) report only seven (less than 4%) that harbor a dozen or more taxa. Cowie et al. (1995) found

that no more than 12 taxa coexisted within approximately 100 m² samples on Hawaiian vegetated lava flows. It will be necessary to expand these analyses to additional landscapes with a greater diversity of geological substrates and ecological histories to determine whether the terrestrial gastropod communities of the Great Lakes are uniquely rich, or if our definition of what constitutes a species-rich community must be expanded.

Little or no variation in richness was recorded over most of the region. Only a very weak longitudinal trends were identified, and only north of UTM 5,000 were strong latitudinal trends observed. When only wooded carbonate outcrops (carbonate cliffs, algific talus slopes, and lakeshore carbonate ledges) were considered, the significant latitudinal trend in richness was restricted to sites falling between UTM 4,900 and 5,000 km N (or roughly 44° N to 45° N). Similarly, the occurrence frequency of high richness sites (using Tattersfield's criteria of 24 or more taxa) was found to only weakly differ between Illinois, Iowa, southern Ontario, New York, and Wisconsin. However, in Minnesota, the Upper Peninsula of Michigan, and Manitoulin Island this ratio was over ten times lower across all

habitats, and at least five times lower on wooded carbonate outcrops.

A number of factors could be responsible for the significantly lower richness levels observed in the northern reaches of the study area. At least some of this decrease in richness may be due to lower-Ca and pH soils associated with igneous (rather than carbonate) bedrock on northern sites. However, this can not explain the significant reductions in richness observed on the northern-most wooded carbonate outcrop sites in the Upper Peninsula and Manitoulin Island. Perhaps the low richness values are related to the greater isolation of these sites, because they are separated from other carbonate outcrops by the waters of Green Bay and Georgian Bay as well as the acidic soils of the Precambrian Shield. Additional research will be necessary to tease apart the differential roles played by contemporaneous and historical processes in determining regional terrestrial gastropod richness patterns.

Habitat-Specific Species Richness Patterns

Significant differences were observed among the 16 sampled habitats, with carbonate cliffs possessing the highest average number of taxa per site. Over one-half of such sites harbored 21 or more species. Other habitats found to harbor rich assemblages of species included algific talus slopes, lakeshore carbonate ledges, rocky woodlands, carbonate glades, calcareous open meadows, white cedar wetlands, and fens. All of these habitats are associated with calcareous substrata, either in the form of exposed bedrock, boulders, talus, wet marl, calcareous alluvium or nutrient-rich peat. The lowest richness habitats were, in general, associated with more acidic substrata such as igneous outcrops, sand dunes, or exposed alluvium. However, this pattern is not without exception as low-richness cobble beach and alvar faunas are developed on carbonate outcrops.

Carbonate Cliffs as Terrestrial Gastropod Diversity Hot Spots

Wooded carbonate cliffs, on average, support the highest number of terrestrial gastropod taxa within any habitat in the study region. The richest 5% of these support 29 or more taxa, with a maximum of 34 taxa being recorded. Such sites appear to be among the richest reported globally from 1 ha or smaller

quadrats. Waldén (1981) observed up to 39 taxa from 1 ha quadrats in wooded talus slopes in Sweden, while Tattersfield (1996) identified up to 33 taxa per one-sixth hectare samples from Kenyan rain forest. Other published reports of terrestrial gastropod richness from 0.1 ha or less quadrats (e.g., Schmid, 1966; Bishop, 1980; Nilsson et al., 1988; Getz & Uetz, 1994; Cowie et al., 1995; de Winter & Gittenberger, 1998) have reported no more than 45 co-occurring taxa. Maximum richness in Great Lakes carbonate cliff sites is also within 25% of the richest known North American site (at Pine Mountain, Kentucky; Ember-ton, 1995).

Further research will be necessary to determine if the richness levels of carbonate cliffs in the Great Lakes region are unique, or if similar levels are present in other landscapes. Research from other regions (e.g., New South Wales, Australia: Stanistic, 1997; Germany: Schmid, 1966; Scotland: Cameron & Greenwood, 1991; Sweden: Waldén 1981) indicates that maximum terrestrial gastropod richness frequently occurs on wooded carbonate outcrops. Based on this current and previous research it seems likely that carbonate cliffs will be found to be among the most important habitats for molluscan biodiversity on a global scale.

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APPENDIX I

Name, location, habitat type, and terrestrial gastropod richness of sample sites

Site Name	Location	Habitat Type	Richness
ILLINOIS			
Calhoun County			
Franklin Hill	90°36'38"W, 39°3'57"N	Carbonate Cliff	28
Jackson County			
Kings Ferry Bluff Base	89°26'16"W, 37°36'36"N	Carbonate Cliff	27
Kings Ferry Bluff Crest	89°26'15"W, 37°36'36"N	Carbonate Cliff	19
Madison County			
Cliffton Terrace	90°13'36"W, 38°54'51"N	Carbonate Cliff	20
Monroe County			
Fults Reserve	90°11'15"W, 38°9'19"N	Carbonate Cliff	20
Fountain Gap	90°15'33"W, 38°22'36"N	Carbonate Cliff	30
Pike County			
Shewhart Bluff	91°6'48"W, 39°39'N	Carbonate Cliff	21
Randolph County			
Prairie du Rocher	90°11'56"W, 38°6'28"N	Carbonate Cliff	20
Chester	89°53'6"W, 37°56'42"N	Carbonate Cliff	25
IOWA			
Bremer County			
Brayton-Horsley	92°6'28"W, 42°48'35"N	Fen	14
Buchanan County			
Rowley West	91°54'39"W, 42°22'15"N	Fen	20
Chickasaw County			
Stapelton Church	92°6'14"W, 43°1'35"N	Fen	17
Clayton County			
Bixby East	91°23'56"W, 42°40'28"N	Algific Talus Slope	16
Buck Creek 4	91°11'23"W, 42°51'35"N	Algific Talus Slope	13
Buck Creek Tributary 1	91°10'55"W, 42°51'47"N	Algific Talus Slope	21
Buck Creek Tributary 2	91°10'55"W, 42°51'56"N	Algific Talus Slope	10
Buck Creek Tributary 3	91°10'55"W, 42°52'5"N	Algific Talus Slope	25
Buck Creek Tributary 4	91°10'58"W, 42°52'1"N	Algific Talus Slope	31
Buck Creek Tributary 5	91°11'2"W, 42°51'52"N	Algific Talus Slope	23
Elkader South	91°23'45"W, 42°50'23"N	Algific Talus Slope	11
South Cedar 2	91°14'38"W, 42°49'58"N	Algific Talus Slope	20
South Cedar 3	91°14'23"W, 42°49'51"N	Carbonate Cliff	30
Delaware County			
Backbone West	91°33'43"W, 42°37'5"N	Carbonate Cliff	26
Elk River East	91°17'27"W, 42°37'42"N	Algific Talus Slope	32
Des Moines County			
Iowa Ammunition Plant	91°17'16"W, 40°46'27"N	Rocky Woods	26
Dubuque County			
Roosevelt Road	90°44'30"W, 42°32'55"N	Carbonate Glade	17

Fayette County			
Brush Creek Canyon 1	91°41'27"W, 42°47'2"N	Algific Talus Slope	11
Brush Creek Canyon 2	91°41'20"W, 42°46'46"N	Carbonate Cliff	27
Floyd County			
Juniper Hill Shale Glade	92°59'2"W, 43°3'10"N	Carbonate Glade	12
Franklin County			
Hampton East Glade	93°8'13"W, 42°43'41"N	Carbonate Glade	14
Howard County			
Larkin Bridge East	92°5'8"W, 43°29'32"N	Algific Talus Slope	21
Jackson County			
Hamilton Glade	90°34'8"W, 42°4'23"N	Carbonate Glade	14
Lytle Creek 6	90°45'21"W, 42°15'34"N	Algific Talus Slope	20
Lytle Creek 16	90°45'28"W, 42°15'40"N	Algific Talus Slope	16
Maquoketa Caves	90°46'22"W, 42°7'3"N	Carbonate Cliff	23
Pine Creek	90°50'41"W, 42°8'27"N	Carbonate Cliff	31
Jones County			
Canton Glade	90°59'52"W, 42°10'46"N	Carbonate Glade	18
Pictured Rocks	91°6'18"W, 42°12'28"N	Carbonate Cliff	23
Linn County			
Dark Hollow	91°30'W, 41°53'54"N	Carbonate Cliff	20
Paris Fen	91°35'41"W, 42°13'39"N	Fen	11
Winneshiek County			
Bluffton West 2	91°55'16"W, 43°24'10"N	Algific Talus Slope	17
Heritage Farm	91°47'59"W, 43°22'55"N	Algific Talus Slope	20

MICHIGAN

Chippewa County			
Bass Cove Cobble Beach	83°32'45"W, 45°55'10"N	Cobble Beach	11
Bass Cove Upland	83°32'46"W, 45°55'24"N	Rocky Woods	11
Hill Lake East	84°30'51"W, 46°7'48"N	Carbonate Cliff	19
Huron Bay	83°45'17"W, 45°57'12"N	Cobble Beach	11
Maple Hill	84°46'55"W, 46°9'34"N	Carbonate Cliff	25
Marble Head Center	83°28'28"W, 45°59'3"N	Carbonate Cliff	18
Marble Head North	83°28'30"W, 45°59'17"N	Carbonate Cliff	19
Marble Head South	83°28'35"W, 45°58'46"N	Carbonate Cliff	22
Maxton Plains Center 1	83°39'48"W, 46°4'26"N	Alvar	8
Maxton Plains Center 2	83°39'24"W, 46°4'44"N	Tamarack Wetland	8
Poe Point	83°38'30"W, 46°6'10"N	Lakeshore Carbonate Ledge	14
Prentiss Bay	84°13'49"W, 45°59'25"N	Tamarack Wetland	13
Scott Bay	83°40'1"W, 46°3'31"N	Fen	12
Scott Quarry	84°50'4"W, 46°10'43"N	Carbonate Cliff	23
Seastone Point	83°45'34"W, 46°1'21"N	Carbonate Cliff	22
Spencers Mountain Lower	84°56'39"W, 46°11'33"N	Rocky Woods	19
Spencers Mountain Upper	84°56'43"W, 46°11'29"N	Carbonate Cliff	16
Tourist Road	83°43'48"W, 46°1'44"N	Carbonate Cliff	18
Delta County			
Ansel's Point	86°34'26"W, 45°48'12"N	Carbonate Cliff	23
Burnt Bluff	86°42'39"W, 45°41'11"N	Carbonate Cliff	19
Cooks Ridge	86°29'27"W, 45°57'59"N	Rocky Woods	20
Fayette St. Park	86°39'46"W, 45°43'40"N	Carbonate Cliff	18
Garden Bluff	86°37'48"W, 45°46'47"N	Carbonate Cliff	17
Garden Corners	86°32'4"W, 45°53'23"N	Tamarack Wetland	11
Garden Peninsula Alvar	86°38'38"W, 45°39'48"N	Alvar	5
Gouly Harbor	86°36'47"W, 45°46'42"N	Carbonate Cliff	21

Jacks Bluff	86°31'33"W, 45°51'10"N	Lakeshore Alluvial Bank	21
Kregg Bay Northwest 1	86°32'10"W, 45°43'13"N	Alvar	9
Kregg Bay Northwest 2	86°31'59"W, 45°42'39"N	Cobble Beach	1
O16 West	86°43'1"W, 45°40'17"N	Rocky Woods	14
Pt. Detour 1	86°37'47"W, 45°38'3"N	Alvar	9
Pt. Detour 2	86°36'21"W, 45°36'25"N	Cobble Beach	15
Pt. Detour 3	86°36'25"W, 45°36'16"N	Lakeshore Carbonate Ledge	19
South River Bay	86°37'19"W, 45°45'22"N	Carbonate Cliff	20
Gogebic County			
Bessemer NE	90°2'24"W, 46°29'31"N	Igneous Cliff	16
Copper Mountain	90°5'16"W, 46°36'5"N	Igneous Cliff	11
Mt. Zion Park	90°10'8"W, 46°28'37"N	Igneous Cliff	2
Keewenaw County			
Agate Point	88°1'4"W, 47°28'38"N	Igneous Lakeshore	14
Brockway Mountain	87°58'15"W, 47°27'48"N	Igneous Cliff	6
Cliff Range North 1	88°15'11"W, 47°23'55"N	Igneous Cliff	11
Cliff Range North 2	88°15'9"W, 47°23'55"N	Igneous Cliff	13
Cliff Range South	88°19'50"W, 47°21'36"N	Igneous Cliff	12
Cliffton	88°19'23"W, 47°21'54"N	White Cedar Wetland	12
Copper Harbor Shore	87°54'15"W, 47°28'36"N	Igneous Lakeshore	10
Copper Harbor Marina	87°54'14"W, 47°28'19"N	White Cedar Wetland	11
Dans Point	87°58'37"W, 47°28'47"N	Igneous Lakeshore	15
Delaware Gap	88°6'57"W, 47°25'29"N	Igneous Cliff	13
Eagle Harbor	88°8'54"W, 47°27'39"N	Igneous Lakeshore	9
Grand Marais Harbor	88°7'9"W, 47°27'37"N	Igneous Cliff	15
Mt. Bohemia	88°0'51"W, 47°23'34"N	Igneous Cliff	12
Luce County			
McLeod Hill	85°15'37"W, 46°15'17"N	Rocky Woods	23
Mackinac County			
Dinkey Line Road	85°14'16"W, 46°10'49"N	White Cedar Wetland	7
Gamble Road	84°45'W, 46°7'42"N	Rocky Woods	21
Greene Cedar	84°51'51"W, 46°1'41"N	White Cedar Wetland	14
Kenneth Road	84°50'34"W, 46°5'50"N	Carbonate Cliff	19
Martineau Creek	84°43'11"W, 45°59'8"N	Fen	14
McCann High School	84°43'36"W, 45°51'48"N	Rocky Woods	12
Point St. Ignace	84°42'34"W, 45°57'8"N	Rocky Woods	18
Round Lake 1	84°52'31"W, 45°57'18"N	Fen	3
Round Lake 2	84°52'31"W, 45°57'11"N	White Cedar Wetland	13
Summerby Swamp	84°47'43"W, 45°58'15"N	Fen	19
Townhall Road	85°10'28"W, 46°8'19"N	White Cedar Wetland	22
Ontonagon County			
Adventure Mountain	89°4'51"W, 46°46'17"N	Igneous Cliff	18
Cloud Peak	89°43'58"W, 46°48'51"N	Igneous Cliff	16
Cranberry River Hill	89°26'28"W, 46°42'47"N	Igneous Cliff	8
Cuyahoga Peak	89°41'59"W, 46°48'56"N	Igneous Cliff	17
Miscowawbic Peak	89°48'47"W, 46°47'N	Igneous Cliff	16
Norwich Mountain	89°22'54"W, 46°39'37"N	Igneous Cliff	12
Rodgers Cedar Swamp	89°44'3"W, 46°33'7"N	White Cedar Wetland	7
Summit Peak Lower	89°46'27"W, 46°44'55"N	Igneous Cliff	3
Summit Peak Upper	89°46'25"W, 46°44'48"N	Igneous Cliff	5
School Craft County			
Manistique North	86°16'W, 46°1'37"N	Carbonate Cliff	17
Merwin Creek	86°5'40"W, 46°2'30"N	Rocky Woods	13
Seul Choix Point 1	85°54'50"W, 45°55'12"N	Cobble Beach	19
Seul Choix Point 2	85°54'53"W, 45°55'14"N	Rocky Woods	11

MINNESOTA

Cook County			
Caribou Lake E	90°40'1"W, 47°42'23"N	Igneous Cliff	9
Caribou Lake N	90°40'40"W, 47°42'46"N	Igneous Cliff	6
Carlton Peak	90°51'22"W, 47°35'9"N	Igneous Cliff	2
Cascade River Cedars	90°31'32"W, 47°44'36"N	Igneous Cliff	14
Cascade River Cliff	90°32'15"W, 47°43'28"N	Igneous Cliff	12
Iceland Fen	90°7'3"W, 47°47'44"N	Tamarack Wetland	11
John Lake	90°3'29"W, 48°3'56"N	Igneous Cliff	9
Lake Cliff	90°58'23"W, 47°29'40"N	Igneous Cliff	5
Lutsen Mountains	90°41'54"W, 47°40'49"N	Igneous Cliff	4
McFarland Lake Cliff	90°5'25"W, 48°3'34"N	Igneous Cliff	6
McFarland Lake Talus	90°5'18"W, 48°3'36"N	Igneous Cliff	6
Moose Mt. Cliff	90°44'9"W, 47°39'7"N	Igneous Cliff	11
Mt. Josephine Cliff	89°39'14"W, 47°58'58"N	Igneous Cliff	14
Mt. Josephine Talus	89°39'14"W, 47°58'50"N	Igneous Cliff	11
Oberg Mountain	90°46'38"W, 47°37'44"N	Igneous Cliff	5
Pine Lake	90°6'7"W, 48°3'3"N	Igneous Cliff	12
Pine River Road	90°18'4"W, 47°54'59"N	Igneous Cliff	10
Poplar River	90°43'15"W, 47°39'55"N	Igneous Cliff	13
Poplar River Overlook	90°43'34"W, 47°40'13"N	Igneous Cliff	6
Port of Entry Cliff	89°37'12"W, 47°59'51"N	Igneous Cliff	9
Port of Entry Talus	89°37'6"W, 47°59'47"N	Igneous Cliff	9
Portage Brook	90°1'24"W, 48°0'2"N	Igneous Cliff	6
Sawbill Road N Cliff	90°51'37"W, 47°35'50"N	Igneous Cliff	10
South Fowl Lake	90°0'28"W, 48°2'27"N	Igneous Cliff	11
Sugarloaf Cove	90°58'50"W, 47°29'12"N	Igneous Cliff	7
Temperance River Road	90°53'3"W, 47°34'6"N	Igneous Cliff	8
Temperance River Upland	90°52'22"W, 47°34'22"N	Igneous Cliff	14
Timber Creek	90°15'19"W, 47°53'53"N	Igneous Cliff	10
Lake County			
Day Hill	91°22'59"W, 47°11'36"N	Igneous Cliff	10
Finland Forest	91°5'25"W, 47°33'13"N	Igneous Cliff	7
Goldeneye Lake Cliff	91°3'55"W, 47°35'53"N	Igneous Cliff	6
Goldeneye Lake Talus	91°3'48"W, 47°35'50"N	Igneous Cliff	7
Manitou River Falls	91°4'27"W, 47°26'44"N	Igneous Cliff	12
Sawmill Creek	91°9'43"W, 47°24'15"N	Igneous Cliff	3
Water Tanks	91°17'45"W, 47°17'42"N	Igneous Cliff	7
St. Louis County			
Chester Bowl	92°5'55"W, 46°48'48"N	Igneous Cliff	10
Hawk Ridge Sanctuary	92°1'55"W, 46°50'49"N	Igneous Cliff	13
Skyline Drive West Cliff	92°10'6"W, 46°45'47"N	Igneous Cliff	5
Skyline Drive West Talus	92°10'6"W, 46°45'43"N	Igneous Cliff	14

NEW YORK

Cayuga County			
Fillmore Glen State Park	76°23'52"W, 42°41'45"N	Shale Cliff	3
Madison County			
Cazenova Gorge 1	75°50'41"W, 42°58'49"N	Carbonate Cliff	25
Cazenova Gorge 2	75°50'49"W, 42°58'49"N	Carbonate Cliff	20
Niagara County			
Gasport Ravine	78°35'2"W, 43°10'52"N	Carbonate Cliff	9
Schuyler County			
Watkins Glen State Park	76°53'24"W, 42°22'16"N	Shale Cliff	14

Wyoming County			
Letchworth State Park	78°1'26"W, 42°35'41"N	Shale Cliff	10

ONTARIO

Bruce County			
Grotto Trail	81°31'19"W, 45°14'38"N	Carbonate Cliff	19
Lions Head	81°13'4"W, 45°0'28"N	Carbonate Cliff	20
Overhanging Point	81°31'51"W, 45°14'40"N	Carbonate Cliff	17
Grey County			
Inglis Falls	80°56'2"W, 44°31'50"N	Carbonate Cliff	28
Metcalfe Rock	80°26'31"W, 44°25'3"N	Carbonate Cliff	22
Skinner's Bluff	80°59'31"W, 44°47'36"N	Carbonate Cliff	30
Halton County			
Crawford Lake	79°56'27"W, 43°28'27"N	Carbonate Cliff	29
Royal Municipality of Hamilton-Wentworth			
Dundas Park	79°58'48"W, 43°14'18"N	Carbonate Cliff	13
Manitoulin District			
Burnt Island	82°56'9"W, 45°49'26"N	Cobble Beach	7
Cooks Dock Lower	82°47'20"W, 45°52'50"N	Carbonate Cliff	12
Cooks Dock Upper	82°47'20"W, 45°52'48"N	Carbonate Cliff	15
Cup & Saucer East	82°6'10"W, 45°51'21"N	Carbonate Cliff	18
Cup & Saucer North	82°5'59"W, 45°51'9"N	Carbonate Cliff	18
Janet Head	82°29'16"W, 45°56'43"N	Carbonate Cliff	22
McLean Park Lowland	81°54'28"W, 45°41'31"N	Lowland Woods	8
McLean Park Upland	81°54'25"W, 45°41'44"N	Rocky Woods	12
Mississagi Lighthouse	83°13'19"W, 45°53'36"N	Lakeshore Carbonate Ledge	13
Niagara County			
Beamers Falls	79°34'1"W, 43°11'16"N	Carbonate Cliff	20
Niagara Whorlpool	79°4'1"W, 43°7'24"N	Carbonate Cliff	20
Peel County			
Devils Pulpit	79°59'27"W, 43°48'4"N	Carbonate Cliff	26
Simcoe County			
Glen Huron	80°11'41"W, 44°21'1"N	Carbonate Cliff	24
Wellington County			
Guelph Jail	80°10'44"W, 43°32'51"N	Carbonate Cliff	8

WISCONSIN

Ashland County			
Beaverdam Lake	90°48'33"W, 46°19'26"N	Igneous Cliff	3
Loon Lake Bluff	90°38'49"W, 46°20'52"N	Igneous Cliff	12
St. Peter's Dome	90°54'39"W, 46°21'5"N	Igneous Cliff	9
Bayfield County			
Rainbow Lake Wilderness	91°20'13"W, 46°26'35"N	Upland Woods	7
Brown County			
Bayshore Park	87°47'59"W, 44°38'12"N	Carbonate Cliff	17
Benderville Wayside	87°50'31"W, 44°36'47"N	Carbonate Cliff	27
Blueberry Marsh	87°53'35"W, 44°31'46"N	Tamarack Wetland	1
Celtis Site	87°50'52"W, 44°36'35"N	Carbonate Cliff	34
Edgewater Villas	87°49'8"W, 44°37'42"N	Carbonate Cliff	14
Escarpment Glade	87°49'4"W, 44°37'9"N	Alvar	12
Fonferik Glen	87°58'15"W, 44°25'34"N	Carbonate Cliff	25
Gibson Alvar	87°50'52"W, 44°35'26"N	Alvar	12

Gravel Pit Road	87°47'59"W, 44°36'7"N	Calcareous Meadow	19
Greenleaf Cliff	88°4'1"W, 44°20'20"N	Carbonate Cliff	29
Greenleaf Talus	88°4'4"W, 44°20'16"N	Carbonate Cliff	21
Hilly Haven	88°2'43"W, 44°20'59"N	Carbonate Cliff	26
Iron Fence Wayside	87°49'40"W, 44°37'14"N	Carbonate Cliff	20
Lily Lake County Park 1	87°51'3"W, 44°25'19"N	Tamarack Wetland	13
Lily Lake County Park 2	87°51'3"W, 44°25'22"N	Lowland Woods	20
Neshota County Park 1	87°48'22"W, 44°24'5"N	Fen	20
Neshota County Park 2	87°48'17"W, 44°24'6"N	Rocky Woods	18
Scray's Hill	88°1'37"W, 44°23"N	Carbonate Cliff	23
UWGB Upland Woods	87°55'22"W, 44°31'33"N	Upland Woods	3
UWGB Cedar Swamp	87°55'29"W, 44°31'33"N	White Cedar Wetland	26
UWGB Escarpment	87°54'21"W, 44°31'48"N	Carbonate Cliff	25
Calumet County			
Calumet County Park	88°19'11"W, 44°6'45"N	Carbonate Cliff	20
Charlesburg Ledge	88°12'3"W, 43°58'10"N	Carbonate Cliff	26
East River Road	88°3'42"W, 44°8'21"N	Tamarack Wetland	12
High Cliff State Park 1	88°17'56"W, 44°9'11"N	Carbonate Cliff	24
High Cliff State Park 2	88°16'44"W, 44°10'13"N	Carbonate Cliff	26
High Cliff State Park 3	88°17'52"W, 44°9'18"N	Rocky Woods	14
High Cliff State Park 4	88°17'49"W, 44°9'14"N	Upland Woods	7
Kiel Marsh	88°3'34"W, 43°53'52"N	Tamarack Wetland	24
Stockbridge	88°17'17"W, 44°3'10"N	Carbonate Cliff	28
Dodge County			
Ledge County Park	88°35'2"W, 43°28'11"N	Carbonate Cliff	22
Mayville South	88°32'24"W, 43°27'27"N	Carbonate Cliff	23
Messner Ledge South	88°35'41"W, 43°37'55"N	Carbonate Cliff	25
Neda Mine	88°32'5"W, 43°25'23"N	Carbonate Cliff	19
Door County			
Bjorklunden	87°7'58"W, 45°1'54"N	Lakeshore Carbonate Ledge	21
Boyer Bluff 1	86°56'2"W, 45°25'10"N	Carbonate Cliff	17
Boyer Bluff 2	86°55'55"W, 45°25'6"N	Carbonate Cliff	16
Brussels Hill North	87°35'45"W, 44°46'13"N	Carbonate Cliff	20
Brussels Hill Radio Tower	87°35'27"W, 44°44'47"N	Carbonate Cliff	19
Carlsville Road	87°22'1"W, 44°57'4"N	Carbonate Cliff	12
Cave Point Shore	87°10'33"W, 44°55'38"N	Lakeshore Carbonate Ledge	9
Cave Point Uplands	87°10'44"W, 44°55'51"N	Rocky Woods	12
Cherry Escarpment	87°9'25"W, 45°11'11"N	Carbonate Cliff	17
Door Bluff Park	87°3'53"W, 45°17'48"N	Carbonate Cliff	18
Ellison Bay Park	87°5'38"W, 45°15'20"N	Carbonate Cliff	26
Fifield Tract	87°3'36"W, 45°6"N	Lakeshore Carbonate Ledge	17
Frey Tract	87°3'45"W, 45°14'34"N	Lakeshore Carbonate Ledge	15
Glidden Drive	87°12'36"W, 44°52'53"N	Lakeshore Carbonate Ledge	13
Hemlock Road 1	86°52'12"W, 45°20'50"N	Lakeshore Carbonate Ledge	23
Hemlock Road 2	86°52'4"W, 45°20'42"N	Rocky Woods	20
Hutter Tract	87°22'55"W, 44°56'5"N	Carbonate Cliff	24
Kangaroo Lake	87°10'4"W, 45°3'12"N	Rocky Woods	13
Kuchar Fen	87°10'51"W, 45°5'12"N	Fen	11
LaSalle Park	87°21'50"W, 44°41'23"N	Lakeshore Alluvial Bank	15
Little Harbor	87°24'7"W, 44°54'45"N	Carbonate Cliff	18
McKnight Escarpment	87°19'47"W, 45°0'43"N	Carbonate Cliff	15

Meridian Park	87°9'57"W, 45°0'22"N	Lakeshore Carbonate Ledge	15
Monument Point South	87°21'35"W, 44°58'35"N	Carbonate Cliff	15
Moonlight Bay	87°4'1"W, 45°4'54"N	Lakeshore Alluvial Bank	4
Mountain Park	86°54'10"W, 45°23'16"N	Carbonate Cliff	24
Mud Lake	87°4'44"W, 45°5'44"N	Lakeshore Carbonate Ledge	13
Newport State Park 1	86°59'13"W, 45°14'52"N	Lakeshore Carbonate Ledge	20
Newport State Park 2	86°59'23"W, 45°15'3"N	Lakeshore Carbonate Ledge	21
Newport State Park 3	86°59'49"W, 45°15'13"N	Lakeshore Carbonate Ledge	21
Peninsula State Park 1	87°13'8"W, 45°9'42"N	Carbonate Cliff	18
Peninsula State Park 2	87°12'7"W, 45°9'45"N	Carbonate Cliff	10
Port de Mort	86°59'31"W, 45°17'50"N	Carbonate Cliff	22
Potawatomie State Park 1	87°25'29"W, 44°52'38"N	Carbonate Cliff	20
Potawatomie State Park 2	87°24'46"W, 44°51'40"N	Carbonate Cliff	18
Potawatomie SW	87°26'31"W, 44°51'45"N	Carbonate Cliff	21
Red River Bluff	87°41'6"W, 44°45'43"N	Carbonate Cliff	12
Ridges Sand Swale	87°7'8"W, 45°4'12"N	Calcareous Meadow	10
Rock Island 1	86°49'44"W, 45°25'20"N	Carbonate Cliff	26
Rock Island 2	86°49'40"W, 45°24'53"N	Upland Woods	15
Rock Island 3	86°49'4"W, 45°25'37"N	Carbonate Cliff	19
Rock Island 4	86°49'8"W, 45°25'31"N	Carbonate Cliff	18
Rock Island 5	86°49'37"W, 45°25'19"N	Carbonate Cliff	28
Rock Island 6	86°49'44"W, 45°25'6"N	Carbonate Cliff	11
Rocky Point	87°19'11"W, 44°46'23"N	Lakeshore Alluvial Bank	13
Sand Dune Park	86°53'52"W, 45°20'14"N	Lakeshore Alluvial Bank	14
Shivering Sands	87°15'57"W, 44°52'30"N	Lakeshore Carbonate Ledge	22
Ski Slope, E-facing	87°14'5"W, 45°6'10"N	Carbonate Cliff	23
Ski Slope, N-facing	87°14'16"W, 45°6'18"N	Carbonate Cliff	15
South Shore Road	86°53'6"W, 45°20'10"N	Lakeshore Carbonate Ledge	21
Standish	87°21'57"W, 45°57'36"N	Carbonate Cliff	22
Thorpe Pond	87°13'40"W, 45°4'20"N	Fen	10
Toft Point 1	87°5'20"W, 45°3'23"N	Upland Woods	13
Toft Point 2	87°6'3"W, 45°4'50"N	Fen	12
Toft Point 3	87°5'52"W, 45°4'43"N	Tamarack Wetland	11
Toft Point 4	87°5'59"W, 45°4'58"N	Lowland Woods	13
Toft Point 5	87°5'52"W, 45°4'22"N	Lakeshore Carbonate Ledge	14
Toft Point 6	87°5'5"W, 45°4'40"N	Lakeshore Carbonate Ledge	19
Toft Point 7	87°5'2"W, 45°3'59"N	Cobble Beach	10
Toft Point 8	87°5'45"W, 45°4'14"N	Rocky Woods	6
Ulman Woods	87°32'3"W, 44°44'58"N	Rocky Woods	11
Werkheiser Escarpment	87°20'27"W, 44°59'39"N	Carbonate Cliff	15
Wilson Escarpment	87°15'43"W, 45°5'3"N	Carbonate Cliff	5
Douglas County			
Flanagan Lookout 1	91°56'9"W, 46°35'8"N	Igneous Cliff	5
Flanagan Lookout 2	91°56'6"W, 46°35'7"N	Igneous Cliff	7
Pattison State Park 1	92°7'26"W, 46°32'14"N	Igneous Cliff	9

Pattison State Park 2	92°7'26"W, 46°32'15"N	Igneous Cliff	8
Pattison State Park 3	92°7'26"W, 46°32'16"N	Igneous Cliff	7
South Range	91°58'26"W, 46°36'15"N	Old Field	4
Fond du Lac County			
Ledge Bar	88°21'3"W, 43°52'4"N	Carbonate Cliff	27
Messner Ledge	88°35'31"W, 43°38'13"N	Carbonate Cliff	23
Messner Ledge North	88°35'24"W, 43°38'25"N	Carbonate Cliff	23
Oakfield Brick Yard	88°33'10"W, 43°40'27"N	Carbonate Cliff	26
Oakfield Ledge	88°34'55"W, 43°38'55"N	Carbonate Cliff	23
Peebles	88°22'40"W, 43°48'32"N	Carbonate Cliff	21
Shley Pond	88°33'39"W, 43°40'8"N	Carbonate Cliff	25
Iron County			
Hurley Visitor Center	90°12'14"W, 46°28'13"N	Igneous Cliff	11
Lake Lavina Bluff 1	90°10'55"W, 46°26'6"N	Igneous Cliff	11
Lake Lavina Bluff 2	90°10'55"W, 46°26'6"N	Igneous Cliff	8
Whitecap Mountain	90°23'49"W, 46°24'15"N	Igneous Cliff	15
Kewaunee County			
Kewaunee Fish Hatchery	87°33'50"W, 44°28'45"N	Lakeshore Carbonate Ledge	19
Lipsky Swamp 1	87°37'15"W, 44°28'57"N	Tamarack Wetland	11
Lipsky Swamp 2	87°37'13"W, 44°28'50"N	Lowland Woods	7
Little Scarboro Creek 1	87°37'26"W, 44°30'39"N	Rocky Woods	18
Little Scarboro Creek 2	87°37'22"W, 44°30'45"N	Cacareous Meadow	16
Mud Lake	87°39'45"W, 44°39'37"N	Tamarack Wetland	19
Stony Creek Woods	87°22'51"W, 44°39'51"N	Lakeshore Alluvial Bank	16
Thiry Daems	87°42'14"W, 44°36'8"N	Calcareous Meadow	19
Tisch Mills	87°38'21"W, 44°20'50"N	Tamarack Wetland	12
Manitowoc County			
Cato Falls County Park 1	87°50'34"W, 44°5'31"N	Carbonate Cliff	16
Cato Falls County Park 2	87°50'49"W, 44°5'29"N	Lakeshore Alluvial Bank	9
Cooperstown Swamp	87°53'12"W, 44°16'16"N	Tamarack Wetland	11
Frelch Road Swamp	87°52'8"W, 44°17'40"N	Lowland Woods	21
Kingfisher Farm 1	87°42'3"W, 43°57'43"N	Lakeshore Alluvial Bank	20
Kingfisher Farm 2	87°42'17"W, 43°57'47"N	Lakeshore Alluvial Bank	6
Kingfisher Farm 3	87°42'3"W, 43°57'51"N	Lakeshore Alluvial Bank	6
Kingfisher Farm 4	87°42'14"W, 43°57'46"N	Upland Woods	2
Kingfisher Farm 5	87°42'10"W, 43°57'51"N	Lowland Woods	3
Kingfisher Farm 6	87°42'21"W, 43°57'48"N	Lowland Woods	9
Kingfisher Farm 7	87°42'25"W, 43°57'49"N	Fen	10
Maribel Caves	87°46'11"W, 44°17'9"N	Carbonate Cliff	17
Point Beach 1	87°31'11"W, 44°12'5"N	Upland Woods	11
Point Beach 2	87°30'39"W, 44°11'52"N	Open Dune	4
SLC Bog 1	87°54'5"W, 43°59'31"N	Calcareous Meadow	7
SLC Bog 2	87°54'3"W, 43°59'21"N	Lowland Woods	5
SLC Bog 3	87°53'50"W, 43°59'31"N	Old Field	9
Tamarack Road	88°0'55"W, 44°12'12"N	Tamarack Wetland	2
Marinette County			
Kimlark Lake	87°50'56"W, 45°39'37"N	Calcareous Meadow	11
Niagara East Bluff 1	87°56'41"W, 45°45'24"N	Igneous Cliff	10
Niagara East Bluff 2	87°56'41"W, 45°45'23"N	Igneous Cliff	15
Pound Roadside	88°1'1"W, 45°7'29"N	Calcareous Meadow	21
Spur Lake 1	88°14'5"W, 45°43'5"N	Fen	15

Spur Lake 2	88°13'58"W, 45°42'59"N	Fen	12
Ozaukee County			
Cedarburg Bog 1	88°0'34"W, 43°23'9"N	Fen	10
Cedarburg Bog 2	88°1'4"W, 43°22'59"N	Tamarack Wetland	16
Harrington Beach 1	87°48'10"W, 43°29'26"N	Old Field	6
Harrington Beach 2	87°47'56"W, 43°29'16"N	Upland Woods	2
Harrington Beach 3	87°47'41"W, 43°29'42"N	Lakeshore Carbonate Ledge	10
Sauk County			
Devils Lake	89°43'57"W, 43°24'30"N	Igneous Cliff	17
Sawyer County			
Pipestone Falls	91°14'12"W, 45°51'19"N	Igneous Cliff	11
Shawano County			
Porter Road	88°30'10"W, 44°44'16"N	Carbonate Cliff	19
Sheboygan County			
Evergreen Park	87°44'29"W, 43°46'56"N	Lakeshore Alluvial Bank	4
Waters Edge	87°46'46"W, 43°34'53"N	Lakeshore Alluvial Bank	7
Mehles Springs	88°1'16"W, 43°51'38"N	Tamarack Wetland	9
Walworth County			
Bluff Creek Fen	88°40'54"W, 42°48'2"N	Fen	19
Washington County			
Allenton Fen	88°18'25"W, 43°22'41"N	Fen	19
Waushara County			
Bass Lake	89°16'58"W, 44°0'15"N	Fen	17

Species	Habitat Type																			Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
<i>Gastrocopta tappaniana</i> (C. B. Adams, 1842)	2			15	4		1	5		6		2			2	1	14	2	1	55
<i>Glyphyalinia indentata</i> (Say, 1823)	51	6	4	2	1	7	1	2	3	1	1	1			9		1			91
<i>Glyphyalinia rhoadsi</i> (Pilsbry, 1899)	15	1				1	1							4						22
<i>Glyphyalinia wheatleyi</i> (Bland, 1883)	5					1								1						7
<i>Guppya sterkii</i> (Dall, 1888)	16		2											2						20
<i>Haplotrema concavum</i> (Say, 1821)	11	7										2			1					22
<i>Hawaia miniscula</i> (A. Binney, 1840)	52	10		5	4		2	1	5	3		1		4		2	1			90
<i>Hawaia</i> n. sp. (<i>sensu</i> Frest, 1990)				4															4	4
<i>Helicodiscus inermis</i> (H. B. Baker, 1929)	4								1											5
<i>Helicodiscus</i> n. sp. (<i>sensu</i> Frest, 1990)				3																3
<i>Helicodiscus notus</i> (Hubricht, 1962)	7																			7
<i>Helicodiscus parallelus</i> (Say, 1817)	34	7	3	4		3		2	3	1					9		6	1	4	77
<i>Helicodiscus shimpei</i> (Hubricht, 1962)	73	19	8	2	1	15	6	2	1	1		5	2		14	1	1	3		154
<i>Helicodiscus singleyanus</i> (Pilsbry, 1890)	7		1											1						9
<i>Hendersonia occulta</i> (Say, 1831)	27	2	16	1		3	9					3			3					64
<i>Mesodon clausus clausus</i> (Say, 1821)	2		2												1					5
<i>Mesodon inflectus</i> (Say, 1821)	3																			3
<i>Mesodon pennsylvanicus</i> (Green, 1827)															1					1
<i>Mesodon thyroideus</i> (Say, 1816)	9		2			1														12
<i>Mesodon zaletus</i> (A. Binney, 1837)	3																			3
<i>Mesomphix cupreus</i> (Ratnesque, 1831)	2															1				3
<i>Mesomphix friabilis</i> (W. G. Binney, 1857)	6																			6
<i>Mesomphix inornatus</i> (Say, 1821)	4																			5
<i>Nesovitrea binneyana</i> (Morse, 1864)	42	52	7	1	1	9	1	1			2	2	1	4	1		4	2	2	129
<i>Nesovitrea electrina</i> (Gould, 1841)	5	9	1	15	2	3	1	3	3	3		4			1	14	5	2		71
<i>Oxychilus cellarius</i> (Müller, 1774)	1																			1
<i>Oxyloma peoriensis</i> (Wolf, in Walker, 1892)																				1
<i>Oxyloma retusa</i> (l. Lea, 1834)	1		1	8	3			1		5		1					1			21
<i>Paravitrea multidentata</i> (A. Binney, 1840)	53	7				12	1				1			11						85
<i>Paravitrea significans</i> (Bland, 1866)	6																			6
<i>Planogyra asteriscus</i> (Morse, 1857)		4		1		2	1							1		1	5			15
<i>Polygyra dorfeuilleana</i> (l. Lea, 1838)	2																			2
<i>Pomatiopsis lapidaria</i> (Say, 1817)	1			2								1								4
<i>Punctum minutissimum</i> (l. Lea, 1841)	68	37		4	5	18	6	4		3	4	3		17	3	5	5	4		186

Species	Habitat Type																			Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
<i>Vertigo "iowaensis"</i> (sensu Frest, 1991)	32		5		4															41
<i>Vertigo mermacensis</i> (Van Devender, 1979)	6		2																	8
<i>Vertigo milium</i> (Gould, 1840)	19		1	8						3	1			1		1	1			35
<i>Vertigo modesta modesta</i> (Say, 1824)	2	5																		7
<i>Vertigo modesta parietalis</i> (Ancey, 18)	1	3		5																4
<i>Vertigo morsei</i> (Sterki, 1894)																				5
<i>Vertigo nylanderii</i> (Sterki, 1909)				4	4							1				8	1			10
<i>Vertigo ovata</i> (Say, 1822)				1	1					6					2	2				16
<i>Vertigo paradoxa</i> (Sterki, 1900)	23	33		3		1				6	1	1		2	1		3			64
<i>Vertigo pygmaea</i> (Draparnaud, 1801)	14			3			2	1		6	1	1		1						29
<i>Vertigo tridentata</i> (Wolf, 1870)	11		6					3												20
<i>Vitina limpida</i> (Gould, 1850)	10	3		1	1		2	2		1		1		5		1	1	3		28
<i>Zonitoides arboreus</i> (Say, 1816)	100	63	6	7	3	18	5	1	4	4		3	5	1	16	1	12	6		255
<i>Zonitoides limatulus</i> (W. G. Binney, 1840)	1																		1	1
<i>Zonitoides nitidus</i> (Müller, 1774)	5			2	2	1	3			1						4	1			19
<i>Zoogenetes harpa</i> (Say, 1824)	7	35				1								3						50
Total sites sampled per habitat	114	72	16	19	7	19	12	6	5	7	3	8	8	1	21	3	16	8	4	349

Habitat Legend:

Habitat	Number	Habitat	Number
Carbonate Cliff	1	Old Field	11
Igneous Cliff	2	Upland Woods	12
Algific Talus Slope	3	Lowland Woods	13
Fen	4	Open Dune	14
Cobble Beach	5	Rocky Woodland	15
Lakeshore Carbonate Ledge	6	Shale Cliff	16
Lakeshore Alluvial Bank	7	Tamarack Wetland	17
Alvar	8	White Cedar Wetland	18
Carbonate Glade	9	Igneous Shoreline	19
Calcareous Meadow	10		

TERRESTRIAL GASTROPOD RICHNESS PATTERNS IN WISCONSIN CARBONATE CLIFF COMMUNITIES

Jeffrey C. Nekola¹ & Tamara M. Smith²

ABSTRACT

The patterns of terrestrial gastropod richness within two species-rich carbonate cliff habitats in eastern Wisconsin were analyzed at two differing sample scales. Up to 23 taxa were found in 1 m² quadrats, and 21 taxa in 0.04 m² quadrats. These observations are among the highest reported globally for 1 ha or smaller samples. At the 1 m² scale, samples collected within 5 m of bedrock outcrops had higher richness than more distant sites. At this scale, only soil pH (not Ca, Mg, N, P, K, percent organic matter, vascular plant species richness, or surface and 20 cm depth soil temperatures) was found to significantly correlate with species richness. At the 0.04 m² scale, the richest sites were restricted to areas within 0.5 m of cliff bases. Comparison of maximum richness levels across varying spatial scales demonstrate that up to a third of the total fauna may co-exist in <0.04 m² regions (alpha diversity), up to half of the fauna may coexist in <100 m² regions (beta diversity), while the remainder of the taxa (gamma diversity) occurs between regions separated by at least 10 km.

Key words: terrestrial gastropods, species richness, diversity patterns, conservation, North America, Niagaran Escarpment.

INTRODUCTION

While a number of studies have documented richness in terrestrial gastropod communities at relatively large (> 100 m²) sample scales (e.g., Paul, 1975; Solem et al., 1981; Waldén, 1981; Cameron, 1986; Nilsson et al., 1988; Emberton, 1995; Tattersfield, 1996; Emberton et al., 1997), fewer have analyzed terrestrial gastropod community structure at smaller scales within sites. The research that has been conducted at this scale has demonstrated terrestrial gastropod community structure can change markedly over limited (e.g., <100 m) spatial extents. For instance, Berry (1966) demonstrated significant changes in faunal composition between moss-covered and moss-free segments on a single limestone cliff. Agócsy (1968) reported substantial differences in species composition and abundance between adjacent limestone and sandstone outcrops. Cameron (1978) reported significant shifts in the faunas found on adjacent vertical and horizontal surfaces. Kralka (1986) demonstrated that over 60% of terrestrial gastropod species clustered significantly within individual boreal forest stands. Small-scale patterns in terrestrial gastropod distribution are also suggested by the control

of soil chemistry on community structure (Outeiro et al., 1993; Hermida et al., 1995), as soil chemistry is known to be highly variable over 1–2 cm distances (Burrough, 1986).

Unfortunately, little is known about small-scale community composition and diversity patterns within the richest known global sites. At the Waipipi Scenic Reserve in New Zealand, Solem et al. (1981) did not quantitatively subsample the fauna, but rather documented total gastropod diversity over the entire 4.2-ha reserve. Emberton (1995) documented total species richness within a 4-hectare region near Manombo, Madagascar without measuring diversity from subsamples within this site. Similarly, Emberton et al. (1997) only documented total richness from entire 4-hectare regions in eastern Tanzania. Diversity gradients at Pine Mountain, Kentucky, the richest North American terrestrial gastropod site, have not been documented (Emberton 1995). The smallest quadrats sampled by Tattersfield (1996) from the Kakamega Forest Reserve in Kenya were 40 × 40 meters in size, while the minimum quadrat size sampled by deWinter & Gittenberger (1998) in southwestern Cameroon was 20 × 20 meters. While Schmid (1966) did measure terrestrial gastropod richness from individual 1 m²

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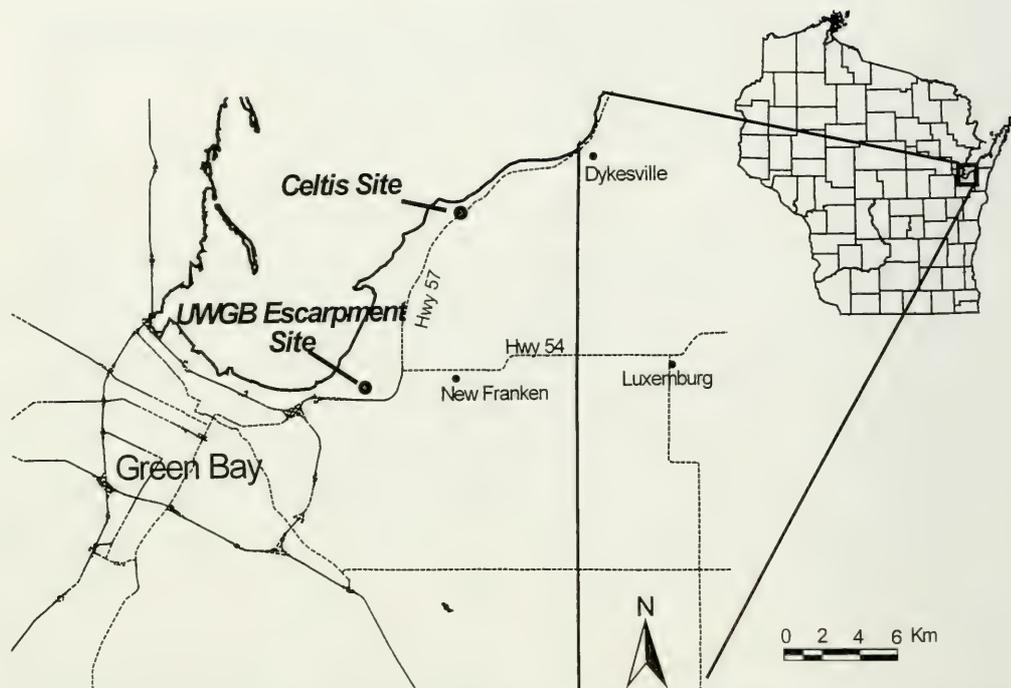


FIG. 1. Location of the Celtis and UWGB Escarpment sites in northeastern Brown County, Wisconsin, USA.

quadrats near Tübingen, Germany, these were not part of a larger, systematic sampling regime and were not used to document diversity gradients within habitats.

Previous analyses (Nekola, 1999) have shown that carbonate cliffs from the Great Lakes region in North America are among the richest terrestrial gastropod communities reported from less than 1 ha scales. However, like other high diversity communities, nothing was known of: (1) diversity gradients within these sites; (2) the environmental factors within sites that correlate with high richness microsites, and (3) the scales of organization for faunal diversity within and between sites. This paper attempts to address these questions by analyzing terrestrial gastropod diversity patterns at two sampling scales (1 m² and 0.04 m²) within two high-richness carbonate cliff sites in northeastern Wisconsin, U.S.A.

MATERIALS AND METHODS

Study Sites

Two wooded carbonate cliffs in Brown County, Wisconsin, with high levels of terres-

trial gastropod species richness were chosen for study (Fig. 1). Both occur along the Niagaran Escarpment, a 1,300 km band of outcropping Silurian-age limestones and dolomites that can be roughly divided into five 200–300 km long regions (northeastern Iowa; eastern Wisconsin through the Garden Peninsula of Michigan; eastern Upper Peninsula of Michigan though Manitoulin Island; Bruce Peninsula though south-central Ontario; and southeastern Ontario to western New York State) which are separated by low areas with little or no bedrock exposure. Within the eastern Wisconsin region, the Niagaran Escarpment is naturally divided into 31 isolated 2–8 km sections of exposed bedrock that emerge above Pleistocene tills and alluvium.

The Celtis site (87°50'52"W, 44°36'35"N) is situated within a 7 km-long Niagaran Escarpment section near the settlement of Benderville. Its canopy is dominated by old-growth sugar maple (*Acer saccharum* Marsh.), white cedar (*Thuja occidentalis* L.), paper birch (*Betula papyrifera* Marsh.) and hackberry (*Celtis occidentalis* L.). The bedrock outcrop at the Celtis site is divided into a 3–6 m primary upland cliff and a 2–5 m secondary cliff associated with a large bedrock block displaced downslope approximately 10 m (Fig. 2). Cool

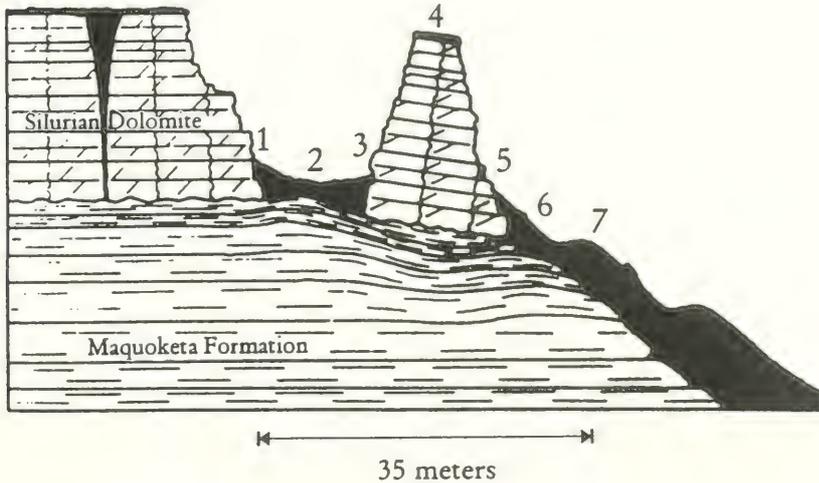


FIG. 2. Schematic profile of the bedrock outcrop at the Celtis site, with location of 1 m² quadrats along each transect. Vertical exaggeration is approximately 4x the linear extent.

air seepage from bedrock fissures and talus occurs throughout the growing season. The presence of large displaced talus blocks, expanded bedrock joints, and an extensive subtending talus indicate that this area was subjected to intense periglacial erosion during the late Pleistocene (Stieglitz et al., 1980). This location harbors the single most diverse terrestrial gastropod assemblage known from the Great Lakes region, with 34 taxa (Nekola, 1999). Included in this fauna are the glacial relicts *Catinella gelida* (F. C. Baker, 1927), *Hendersonia occulta* (Say, 1831), *Vallonia gracilicosta albula* (Sterki, 1893), and *Vertigo hubrichti* Pilsbry, 1934 (Nekola et al., 1996).

The University of Wisconsin—Green Bay (UWGB) Escarpment site (87°54'21"W, 44°31'48"N) is located within a 4-km Niagaran Escarpment section located near the Bay Settlement community. This site consists of a single 2–5 m tall upland cliff within a white cedar and box elder (*Acer negundo* L.) canopy. Large displaced talus blocks are absent. This site was extensively modified by small-scale quarrying for lime and building material approximately a century ago (Stieglitz et al., 1980). As these activities were carried out with hand tools and were spatially limited, this site consists of a mixture of modified and unmodified cliff segments. Unmodified segments appear essentially identical to the upland outcrop at the Celtis site. A total of 25 terrestrial gastropod taxa have been located here, including the glacial relicts *Vallonia gra-*

cilicosta albula, *Vertigo hubrichti*, and *Vertigo* n. sp. ("*V. iowaensis*" of Frest, 1991).

Data Sets

A representative and relatively undisturbed section of exposed cliff was identified at each site. From a random starting point within these sections, five transects were laid out perpendicular to the cliff face at 5-m intervals. Along each transect both 1 m² and 0.04 m² samples were sampled:

1 m² Quadrats: At the Celtis Site, seven quadrats, each separated by 5-m distances, were collected along each transect (Fig. 2) for a total of 35. At the UWGB Escarpment site, three quadrats, separated by 5-m distances, were collected from each transect for a total of 15. Only three samples per transect were gathered from the UWGB Escarpment site to avoid highly disturbed forest and recreational trails occurring at greater distances from the cliff base.

The vascular plants growing on or over each quadrat were recorded and their species richness calculated. Soil temperatures at the ground surface and at 20 cm depth were measured using a thermocouple thermometer. A 100 gm dry weight soil sample was collected by subsampling the corners and center of each quadrat. These samples were sent to the Wisconsin State Soils Lab at the University of Wisconsin—Madison for analysis of

percent organic matter, N, P, K, S, Ca, Mg, and pH, using methods outlined in Dahnke (1988).

Terrestrial gastropod assemblages were determined by collecting a total of 4–5 deciliters of soil litter from the corners and center of each quadrat. Samples were slowly and completely dried in either a low-temperature soil oven (approx. 80–95°C) or in full sun in a greenhouse. Dried samples were then soaked in water for 3–24 h, and subjected to careful but vigorous washing through a standard sieve series (ASTME 3/8" (9.5 mm), 10 (2.0 mm), 20 (0.85), and 40 (0.425 mm) mesh screens). The washed fractions were re-dried and then re-sifted through the original sieve series. The dry, resorted fractions were hand picked against a neutral-brown background using a small sable brush. All shells and shell fragments were removed.

Recovered, identifiable shells were assigned to species (or subspecies) using the author's reference collection and the Hubricht Collection at the Field Museum of Natural History. From this, species composition and richness per quadrat was calculated. All specimens are housed in collections maintained at the University of Wisconsin—Green Bay.

0.04 m² Quadrats: 20 × 20 cm quadrats were collected adjacent to 1 m² quadrats along each of the five established transect lines. Quadrats were sampled at distances of 0, 0.5, 1.0, 1.5, and 2.0 meters from cliff bases. The primary and secondary cliffs at the Celtis site (located at positions 1 and 5, respectively, on Fig. 2), and the primary cliff at the UWGB Escarpment were analyzed in this fashion for a total of 75 observations. For each quadrat, transect position and distance from the cliff base were recorded, and a 2–3 deciliter soil litter sample collected. These litter samples were subjected to the same laboratory procedures described for the 1 m² samples to determine terrestrial gastropod composition and richness.

Comparison of Maximum Richness Levels: The maximum richness of 0.04 m² and 1 m² samples from each site were compared with known richness values from a series of nested samples of increasing sample grain. These grains include each site (observed from a 100 m² quadrat), escarpment section, escarpment region, Brown County, and the state of Wisconsin. Richness values at these increasing scales of observation are based upon species lists from other sites (summarized in Nekola,

1999), augmented by other published records (Levi & Levi, 1950; Teskey, 1954; Jass, 1986). Richness estimations were limited to carbonate cliff habitats for Escarpment sections and regions, while those for Brown County and the state of Wisconsin included all habitat types.

To compare the maximum richness values from this study with other reported maximum richness values, a survey was made of the published literature to identify other datasets in which both terrestrial gastropod richness and sample grain were reported. If multiple examples of such data were found from a single paper, only the richest were entered for a given sample size. Through this process, a total of 35 records from four continents (Africa, Australia, Eurasia, and North America) were recorded (Burch, 1956; Schmid, 1966; Agócsy, 1968; Mason, 1970; Berry, 1973; Paul, 1975; Uminski & Focht, 1979; Bishop, 1980; Solem et al., 1981; Van Es & Boag, 1981; Waldén, 1981; Nilsson et al., 1988; Cameron & Greenwood 1991; Young & Evans, 1991; Cameron, 1992; Outeiro et al., 1993; Getz & Uetz, 1994; Cowie et al., 1995; Emberton, 1995; Wardhaugh, 1995; Tattersfield, 1996; Emberton et al., 1997; de Winter & Gittenberger, 1998).

Statistical Analyses

1 m² Quadrats: Analysis of the effect of quadrat position and site on richness was conducted via ANOVA. These data were graphically represented using box plots (Velleman & Hoaglin, 1981). In box plots, the central line represents the median of the sample, the margins of the box represent the interquartile distances, and the fences represent 1.5 times the interquartile distances. For data having a Gaussian distribution, approximately 99.3% of the data will fall inside of the fences. Outliers falling outside of the fences are shown with asterisks. Identification of the environmental variables that best predict observed richness was accomplished through multiple linear regression using a backwards stepwise selection procedure. Beginning with the most non-significant, variables were removed from the model until all remaining p-values fell below the 0.05 level. Analysis of residuals and individual variable distributions indicated that data transformations were not necessary.

0.04 m² Quadrats: Analysis of the effect of quadrat position, cliff position, and site location on richness was conducted via

ANOVA. These data were graphically represented for each of the three sampled cliffs (Celtis Site primary and secondary, and UWGB Escarpment primary) using box plots. A full ANOVA with all interaction terms was not conducted as a secondary cliff was not present at the UWGB Escarpment site.

Comparison of Maximum Richness Levels:

The percent of total richness from the five nested larger sample grains overlying each maximally-rich 0.04 m² and 1 m² quadrat was calculated for each site. The richness of these different sample areas was natural-log transformed and regressed against natural log-transformed estimates of habitat area. Habitat size estimates for Niagara Escarpment sections and regions were generated by multiplying average cliff-base habitat width (approx. 5 m) by habitat length. The natural log of maximum richness vs. the natural log of sample size was also plotted for the 35 literature richness records and for the maximum richness 0.04, 1, and 100 m² quadrats from the Celtis and UWGB Escarpment sites. While the limited number of samples prevented use of inferential statistics to test for significant differences between the maximum species area curves for carbonate cliffs ($n = 3$ for each site) and other habitats, a qualitative assessment was made.

RESULTS

1 m² Quadrats

Richness of terrestrial gastropods at the two sites ranged between 0 and 23 taxa (Tables 1, 2), with a mean of 6.6 taxa/ quadrat. Across both sites, mean richness was 9.9 from quadrats collected at transect position 1, 7.1 at position 2, 7.2 at position 3, 6.8 at position 4, 5.8 at position 5, 2.4 at both positions 6 and 7 (Fig. 3). ANOVA of these data demonstrated that this variation was weakly significant ($p = 0.04$; Table 3). Further ANOVA tests demonstrated that no significant differences were present between the mean richness of transect positions 1–5 ($p = 0.326$), or between the Celtis and UWGB Escarpment sites ($p = 0.646$). No interaction between transect position and site on richness was observed ($p = 0.947$). Backwards stepwise linear regression of ten environmental variables on richness demonstrated that only pH ($p < 0.0005$) and P ($p = 0.04$) were significant predictors (Table

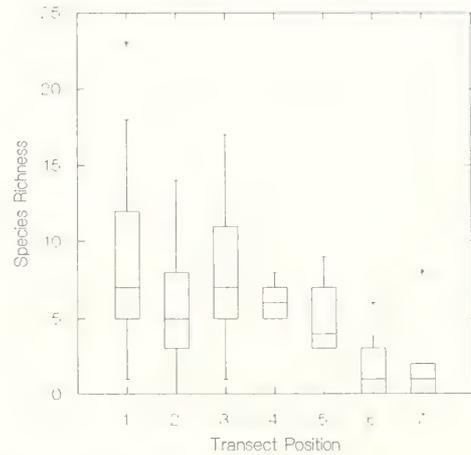


FIG. 3. Box-plot diagram of terrestrial gastropod richness at 1 m² scales with increasing distance from the primary upland cliff.

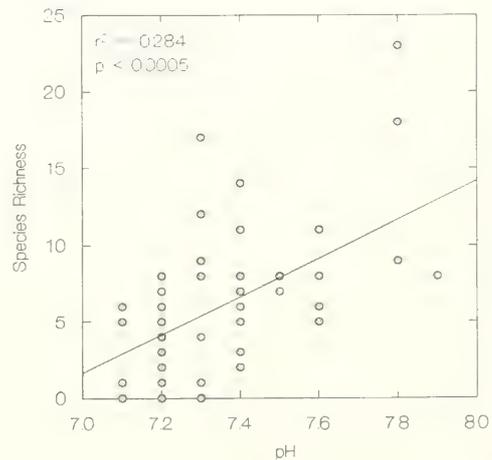


FIG. 4. Scatterplot of terrestrial gastropod richness in 1 m² quadrats vs. soil pH, with best-fit line. Twelve of the data points are not apparent in this graph as they overlap previously plotted values.

4), accounting for almost 30% of observed richness variation. However, this level of significance of P appears to be based upon a single outlier. When this observation was removed from analysis, the p value for P in a multiple linear regression of pH and P on richness dropped to 0.266. The amount of variation in richness accounted for by pH alone was found to exceed 28% (Fig. 4).

TABLE 2. Abundance and richness of terrestrial molluscs in 15 1 × 1 m² quadrats at the UWGB Escarpment site

Transect	A	A	A	B	B	B	C	C	C	D	D	D	E	E	E	
Quadrat	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Auguipira alternata</i> (Say, 1817)	2			6			1						3	1	3	2
<i>Carychium exile</i> H. C. Lea, 1842	12	6	11	8	15	8	18	10	3	2			6	2	6	5
<i>Catinella avara</i> (Say, 1824)								1								
<i>Cochlicopa lubrica</i> (Müller, 1774)		2	1	3	8	7	5	5	4	3	11	1	2	3		
<i>Cochlicopa lubricella</i> (Porro, 1838)				1												1
<i>Discus cronkhitei</i> (Newcomb, 1865)	1	1		1	3	1			3		3	1				
<i>Gastrocopta armifera</i> (Say, 1821)				1												1
<i>Gastrocopta holzingeri</i> (Sterki, 1889)										3			1	1	1	1
<i>Hawaii miniscula</i> (A. Binney, 1840)			2	1								4	1	1		2
<i>Helicodiscus shimeki</i> Hubricht, 1962								1	1							
<i>Strobilops labyrinthica</i> (Say, 1817)			1	1			1							1	1	
<i>Succinea ovalis</i> Say, 1817	2		4	8	2				3	2	2	1			1	2
<i>Triodopsis multilineata</i> (Say, 1821)									1							
<i>Vallonia costata</i> (Müller, 1774)		1		6	19	14	5	16	7	8	30	10	20		2	
<i>Vallonia gracilicosta</i> Reinhardt, 1883										3				1		
<i>Vallonia pulchella</i> (Müller, 1774)					1				1							
<i>Vertigo gouldi</i> (A. Binney, 1843)					1						1			2		
<i>Vertigo hubrichti</i> (Pilsbry, 1934)														5		
<i>Vertigo "lowaensis"</i>													1			
<i>Vertigo pygmaea</i> (Draparnaud, 1801)	4		2	2	1	3	2	9	1	1	4	1	7			
Immature Individuals	1	2		4	2	3	5	3		2		2	13	3		
Total Individuals	22	12	21	42	52	36	37	45	23	25	55	26	57	22	12	
Richness	5	4	6	11	8	5	6	6	8	8	7	8	12	9	5	

TABLE 3. Summary statistics for ANOVA of terrestrial gastropod species richness in 1 m² quadrats vs. quadrat distance from base of primary cliff at both the Celtis and UWGB Escarpment sites.

Source	Sum-of-Squares	df	F-Ratio	p
Distance from base	313.48	6	2.688	0.026
Error	835.90	43		
Squared multiple r:	0.273			

TABLE 4. Results of backwards stepwise linear regression of 10 environmental variables on terrestrial gastropod species richness at 1 m² grains. Variables are listed in the order in which they were removed from the model. The p-values reported are those immediately prior to removal of that variable from the model.

Variable	p-value
Mg	0.968
Percent Organic Matter	0.783
Soil Temperature at 20 cm Depth	0.406
K	0.314
Vascular Plant Species Richness	0.293
Ca	0.283
N	0.167
Surface Soil Temperature	0.121
P	0.040
pH	< 0.0005

0.04 m² Quadrats

Terrestrial gastropod richness varied from 0 to 21 taxa on all three sampled cliffs (Tables 5–7). At the primary upland cliff at the Celtis site, average species richness was 16.8 at the cliff base, 9.4 at 0.5 m, 3.2 at 1 m, 2.8 at 1.5 m, and 2.2 at 2 m distances from the cliff. At the secondary cliff at the Celtis site, average species richness was 14.4 at the cliff base, 11.2 at 0.5 m, 5.2 at 1 m, 2.8 at 1.5 m, and 2.8 at 2 m distances from the cliff. At the UWGB Escarpment site, average species richness was 8.0 at the cliff base, 8.0 at 0.5 m, 6.0 at 1 m, 5.8 at 1.5 m, and 6.4 at 2 m distances from the cliff (Fig. 5). ANOVA of these data (Table 8) demonstrated that distance from the cliff base and the interaction between this variable

TABLE 6. Abundance and richness of terrestrial molluscs in 25 20 × 20 cm² quadrats at the Celtis site secondary cliff

Transect	Quadrat																									E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5																							
<i>Anguispira alternata</i> (Say, 1817)	10	20	1			8	2														24	4	1	2		23	6	1	2	3																		
<i>Carychium exile</i> H. C. Lea, 1842	2	4	2			2															30	6				67	23	3	1																			
<i>Catinella gelida</i> (F. C. Baker, 1927)							1	2													1	1	2			3	3																					
<i>Cochlicopa lubrica</i> (Müller, 1774)	1	1	1				1														1	1	1			3																						
<i>Deroceras laeve</i> (Müller, 1774)	1	1	1				1														1	1	1			3																						
<i>Discus catskillensis</i> (Pilsbry, 1898)	4					1	1														3					1																						
<i>Euconulus fulvus</i> (Müller, 1774)							1														3					1																						
<i>Euconulus polygynatus</i> (Pilsbry, 1899)	4																				2	1				3																						
<i>Gastrocopta armifera</i> (Say, 1821)																					11																											
<i>Gastrocopta contracta</i> (Say, 1822)	4	2																			2	1				3																						
<i>Gastrocopta corticaria</i> (Say, 1816)	5	2	1	1																	2					14	8	1	1																			
<i>Gastrocopta holzingeri</i> (Sterki, 1889)	1	1	2																		3	1				1																						
<i>Gastrocopta pentodon</i> (Say, 1821)	5	1	1			1															40	21	1	1		58	33	12	10	5																		
<i>Glyphyalinia indentata</i> (Say, 1823)																					4																											
<i>Glyphyalinia rhoadsi</i> (Pilsbry, 1899)	1																				1																											
<i>Guppya sterkii</i> (Dall, 1888)																					1																											
<i>Hawaiiia minuscula</i> (A. Binney, 1840)	1	1	4																		2					16	9	1	1																			
<i>Helicodiscus parallelus</i> (Say, 1817)																					1																											
<i>Helicodiscus shimeki</i> Hubricht, 1962	19	5	3																		3					11	3																					
<i>Hendersonia occulta</i> (Say, 1831)	2	8	1	3	1	8															2	5	1	1		2	1			2																		
<i>Nesovitreia binneyana</i> (Morse, 1864)	1																				1																											
<i>Paravitrea multidentata</i> (A. Binney, 1840)	4	1				1															1																											
<i>Punctum vitreum</i> H. B. Baker, 1930	1																				1																											
<i>Strobilops labyrinthica</i> (Say, 1817)	2	1	1			1															4	1				6	5			1																		
<i>Vertigo gouldi</i> (A. Binney, 1843)	1	3	1	1																	1	1	1			5	1																					
<i>Vertigo hubrichti</i> (Pilsbry, 1934)																					1	13	5	2		2	29	35	4	4																		
<i>Vertigo pygmaea</i> (Draparnaud, 1801)																																																
<i>Zonitoides arboreus</i> (Say, 1816)	24	6				3															6					5																						
Immature																																																
Total	91	58	25	3	3	18	25	2	1	6	81	13	1	0	3	151	52	6	4	2	280	148	26	21	22																							
Richness	18	15	12	3	1	8	11	1	1	3	12	4	1	0	2	20	13	5	3	1	15	12	8	5	8																							

TABLE 7. Abundance and richness of terrestrial molluscs in 25 20 × 20 cm² quadrats at the UWGB Escarpment cliff

Transect	A	A	A	A	A	B	B	B	B	B	C	C	C	C	C	D	D	D	D	D	D	E	E	E	E	E
Quadrat	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	
<i>Anguispira alternata</i> (Say, 1817)	3	1			1	1	1	1	2	1	1	2				2		1			3					
<i>Carychium exile</i> H. C. Lea, 1842	4	5	13	10	9	16	24	12	29	11	93	23	13	18	1	7	9	8	1	18	49	29	36			
<i>Catinella avara</i> (Say, 1824)										1															1	
<i>Cochlicopa lubrica</i> (Müller, 1774)	1	2	3		1	1			1	3	2				9	7	1	2	3	2	4	4	2	5		
<i>Cochlicopa lubricella</i> (Porro, 1838)											1	1			2	2						1				
<i>Discus cronkhitzi</i> (Newcomb, 1865)					1			1			1				1	5					2	1				
<i>Gastrocopta armitera</i> (Say, 1821)						1				1	1				1	1		2								
<i>Gastrocopta contracta</i> (Say, 1822)																										
<i>Gastrocopta holzingeri</i> (Sterki, 1889)																1	1	1		1						
<i>Hawaiiia miniscula</i> (A. Binney, 1840)																										
<i>Helicodiscus shimiki</i> Hubricht, 1962																										
<i>Strobilops labyrinthica</i> (Say, 1817)																										
<i>Succinea ovalis</i> Say, 1817	1	3	1	2	1	2	2	2	2	1	2	1			1	2	2	1	1	2	3	1	7			
<i>Triodopsis multiinata</i> (Say, 1821)																										
<i>Vallonia costata</i> (Müller, 1774)																										
<i>Vallonia gracilicosta</i> Reinhardt, 1883	3	3	2	1	5	5	2	2	2	3	2			1	8	20	7	3	2	7	9	1	2			
<i>Vallonia pulchella</i> (Müller, 1774)																										
<i>Vertigo gouldi</i> (A. Binney, 1843)	1					1																				
<i>Vertigo hubrichti</i> (Pilsbry, 1934)																										
<i>Vertigo "lowaensis"</i>																										
<i>Vertigo milium</i> (Gould, 1840)	1				1																					
<i>Vertigo pygmaea</i> (Draparnaud, 1801)	5	3	2	1	1	5	2	2	2	5	2	4	1		6	6	2	5	7	4	4	5	6			
Immature	4	3		5	6	5	1	10	7	1	7	9			4	25	18	18	26	10	12	18	9			
Total	23	22	22	22	25	19	26	39	26	38	29	107	41	17	26	56	65	38	49	32	31	68	74			
Richness	8	8	6	7	7	6	6	4	5	6	10	5	6	4	5	10	10	6	7	6	6	12	8			

TABLE 8. Summary statistics for ANOVA of terrestrial gastropod species richness in 0.04 m² quadrats vs. quadrat distance from cliff base, cliff position, and site location.

Source	Sum-of-Squares	df	F-Ratio	<i>p</i>
Site	0.08	1	0.008	0.928
Cliff position	2.88	1	0.296	0.589
Distance from base	406.08	4	10.423	< 0.0005
Distance * Site	271.72	4	6.974	< 0.0005
Distance * Cliff	25.12	4	0.645	0.633
Error	584.04	60		

Squared multiple *r*: 0.699

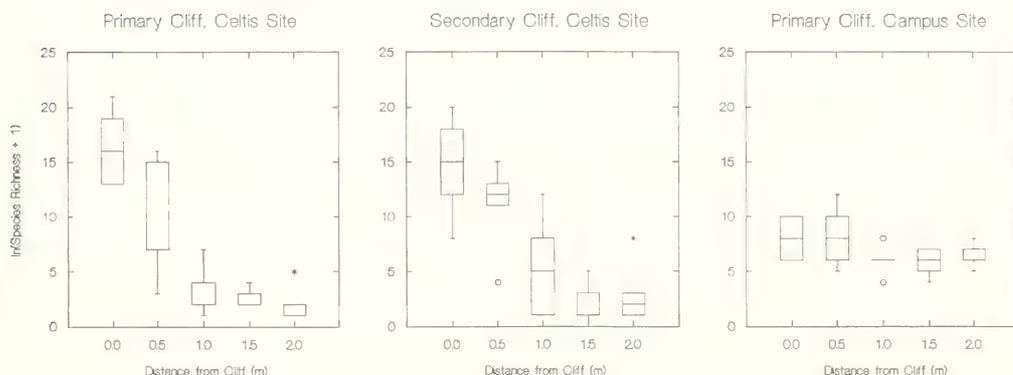


FIG. 5. Box-plot diagram of terrestrial gastropod richness from 0.04 m² scales at increasing distances from three cliff exposures at the Celtis and UWGB Escarpment sites.

and site were both highly significant ($p < 0.0005$). In all, distance from cliff base and site accounted for 70% of the observed variation in richness. Mean richness was not affected by cliff type (primary or secondary; $p = 0.649$) and cliff type did not interact with the rate of richness decrease from the cliff base ($p = 0.518$).

Comparison of Maximum Richness Levels

At the UWGB Escarpment, a maximum of 12 taxa were observed from single 0.04 and 1 m² quadrats. As this site represents the lone surviving carbonate cliff community within the 6 km Bay Settlement section, the total richness of this Escarpment section equals site richness (25 taxa). For the Celtis site, all small-scale richness values were higher, with a maximum richness of 21 taxa occurring in a single 0.04 m² sample, and 23 taxa in a single 1 m² sample. Carbonate outcrops along the 8 km Benderville Escarpment Section (within which the Celtis site occurs) support 38 taxa. All carbonate cliffs along the 350 km Eastern Wisconsin-Garden Peninsula Escarpment Re-

gion support 62 taxa. The total richness of Brown County terrestrial gastropods, across all habitat types, is 65 taxa, while a total of 95 taxa have been documented across all habitats in the state of Wisconsin (Table 9).

The faunas of maximum richness 0.04 m² quadrats at the Celtis site thus account for up to 91% of maximum 1 m² richness, 62% of site richness, 55% of Escarpment section richness, and 34% of Escarpment region richness. Individual 0.04-m² quadrats also harbored up to 32% of the entire county fauna, and 22% of the entire state fauna. Given their similar maximum richness, almost identical results are present for maximum richness 1 m² quadrats, which can harbor up to 68% of the entire site fauna, 61% of the Escarpment section fauna, and 37% of the entire Escarpment region fauna. Individual 1-m² quadrats can also harbor up to 35% of the entire county fauna, and 24% of the entire state fauna. Because of the lower richness levels at the UWGB Escarpment Site, these numbers tended to be lower by almost 30–50% from Celtis site levels. Regression analysis demonstrates that a high correlation ($p < 0.0005$; $r^2 =$

TABLE 9. Percent of site, escarpment section, escarpment region, county, and state terrestrial gastropod faunas contained within maximum diversity 0.04 and 1 m² quadrats.

UWGB Escarpment Site			
Sample Grain	Richness	Percent Overlap	
		0.04 m ²	1 m ²
0.04 m ²	12		
1 m ²	12	100	
Site (100 m ²)	25	48	48
Niagara Escarpment			
Bay Settlement Section	25	48	48
Eastern Wisconsin Region	62	19	19
Brown County	65	18	18
Wisconsin	95	13	13

Celtis Site			
Sample Grain	Richness	Percent Overlap	
		0.04 m ²	1 m ²
0.04 m ²	21		
1 m ²	23	91	
Site (100 m ²)	34	62	68
Niagara Escarpment			
Benderville Section	38	55	61
Eastern Wisconsin Region	62	34	37
Brown County	65	32	35
Wisconsin	95	22	24

0.839) exists between natural log-transformed richness and sample area, with the best-fit line having an intercept of 2.93 and a slope of 0.063 (Fig. 6). This translates to an average richness of 18.7 taxa per 1 m² and 33.4 taxa per hectare.

Comparison of maximum richness from 0.04 and 1 m² quadrats to other reported maximum richness values demonstrates the Celtis site site is among the richest reported globally from small observational scales (Fig. 7). Maximum richness levels at the Celtis site compare favorably with the richest reported 1–400 m² samples in Germany, Sweden, and Scotland (Schmid, 1966; Waldén, 1981; Cameron & Greenwood 1991). The richest global terrestrial gastropod faunas, collected over larger areas (400–40,000 m²) in New Zealand, Madagascar, Tanzania, and Cameroon appear to fall along the same maximum species-area curve defined from the Celtis Site. While maximum richness at the UWGB Escarpment is lower, these observations still lie within the upper half of previously reported maximum richness levels for the given range of sample scales.

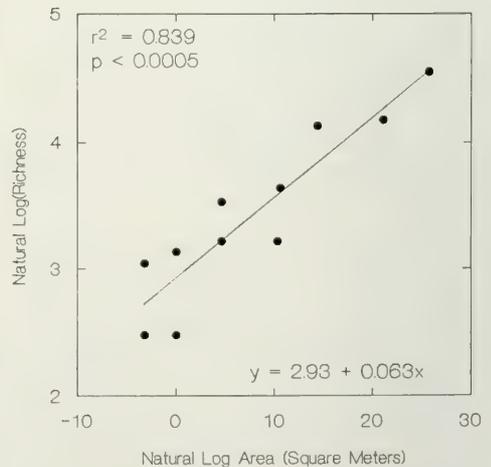


FIG. 6. Scatterplot of natural log-transformed terrestrial gastropod richness vs. natural log-transformed sample area for Wisconsin carbonate cliff land snail faunas. The scales of observation include: maximum-richness 0.04, 1, and 100 m² quadrats from the Celtis and UWGB Escarpment sites; the respective escarpment sections for each; escarpment region; county; and state.

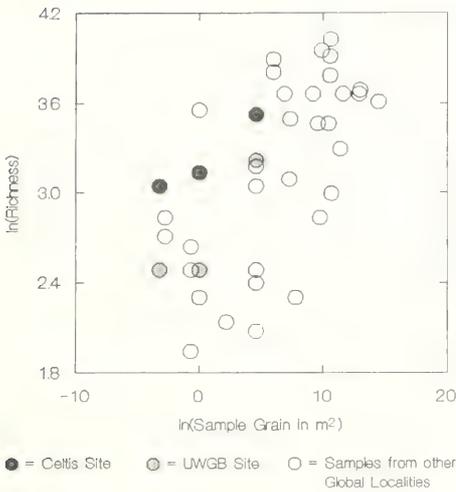


FIG. 7. Species-area plot of maximum-richness 0.04, 1 and 100 m² quadrats from the Celtis and UWGB Escarpment sites compared to other maximum richness levels reported from other global sites.

DISCUSSION

Diversity Patterns Within Carbonate Cliff Communities

The high richness of terrestrial gastropods within carbonate cliff habitats occurs down to very limited spatial scales, with up to 62% of site richness (and up to 22% of total state richness) being found within single 0.04 m² areas along cliff bases. The limitation of high-diversity assemblages to the immediate vicinity of vertical bedrock outcrops is striking, with richness decreasing by almost six-fold from 0 to 1 m from cliff bases. This rapid and drastic reduction in richness helps explain why, on average, 0.04 m² quadrats at cliff bases harbor more species than adjacent 1 m² quadrats. As the 1 m² samples consisted of pooled subsamples taken from the corners and center of each quadrat, only two subsamples per quadrat were thus collected from mollusc-rich microsites. This had the unintended effect of diluting snail density and richness. However, the 0.04 m² cliff-base quadrats, which consisted to a similar total volume of soil litter, were collected entirely from the richest microsites, so that no dilution in snail density or richness occurred.

While diversity at the 0.04 m² scale was markedly higher adjacent to cliffs at the undis-

turbed Celtis site, such spatial limitation of richness was not observed at the UWGB Escarpment. In addition, maximum (but not mean) richness at both 0.04 and 1 m² scales at the UWGB Escarpment site was roughly one-half that recorded at the Celtis Site. It is probable that these lower maximum diversity levels, and lack of strong micro-scale diversity gradients, are related to this site's past quarrying history, which may have simplified the range of microhabitats present along the cliff base.

The exact mechanisms that lead to high levels of terrestrial gastropod richness at micro-scales within these carbonate cliff sites have not been documented. First, it is not known how many of these shells originate from individuals living in the quadrat versus shells that have been deposited from nearby areas, such as adjacent vertical rock faces. If this latter process is important, levels of micro-scale sympatry could be substantially lower than the observed shell richness suggests. However, preliminary observations of living individuals on cliff bases and adjacent vertical faces suggest that the majority of shells originate from within quadrats. Additionally, most species observed from cliff-base quadrats are represented by at least one living snail or recently dead shell, while species reported from more distant quadrats are almost always represented by long-dead shell fragments.

Second, even if shells from high-richness microsites are locally derived, observed richness may be exaggerated if shells persist in the soil for long periods. In this case, species lists will represent an integration over the persistence-time of shells. This could lead to an overestimate of microsympatry if local faunas are in a state of constant flux. Use of radioisotope dating on shells could provide a possible test for shell half-life, which would help set the temporal scales of integration for such shell-banks in carbonate cliff soils. However, the fact that most taxa are represented in samples by at least one live or recently dead shell suggests that high levels of sympatry are likely maintained at both limited temporal and spatial extents.

The existence of diverse terrestrial gastropod assemblages at very small scales on or adjacent to carbonate outcrops is also likely not unique to the two sites chosen for analysis. Qualitative observations of other carbonate cliffs in Illinois, Iowa, New York, Ontario, Wisconsin, and southwestern England sug-

gest that the co-occurrence of 20 or more taxa at 1 m² or smaller grains may be typical in undisturbed sites.

The limitation of high richness terrestrial gastropod assemblages to the immediate proximity of cliffs also indicates that these microhabitats must be afforded special protection if their biodiversity is to be protected. Unfortunately, planned and spontaneous recreational trails in reserves are often routed through these exact areas as they are aesthetically pleasing and provide access to charismatic natural features such as caves, fissures, and rock walls. Such trails may place any high-richness terrestrial gastropod assemblages in serious jeopardy. For instance, the cliff base at Bayshore County Park, 6 km north of the Celtis Site, has been turned into a graveled trail which now lacks a terrestrial gastropod fauna (Nekola, unpublished data).

Environmental Controls on Small Scale Richness Patterns

The literature regarding the environmental controls of terrestrial gastropod richness and abundance is very conflicting. Burch (1955) suggested that in eastern Virginia snail abundance (and presumably diversity) was related to soil organic matter, Ca, Mg, and K, but not pH. Lack of correspondence between terrestrial gastropod distribution and pH has also been demonstrated in southwestern Ireland (Bishop, 1977) and the Italian Alps (Bishop, 1980). However, Gleich & Gilbert (1976), stated that in central Maine soil moisture, but not soil Ca, was the most important determinant of snail abundance. Outeiro et al. (1993) demonstrated that soil texture and pH were the most important factors effecting terrestrial gastropod distribution in central Spain. Soil pH was also identified as an important determinant of terrestrial gastropod density and diversity by Waldén (1981) and Gårdenfors (1992) in southern Sweden, and by Bishop (1976) in Somerset, England. Getz (1974) and Getz & Uetz (1994) identified soil moisture, tree diversity, and leaf litter diversity as major determinants of diversity in the Great Smoky Mountains. However, Locasciulli & Boag (1987) demonstrated in Alberta forests that terrestrial gastropod abundance was not related to vegetation.

Within eastern Wisconsin carbonate cliff habitats, only soil pH (and not soil Ca, Mg, N, P, K, percent organic matter, soil temperature, or vascular plant richness) was found to cor-

relate significantly with terrestrial gastropod richness at the 1 m² scale. This result is consistent with the analysis of Bishop (1980), who stated that soil pH will only be an important environmental correlate of terrestrial gastropod assemblages when soil Ca levels are high. Interactions between environmental variables may explain why such a diversity of factors exist that have (and have not) been shown to influence terrestrial gastropod richness across various habitats and regions. Thus, extrapolation of these results to other habitats or regions may be risky. These results do, however, provide insight into the environmental correlates of terrestrial gastropod richness within Ca-rich carbonate cliffs.

Spatial Scales of Terrestrial Gastropod Coexistence

A complete assessment of the scales over which terrestrial gastropod coexistence is mediated is not possible because the current data are restricted to five discrete spatial scales (0.04 m², 1 m², 100 m², approx. 3.5 ha, and approx. 1750 ha). However, given the wide total range covered, some preliminary insights into this issue can be made.

Three of the measured scales appear to harbor the bulk of terrestrial gastropods. Up to one-third of the total regional fauna may occur within individual 0.04 m² regions. Over 50% of the total regional fauna may occur within 100 m² regions on individual sites. The remainder of the fauna (almost 50% of the regional total) is largely found between sites on different Escarpment Sections (e.g., sites 10 km or more apart, corresponding to 3.5 + ha of cliff base habitat). However, little increase in richness was observed between 0.04 m² and 1 m² extents within sites, and between sites within the same Escarpment Section (100 m²–3.5 km of cliff base habitat).

One tentative conclusion that can be drawn from these results is that alpha diversity (*sensu* Whitaker, 1975) in these sites is best measured at scales no larger than 0.04 m², beta diversity (*sensu* Whitaker, 1975) is best measured at scales no larger than 100 m², and gamma diversity (*sensu* Cody, 1986) is best measured between sites at least 10 km distant from one another. Although Emberton (1995) states that distinctions between alpha, beta, and gamma diversity are hazy for terrestrial gastropod communities, these data suggest that such problems in resolution may be due to poorly chosen observational scales.

For instance, if the Celtis and UWGB Escarpment sites had been sampled at typical malacological sampling scales of 0.1 ha or larger, distinctions between alpha and beta diversity would be impossible to make, as sample resolution would be at least 1000 times greater than the scale at which alpha diversity likely exists.

Additional research will be necessary to document the mechanisms that allow for species coexistence at these differing scales. Ecological theory suggests that alpha diversity levels may be related to levels of niche partitioning between species (Auerbach & Shmida, 1987). However, competition and predation have only rarely been shown to influence terrestrial gastropod distribution and abundance (Cain, 1983; Cowie & Jones, 1987; Smallridge & Kirby, 1988). The high density of shells (up to 330 per 0.04 m² quadrat) further suggests that resource levels are also high, cautioning against use of resource-ratio models (e.g., Tilman, 1988). Identification of the small-scale coexistence mechanisms in these habitats may be of broad ecological importance, as it is rare for alpha diversity to constitute such a high proportion of regional diversity, and thus for the rate of species accumulation with increasing sample size to be so low (Rosenzweig, 1995).

At larger scales of observation, habitat heterogeneity may be important in determining levels of species richness (Auerbach & Shmida, 1987; Rosenzweig 1995). The few additional species added between site and escarpment section scales suggests that the universe of microenvironments found within a given carbonate cliff site may be very similar to those present within an entire Escarpment section. At the largest scales, coexistence of terrestrial gastropods will likely be mediated by large environmental gradients (including climate), differential colonization histories of habitats (Ricklefs & Schluter, 1993; Nekola, in press), and the incomplete dispersal of species between sites (Auerbach & Shmida, 1987).

It is important to note that the spatial scales of coexistence for terrestrial gastropod communities, and hence the optimal scales of observation, may differ between systems and landscapes. For instance, it is not clear that alpha diversity will always be confined to such small scales in habitats that support substantially lower densities of individuals and taxa. Gamma diversity will also likely vary between landscapes, as preliminary analyses have

documented for Niagaran Escarpment carbonate cliffs in which rates of community turnover may vary by an order-of-magnitude (Nekola, unpublished data). Such observations suggest that additional research will be necessary within and between a diversity of habitats and landscapes to determine if any general rules exist to guide the ecological sampling of terrestrial gastropod communities.

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SAMPLING TERRESTRIAL GASTROPOD COMMUNITIES: USING ESTIMATES OF SPECIES RICHNESS AND DIVERSITY TO COMPARE TWO METHODS

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ABSTRACT

Terrestrial gastropods are sometimes sampled for ecological and parasitological studies using simple traps of cardboard placed on the ground. This method enables the collection of large numbers of individuals with relatively little effort. However, the violation of assumptions of the trap method may mean that samples are biased. I examined the reliability of this method by comparing species composition, richness and diversity of gastropod collections from traps to paired hand-searched plots in Algonquin Park, Ontario, Canada. Ten samples for each method were made from five replicate sites in three habitat types, white birch, hardwood and logged forest stands. Of the 18 species found overall, 17 and 16 species were found using hand search and trap methods respectively. Some species, especially *Arion circumscriptus*, were consistently over-represented using traps, while most other species had greater representation in hand-search collections. In general, hand-search collections had significantly greater species richness and diversity than trap collections. Further, the similarity in species composition of the two collections seemed to depend, at least in white birch and hardwood habitats, on the level of site diversity; as site diversity increased the percent similarity decreased. However, estimated richness, calculated using a jackknife estimator that accounts for heterogeneity in species detection, was not different for the two collection methods. There was no difference in average species' detection per sample between collections; the probability of sampling a species using a single sample within a site was low for both methods (16–34% of species). However, overall detection of species on a site (average across all samples within a site) tended to be higher for hand-searched plots, especially in more complex habitats. Thus, as trap sampling does not provide representative gastropod collections, this method may not be appropriate to use when physical collections or detailed information on species abundances are required. Conversely, as there is no difference between methods in estimated richness, traps may be suitable to estimate gastropod community metrics, provided appropriate estimation models are used.

Key words: Gastropoda, land Mollusca, community ecology, species richness, diversity, sampling techniques, richness estimation.

INTRODUCTION

Terrestrial gastropods are often sampled using simple traps of moistened cardboard, masonite or tile, particularly for parasitological studies (e.g., South, 1965; Gleich & Gilbert, 1976; Kearney & Gilbert, 1978; Boag, 1982; Samuel et al., 1985; Strayer et al., 1986). This method is relatively simple and time efficient, and has been considered an effective means of sampling to estimate relative population density and compositional changes of gastropod communities, both spatially and temporally (Boag, 1982). This method assumes that (1) all species and age classes are equally attracted to the traps at the times when the traps are typically examined, dawn or dusk, and (2)

that individuals are only attracted vertically from the litter layers beneath and not horizontally to the trap. However, there are interspecific differences in gastropod activity (Blinn, 1963; Cameron, 1970, 1978; Cain & Cowie, 1978; McCoy & Nudds, 1997), and in the use of these traps (Boag, 1990). Further, some gastropod species are thought to have large home ranges (Blinn, 1963; Thomas, 1944; Cook, 1979) such that individuals may be attracted to traps for shelter or food from an area of unknown size; there is now evidence that such horizontal movement to traps exists (Boag, 1990; Hawkins et al., 1997). Thus, the violation of the assumptions of trap sampling could result in biased gastropod collections both in terms of the number of individuals per

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species collected and the number of species represented (species richness).

The extent to which any collection technique accurately represents the gastropod community in a given area is difficult to determine as there are limitations associated with all techniques (e.g., Bishop, 1977; Emberton et al., 1996). Bishop (1977) suggested that the least biased way to sample terrestrial gastropods would use stratified random quadrats in which litter is either searched, or removed and searched in the laboratory. Likewise, Newell (1971) reviewed numerous direct and indirect methods and found that hand sorting soil samples, while slow and laborious, provided more accurate estimates of abundance of some species than did surface counts alone. He recommended the use of indirect methods (such as trap sampling) only for those animals that could not be reliably estimated by a direct method. Conversely, Van Es & Boag (1981) compared hand-searched field samples to samples sorted in the laboratory after litter was dried and sieved and found that hand-searched collections contained fewer individuals than did litter samples and were biased towards larger specimens. Emberton et al. (1996) suggested that combining direct, timed searches with litter-plus-soil samples was the most efficient means of quantifying microgastropods (< 5 mm) in tropical rainforests. Others have recommended using indirect trap sampling, within certain climatic limits, due to its efficiency in collecting (Hawkins et al., 1997). Thus, depending on the nature of ecological question under consideration and the degree of sampling bias in the technique, a less laborious method such as traps could provide a viable alternative to labour-intensive methods such as hand searching individual quadrats.

Bias in gastropod sampling can be problematic for numerous reasons. First, terrestrial gastropod fauna have been collected for various ecological studies: examining patterns of species distributions and biogeographical relationships (e.g., Burch, 1956; Roth & Lindberg, 1981; Van Es & Boag, 1981; Cameron, 1986; Gascoigne, 1994; Tattersfield, 1996), evaluating biodiversity and community changes (e.g., Nilsson et al., 1988; Niemelä, 1997), and studying population dynamics (e.g., Williamson et al., 1977; Uminski & Focht, 1979). Bias in sampling methods may mean that ecological inferences drawn from collected gastropods are incorrect. Likewise, terrestrial gastropods are commonly

sampled to estimate the prevalence of parasites for whom they act as intermediate hosts. These collections are typically used to infer the relative importance of different species in transmission (e.g., Lankester & Anderson, 1968; Kearney & Gilbert, 1978) and to predict the risk of infection to vertebrate hosts (e.g., Boag, 1985). Nonrepresentative samples of gastropods can lead to inaccurate assessments of parasite prevalence and misconceptions about transmission dynamics. If surveys are to provide useful ecological information, a rigorous approach to sampling is required, and evaluations of existing procedures need to be performed to improve sampling protocol.

In this study, I used a stratified, paired-plot design to directly compare trap and hand-search collection techniques for sampling terrestrial gastropod communities in three different habitat types to determine whether traps provide representative samples of gastropod species composition, richness and diversity. In order to partially overcome the size bias inherent in hand-searched collections and to provide a more representative collection for comparison, I combined hand searches in the field with litter-plus-soil subsamples hand sorted in the laboratory. Further, to evaluate the effectiveness of trap sampling for different types of ecological studies, I compared estimates of species richness calculated using both direct counts and the jackknife estimator of Burnham & Overton (1979). The jackknife estimator of species richness specifically takes into account heterogeneity in species detectability, that is, differences among species in their probability of being sampled given that they are present at a given sampling location.

MATERIALS AND METHODS

Field Methods

The study took place in Algonquin Park, Ontario, Canada (45°35'N, 78°30'W), in June and July 1996. As this work was carried out in connection with a broader study of the transmission of a nematode parasite (*Parelaphostrongylus tenuis*) of white-tailed deer (*Odocoileus virginianus*), three habitat types commonly used by white-tailed deer were chosen to compare collection methods: white birch (*Betula papyrifera*), mixed hardwood and selectively logged sites (Kohn & Mooty, 1971; Kearney & Gilbert, 1978). Five sites for each habitat type were randomly selected

from a list of suitable sites. Suitable sites were determined by stand content (greater than 60% white birch or hardwood content), accessibility and, for the logged sites, time since disturbance (logged between 1990 and 1994). The order in which the 15 sites were examined was determined by lottery.

At each site, two transects, each with five plot markers, were set up. The plot markers were spaced 10 m apart and there was 20 m between the two transect lines. On either side of the transects, 10 m from each plot marker, a 1 × 1-m piece of moist cardboard was set out. Therefore, at each site, a total area of 40 × 40 m was examined using ten plots and ten paired traps. As traps are typically left out for a period of time before snails are collected from them (e.g., Gleich & Gilbert, 1976; Kearney & Gilbert, 1978), the boards were set out seven days before the site was scheduled to be searched. Pieces of woody debris were placed on the top of the boards to keep them in place and to help maintain the moisture beneath.

All sites were examined between 0600h and 0900h. At each plot marker, a 1 × 1-m area was outlined using a wooden frame such that the marker was in the center of the plot. Two random subsamples were taken from within each plot using a corer of 15 cm diameter. These subsamples included leaf litter, soil to at least a 5 cm depth, and any plants and debris from within the subsample area. Each subsample was later handsorted in the laboratory. Once subsamples were removed, the remainder of the plot was searched for 10 min for gastropods; all vegetation and possible shelters were examined and leaf litter and soil were sifted by hand. Although it is clear that not all gastropod individuals were found using this method, the 10-min period was sufficient to thoroughly examine the entire plot once. After a plot was searched, its corresponding cardboard trap was examined for gastropods and all specimens on and under the board, and on the debris on top of the board were collected.

The subsamples removed from each plot were examined the same day they were collected. To examine for gastropods, a subsample was emptied into a large, light-coloured sorting pan. During a 10-min period, each leaf or piece of debris was individually examined, soil was spread and sifted by hand and all gastropods found were removed. Because the volume of each subsample was relatively small (1.0–1.5 L), the 10-min period allowed

an exhaustive examination of all material. Nevertheless, to ensure greater representation, particularly of microgastropods, the two subsamples from a single plot were sorted by different observers.

All collected gastropods were counted and identified in the lab. Species identifications were made using keys by Pilsbry (1946) and Burch (1962). Identifications were aided by a zoogeographical study by Oughton (1948) and were confirmed using voucher specimens at the Royal Ontario Museum, Canada.

Statistical Methods

Richness for each collection method was calculated two ways: (1) as a direct count of the number of species found at a site and, (2) because it was unlikely that all species present at a site were sampled, as estimated by the jackknife estimator of Burnham & Overton (1979) using the program CAPTURE (species richness values can also be calculated using subprogram SPECRICH from software COMDYN available on the Internet—<http://www.mbr-pwrc.usgs.gov/comdyn.html>).

The jackknife estimator for closed populations calculates species richness using the pattern of observed species occurrences (presence/absence) across replicated samples within a site (Nichols & Conroy, 1996) and is based on a capture-recapture model (model M_h) that takes into account heterogeneity in the probability of capture among species. The program CAPTURE has a built-in selection procedure that chooses the appropriate capture-recapture model for the presented data. There are several different models possible, each with different assumptions regarding the source of variation in detection probabilities (Boulinier et al., 1998, provides an overview). Nonetheless, here I used only the richness estimates provided by model M_h as this model has been found to be relatively robust to other factors affecting detection probabilities (e.g., observer influence, quadrat heterogeneity) (Otis et al., 1978) and as heterogeneity in species detection is likely to be the main source of variation in detection probability when estimating species richness (Boulinier et al., 1998). Detectability refers to the probability of sampling at least one individual of a given species in a plot (the sampling unit) given that the species is present in that plot (Boulinier et al., 1998).

It is well recognized that terrestrial gastropods tend to be heterogeneously distrib-

uted in space (e.g., Cameron, 1986; Ember-ton et al., 1996). Thus, for estimating species richness, the assumption of closure (i.e., that the community being sampled does not change among replicated sample locations) (Burnham & Overton, 1979) is likely to be violated at some spatial scale. For this study, sampling methods equally covered a small (40×40 m), uniform area of forest. As terrestrial gastropods should be relatively mobile at this scale, I felt it was safe to assume that, at each plot or trap within a site, I sampled from the same gastropod community.

The program CAPTURE also provides an estimate of the average instantaneous detectability of species for a given site (or the average probability of sampling species on a plot) based on the number of species captured on each plot within the site. From the values of estimated species richness, the overall detectability of species (observed # species/estimated # species) on a site can also be calculated. Thus, the average instantaneous detectability gives an indication of the probability of capturing all species at a single sample location within a site (average probability per plot), and the overall detectability describes the probability of capturing all species across all sampling locations within a site (probability across ten plots). The jackknife estimator can be biased if there is a low probability of detecting species but regardless is considered to provide a better estimate of richness than counts alone (Nichols & Conroy, 1996).

The diversity at each site was calculated separately for each collection method using DIVERS (Krebs, 1989). The Shannon-Wiener diversity index was considered the most appropriate diversity index to use for gastropod communities, as it makes no assumptions about the shape of the underlying distribution of species-abundance and is relatively insensitive to changes in the dominant species (Krebs, 1989). This index will likely be biased to different degrees depending on the species present in different sites, but it is still appropriate here as I compare the relative diversity of the two collection methods for the same locations where the same species are available to be sampled. As microgastropods from hand-searched subsamples in the laboratory were directly included in the list of species for a given plot without extrapolating the numbers to the entire area of the plot, I considered tests for differences in diversity between methods to be conservative.

If the two collection methods sample different species at a given location, we might not find differences between methods using such measures as richness or diversity. Thus, I calculated the average similarity in species composition of methods on a site using the % similarity (Renkonen) coefficient for each plot pair (a hand-searched plot and its corresponding cardboard trap). This coefficient uses the abundance of each species on a plot and can be calculated using the program SIMILAR (Krebs, 1989). It can be biased if sample sizes are small and diversity is high (Wolda, 1981), but should still perform well in this case as gastropod diversity is relatively low in the study area considered and sample sizes are equal. Further, because the effectiveness of the two sampling methods could change with varying levels of diversity/richness at different sampling sites, I regressed the similarity in species composition of the two methods at a site against diversity and estimated species richness. As there were two estimates of diversity/richness for a given site (one for each method), the diversity/richness estimate used for regressions was the higher of the two estimates.

Calculated estimates for the two collection methods were statistically compared using analysis of variance and paired t-tests (Zar, 1984). All tests were performed using SAS (SAS Institute, 1996) and were considered significant at the 0.05 level.

RESULTS

Across all three habitat types a total of 18 gastropod species were sampled; 17 and 16 species were found using hand-search and trap methods, respectively. The average number of individuals of each species collected using each method is summarized in Table 1. One slug species, *Arion circumscriptus*, was consistently collected in greater numbers by the trap method in all habitats. Six other species (*Anguispira alternata*, *Discus cronkhitei*, *Zonitoides arboreus*, *Deroceras laeve*, *Mesodon sayanus* and *Stenotrema fraternum*) also tended to be found in higher numbers in trap collections, although the difference between methods changed depending on habitat type; all other species had similar or greater representation using hand searches (Table 1). Two unique species (*Paravittrea multidentata* and *Columella edentula*) were found only by hand searching; individuals of *C. edentula* were only

TABLE 1. Number of individuals of each gastropod species collected in white birch, hardwood and logged sites using the hand-search collection method (Hand) and the trap-collection method (Trap).

Family Species	White Birch		Hardwood		Logged		Total	
	Hand	Trap	Hand	Trap	Hand	Trap	Hand	Trap
Arionidae								
<i>Arion circumscriptus</i> (Johnston)	222	349	104	307	57	261	383	917
Endodontidae								
<i>Discus cronkhitei</i> (Newcomb)	82	88	16	78	27	97	125	263
<i>Anguispira alternata</i> (Say)	0	3	0	0	1	26	1	29
<i>Helicodiscus parallelus</i> (Say)	42	0	9	1	26	0	77	1
<i>Punctum minutissimum</i> (Lea)	38	0	20	1	45	1	103	2
Limacidae								
<i>Deroceras laeve</i> (Müller)	8	12	3	13	2	10	13	35
Philomycidae								
<i>Palifera dorsalis</i> (Binney)	9	3	11	11	17	1	37	15
Polygyridae								
<i>Mesodon sayanus</i> (Pilsbry)	1	2	1	4	7	28	9	34
<i>Stenotrema fraternum</i> (Say)	0	1	0	6	3	11	3	18
<i>Triodopsis dentifera</i> (Binney)	0	0	0	0	0	3	0	3
Pupillidae								
<i>Columella edentula</i> (Draparnaud)	4	0	0	0	0	0	4	0
Strobilopsidae								
<i>Strobilops labyrinthica</i> (Say)	77	5	14	1	2	1	93	7
Succineidae								
<i>Succinea ovalis</i> (Say)	6	5	11	18	9	10	26	33
Vallonidae								
<i>Zoogenetes harpa</i> (Say)	10	2	1	0	0	0	11	2
Zonitidae								
<i>Retinella binneyana</i> (Morse)	22	0	3	3	13	1	38	4
<i>Euconulus fulvus</i> (Müller)	9	2	15	5	18	1	42	8
<i>Paravitrea multidentata</i> (Binney)	6	0	0	0	3	0	9	0
<i>Zonitoides arboreus</i> (Say)	4	9	12	24	39	160	55	193
TOTAL	540	481	220	472	269	611	1,029	1,564

detected in white birch stands. On logged sites, one unique species (*Triodopsis dentifera*) was collected by traps and was not found during hand searches (Table 1).

Overall, for both richness and diversity, hand-searched samples had significantly greater estimates than did samples from traps ($F_{1,14} = 4.75$, $P = 0.047$; $F_{1,14} = 7.04$, $P = 0.019$; Tables 2,3). Richness on a site varied from 4 to 13 species and diversity from 0.28 to 2.38 for hand searches. For trap collections, from 1 to 12 species were found on a site and diversity levels varied from 0 to 1.73. By habitat type, white birch and logged stands showed greater differences between methods in both components than hardwood stands, which showed very little (Table 2). Overall, there was no difference among habitat types in either richness or diversity, but there was significant variation in both measures among different sites within the same habitat (Table 3).

Unlike for observed richness, there was no

significant difference in species richness of the two collections as estimated by the jack-knife ($F_{1,14} = 0.27$, $P = 0.61$; Tables 2,3). There was also no difference in average instantaneous detectability between the two methods (hand: 0.28 ± 0.03 , trap: 0.23 ± 0.03 ; $t = 1.11$, $P = 0.28$). Over all habitat types and methods, estimates of average instantaneous detectability were relatively low (between 16 and 34%), suggesting only a small proportion of the community is sampled using one plot or trap on a site. However, the overall detectability at a site was higher, ranging between 10% and 100%, with averages of 80% and 70% for hand and trap methods respectively (Table 2). As there was a significant interaction in overall detectability between habitat and collection technique (Table 3), the effect of technique for this variable was examined for each habitat type independently. The overall detectability of species was higher for hand searches than for traps for two of the three habitats (Table 2); however, this differ-

TABLE 2. Summary of community estimates for two gastropod collection methods across three habitat types. Hand refers to the average estimate value (\pm standard error) based on collections sampled by quadrat based plot searches and trap refers to the average estimate value (\pm standard error) based on collections sampled using cardboard traps.

		White birch	Hardwood	Logged	All sites
Richness	Hand	8.8 \pm 1.83	8.0 \pm 1.05	10 \pm 0.63	8.9 \pm 0.71
	Trap	6.4 \pm 1.03	7.8 \pm 1.07	7.4 \pm 1.89	7.2 \pm 0.76
Diversity	Hand	1.32 \pm 0.40	0.99 \pm 0.15	1.29 \pm 0.20	1.20 \pm 0.15
	Trap	0.60 \pm 0.14	0.86 \pm 0.17	0.95 \pm 0.30	0.80 \pm 0.12
Est. Richness	Hand	9.2 \pm 1.74	15.4 \pm 4.45	11 \pm 0.84	11.87 \pm 1.65
	Trap	8.4 \pm 1.69	8.4 \pm 1.03	15 \pm 5.55	10.6 \pm 2.0
Overall Detectability	Hand	0.86 \pm 0.12	0.67 \pm 0.34	0.87 \pm 0.09	0.80 \pm 0.22
	Trap	0.76 \pm 0.18	0.88 \pm 0.10	0.47 \pm 0.32	0.70 \pm 0.27

TABLE 3. Summary of ANOVAs for gastropod community estimates from trap and hand search methods across three habitat types. Sources were considered significant at $P < 0.05$.

	Source	df	Type III	F value	P value
Richness	Habitat	2	6.47	0.68	0.522
	Site(habitat)	12	154.40	2.71	0.039
	Technique	1	22.53	4.75	0.047
Diversity	Habitat	2	0.22	0.65	0.537
	Site(habitat)	12	5.28	2.57	0.048
	Technique	1	1.20	7.04	0.019
Est. Richness	Habitat	2	94.87	1.07	0.369
	Site(habitat)	12	697.00	58.08	0.310
	Technique	1	12.03	0.27	0.610
Overall Detectability	Habitat	2	0.10	1.44	0.275
	Site(habitat)	12	0.69	1.60	0.215
	Technique	1	0.071	1.96	0.187
	Habitat*technique	2	0.46	6.34	0.013

ence was significant only on logged sites ($t = 3.621$, $P = 0.022$).

Similarity in species composition of the two collections varied from 0 to 86% across sites. White birch and hardwood sites showed related levels of similarity [53.30% (± 21.28) and 52.56% (± 17.75) respectively], but average similarity was much lower on logged sites [25.37% (± 25.00)]. Regressions of diversity and similarity, both across all habitat types and within each habitat type, were not significant at the 0.05 level. However, data from white birch and hardwood showed similar trends. When I combined the data from only these two habitat types, I found that as site diversity increased, the two methods had more dissimilar compositions (Fig. 1; % similarity = $(-18.93)\text{diversity} + 75.65$, $F_{1,8} = 6.52$, $P =$

0.034, $R^2 = 0.449$). The regressions of estimated richness and similarity showed similar trends, but for this measure the regression using only white birch and hardwood sites was not significant ($F_{1,8} = 2.80$, $P = 0.133$). For all regressions performed, however, the power of significance tests were low (between 6% and 30%), indicating a high potential to commit Type II errors. Further, when the composition of collections were highly dissimilar at a site, hand searches always had higher diversity than did trap collections.

DISCUSSION

If we assume that the hand-search collection method provides a relatively accurate

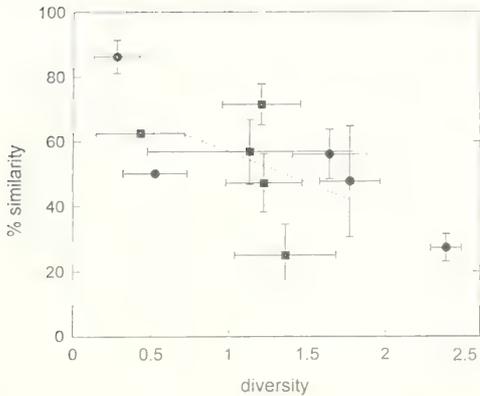


FIG. 1. Regression of diversity of terrestrial gastropods versus percent similarity in species composition of hand search and trap collections. Regression line is based on data from white birch (solid circles) and mixed hardwood (solid squares) sites.

representation of the gastropod community, then the trap collection method using cardboard appears to be generally biased in terms of both observed species richness and diversity. There were evident differences between methods in observed richness and diversity for white birch and logged sites, but very little for hardwood stands. Further, there was a much lower similarity in species composition between the two collections in logged sites compared to the other two habitats. Regressions of diversity and similarity in collection methods, while only significant when data from logged sites were removed, implied that the methods yield relatively similar lists of species when community diversity is low and become increasingly dissimilar as diversity increases. Because of high variation in estimates among sites, even within the same habitat, there was a high probability of committing a Type II error in all regressions performed; further replicates would be required to examine the relationship between similarity in collections and diversity more completely. Nonetheless, traps seemed to perform best, in terms of consistency, in hardwood stands and worst in logged sites.

The observed differences among habitats in the degree of bias in trap collections could be related to either direct differences in the species present in the three habitats or, more indirectly, could be associated with differ-

ences in habitat spatial complexity. For example, there tends to be less understory vegetation and debris in hardwood stands compared to white birch or logged sites (pers. obs.). Where there are fewer potential refuge sites, gastropods may be more readily attracted to traps. Conversely, where there are erratic areas of potential refuge (as in recently logged sites), the ability to collect using traps will depend on the placement of the trap and the performance will be less predictable.

The distance across which gastropods might be attracted to cardboard traps is largely unknown. In a study of dispersal of *Agriolimax reticulatus* (= *Deroceras reticulatum*) on a fallow field, South (1965) found that slugs travelled a mean distance of 1.13 m in seven days. However, *Limax maximus* has been known to move directly towards a food source from up to 7.5 m away (Cook, 1979). The slugs *Arion circumscriptus* and *Deroceras laeve*, and five snail species (family Polygyridae: *Anguispria alternata*, *Mesodon sayanus*, *Stenotrema fraternum*; family Endodontidae: *Discus cronkhitei*; family Zonitidae: *Zonitoides arboreus*), tended to be collected in higher numbers from cardboard traps. All of these species were, with the exception of *A. alternata*, consistently found during hand searches and most are relatively large, obvious gastropods that can be easily spotted during searches. Such aspects suggest that these species are over-represented in trap collections. Because most of these species are robust and all are active in the leaf litter (McCoy & Nudds, 1997), they may "find" cardboard traps from greater distances and aggregate there. Other species, such as *Strobilops labyrinthica* (family Strobilopsidae), and some members of families Endodontidae (*Helicodiscus parallelus*, *Punctum minutissimum*) and Zonitidae (*Paravitrea multidentata*, *Euconulus fulvus*) appear to be underrepresented in cardboard trap collections. All of these species are relatively small (< 3.5 mm), and while some (such as *H. parallelus*) are more subterranean in nature, at least one has been found on tree trunks (pers. obs.) and is, therefore, relatively mobile.

It is not clear why some species would be attracted to cardboard traps and others would not. It was previously shown that different species show different affinities for traps. For example, Boag (1990) demonstrated that *Discus cronkhitei* used artificial masonite shelters consistently less often than did *Euconulus ful-*

vus. I found the opposite: *D. cronkhitei* was sampled relatively more frequently than *E. fulvus* by cardboard traps. This further suggests that the affinity of different species for traps can change depending on the trap material used. In addition, considerable variation has been found in the frequency of snails adhering to shelters over time and depending on ambient temperature and moisture conditions (Boag, 1990; Hawkins et al., 1997). Likewise, there are substantial differences in activity and use of different micro-sites by species found at the same location (Cain & Cowie, 1978; Cameron, 1978). These aspects imply that the quantitative characteristics of gastropod populations can vary greatly depending on both the sampling technique and material used for collection, and on the weather conditions at the time collections are performed.

I estimated species richness using a capture-recapture model (model M_{th}) that incorporated variation in detection probabilities among species in the community. This model was most frequently selected in bird community studies, emphasizing the potential importance of heterogeneity in species' detection (Boulinier et al., 1998). While the model selection procedure of CAPTURE did not select model M_{th} more frequently than other capture-recapture models for the gastropod collection data, this model did fit more than 80% of the time for both methods, suggesting that heterogeneity in species detection exists among gastropod species.

Overall species detection at a site (i.e., probability of capturing a species at least once across all sampling locations) tended to be higher for hand-searched plots compared to traps, but was typically between 70% and 80%, meaning that most species were seen by both methods when ten replicates within a site were used. At the scale of the plot, however, the average instantaneous detectability of gastropod species was low. A low probability of detecting species on plots means that estimated richness could be slightly biased. Nonetheless, previous work suggests that it should still be closer to the actual number of species than simple counts alone (Nichols & Conroy, 1996). For this study, the estimated richness values found match well with the number of species recorded for the area. For example, I observed an average of nine species per site, but calculated a estimated average richness of 12 species. As 18 species were found overall and 27 species have been previously reported in the Algonquin re-

gion (Oughton, 1948), I expect that a few species were never sampled on a site. Thus, the low probability of detecting species on a plot underlines the potential problems associated with using simple counts for determining species richness, regardless of the sampling technique used and demonstrates the usefulness of species richness estimators. These types of estimators are now being more frequently employed in community-level studies (e.g., Derleth et al., 1989; Morrison, 1996; Boulinier et al., 1998; Nichols et al., 1998a).

The differences I found between the collection methods can have important implications for studies that require representative samples of the gastropod community. For example, this study was performed as part of a larger project examining the transmission of *Parelaphostrongylus tenuis*, a common nematode parasite of white-tailed deer (*Odocoileus virginianus*) in northeastern North America. Terrestrial gastropods act as intermediate hosts for this and other such parasites (Anderson & Prestwood, 1981). To assess the potential risk of transmission of these parasites and to study the transmission ecology in general, gastropods are commonly collected using traps (Kearney & Gilbert, 1978; Samuel et al., 1985; Upshall et al., 1986; Robb & Samuel, 1990). If certain species are missed or misrepresented in samples, calculated estimates of prevalence may be incorrect. For example, many of the species I found to be over-represented in trap collections (e.g., *D. cronkhitei*, *Z. arboreus* and *D. laeve*) are typically considered to be important intermediate hosts of *P. tenuis* based on their relative abundance and prevalence of infection (e.g., Lankester & Anderson, 1968; Platt, 1989). Further, the role in parasite transmission played by many of the species I found to be commonly underrepresented in trap collections has never been assessed. In addition, the bias in the trap method appears to be stronger in high diversity sites, such as white birch stands, and these stands have been considered to be potential transmission foci (Kearney & Gilbert, 1978). Thus, due to sampling technique, estimates of prevalence and our understanding of transmission of parasites such as *P. tenuis* could be inaccurate.

Richness, based on simple counts, and diversity were significantly different for the two collection methods, but there was no difference between methods in estimated richness. This suggests that for the examination of gastropod community richness alone, collections

from cardboard traps can be as representative as collections based on hand searches. Estimates of richness are essential in many ecological studies, and invertebrates are only now being considered more in studies of biodiversity (e.g., Dobyns, 1997; Niemelä, 1997). Therefore, while cardboard traps are not reliable for physically obtaining a representative gastropod collection, they may be sufficient for establishing the pattern of species occurrences such that estimates of richness are similar to what they would be if a more laborious and time consuming method of searching individual quadrats was performed. Other studies on invertebrates have also found that certain species were missed by different collection methods and that estimation techniques were useful for determining overall richness (Morrison, 1996; Dobyns, 1997). Nevertheless, estimation techniques are still limited in that they can not necessarily provide information about specific species changes within the community as some species are never actually detected. However, methods to make inferences about such community attributes are now being developed (Hines et al., 1999; Nichols et al., 1998a, b).

Various methods have been developed and used to sample terrestrial gastropods. However, as there are problems and biases associated with all sampling techniques, the most appropriate method will depend on the question being asked. If the goal is to estimate the richness of a gastropod community, it is appropriate to use simple cardboard traps to sample as long as the estimation model used takes into account potential heterogeneities inherent in the system. However, if the goal is, for example, to determine the relative abundance of species over space and time and/or to estimate the prevalence of a parasite (such as *P. tenuis*), traps may not be sufficient; potentially important components of the community may be missed or mis-represented in inconsistent manners. I recommend collecting gastropods by hand searching quadrats, using sorted subsamples, whenever this method can practically be undertaken.

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POPULATION STRUCTURE IN A SNAIL SPECIES FROM ISOLATED MALAYSIAN LIMESTONE HILLS, INFERRED FROM RIBOSOMAL DNA SEQUENCES

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ABSTRACT

We sequenced the first internal transcribed spacer (ITS-1) of the ribosomal DNA in nine populations of the vertiginid *Gyliotrachela hungerfordiana*, which lives on isolated (and threatened) limestone hills in the Malaysian peninsula. Current data suggest that the species is an obligate calcicole. The application of a tentative molecular clock suggests a Quaternary divergence for the *G. hungerfordiana* populations. A strong positive correlation between genetic and geographic distance was observed, which, combined with geological data, suggests that the hill populations may be interconnected by as yet unsampled populations.

Key words: internal transcribed spacer, ITS-1, Gastropoda, Pulmonata, Vertiginidae, *Gyliotrachela*, gene flow, Southeast Asia.

INTRODUCTION

Land snails have proverbially poor abilities for dispersal (e.g., Cowie, 1984; Schilthuizen & Lombaerts, 1994), which causes them to show evolutionary patterns at much smaller spatial scales than many other organisms of similar size. As a result, strong geographic structuring of populations is common in snails (e.g., in *Liguus*; Hillis et al., 1987). Another consequence is endemism, which is seen, for example, in the Mediterranean causiiliid genus *Albinaria*, of which almost 30 species are endemic to the island of Crete, with distribution areas of sometimes only one kilometer across (Gittenberger, 1991; Welter-Schultes, 1998).

An impressive situation of high endemism and geographic structuring of land snails in a strongly fragmented habitat exists in peninsular Malaysia. Here, limestone is exposed in the form of "tower karst" and other karstifications, limited to about three hundred hills, scattered over the peninsula. These hills are often very small, the largest with a diameter of a few kilometers, but most measuring only a few hundred meters across. In spite of their small size, the hills are a prominent feature of the landscape, because they usually stand isolated, are riddled with caves and are bounded by precipitous cliffs.

For more than a century, malacologists have been interested in the rich malacofauna that the hills support (de Morgan, 1885). High numbers of species are found, and the morphologies of some Diplommatinidae foreshadow the bizarre and extravagant forms found in this group in Borneo (Vermeulen, 1993, 1994; Gittenberger, 1995). But especially fascinating is the staggering degree of endemism in these calcicolous snails. Tweedie (1961) gave an overview of six taxa containing many obligate calcicoles (*Diplommatina*, *Opisthostoma*, Vertiginidae, *Discartemon*, *Oophana*, and *Sinoennea*). He listed the presence of 106 species on 28 hills or hill-clusters, of which 70 are endemic to only one locality. Some calcicolous species, however, are widespread and occur on almost all hills without a trace of morphological differentiation (e.g. *Gyliotrachela hungerfordiana* and some *Alycaeus* species).

Geologically, the hills form the exposed parts of a number of larger paleozoic limestone deposits, which are elsewhere overlain by non-calciferous alluvial deposits (Gale, 1986; Crowther, 1986). Some hills may thus have been connected in the past, while others have always been separate. Consequently, the hills form virtual "islands" for obligately calcicolous land snails, which they may reach by incidental dispersal. Alternatively, the pop-

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ulations on the hills may be relicts from a time when the hills were part of large continuous plateaus, which were subsequently fragmented.

In this paper, we examine a relatively widespread representative of the peninsular Malaysian hill malacofauna, using molecular and geological data, to answer the following questions: (1) what pattern of phylogeographic relationships exists among the populations of this widespread species, and (2) how has the population structure been shaped, that is, what are the relative influences of dispersal and habitat fragmentation over geological time?

By analyzing the variance in a noncoding nuclear DNA marker, we attempt to differentiate between various alternative population structures. In the case of ancient vicariance, we expect to find genetic distances that reflect the age of fragmentation of the limestone hills, while dispersal would result in genetic distances more or less related to geographic distance. Under the latter hypothesis (dispersal), indications of the type and frequency of dispersal may be gleaned from the degree of correlation between genetic and geographic distance; if dispersal is randomly oriented (i.e., corresponding to an island model of population structure; Wright, 1931), stochasticity would result in a poor fit, while dispersal occurring mainly among neighboring hills (i.e., corresponding to a stepping-stone model; Kimura, 1953) would be revealed by a strong correlation (Kimura & Weiss, 1964).

Sadly, there are other motives for working on this fauna. The hills of peninsular Malaysia are disappearing and becoming depauperate at an alarming rate. Forest clearing has destroyed the vegetation on some hills; and in the densely populated areas near Ipoh and Kuantan, many hills are being removed by quarrying. The true rate of species loss can only be guessed at, but the extinction of at least one endemic snail species, *Opisthostoma sciaphilum*, from Bukit Panching, has been documented (Schilthuizen et al., unpubl.).

MATERIAL AND METHODS

Selection of Taxa

We selected the widespread and morphologically uniform vertiginid *Gyliotrachela hungerfordiana* for study (Fig. 1). The related

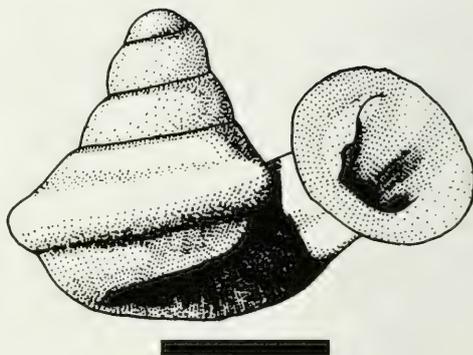


FIG. 1. *Gyliotrachela hungerfordiana* (von Möllendorff). Scale bar = 1 mm.

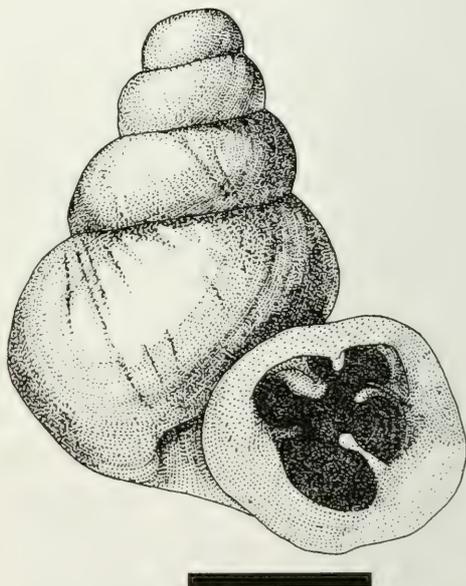


FIG. 2. *Gyliotrachela frequens* van Benthem Jutting. Scale bar = 1 mm.

species *G. frequens* (Fig. 2) was selected to serve as an outgroup in the phylogenetic analysis.

Collecting

In July 1997, the first author visited 22 limestone hills in the West-Malaysian states of Pahang, Kelantan, Perak and Perlis. Living snails were discovered by eye using two strategies: (a) close inspection of limestone rock faces,

either damp or dry, bare or covered in algae, mosses and lichens; and (b) sifting through damp and decaying leaf litter on limestone rocks or at the base of the limestone cliffs. All snails were put in 100% ethanol on the spot and kept at ambient temperatures until arrival in the laboratory for further processing. Identification of the material was carried out by the second author while the material remained in alcohol. *Gyliotrachela hungerfordiana* was collected from nine of the 22 localities (Fig. 3): loc. 5, State of Pahang: Gua Bama (ca. 10 km W of Kuala Lipis); loc. 8, State of Kelantan: Gua Musang, southern of the two hills that the road to Kuala Kerai passes between; loc. 9, State of Kelantan: rocks 59 km in the direction of Gua Musang, measured along the road from Kuala Krai; loc. 16, State of Perak: Bukit Tambun (ca. 6 km E of Ipoh); loc. 22, State of Perak: hill directly east of Sungai Siput Utara hospital; loc. 23, State of Perlis: hill ca. 1 km S of Kangar; loc. 24, State of Perlis: 9 km along the road from Kangar to Kaki Bukit; loc. 25, State of Perlis: Gua Kelam at Kaki Bukit; loc. 26, State of Perlis, Timah Tasoh (ca. 16 km NE of Kangar). All samples were taken between 27.vi.1997 and 17.vii.1997. *Gyliotrachela frequens* was taken only from locality 8. Voucher specimens have been deposited in the collection of the National Museum of Natural History "Naturalis", Leiden.

Molecular Techniques

DNA was isolated from pools of between one and five complete snails with their shells, using either a phenol/chloroform extraction as described previously (Schilthuisen et al., 1998a) or a sucrose-based protocol (van Moorsel & van Nes, unpublished), which can be briefly summarized as follows. Snails were ground in 200 μ l of sucrose-buffer (0.1 M Tris; 0.02 M NaCl; 0.2 M sucrose; 0.05 M EDTA) and centrifuged. The pellet was incubated at 65°C for 60 min in 200 μ l SDS-buffer (0.02 M Tris; 0.01 M EDTA, 1.25% SDS), 15 μ l of cold KAc was added, and the mixture was incubated on ice for 60 min and centrifuged. The DNA was precipitated from the supernatant by the addition of two volumes of 100% ethanol and incubation at -20°C for 30 min. The DNA was dried and treated with 200 ng of RNase. Full details can be obtained from M.S. on request. Homogenization was always done with a sterile, disposable plastic pestle. The DNA was dissolved in 50 μ l of Tris-EDTA buffer (phenol protocol) or 30 μ l of ddH₂O (sucrose

protocol) and stored at -20°C. The first internal transcribed spacer of the nuclear ribosomal DNA was amplified with the SuperTag enzyme (HT Biotechnology, Cambridge, England) as described previously (Schilthuisen et al., 1995) and isolated using the "freeze-squeeze" technique (Tautz & Renz, 1983). Because PCR-amplification was at times too weak for direct sequencing, we resorted to cloning (PCR-based error is usually not a concern with this methodology; Schilthuisen et al., 1998b). After isolation, the fragments were ligated into Promega or Invitrogen T-tailed vectors, following the manufacturer's instructions. Colonies were screened for the presence of the correct insert by PCR. Plasmid DNA was isolated from the bacteria using QIAPrep spin columns (QIAGEN). One or two clones per sample were sequenced in both directions on an ABI automated sequencer.

Alignment

Before alignment, all chromatograms were checked and reading errors were corrected blindly where necessary (this never amounted to more than three corrections in a single sequence). Vector and primer sequences were removed. Sequences in the ingroup were sufficiently similar to allow manual alignment. Wherever alignment with the outgroup was ambiguous, missing data were introduced into the outgroup sequence.

Phylogenetic Analysis

Phylogenetic analyses of the data set were performed in PAUP3.1 (Swofford, 1993). Gaps were treated as missing data. Searches for the most parsimonious trees were carried out with the branch-and-bound option. Bootstrap replicates were carried out 100 times, using heuristic searches. In addition, Bremer (1988) support was determined. Kimura's 2-parameter genetic distances (Kimura, 1980) were calculated with the DNADIST program of the PHYLIP package (Felsenstein, 1995).

RESULTS

PCR-products ranged in length from 755 to 772 bp, including primers (52 bp), and the flanking regions of 18S (146 bp) and 5.8 S (87 bp). These lengths correspond well with other ITS-1 lengths reported in mollusks (Anderson

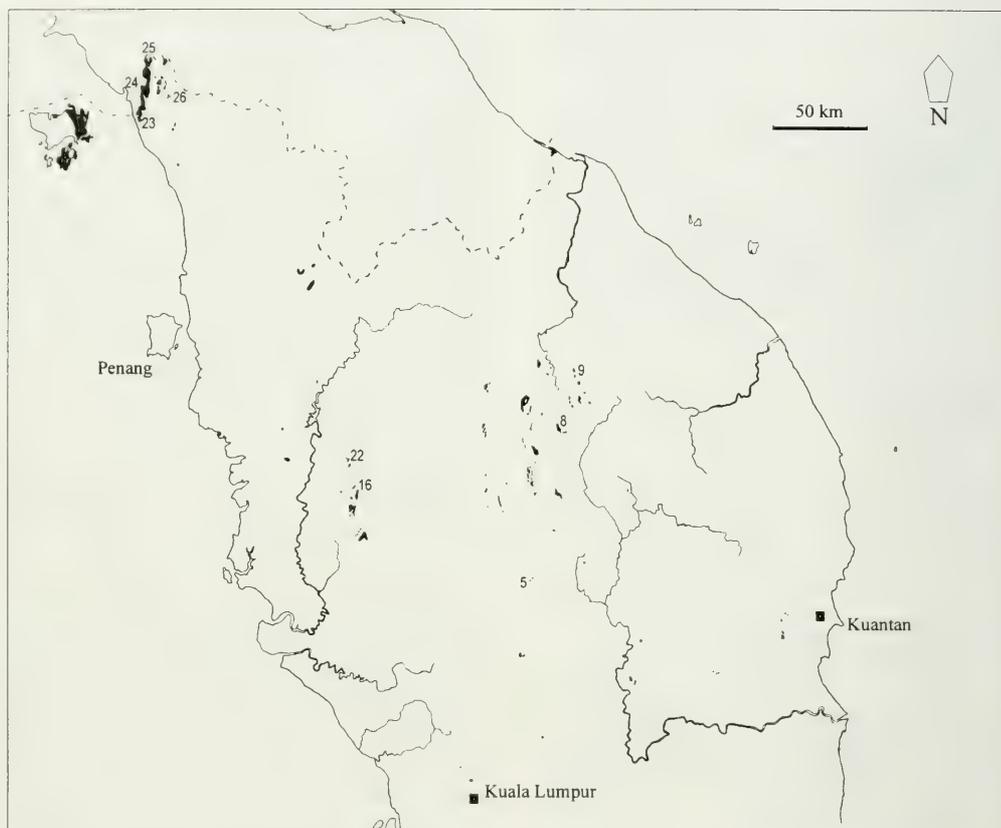


FIG. 3. A map of the northern part of Peninsular Malaysia, with the limestone hills drawn in black (modified after Gobbett, 1965). The numbers refer to localities where *Gylotrichela hungerfordiana* and *G. frequens* were collected (see text for further details).

& Adlard, 1994; Schilthuizen et al., 1995; Armbruster et al., unpubl.).

We obtained sixteen sequences from *G. hungerfordiana* and one sequence for the out-group, *G. frequens* (Appendix, Table 1). They have been deposited in GenBank under accession numbers AF118000-AF118016. Only small genetic distances were found among the *G. hungerfordiana* sequences, the largest being 0.048 between sequence *a* from locality 5 and sequence *b* from locality 23. A comparison between pairwise genetic distances and pairwise geographic distances between sequences revealed a strongly significant ($p < 0.005$) positive correlation (Fig. 4, Appendix, Table 2). The phylogenetic analysis produced 18 most parsimonious trees (length = 89 steps, RI = 0.95), which showed two

alternative topologies for three monophyletic groups of sequences, and otherwise only minor differences in topology within each of these three monophyletic groups (Figs. 5, 6). The fact that duplicate sequences from a single locality always formed monophyletic groups might justify the small sample sizes. Geographic structuring is apparent in the trees also, as these show monophyly for the sequences derived from populations in Perlis, Pahang + Kelantan, and Perak.

DISCUSSION

Unfortunately, it is difficult to estimate reliably from the molecular data the time since divergence. Unlike the situation for mitochon-

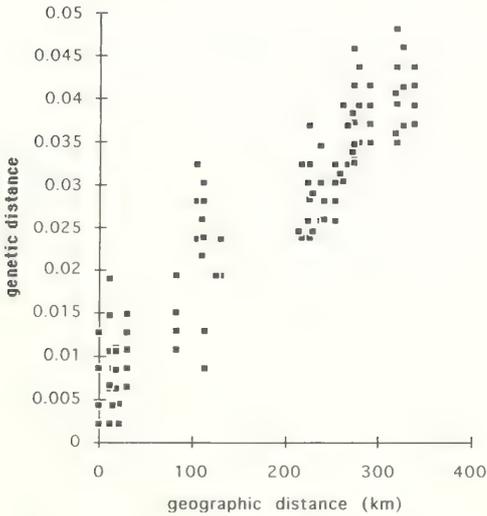


FIG. 4. The relationship between geographic distance and Kimura's 2-parameter distance for the sequences of *Gylotrichela hungerfordiana*.

drial DNA, corroborated molecular clocks for the ITS regions are hardly available yet, and where they are, they differ by orders of magnitude among taxonomic groups. In the angiosperm families Cucurbitaceae and Winteraceae, substitution rates of 3.62×10^{-3} and 3.4×10^{-4} per site per million years (MY) were calculated, respectively (Jobst et al., 1998; Suh et al., 1993), while in Chlorophyta, a rate of $0.8 - 2.0 \times 10^{-2}$ was estimated (Bakker et al., 1995). In animals, rates of substitution in ITS appear to be somewhat higher. Schlötterer et al. (1994) give a figure of 1.2×10^{-2} for *Drosophila*, and preliminary data for clausiliid land snails from Greek islands indicate a similar rate (van Moorsel, unpublished data).

Here, we will adopt a substitution rate of 1×10^{-2} per site per MY as a very rough molecular clock. Applying this rate to the average genetic distance between sequences on either side of the node basal to all *G. hungerfordiana* sequences in the trees, we obtained an estimated divergence time of 1.8 MYA for the populations of *G. hungerfordiana*. It should be stressed that, given the lack of agreement in the few calibrated molecular clocks available, not too much confidence should be placed on this date. However, it may be safe to assume a Late Tertiary or Quaternary origin for *G. hungerfordiana*.

Given the low degree of genetic divergence among the *G. hungerfordiana* populations, it seems unlikely that vicariance has played an important role; hills which have been studied geologically are thought to be older than Late Tertiary/Quaternary (Gale, 1986). However, in view of the uncertainty about the calibration of the ITS-1 molecular clock, this reasoning may be little meaningful. More importantly, geological data indicate that most of the hills from which the species was sampled have never been part of one continuous plateau (Paton, 1961). It is for this reason not likely that vicariance events have been important in its distribution pattern. Rather, the limestone hills on which it lives now must have been colonized after dispersal.

Several mechanisms for passive dispersal in small snails have been suggested, including wind and water mediated dispersal. In reference to *Gylotrichela* and similar snails, Tweedie (1961) has suggested that flooding may be important in producing dispersal among hills that are situated close together. However, the drainage patterns in the peninsula preclude any long-range dispersal by this mechanism. Stagnant water may also provide means of dispersal, and geological data (Gale, 1986; Crowther, 1986) indicate that lacustrine conditions have prevailed around several limestone hills in the past. But here, too, dispersal would be across very small distances. Another possibility is wind-dispersal. Kirchner et al. (1997) demonstrate how *Truncatellina*, a vertiginid very similar in size to *G. hungerfordiana*, could be blown over distances of several kilometers during storms.

Some additional characteristics of dispersal may be gleaned from Figure 4, which suggests a linear relationship between geographic and genetic distance. If dispersal from one hill to another were infrequent and undirected (i.e., a population structure corresponding to Wright's [1931] island model, where all possible pairs of subpopulations are equally likely to exchange migrants), such a clear relation would not be expected. The fact that genetic distance is reliably predicted ($r^2 = 0.77$) by geographic distance, suggests that a structured network of dispersal connects the hills. This corresponds to a stepping-stone model (Kimura, 1953). Under such a model, genetic similarities drop steeply with increasing numbers of intervening populations (Kimura & Weiss, 1964). The fact that we observe a strong relationship with geographic distance, suggests that the hill popu-

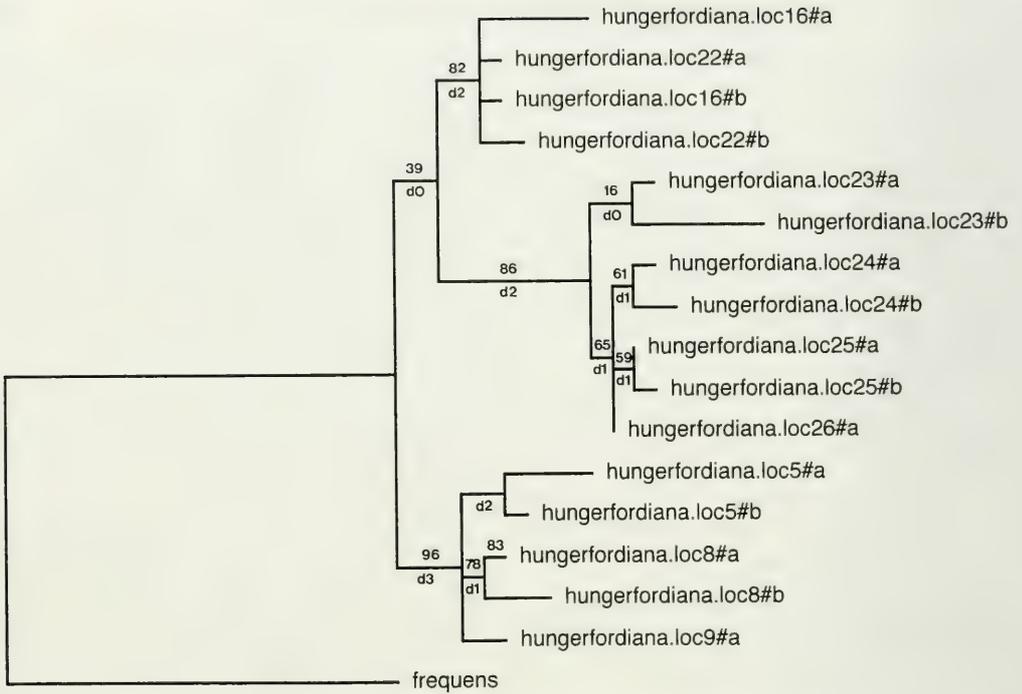


FIG. 5. A representative most parsimonious tree of the *Gylotrachela* sequences. Bootstrap percentages and decay indices have been indicated on the branches.

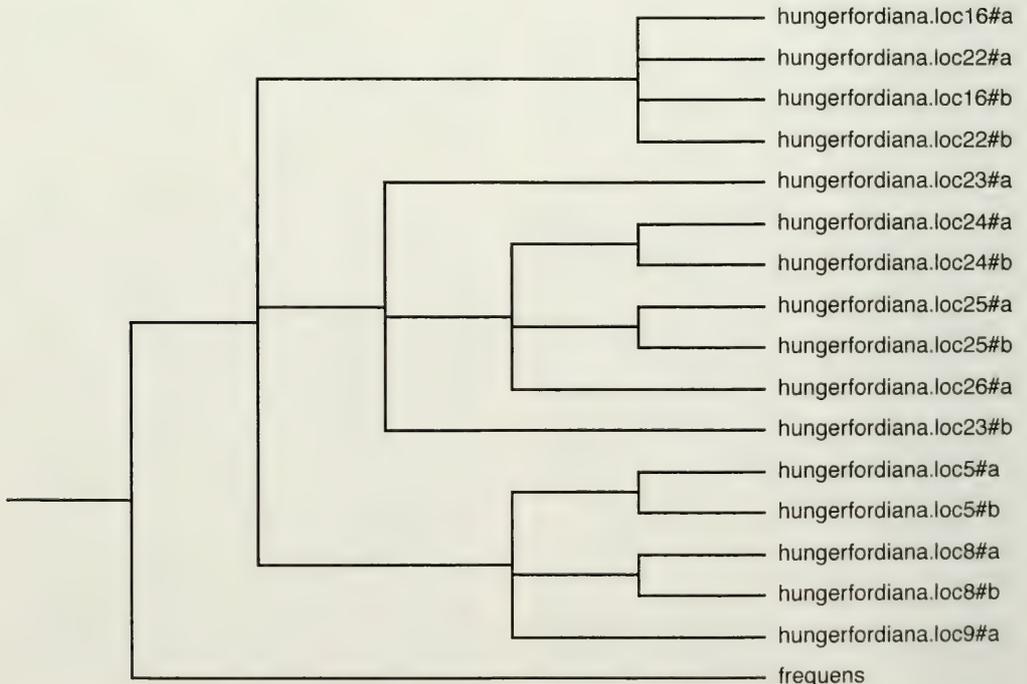


FIG. 6. Strict consensus over all 18 most parsimonious trees.

lations cannot represent directly adjacent populations in a two-dimensional stepping-stone lattice. Rather, to obtain this result, it is necessary to postulate unsampled populations in between. Unfortunately, the population genetics of ribosomal DNA are as yet far from clear (Hillis et al., 1991; Rich et al., 1997), which makes a quantitative analysis of dispersal parameters and spatial details of the population structure impossible. Therefore, it is not possible to tell whether the hills that separate our sample sites (e.g., the six or more hills between sites 8 and 9) will suffice as additional stepping stones. This might be tested, for instance, by exhaustively sampling the hills in a given subregion.

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APPENDIX

TABLE 1. Aligned sequences for *Gylotrichela hungerfordiana* and *G. frequens*. The 5' end of the 18S region is at position 146, the 3' end of the 5.8S region is at position 694.

	10	20	30	40	50
<i>hungerfordiana</i> .loc5#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc5#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc8#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc8#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc9#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc16#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc16#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc22#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc22#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc23#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc23#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc24#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc24#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc25#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc25#b	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>hungerfordiana</i> .loc26#a	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
<i>frequens</i>	AGCGGTTACAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
	60	70	80	90	100
<i>hungerfordiana</i> .loc5#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc5#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc8#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc8#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc9#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc16#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc16#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc22#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc22#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc23#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc23#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc24#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc24#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc25#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc25#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>hungerfordiana</i> .loc26#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
<i>frequens</i>	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
	110	120	130	140	150
<i>hungerfordiana</i> .loc5#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc5#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc8#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc8#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc9#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc16#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc16#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc22#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc22#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc23#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc23#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc24#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc24#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc25#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc25#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>hungerfordiana</i> .loc26#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
<i>frequens</i>	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG

TABLE 1. Continued.

	160	170	180	190	200
hungerfordiana.loc5#a	TATAAT----	-----CATCA	GGCTGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc5#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc8#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc8#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc9#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc16#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc16#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc22#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc22#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc23#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc23#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc24#a	TACAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc24#b	TACAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc25#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc25#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCCGAG	CGGCTTATGA
hungerfordiana.loc26#a	TATAAT----	-----CATCA	GGCAGC	-----	-GGCTTATGA
freqs	TATTATTACA	AAATACGTCA	GGC-----	-----	-----ATGA
	210	220	230	240	250
hungerfordiana.loc5#a	TGAAATTA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc5#b	TGAAATTA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc8#a	TGAAATTA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc8#b	TAAAATTA--	---TGCTGGT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc9#a	TGAAATTA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc16#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc16#b	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc22#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc22#b	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc23#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc23#b	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc24#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc24#b	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc25#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc25#b	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
hungerfordiana.loc26#a	TGAAATA--	---TGCTGAT	TGAACGCTCG	TC-----	-----
freqs	TG--TATAGA	TAATGCTGAT	GGAACGCTGTC	TCGTCTCGTC	TCGTCTCGTC
	260	270	280	290	300
hungerfordiana.loc5#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc5#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc8#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc8#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc9#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc16#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc16#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc22#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc22#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc23#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc23#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc24#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc24#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc25#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc25#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
hungerfordiana.loc26#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG
freqs	TCGTCTCATT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCC	CGGTCGGTTG

TABLE 1. Continued.

	310	320	330	340	350
hungerfordiana. loc5#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc5#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc8#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc8#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc9#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc16#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc16#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc22#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc22#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc23#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc23#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc24#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc24#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc25#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc25#b	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana. loc26#a	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
frequens	ACCCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
	360	370	380	390	400
hungerfordiana. loc5#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc5#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc8#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc8#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc9#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc16#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc16#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc22#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc22#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc23#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc23#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc24#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc24#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc25#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc25#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
hungerfordiana. loc26#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
frequens	CCACGGTGAC	GGCATGAGCT	CTCAGCTCGC	CGGGTCGTCA	GGCTAAGGA
	410	420	430	440	450
hungerfordiana. loc5#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGAGG
hungerfordiana. loc5#b	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGAGG
hungerfordiana. loc8#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana. loc8#b	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana. loc9#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana. loc16#a	GCGTGCTCC	GACTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana. loc16#b	GCGTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana. loc22#a	GCGTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana. loc22#b	GCGTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana. loc23#a	GCGTGCTCT	GATTGCCCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc23#b	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc24#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc24#b	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc25#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc25#b	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana. loc26#a	GCGTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
frequens	GCGCGCTCT	GACTGCTCTA	TGAGCGGCGC	CGCCCCGGTA	GTTG-TGTGG

TABLE 1. Continued.

	460	470	480	490	500
hungerfordiana.loc5#a	-ATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc5#b	-ATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc8#a	-ATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc8#b	-ATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc9#a	-ATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc16#a	GATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc16#b	GATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc22#a	GATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc22#b	GATAATGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGT-CTGC
hungerfordiana.loc23#a	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc23#b	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc24#a	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc24#b	-ATGACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc25#a	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc25#b	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
hungerfordiana.loc26#a	-ATAACGGAG	G-----	---GTACCTG	TGCCTCGAC	CCGTCTGC
frequens	-ATCAAGGAG	GCAAGGCCG	AGGTACCTG	TGCCTCGAC	CG-CT-CTGC
	510	520	530	540	550
hungerfordiana.loc5#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc5#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc8#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc8#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc9#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc16#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc16#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc22#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc22#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc23#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc23#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc24#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc24#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc25#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc25#b	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc26#a	TGGGCGGATC	CGGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
frequens	TGGGCGGATC	TGGTGCAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
	560	570	580	590	600
hungerfordiana.loc5#a	GCTTAAAGA?	GTCGGCC-GT	A-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc5#b	GCTTAAAGA?	GTCGGCC-GT	A-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc8#a	GCTTAAAGA?	GTCGGCC-AT	A-TATATGCT	CG?GCA?ACC	CGCCCGTCC
hungerfordiana.loc8#b	GCTTAAAGA?	GTCGGCC-AT	A-TATATGCT	CG?GCA?ACC	CGCCCGTCC
hungerfordiana.loc9#a	GCTTAAAGA?	GTCGGCCAT	A-----TGCT	CGAGCG?ACC	CGCCCGTCC
hungerfordiana.loc16#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc16#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc22#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc22#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc23#a	GCTTAAAGA?	GTCGGCC-AC	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc23#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?A-C	CGCCCGTCC
hungerfordiana.loc24#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc24#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc25#a	GCTTAAAGA?	GTCGGCC-AA	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc25#b	GCTTAAAGA?	GTCGGCC-AA	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
hungerfordiana.loc26#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGTCC
frequens	GCTTAAAGA?	GTCGGCC-AT	GCTCGGGCT	-GACCC-GCC	CGCC--T--

TABLE 1. Continued.

	610	620	630	640	650
hungerfordiana.loc5#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc5#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc8#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc8#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc9#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc16#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTTGT	CTGTCTTATT
hungerfordiana.loc16#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc22#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc22#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc23#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc23#b	GCCTTCCT--	CATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc24#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc24#b	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc25#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc25#b	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
hungerfordiana.loc26#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCTTATT
frequens	GTC-TCCTCT	CATTTTATTT	TGTTACGCT-	-----	-TGTCCGAT-
	660	670	680	690	700
hungerfordiana.loc5#a	TGTCAGTTAC	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc5#b	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc8#a	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc8#b	TGTCAGTTAT	CGAAAAAG----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc9#a	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACGAC
hungerfordiana.loc16#a	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc16#b	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc22#a	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc22#b	TGTCAGTTAT	CGAAAAA----	-----C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc23#a	TGTCAGTTAT	CGAAAAA--A	AAAACAAA-C	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc23#b	TGTCAGTTAT	C?AAAAA	AAAAC?AAAC	AA?ATTGCCT	GTCGT?CAAC
hungerfordiana.loc24#a	TGTCAGTTAT	CGAAAAA	AAAA-----	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc24#b	TGTCAGTTAT	CGAAAAA	AAA-----	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc25#a	TGTCAGTTAT	CGAAAAA	A-----	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc25#b	TGTCAGTTAT	CGAAAAA	A-----	AAGATTGCCT	GTCGTACAAC
hungerfordiana.loc26#a	TGTCAGTTAT	CGAAAAA----	-AAACAAAC	AAGATTGCCT	GTCGTACAAC
frequens	----GGTTAT	CGAAAAA-CC	AAAACAAAA-	----TGCTT	GTCGTACAAC
	710	720	730	740	750
hungerfordiana.loc5#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAACCAGC
hungerfordiana.loc5#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc8#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc8#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc9#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc16#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc16#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc22#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc22#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc23#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCGGCCGGC
hungerfordiana.loc23#b	TTT??????	?????????	?????????	?????????	?????????
hungerfordiana.loc24#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc24#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc25#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc25#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc26#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC
frequens	TTTGAGCGGT	GGATCACTCG	GCTCGTGCCT	CGATGAAGAG	CGCAGCCAGC

ALLOZYME ANALYSES TEST THE TAXONOMIC RELEVANCE OF RIBBING IN CHINESE *ONCOMELANIA* (GASTROPODA: RISSOACEA: POMATIOPSIDAE)

George M. Davis¹, Yi Zhang², Xingjiang Xu³ & Xianxiang Yang³

ABSTRACT

The Tropical Medical Research Center of the Institute of Parasitic Diseases, Shanghai, China, is studying the genetics of *Oncomelania* snail populations throughout China relative to the transmission of the human blood parasite *Schistosoma japonicum* by genetically competent populations of *Oncomelania*. On the basis of allozymes and CO1 gene sequence data, *Oncomelania* has diverged significantly on a regional basis. There are three groups of populations we classify as subspecies: *O. h. robertsoni* in Yunnan and Sichuan above the Yangtze River Three Gorges (small, smooth and varix-less shells); *O. h. tangi* of Fujien Province, removed from the Yangtze River drainage (small, smooth squat snails with a double thick varix); and *O. h. hupensis* in the Yangtze River drainage below the Three Gorges (large shells, always with varix, primarily ribbed). These three clusters of populations differ from each other by Nei's "D" (allozymes) of 0.267 to 0.405. Thus, there is considerable genetic divergence among the subspecies.

There is the residual problem of what, genetically and taxonomically, are large smooth shelled *Oncomelania*, with varix, found below the Three Gorges in the Yangtze drainage? It is well known that in the floodplains of the Yangtze, *Oncomelania* populations are ribbed; above the effects of the annual flooding, the snails are smooth. Some taxonomists have called these smooth-shelled populations with varix *O. fausti*. The Miao River of Hubei Province offers a natural experiment to resolve this problem. In a small river there are four population centers that are affected by flooding; they have ribbed shells (RSP); above the flood zone there are several population centers where the shells are smooth but with pronounced varix (SSP).

Allozyme data (35 loci, 72 alleles; mean samples per locus, 44 to 122) resolve the problem. Mean Nei's D for RSP = 0.045 ± 0.036 ; for SSP = 0.024 ± 0.016 ; comparing RSP and SSP = 0.038 ± 0.035 . There are no significant differences among these populations; they are all *O. h. hupensis*. Ribbing is genetically controlled by a single gene with multiple alleles (Davis & Ruff, 1973). It thus appears that ribbing is a genetically controlled adaptation for dealing with annual flooding and survival by water transport. It also appears that terminal varix formation is controlled by a second gene. We predict that there will be no difference among all populations in the Miao River for the capacity to transmit the same genetic strain of *S. japonicum*. The role of unique alleles and gene flow are addressed.

Key words: systematics, *Oncomelania*, China, allozymes, population genetics, ribbing, Yangtze River, flooding, Hardy-Weinberg equilibrium.

INTRODUCTION

Oncomelania is one of eight Gondwanian genera of the rissoacean family Pomatiopsidae. The genus is comprised of a morphostatic radiation (defined in Davis, 1994) with only two species, *O. hupensis* Gredler, 1881, and *O. minima* Bartsch, 1936. The former, an amphibious species, is distributed from Burma (fossil) throughout southern China, Taiwan, the Philippines, Sulawesi and Japan, with a series of geographically isolated subspecies; the latter, an aquatic species, is found in

northern Japan. *Oncomelania hupensis* is of particular importance to humans as it is the intermediate host for the human blood fluke *Schistosoma japonicum*. The Tropical Medical Research Center of the Institute of Parasitic Diseases, Shanghai, China, is studying the coevolution of *Oncomelania* snail populations with *Schistosoma japonicum*. The hypothesis is: "as snail populations diverge genetically, so must the associated schistosomes; genetic divergence of schistosomes may have an important impact on human pathology and vaccine development for anti-

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FIG. 1. General position of the Miao River with respect to The Yangtze River and Wuhan City. Locations for control populations HB 22 and HB 6 are shown.

Schistosoma vaccines". We have used allozyme electrophoresis and gene sequencing of the mt CO1 and CYTb genes to begin to address the hypothesis. On the basis of allozymes and CO1 data, *Oncomelania* has diverged significantly on a regional basis. There are three primary groups we call subspecies: *O. h. robertsoni* in Yunnan and Sichuan, above the Yangtze River Three Gorges, with small, smooth and varix-less shells; *O. h. tangi* of Fujian Province, removed from the Yangtze River drainage, with small, smooth squat snails with a double thick varix; *O. h. hupensis* in the Yangtze River drainage below the Three Gorges with large shells, always with varix, primarily ribbed (Davis et al., 1995). These three clusters of populations differ from each other by Nei's "D" (allozymes) of 0.267 to 0.405; by 0.28 (28%) CO1 sequence divergence (over 578 nucleotide positions). There is great genetic divergence among the subspecies!

There is the residual problem of what, genetically and taxonomically, are large smooth-shelled *Oncomelania*, with varix, found below the Three Gorges in the Yangtze drainage? It is well known that in the floodplains of the Yangtze, *Oncomelania* populations are ribbed; above the effects of the annual flooding, the snails are smooth. Some taxonomists have called these smooth-shelled populations with varix *O. fausti* (reviewed in Davis et al., 1995). The Miao River of Hubei Province (Figs. 1, 2) offers a natural experiment to re-



FIG. 2. Localities along the Miao River and the relationship of the Miao River to the Yangtze river. A-C populations have ribbed shells and are below the bridge. Populations D-G have smooth shells and are above the bridge.

solve this problem. In this short river, there are four population centers that are affected by flooding; they have ribbed shells (RSP); above the flood zone there are several population centers where the shells are smooth but with pronounced varix (SSP). The purposes of this paper are to determine the following: (1) Are the RSP and SSP populations signifi-

cantly different genetically? (2) Are there indications of gene flow or allele gradients? (3) Are there populations with unique alleles? (4) Are the populations subject to annual flooding genetically unstable, that is, with polymorphic loci in considerable Hardy-Weinberg disequilibrium due to immigration of snails floated into the river by the annual flooding of the Yangtze from various other population centers (in contrast to genetically stable populations above the effects of the flooding)? (5) Are the Miao River populations significantly different from control populations from other parts of Hubei Province?

MATERIALS AND METHODS

Locality Data

All populations collected in 1994 are from Hubei Province, except the universal control, GC, from Anhui Province (Figs. 1, 2). GC, HB6 and HB22 are control populations. GC is the universal control population used with all populations of *Oncomelania* studied from throughout China. HB6 is the control population for Hubei Province. HB22 is a population outside the Miao River basin but not distant from the Miao River. Shells of these populations are shown in Figure 3.

HB6 (Figs. 1, 3l): 28 Sept. 1994; Jian Li County; Mao Shi Township; Su Hu Canal; Su Jia Yuan Section of the canal. The snails were collected from the banks of the Grand Canal about 1.5 m from the edge of the water under dense grass. The grass indicated that the area was not often flooded; at the edge of the water there were very narrow beaches of sand-shell perhaps a foot to 2 ft. wide in places. The greatest density of snails was in a narrow band. Above the band the soil was drier and snails correspondingly fewer. The slope was about 15°; at the upper edge about 15 ft. from the water it was about 25°. With the *Oncomelania* were *Parafossarulus*, and some dead shells of *Semisulcospira*. There were the same millipedes as in previous localities with *Oncomelania*. High humidity under the grass. Snails were in pockets of two to ten per pocket. We collected about 1,200 snails. The snails were numerous in this locality; all snails came from a 15.2 m-long stretch of bank. The canal had high density of Viviparidae [*Belamya quadrata* type]; shells of *Anodonta* were abundant.

The canal was built in 1959 to 1962; ac-

cording to the Hubei Institute of Schistosomiasis Control, in 1962 there were no *Oncomelania* in the canal; they came down from the three lakes region at the head of the canal. The shells are longer and more slender than those from Gui Chi (contrast Fig. 3 I and H), and they are not as heavily ribbed.

HB11 (A, Figs. 2, 3): 1 Oct.; Song Zi County; Xin Jiang Kou Township; De Shun Village. Miao River about 0.4 km above the mouth of the river. Snails were collected from the moist to wet mud under grasses of small ponds in the narrow flood plain of the river. The snails were ribbed and with a thick varix. Snail density was great. All snails were heavily ribbed.

HB12 (not on Fig. 2): 1 Oct.; Song Zi County; Cheng Dian Township; Ten Zi Qiao Village; Miao River 13 km upstream from HB11. Snails, very few, were collected from a small stream flowing over a small dam into the Miao River some 50 m above the bridge crossing the Miao. Snails came from the wet mud under thick grass at the edges of the stream near the dam and rapidly flowing water. No snails were found on the banks of the main river that had a rapid flow over cobbles. There were too few snails for electrophoresis. Snails had smooth shells.

The bridge (Fig. 2, bridge) has great significance. For some 60 m up- and downstream the snails are reputed to have a mixture of phenotypes from ribbed to smooth. One km downstream one begins to get ribbed snails that transmit *Schistosoma japonicum*. There is apparently no transmission, at this time, by smooth snails at the bridge or above it. The bridge marks the uppermost limit of the Yangtze flood during the rainy season. The bridge is about 13 km from the mouth of the river.

HB13 (D, Figs. 2, 3): 1 Oct.; Song Zi County; Cheng Dian Township; Ten Zi Qiao Village; Miao River 1.0 km above the bridge and HB12. Snail population density high; snails came from behind a natural dike or bank between the river and the true bank of the river. This backwater has water flowing through it cutting through the secondary bank into the river. A little upstream, the river bed widens and this ridge separates the river from the natural river bank by some 40 m with filled in land with crops. The snails came from mud under thick grasses at the edge of the water filling in the backwater. The snails were smooth.

HB14 (F, Figs. 2, 3): 1 Oct.; Song Zi County; Cheng Diam Township; Ten Zi Qiao Village; Miao River 1.5 km above bridge. At this local-

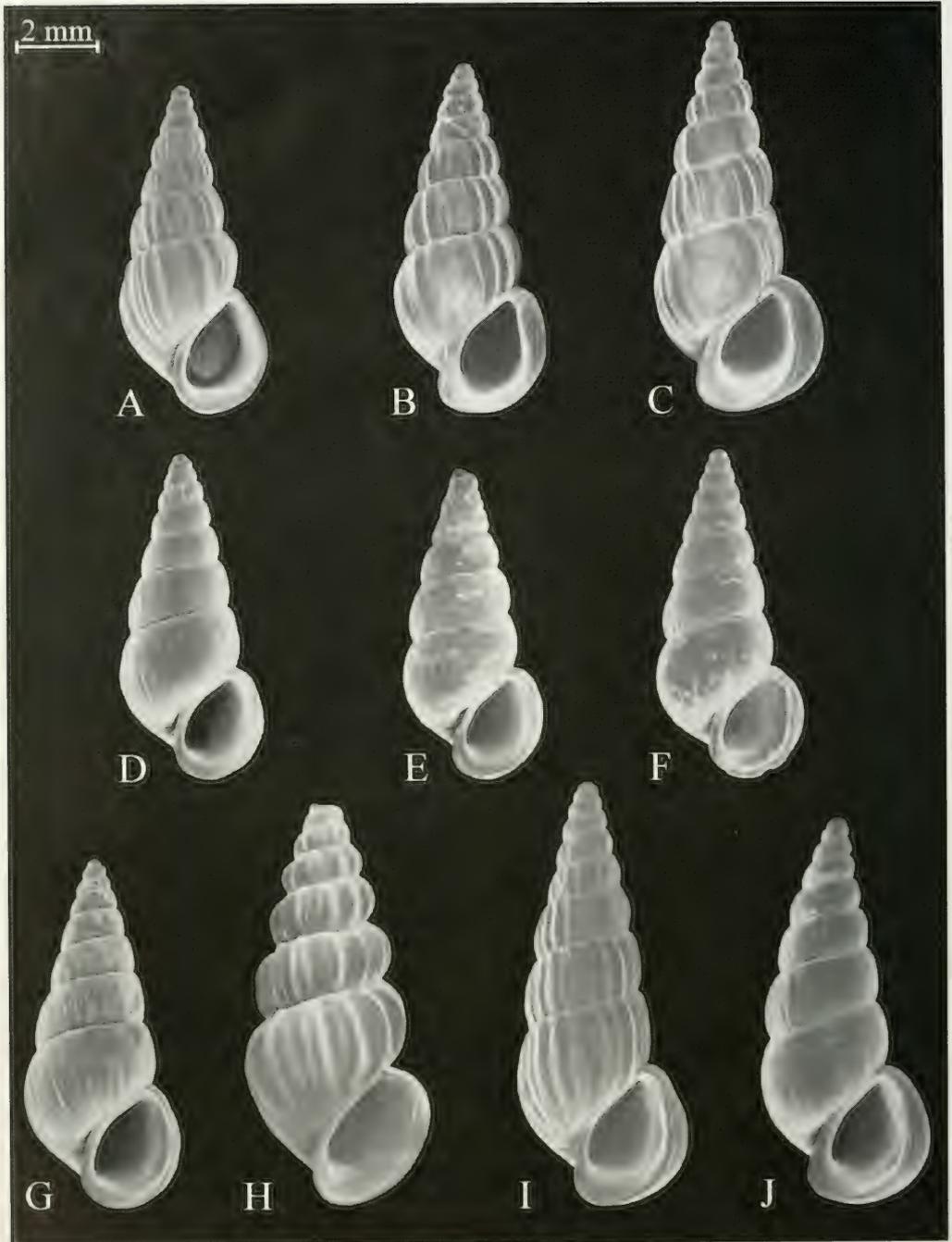


FIG. 3. Representative shells from the populations of this study. Figs. A–G are from populations A–G in Fig. 2. Fig. H is a shell from GuiChi; I is from Jian Li; J is from Jing Men.

ity two branches of the river bend around and are separated from each other by only the road's width of some 14 m. On the south side is a dam and spillway. Behind the spillway there is no discernable current. The water is choked with aquatic plants. Extensive marshy areas are at the margins. Snails are dense on the mud under the grass at the water's edge. HB14 is on the exact opposite side of the road from the north branch. Snails are smooth.

HB15 (E, Figs. 2, 3): 1 Oct.; Song Zi County; Miao River 1.5 km above bridge. Opposite HB14 from the north branch of the river. Wide marshy areas on each side of the slow flowing stream harbor dense snail populations. Snails come from the wet mud under dense grass cover. Snails are smooth.

HB16 (G, Figs. 2, 3): 1 Oct.; Song Zi County; Cheng Diam Township; Long Tan He Village; Miao River; 7 km above the bridge and 5 km from the top of the river; elevation about 47 m. The bridge elevation is 42 m. Snails numerous on mud under dense grass cover in the marshy margin of the river. Marshy border some 4.6 m wide. Stream banks some 15 m apart. Free flowing water about 4.6 m wide and choked with aquatic vegetation. Current very slow flow. Riverbanks 1.8 m high on S side; 1.2 m high on N side. Snails are smooth.

HB17 (B, Figs. 2, 3): collected by the schistosomiasis control station about 30 Oct. Song Zi County; XinJiang Kou Township; De Shun Village; Miao River main branch; Lower Miao River 3 km upstream from HB11. Snails with ribbed shells.

HB18 (C, Figs. 2, 3): 26 Sept.; Song Zi County; Lao Cheng Township; Yu Jia Du Village. Miao River mainstream. Collected by Schistosomiasis Control Unit. 2.0 km downstream from the bridge. 8.5 km upstream from HB17. Snails with ribbed shells.

HB22 (Figs. 1, 3J; Control; not Miao River): 6 Oct.; Jing Men City; 50 km SE of the city; Sha Yang Agricultural Management; Mia Liang State Farm; group 13. Snails came from a drainage canal bounded by cotton fields on one side and a road on the other with rice fields beyond that. The ditch was about 5.2 m wide from bank top to bank top; about 1.2 m to 1.5 m deep with sides about 40°. Snails were on the mud at the water level of the ditch up about 0.3 m under grass cover. Density was high. Shells smooth.

GC (Control) (Fig. 3H): Anhui Province; Gui Chi City; 117° 20.6' E; 30° 30' N. collected in 1994. Control population. The snails came from the flood planes of the Yangtze River.

Electrophoresis

Horizontal starch gel electrophoresis of tissue proteins from ten populations was followed by staining for the following 35 loci: AAT-1, AAT-2 (aspartate aminotransferase, 2.6.1.1); AK-1 and 2 (adenosine kinase, 2.7.1.20); AO (aldehyde oxidase, 1.2.3.1); ACPH-1 and 2 (acid phosphatase, 3.1.3.2); APH (alkaline phosphatase, 3.1.3.1); CK-1 and 2 (creatine kinase, 2.7.3.2); EST-1, EST-2, EST-3 (esterase, 3.1.1.1); GDH (glutamate dehydrogenase, 1.4.1.2); G6PD (glucose-6-phosphate dehydrogenase, 1.1.1.49); GPI (glucose-6-phosphate isomerase, 5.3.1.9); HBD (hydroxybutyrate dehydrogenase, 1.1.1.30); ISDH-1, ISDH-2 (isocitrate dehydrogenase, 1.1.1.42); LDH (L-lactate dehydrogenase, 1.1.1.27); MDH (malic dehydrogenase, 1.1.1.37); ME-1, ME-2 (malic enzyme, 1.1.1.40); MPI (mannose-6-phosphate isomerase, 5.3.1.9); NADD-1 and 2 (NADH dehydrogenase, 1.6.99.3); 6PGD-1 and 2 (phosphogluconate dehydrogenase, 1.1.1.44); PGM-1, PGM-2 (phosphoglucomutase, 5.4.2.2); OCT (octopine dehydrogenase, 1.5.1.11); SDH-1; SDH-2 (sorbitol dehydrogenase, 1.1.5.1.1); SOD (SOD (superoxidismutase, 1.15.1.1); XDH (xanthine dehydrogenase, 1.1.1.204). Procedures are those of Ayala et al. (1973) as modified by Dillon & Davis (1980), Davis (1983), Davis & Fuller (1981), Davis et al. (1981, 1988), and most recently for *Oncomelania*, by Davis et al. (1994, 1995).

Genetic parameters were calculated using BIOSYS-1 (Swofford & Selander, 1981). Hardy-Weinberg equilibrium was analyzed for all polymorphic loci. Nei's (1978) genetic distance and Wright's (1978) modified Rogers distance were calculated and phenograms constructed using the UPGMA method. An unrooted tree based on Wright's D was also constructed using the FITCH program of PHYLIP version 3.4 (Felsenstein, 1989). This phylogenetic analysis program does not assume equal rates of evolution. Some 50 repetitions of FITCH were run with randomized input order and optimization by global branch rearrangement. We used both Nei's D and Wright's modified Rogers D as explained in Davis (1994). The former is traditional and widely used, and as such sets a standard for comparisons. However, it is not metric, and closely related populations are rather compacted towards the 0.01 end of the range. With Wright's D, a metric, the closely com-

TABLE 1. Shell length and smooth/ribbed characteristics. N = 5 unless stated otherwise.

Population	No. Whorls	Shell Length	Length Body Whorl	No. Ribs
CONTROLS				
GUICHI	6-7 eroded	9.6 ± 0.3	5.3 ± 0.2	14 ± 1
JING MEN	8.5-9.0	8.4 ± 0.6	4.0 ± 0.2	SMOOTH
JIAN LI	9.0-9.5	9.1 ± 0.3	4.3 ± 0.1	18 ± 2
MIAO RIVER				
BELOW BRIDGE				
A	8.5-9.0	8.2 ± 0.7	4.2 ± 0.3	19 ± 2
B	8.5-9.0	8.1 ± 0.4	4.1 ± 0.2	18 ± 2
C	9.0-10.0	9.4 ± 0.2	4.6 ± 0.3	21 ± 4
ABOVE BRIDGE				
D	8.5	7.0 ± 0.5	3.7 ± 0.2	SMOOTH
E	8.5	6.8 (N = 2)	3.6	SMOOTH
F	8.5-9.0	7.0 ± 0.5	3.6 ± 0.1	SMOOTH
G	8.5	7.4 ± 0.5	3.8 ± 0.3	SMOOTH

pacted Nei's values are more spread out and better reflect the actual differences among populations in terms of a finite limit.

RESULTS

Table 1 provides basic shell data. Shells above the bridge (Fig. 3, D-G) are of the same whorl number (possibly excepting population C) as populations below the bridge (Fig. 3, A-C) but are significantly smaller. The Gui Chi controls (Fig. 3, H) are significantly larger than the other ribbed populations (based on length of body whorl, because shell length cannot be calculated due to the eroded condition of the apices of all shells). The Gui Chi population also has significantly fewer ribs per whorl than do the other ribbed populations.

Table 2 lists the genotype frequencies for the ten populations listing the 16 of 35 loci (46%) that are either polymorphic or have alternative alleles; these involve 71 alleles. The Miao River populations are arranged in the table from downstream (left side) to upstream (right side). Indices of genetic variability are given in Table 3. Mean sample sizes per locus ranged from 44.4 to 122.3. The percentage of polymorphic loci ranged from 11.4 to 22.9, the value not correlated with position along the river. The highest values were from terminal populations at the head and mouth of the river. Heterozygosity was low, ranging from 0.014 to 0.059 (direct count). The mean number of alleles per locus was low with the average for all populations = 1.36 ± 0.09 .

Populations are scored for the number of polymorphic loci (out of 12 such loci) and number of loci considered to differ significantly from Hardy-Weinberg equilibrium (Hwe) (Table 4). All but population A had some loci significantly different from HWe (range of 0-4), with the highest number occurring in the Anhui, Gui Chi population, the middle population below the bridge (B), and the uppermost population above the bridge (G). The actual loci involved are scored in Table 5, where it is seen that the EST1, AAT1, ME1, and PGM1 were the loci most often out of HWe (from 4 to 7 populations). The ME1 locus was only out of HWe in populations above the bridge. Probabilities and fixation indices (F) and direction and index of deviation (D) are given for all relevant loci for all populations in Table 6. Heterozygote deficiency was the predominant phenomenon. We examined the populations to see if there were discernible patterns of gene flow. Table 7 lists cases of alternative alleles (in monomorphic loci where populations have different alleles), unique alleles to *Oncomelania*, and alleles unique to the Miao River. Pie diagrams showing the frequencies of alleles (alternative alleles or polymorphisms) are given in Figure 4, with columns arranged in order from downstream (right end) to upstream (to the left). There are alternative alleles or unique alleles in all but one Miao River population. Two alternative alleles are found in population A at the mouth of the river, while one alternative allele is found in population D just above the bridge. Of particular interest are the unique alleles found in populations E and F, found only some 20 m

TABLE 2. Allele frequencies for the 16 loci (46%) that are polymorphic or with alternative alleles among the 10 populations. Populations GC, HB6 and HB22 are not in the Miao River drainage. There are 71 alleles total. For A-G, see Fig 2.

Locus	GC	HB6	HB22	HB11 A	HB17 B	HB18 C	HB13 D	HB14 F	HB15 E	HB16 G
AAT1										
(N)	100	80	50	50	50	50	50	70	50	160
A	0.985	0.938	1.000	1.000	0.980	1.000	1.000	1.000	1.000	0.972
B	0.015	0.044	0	0	0	0.020	0	0	0	0.022
D	0	0.019	0	0	0	0	0	0	0	0.006
AAT2										
(N)	100	80	50	50	50	50	50	70	50	160
A	1.000	0.906	1.000	1.000	0.970	1.000	1.000	1.000	1.000	1.000
B	0	0.013	0	0	0.020	0	0	0	0	0
C	0	0.081	0	0	0.010	0	0	0	0	0
ACPH2										
(N)	60	65	50	50	50	70	50	60	50	110
A	1.000	1.000	1.000	1.000	1.000	1.000		1.000	1.000	1.000
D							1.000			
AK1										
(N)	60	65	50	50	50	70	50	60	50	110
A	1.000	0.923	1.000	0	1.000	1.000	1.000	1.000	1.000	1.000
B	0	0	0	1.000	0	0	0	0	0	0
E	0	0.077	0	0	0	0	0	0	0	0
APH										
(N)	100	80	50	50	50	60	50	70	50	160
A	1.000	1.000	1.000	1.000	0.980	1.000	1.000	1.000	1.000	1.000
B	0	0	0	0	0.020	0	0	0	0	0
CK1										
(N)										
A	1.000	1.000	1.000	0	1.000	1.000	1.000	1.000	1.000	1.000
D	0	0	0	1.000	0	0	0	0	0	0
EST1										
(N)	100	80	50	50	44	50	50	70	50	159
A	0.175	0.788	0.930	0.450	0.602	0.610	0.780	0.679	0.940	0.755
B	0.115	0.087	0.070	0.310	0.205	0.190	0.070	0.050	0.030	0.097
C	0.020	0	0	0.240	0.080	0	0	0.007	0.020	0.038
D	0.150	0.125	0	0	0.114	0.200	0.150	0.257	0	0.110
E	0	0	0	0	0	0	0	0.007	0	0
F	0	0	0	0	0	0	0	0	0.010	0
EST2										
(N)	100	80	50	50	50	50	50	50	100	153
A	0.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990
C	0.005	0	0	0	0	0	0	0	0	0.010
GPI										
(N)	80	30	50	50	50	50	50	70	50	160
A	0.656	0.883	1.000	0.760	0.740	0.770	0.930	0.871	0.980	0.962
B	0.262	0.113	0	0.060	0.220	0.190	0.060	0.036	0	0.031
C	0.044	0	0	0.100	0	0	0.010	0.093	0.020	0
E	0.038	0.033	0	0.040	0.040	0.040	0	0	0	0.006
H	0	0	0	0.040	0	0	0	0	0	0
HBD1										
(N)	50	6	50	50	50	60	50	10	50	90
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.444
C	0	0	0	0	0	0	0	0	0	0.556
ME1										
(N)	80	30	50	50	50	50	50	70	50	160
A	1.000	1.000	1.000	1.000	1.000	1.000	0.700	0.957	0.570	0.956
B	0	0	0	0	0	0	0.020	0	0	0.006
C	0	0	0	0	0	0	0.140	0.029	0.290	0.019
D	0	0	0	0	0	0	0.100	0.014	0.140	0.019
E	0	0	0	0	0	0	0.040	0	0	0

(continued)

TABLE 2. (Continued)

Locus	GC	HB6	HB22	HB11 A	HB17 B	HB18 C	HB13 D	HB14 F	HB15 E	HB16 G
OCT										
(N)	86	80	50	47	50	50	50	70	50	152
A	0.547	0.444	0.620	0.649	0.680	0.530	0.940	0.557	0.550	0.651
B	0.064	0.019	0	0.021	0.010	0	0	0	0	0.010
C	0.314	0.525	0.230	0.277	0.260	0.360	0.030	0.279	0.120	0.204
D	0.076	0	0.150	0.053	0.050	0.110	0.030	0.136	0.300	0.135
E	0	0.013	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0.029	0.030	0
PGM1										
(N)	70	20	50	50	50	50	50	70	50	143
A	0.679	1.000	0.530	0.610	0.810	0.530	0.930	0.740	0.790	0.745
B	0.007	0	0.030	0.010	0	0.030	0	0	0	0.003
E	0.071	0	0	0.080	0.130	0.020	0.060	0.214	0.180	0.105
F	0.229	0	0.440	0.300	0.060	0.420	0.010	0.043	0.030	0.147
G	0.014	0	0	0	0	0	0	0	0	0
PGM2										
(N)	80	30	50	50	50	50	50	70	50	160
A	0.975	0.983	0.980	1.000	0.940	1.000	1.000	0.993	1.000	1.000
B	0.025	0.017	0	0	0.060	0	0	0.007	0	0
D	0	0	0.020	0	0	0	0	0	0	0
XDH										
(N)	60	65	50	50	50	60	50	60	50	110
A	1.000	0	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
D	0	1.000	0	0	0	0	0	0	0	0

TABLE 3. Population genetic indices.

Populations	Mean sample No. per locus	Mean no. alleles per locus	% loci polymorphic	Mean heterozygosity	
				Direct count	Hdywbg expected
CONTROLS					
GC1-Gui Chi	66.7 (3.3)	1.5 (0.2)	20	0.039 (0.020)	0.061 (0.028)
HB-6-Jian Li	54.7 (3.9)	1.4 (0.1)	20	0.030 (0.017)	0.047 (0.020)
HB-22 Jing Men City	50.7 (0.7)	1.2 (0.1)	11.4	0.034 (0.021)	0.036 (0.022)
MIAO RIVER					
HB-11 = A	47.1 (1.9)	1.3 (0.02)	11.4	0.059 (0.029)	0.060 (0.029)
HB-17 = B	49.8 (0.20)	1.4 (0.10)	20	0.034 (0.016)	0.057 (0.025)
HB-18 = C	56.6 (1.30)	1.3 (0.10)	14.3	0.046 (0.022)	0.060 (0.028)
BRIDGE					
HB-13 = D	48.6 (1.7)	1.3 (0.20)	14.3	0.014 (0.008)	0.035 (0.018)
HB-14 = F	55.8 (3.1)	1.4 (0.2)	17.1	0.038 (0.019)	0.052 (0.024)
HB-15 = E	44.4 (2.7)	1.3 (0.1)	14.3	0.023 (0.015)	0.048 (0.025)
HB-16 = G	122.3 (4.6)	1.5 (0.2)	22.9	0.027 (0.014)	0.059 (0.025)

from each other but in different branches of the river (Fig. 2). With perhaps the exception of allele B in EST-1, there is no discernable pattern of gene flow or allele frequency gradation among populations. Allele B in the case cited decreases from the mouth of the river to 0 in HB15; however, it is present in low frequency in HB-4 and 16.

Nei's and Wright's modified Rogers' genetic distances are given in Table 8; the relevant

phenograms are given in Figures 5 and 6. The respective cophenetic values are 0.995 and 0.978. The overall genetic distances involved (clustering at <0.08, Nei's D) clearly shows conspecificity (for species concepts involving *Oncomelania*: Davis 1994; Davis et al., 1995). There is no separate cluster of ribbed snails or separate cluster for smooth snails. The ribbed control from Anhui Province (GC) clusters most closely with ribbed populations B, C

TABLE 4. Populations scored for number of polymorphic loci and number of loci significantly differing from Hardy-Weinberg equilibrium where significance using exact probabilities is set at 0.06. There are 12 polymorphic loci total.

Population	No. Polymorphic loci	No. Significantly different	% Significantly different
Controls			
Gui Chi	7	3	43
HB-6	7	4	57
HB-22	4	1	25
Miao River			
Below Bridge			
A = HB-11	4	0	0
B = HB-17	7	4	57
C = HB-18	5	2	40
Above Bridge			
D = HB-13	5	2	40
E = HB-15	5	2	40
F = HB-14	6	3	50
G = HB-16	7	4	57

TABLE 5. Chi-square tests for deviation from Hardy-Weinberg Equilibrium: N = not polymorphic, S significance is derived from using exact probabilities; S = $P < .06$. [] = number of loci differing significantly from H-W. P values are given.

Locus:	AAT1	AAT2	AKI	APH	EST1	EST2	GPI	HBD1	ME1	OCT	PGM1	PGM2
Population												
GuiChi [4]	S	N	N	N	S	1.000	0.218	N	N	0.831	S	S
HB-6 [4]	S	S	S	N	S	N	1.000	N	N	1.000	N	1.000
HB-22 [1]	N	N	N	N	1.000	N	N	N	N	0.559	0.579	S
MIAO RIVER BELOW BRIDGE												
A = HB-11 [0]	N	N	N	N	0.774	N	0.249	N	N	0.754	0.338	N
B = HB-17 [4]	N	S	N	S	S	N	0.269	N	N	0.210	S	1.000
C = HB-18 [2]	S	N	N	N	0.388	N	0.254	N	N	0.267	S	N
MIAO RIVER ABOVE BRIDGE												
D = HB-13 [3]	N	N	N	N	S	N	1.000	N	S	1.000	S	N
E = HB-15 [2]	N	N	N	N	1.000	N	1.000	N	S	0.097	S	N
F = HB-14 [3]	N	N	N	N	S	N	0.588	N	S	0.628	S	1.000
G = HB-16 [4]	S	N	N	N	S	1.000	1.000	N	S	0.110	S	N

and smooth population F. Population A at the mouth of the river stands slightly apart from all others. Table 9 provides inter-population comparisons of values of Nei's D. There is no significant difference between the average D for all Miao River populations (0.038 ± 0.035) and the separate average D for populations above the bridge (0.024 ± 0.016) or below the bridge (0.045 ± 0.036).

The unrooted FITCH tree is given in Figure 7, freed from the constraints of phenogram construction and based on a metric distance measure. There are no two distinct clusters of populations representing smooth and ribbed shells. Populations A, Jian Li, and D indicate their unique genetic structure and divergence from all the other populations. Note that A differs from D by a Nei's D of 0.106 (Table 8), the

greatest divergence among Miao River populations.

DISCUSSION

Do Smooth and Ribbed Snails Deserve Separate Taxonomic Rank?

The question appears resolved. Based on this natural experiment, populations of *Oncomelania* within the Yangtze River drainage below the Three Gorges of the Yangtze River that are smooth (but with varix), and with the same allometry as snails of ribbed populations in the lower Yangtze drainage, are one subspecies, *O. hupensis hupensis*. Wang et al. (1998) support this concept on the basis of a

TABLE 6. Deviation from Hardy-Weinberg and heterozygote deficiency for all populations. P is based on exact probabilities and significance is set at 0.06. Population A is at the mouth of the Miao River; G is farthest upstream.

Population	locus	P	Fixation index (F)	D	
Controls					
Gui Chi	AAT1	0.02	0.662	-0.663	
	EST1	0.00	0.713	-0.714	
	GPI	0.22	0.220	-0.225	
	OCT	0.83	0.137	-0.142	
	PGM1	0.05	0.318	-0.323	
Jian Li	PGM2	0.00	1.000	-1.000	
	AAT1	0.00	0.790	-0.791	
	AAT2	0.00	0.927	-0.928	
	AK	0.00	1.000	-1.000	
Jing Men	EST1	0.00	0.369	-0.373	
	PGM2	0.01	1.000	-1.000	
MIAO RIVER					
Below Bridge					
A (HB-11)	GPI	0.25	-0.134	0.123	
	PGM1	0.39	0.172	-0.180	
B (HB-17)	AAT2	0.03	0.659	-0.662	
	APH	0.01	1.000	-1.000	
	EST1	0.00	0.645	-0.649	
	GPI	0.27	0.205	-0.213	
	OCT	0.21	0.144	-0.153	
C (HB-18)	PGM1	0.00	0.567	-0.571	
	AAT1	0.01	1.000	-1.000	
	EST1	0.39	0.166	-0.175	
	GPI	0.25	0.188	-0.196	
	OCT	0.27	0.169	-0.177	
Above bridge	PGM1	0.01	0.335	-0.342	
	D (HB-13)	EST1	0.00	0.451	-0.456
		ME1	0.00	1.000	-1.000
		PGM1	0.00	0.848	-0.849
	E (HB-15)	ME1	0.00	0.965	-0.965
OCT		0.10	0.122	-0.131	
PGM1		0.00	0.708	-0.711	
F (HB-14)	EST1	0.00	0.393	-0.660	
	GPI	0.59	-0.115	0.107	
	ME1	0.00	1.000	-1.000	
	OCT	0.63	0.108	-0.115	
	PGM1	0.010	0.358	-0.362	
G (HB-16)	AAT1	0.00	0.659	-0.660	
	EST1	0.00	0.444	-0.446	
	HBD1	0.00	1.000	-1.000	
	ME1	0.00	1.000	-1.000	
	OCT	0.11	0.235	-0.237	
	PGM1	0.00	0.458	-0.460	

few enzymes (esterases and MDH, slab PAG), and a few specimens of ribbed and smooth-shelled *Oncomelania* from three counties (five populations) in Hubei Province. They found very little difference among populations (data not sufficient or scoreable for population genetic analysis).

Ribbing is associated with annual flooding of the Yangtze River and its tributaries. As is

well known to Chinese field workers attempting to monitor and control *Oncomelania* snails (Liu et al., 1981), snails from any elevation, or a man-made situation that removes a population from the annual floods, attain a smooth shell but still retain the varix. Molecular genetic data do not support the concept of different taxonomic status for these two shell types. Accordingly, *Katayama fausti* Bartsch,

TABLE 7. Distribution of unique and alternative alleles among the 10 populations.

Population	Alternative alleles	Unique alleles	Alleles unique to the Miao River
CONTROLS			
Gui Chi	none	none	
Jian Li	XDH/d	AAT1/d (0.019) AK-1/e (0.077)	
Jing Men	none	none	
MIAO RIVER			
Below Bridge			
A	AK1/b CK1/d	none	GPI/h (0.040)
B	none	none	AAT2/b (0.020) AAT2/c (0.010) APH/b (0.020)
C	none	none	none
Above Bridge			
D	ACPH2/d	none	ME1/e (0.040)
E	none	none	EST1/f (0.010)
F	none	none	EST1/e (0.007) PGM2/b (0.007)
G	none	HBD/c (0.556)	AAT1/d (0.006) EST2/c (0.010)

1925, is a synonym of *O. hupensis hupensis* Gredler, 1881. (*Katayama* used to be used as a genus to include all smooth forms of *Oncomelania hupensis*.)

Davis & Ruff (1973) employed breeding experiments and showed that ribbing in *Oncomelania hupensis* from mainland China is controlled by a single gene with multiple alleles. Davis (1979) considered the smooth shelled condition to be primitive and that ribbing is the derived condition. We argue that the earliest ecology and shell morphology of *Oncomelania hupensis* is that seen in Yunnan and Sichuan, areas into which pomatiopsine snails were introduced through a trajectory from the Indian Plate into northern Burma and Yunnan, China, in the Miocene (Davis, 1979). The habitat involves marshy ecotones in hilly regions not subjected to annual flooding. It is noteworthy that these Yunnan and Sichuan snails, *O. hupensis robertsoni*, are smooth and have no varix. The natural course of evolution would have been through dispersal down the evolving Yangtze River. With the geological formation of the Three Gorges section of the Yangtze creating an effective barrier to passive movement of snails up or down the Yangtze River, snails living in the flood planes and on the islands of the Yangtze River, through mutation, developed ribs and a varix. The hypothesis is that ribbing contributes greatly to survival by significantly increasing shell surface area, facilitating floatation and

dispersal during flooding. Additionally, ribbing may also increase shell strength as well as surface area. Greater strength would abet survival during flotation and vagaries of being swept into solid objects. The loss of ribbing but not the varix indicates that there is a different gene(s) governing varix formation.

Floatation during Yangtze River flooding is a major source of dispersion for *Oncomelania hupensis hupensis* and the schistosomes they transmit. This phenomenon is apparently not known outside China. During the floods, snails are lifted off the islands in the Yangtze and floodplains and floated by the millions down the river to be deposited on downstream floodplains or swept into canals when the flood gates are opened. The impact on importation into the canals of Hubei Province has been documented by Xu & Fang (1988) and Xu et al. (1989, 1993), Yang et al. (1992). While snails on the islands either float off or drown, snails on the floodplains often escape flooding by climbing tree trunks, often to heights of more than three meters. With reference to drowning, *Oncomelania hupensis* is an amphibious species. The young stay submerged during their early stages of development, often floating upside down, feeding on the surface of quiet water. As adults, the snails are found out of but near water on the banks of irrigation ditches and swamps, on shaded, moist soil. During drought, the adults move down into the soil and aestivate. Adults

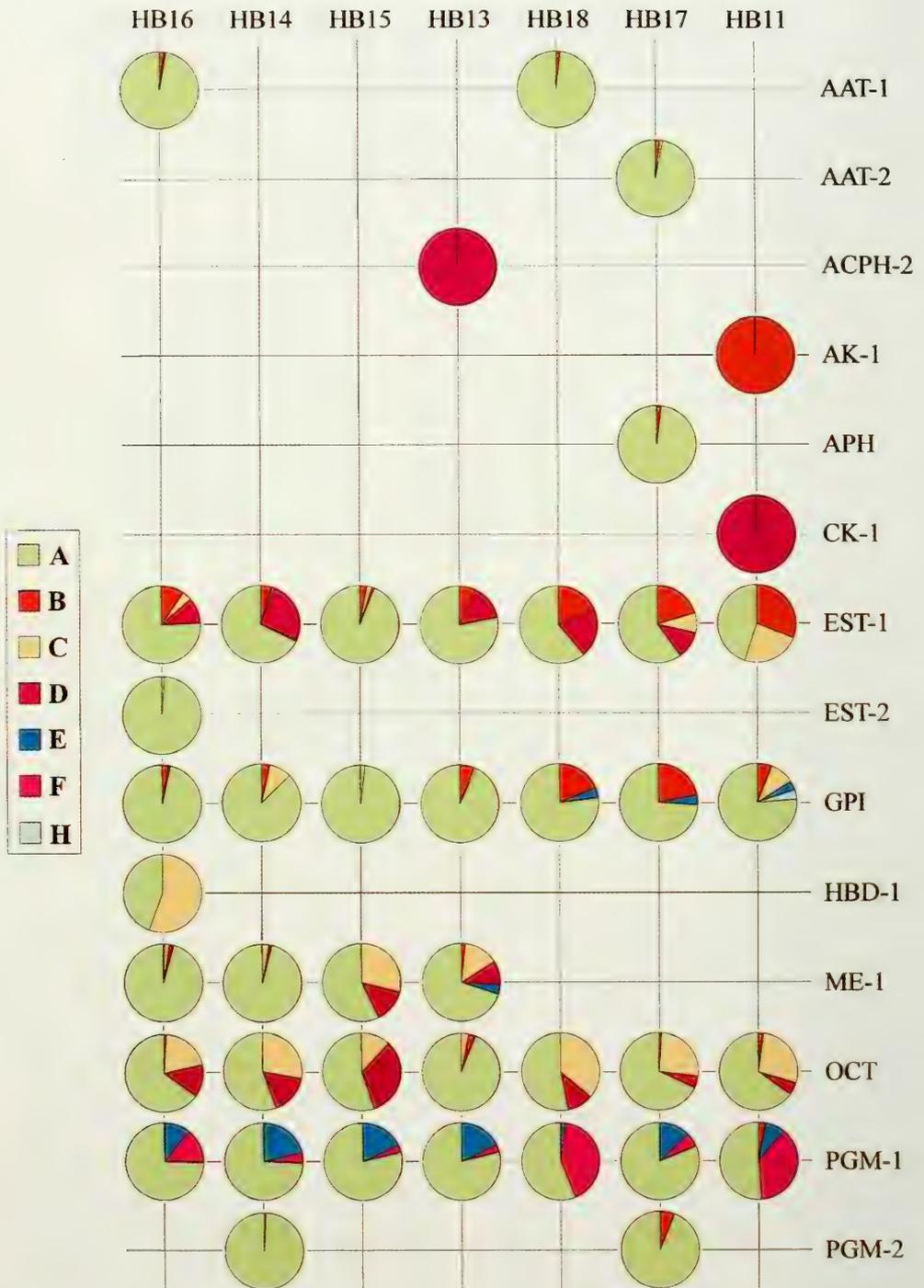


FIG. 4. Pie diagrams showing allele frequencies and alternative alleles for each population. Populations are in rows with HB 11 (=A) at the mouth of the river and HB 16 (=G) at the head of the river. The colors are coded A, B, etc. for alleles A, B, etc. listed in Table 2. For example, solid orange in Fig. 4, HB11 indicates an alternative allele B. The dominant allele is A.

TABLE 8. Matrices of Nei's 1978 genetic distance (above the diagonal) and Wright's modified 1978 Rogers genetic distance (below the diagonal).

Population	GC	JIAN LI	A	D	F	E	G	B	C	JING MIN
GC		0.035	0.066	0.039	0.003	0.010	0.012	0.001	0.001	0.005
JIAN LI	0.182		0.103	0.072	0.035	0.042	0.045	0.035	0.038	0.040
A	0.247	0.305		0.106	0.068	0.077	0.077	0.066	0.066	0.069
D	0.193	0.258	0.310		0.036	0.035	0.044	0.036	0.043	0.040
F	0.052	0.181	0.250	0.185		0.006	0.010	0.002	0.004	0.006
E	0.100	0.198	0.265	0.184	0.080		0.014	0.010	0.013	0.009
G	0.108	0.205	0.265	0.203	0.100	0.118		0.011	0.013	0.009
B	0.041	0.181	0.246	0.185	0.048	0.098	0.105		0.004	0.007
C	0.040	0.190	0.245	0.201	0.067	0.113	0.113	0.064		0.004
JING MIN	0.071	0.195	0.253	0.196	0.077	0.093	0.108	0.087	0.063	

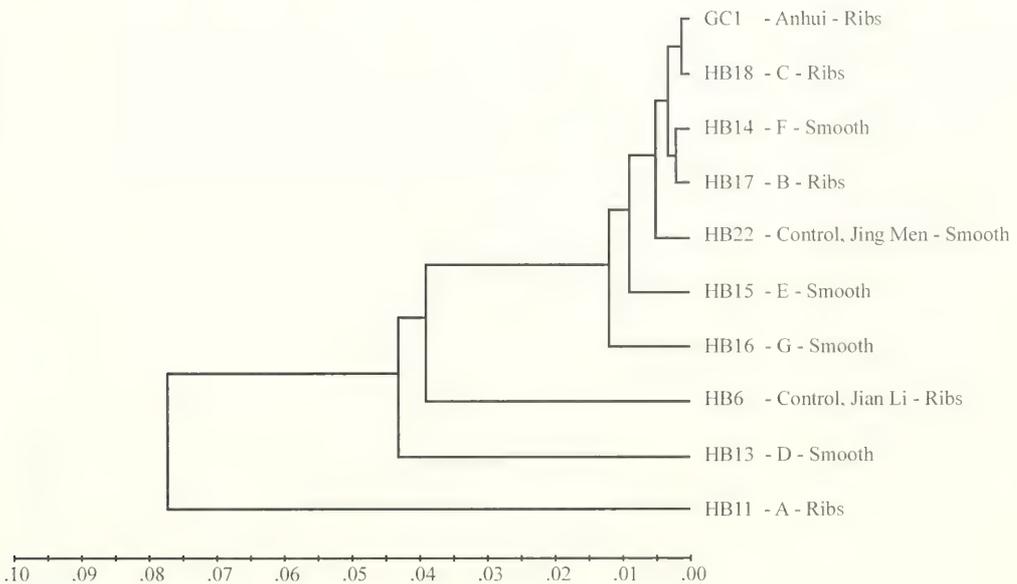


FIG. 5. Phenogram based on Nei's genetic distance.

cannot withstand continual submersion; they will drown. Because of this, drowning is a method used to control schistosomiasis in some areas of China, including the Miao River. During the dry season, a control dam is closed at times, flooding a large area of grazing land with numerous marsh-edged pools harboring large populations of *Oncomelania*. Our site B is in this section of river. The flooding does reduce the sizes of populations inundated (personnel communication; Dr. Yang, Xian-Xiang, Director, Hubei Institute of Schistosomiasis Control).

After revisiting and collecting snails on Lao Zhou Island in the Yangtze River in Tong Ling County, Anhui Province, we now understand

why three populations, including the Lao Zhou, snails did not group with the clusters we classified as *O. h. hupensis*, *O. h. tangi*, and *O. h. robertsoni* (Davis et al., 1995) in the Fitch tree based on allozyme data from 14 *Oncomelania* populations. The other populations were Gui Chi (one of our control populations) from Anhui, and Jian Li from Hubei Province. These three localities are heavily flooded and swept during the annual floods. Snails found in these locations are not populations, really, but aggregates of snails imported from diverse areas and deposited with the receding floodwaters. In Davis et al. (1995), these three populations were considered as possible "hybrid" populations, because of alleles shared with

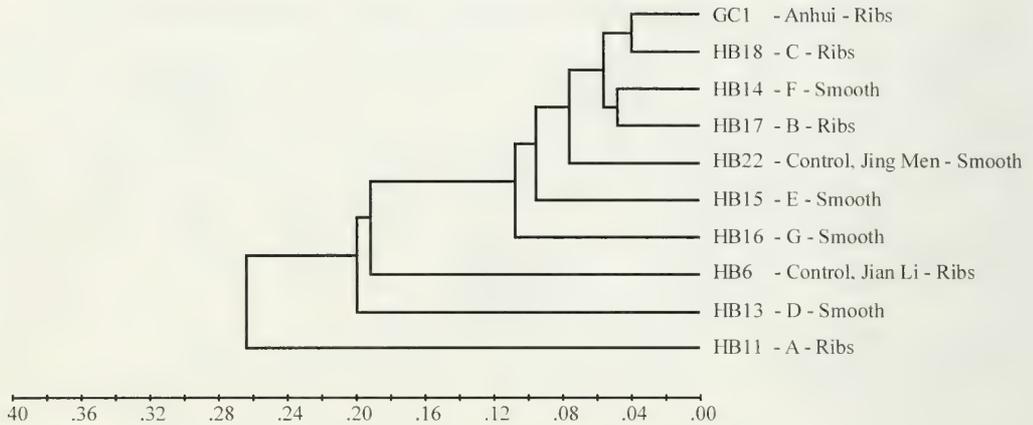


FIG. 6. Phenogram based on Wright's modified Rogers genetic distance.

TABLE 9. Comparison of Nei's D among populations.

Populations	Mean and S.D.	No.
Above bridge × Below Bridge	0.038 (±0.035)	12
Below Bridge	0.045 (±0.036)	3
Above Bridge	0.024 (±0.016)	6
Controls × Below Bridge	0.041 (±0.039)	6
Controls × Above Bridge	0.032 (±0.023)	8

other populations and subspecies, especially with the upstream *robertsoni* subspecies. We here call such populations "genetically unstable" populations where true population structure is not reached and HWe is not attained in most polymorphic loci.

Genetically stable populations, theoretically, would be defined as those with normal panmixia, little or no immigration, and all loci in HWe, that is, populations that are large and have had stable population structure for many years. There are three populations that could arguably fit these criteria; HB-11, HB-22 with one locus not in HWe due to very low frequency of one allele, HB-18, and HB-15 with 2 of 5 polymorphic loci not in HWe. The population at the head of the river, HB-16 should be genetically stable but equals GuiChi in having 57% polymorphic loci not in Hwe.

Theoretically, population HB-11 at the mouth of the Miao River should be "unstable" due to annual flooding, but on the basis of H-W, this is not the case. The mouth of the Miao River is not on the Yangtze, but on a loop that branched off the main channel of the Yangtze.

While the lower Miao River is flooded annually, it may not receive many introductions of snails from upstream localities. However, as clearly seen in Figures 5-7, population A is clearly divergent from all the others.

The Question of Heterozygote Deficiency

Four reasons could account for heterozygote deficiency such as seen here: (1) organisms came from two or more populations with different allele frequencies (Wahlund effect), (2) natural selection acts against heterozygotes at some loci, (3) there is inbreeding, or (4) our scoring is in error and is biased against heterozygotes (Ayala et al., 1973). Error in scoring may result in one, or perhaps two loci being considered not in HWe, especially where EST is involved and gels can be difficult to score. However, we consider that 1 and 3 above are probably mostly responsible, although we cannot discount selection against heterozygotes.

How often is heterozygote deficiency found in molluscs overall, and such rissocean snails as *Oncomelania*, in particular? It is important to place *Oncomelania*, a rissocean snail, in context with other molluscs with regard to what one might expect in terms of heterozygosity and heterozygote deficiency. Heterozygote deficiency is common among molluscs, especially bivalves where heterozygosity mostly exceeds 0.250 (see references below). Numerous authors have discussed this phenomenon in the Bivalva (for example, Ayala et al., 1973; Mitton & Koehn, 1973; Lassen & Turano, 1978; Wilkins, 1978;

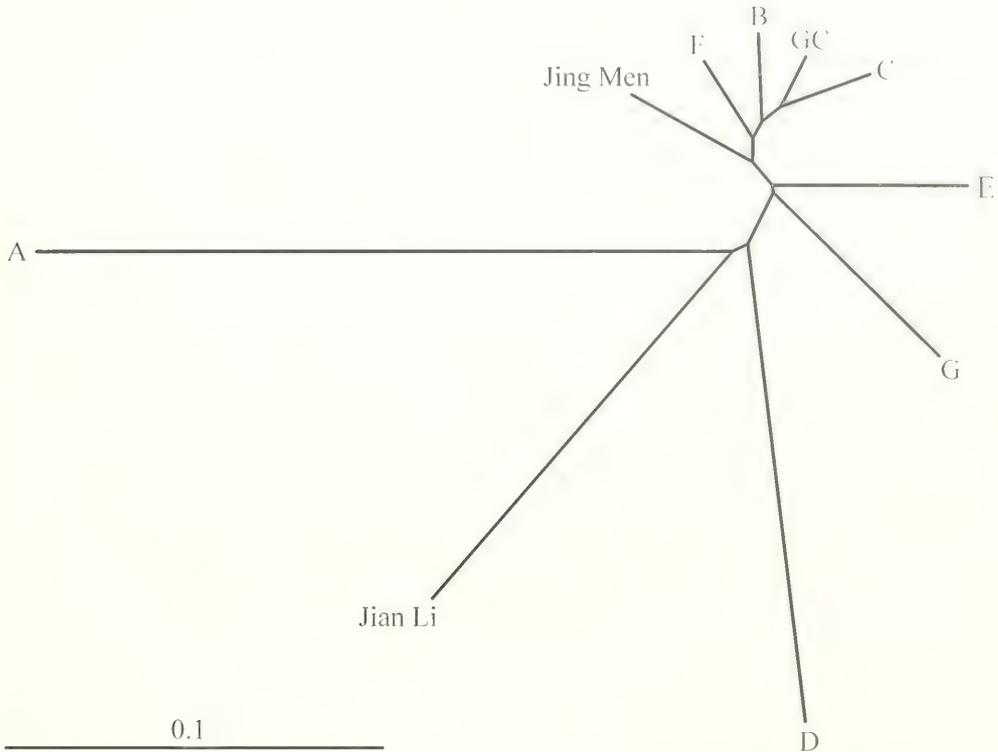


FIG. 7. An unrooted FITCH tree based on Wright's D. Line lengths are proportional to branch lengths.

Gosling & Wilkins, 1981; Mallet & Haley, 1983; Skibinski et al., 1983; Adamkewicz et al., 1984; Hedgecock & Okazaki, 1984; Singh & Green, 1984; Koehn & Gaffney, 1984; Zouros & Foltz, 1984; Diehl & Koehn, 1985; Mallet et al., 1985; Hoagland, 1986; Volckaert & Zouros, 1989; Gaffney et al., 1990; Beaumont, 1991; Nirchio et al., 1991; Bricelj & Krause, 1992; Gaffney et al., 1992; Liu et al., 1995; Gardner et al., 1996; Marsden et al., 1996; Michinina & Rebordinos, 1997). Significant deviation from HWE in bivalves has been attributed to Wahlund effect in limited cases, and natural selection on young just post settlement in many instances.

In bivalves generally, heterozygosity is much greater than that found in gastropods. Selander & Ochman (1983) summarized allozyme data relating to the genetic structure of about 100 species of gastropods. Heterozygosity of amphimictic species averaged about 0.12 and ranged from 0.004 to 0.198. Issues of selfing and parthenogenesis involve both aquatic and terrestrial pulmonates, especially certain slugs in which there is little or

no heterozygosity, or in which there is considerable heterozygote deficiency do not pertain to *Oncomelania*. Foltz et al. (1982 a, b, 1984) provided data on slugs. There are also strong indications of self fertilization in certain basommatophoran freshwater pulmonates of the genera *Biomphalaria* and *Bulinus*, taxa involved in the transmission of schistosomes infecting humans in Africa and South America (*Biomphalaria*) (Bandoni et al., 1990; Mimpfoundi & Greer, 1990a, b; Bandoni et al., 1995; Doumas et al., 1996; Mukaratirwa et al., 1996). In stylommatophoran pulmonate land snails, selection seems to operate on some loci. Emberton (1993) found over-representation of some rare alleles in the young of widespread populations of some polygyrids. Falniowski et al. (1998) found only one of many loci of *Bradybaena fructicum* not to be in HWE.

Among the "prosobranch" grade, non-hydrobioid gastropods, populations of some species of some genera have been found to be in HWE (all loci in HWE): *Nassarius* (Gooch et al., 1972; Sanjuan et al., 1997), *Patella*

(Wilkins, 1977), *Melanoides* (Livshits & Fishelson, 1983), and some populations of *Littorina* (Rolan-Alvarez et al., 1995). Some have been out of HWe, including some *Littorina* (Rolan-Alvarez et al., 1995) and *Siphonaria* (Johnson & Black, 1984), both attributed to the Wahland effect.

Papers involving hydrobioid snails and allozymes have been few, and most of these have been concerned primarily with genetic distances and taxon relationships. Heterozygosity in hydrobioid populations is lower than the average for snails overall; in *Oncomelania* it was 0.052 ± 0.030 ($N = 14$) (Davis et al., 1994, 1995). Heterozygosities reported for two Australian genera were 0.038 ± 0.016 ($N = 26$ populations) and 0.043 ± 0.018 ($N = 48$ populations) (Ponder et al., 1996; also see Ponder et al., 1994, 1995). Heterozygosity for *Hydrobia* was equally low, reported as $0.008 - 0.074$ (Davis et al., 1988, 1989). Haase (1993) studied one population each of three species of *Hydrobia sensu lato* and found that for one there were heterozygote deficiencies at virtually all polymorphic loci, whereas in the other two populations there was low or no variability. Haase attributed the heterozygote deficiencies to selection due to parasite pressure; the cases of low to no heterozygosity to genetic drift and bottlenecks.

To what do we attribute heterozygote deficiencies in this study? There are two probable explanations. (1) There appears to be a Wahland effect in "unstable" populations such as Gui Chi and Jian Li, localities swept by the Yangtze River annual flooding. There is a mixture of alleles from snails of diverse localities. Because there are no diagnostic loci that serve to separate the subspecies, the use of multidimensional scaling and a Prim network, or a FITCH unrooted tree aides one to identify unstable populations. Such trees are freed from the constraints imposed in a UPGMA phenogram. For example, Davis et al. (1995: fig. 9) present a FITCH tree in which there are three clusters of populations attributed to three subspecies, but three populations did not fit into the clusters; on further investigation, it was apparent that these three acted as hybrid populations but were actually genetically unstable populations.

(2) There have been extensive efforts along the Miao River to control snails using mollusciciding and by flooding to drown snails. However, the snails have not been eradicated, and we have been able to collect snails from these same localities over several years.

The life span of *Oncomelania hupensis* is three to four years (Davis, 1967; van der Schalie & Davis, 1968), and a female, fertilized just once, can store viable sperm over the course of her lifetime. Accordingly, as females produce quantities of eggs it is possible for one or two females to totally repopulate a locality. Accordingly, we attribute the heterozygote deficiencies in the upper Miao River, in localities where there should be "stable" populations, to extensive inbreeding. As *Oncomelania hupensis* is under continual assault in efforts to control schistosomiasis, and habitats for these snails are ever changing due to land-use changes in China, it may be difficult to locate genetically stable populations using allozymes.

We are now testing an alternative to allozymes, CO1 gene sequences. They serve very well to clearly demonstrate the divergence of the subspecies of *Oncomelania* (Davis et al., 1998) but are much more conservative within populations. We have come to expect variation of 0 to 2 base substitutions in a sequence length of 648 base pairs (0.00 to 0.31%) in isolated and genetically stable populations, but 4 to 8 (0.62 to 1.23%) or more base substitutions in unstable populations, indicating the import of differing genotypes with flooding. A study on this phenomenon is in progress.

The Subspecies Question in Mainland China

As discussed above, smooth shelled snails in the Yangtze River drainage previously classified as *Oncomelania fausti* belong to *Oncomelania hupensis hupensis*. The genetic relationships among the three subspecies found on the mainland are graphically portrayed in Figure 8 (data from Davis et al., 1995) (justification for using the polytypic species designation given in Davis, 1994, and Davis et al., 1995). All the populations of this study fall within the variance around the mean value for *O. hupensis hupensis*.

There is one more name that requires some discussion, *Oncomelania hupensis guangxiensis*, named recently by Liu et al. (1981) from the Xun Jiang (Xun River) drainage, which becomes the Xi Jiang flowing into Guangdong Province from Guangxi Province. The Xi Jiang does not flow to the Yangtze River, but to the South China Sea near Macao. Snails classified as *guangxiensis* are generally smooth but with sporadic, irregular appearance of low ribs (Davis et al., 1995: figs. 2,

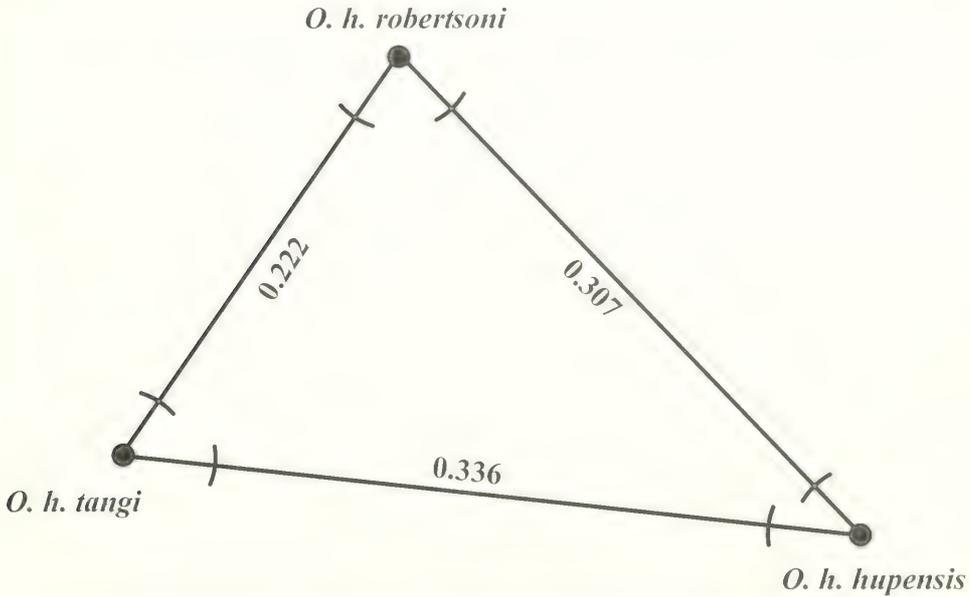


FIG. 8. Graphic relationships, in scale, among the three subspecies of *Oncomelania hupensis* on the mainland of China. The standard deviations are marked (curved lines).

4A). The shells have a strong varix. We place this nominal subspecies in the synonymy of *O. hupensis hupensis* because snails studied electrophoretically by Davis et al. (1995) are within the variance of *O. h. hupensis* (Davis et al., 1995: fig. 9) We hypothesize that these snails reached Guangxi Autonomous Region via a canal dug some centuries ago connecting the Xiang Jiang River in Hunan Province to Guangxi, thus connecting the snail-rich Tong Ting Lake district of Hunnan to the southern coast. On the basis of allozymes, the Gui Ping County Guangxi snails from near the confluence of the Qian Jiang and Yu Jiang that forms the Xun Jiang are most closely related to a population from Hunan (Nei's mD of 0.150 from Yue Yang, Hunan).

There are still some unresolved problems involving smooth shells with a strong varix. While it seems clear that *Oncomelania hupensis hupensis* living along the Yangtze and up into the lower stretches of rivers flowing into the Yangtze belong to the same subspecies, whether they have ribbed or smooth shells, there are some populations in the hills in eastern China of coastal provinces, Jiangsu and Zhejiang, isolated from the Yangtze River drainage, that are problematic.

Zhou et al. (1995) studied the allozymes of

34 populations from nine provinces They used 16 loci, of which 5 were esterase loci. In UPGMA clustering of Nei's (1978) "D", they also found that Sichuan and Yunnan snails clustered apart from *O. hupensis* (*sensu* Davis et al., 1995), as did the one population they had from Fujian (*O. hupensis tangi*). One population with a smooth shell and varix, from Anhui) clustered with the ribbed-shelled populations. Using Fitch-Margoliash least-squared cluster analysis, all smooth-shelled *Oncomelania* clustered together apart from ribbed-shelled populations, but the Anhui smooth-shelled population was basal and distinctly apart, rather intermediate between the smooth and ribbed-shelled populations. Zhou et al. (1995) concluded that there were two taxonomically distinct groups: ribbed and smooth-shelled types. Aside from the Sichuan, Yunnan and Fujian populations, they examined only three other smooth-shelled populations, the one from Anhui and two from Jiangsu provinces. They did not consider the shell morphological and biogeographical differences that separate *hupensis*, *robertsoni* and *tangi*. They did consider that smooth-shelled population groups might be separated into subspecies, that is, those from Fujian, Yunnan, Sichuan, and the hilly region of Jiangsu

Province at the extreme eastern edge of China.

Davis et al. (1994) had studied three populations of *Oncomelania* from Zhejiang Province, like Jiangsu, a coastal province. Two populations were ribbed and one smooth. One ribbed and the smooth population were very close to each other geographically, whereas the second ribbed population was a considerable distance from the others. On the basis of Nei's (1978) "D", the smooth-shelled population was highly divergent from the ribbed snails, a result similar to that later found by Zhou et al. (1995) for the two smooth-shelled from hilly areas of Jiangsu Province. All these smooth-shelled populations had a strong varix. Davis et al. (1995) found that one smooth-shelled population from Zhejiang Province clustered closely with the Yunnan and Sichuan *O. h. robertsoni*; it had the least genetic distance with the Sichuan populations considering all pair-wise comparisons among populations. Davis et al. (1995) classified this Zhejiang population as *robertsoni*.

It has yet to be determined how these eastern hill-dwelling populations relate to smooth-shelled Miao River snails studied here. These few special populations require intense study. Are they truly genetically divergent from *O. h. hupensis*? Are they part of the *robertsoni* complex but have independently evolved a varix? Are they a distinct subspecies?

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MALACOLOGIA

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INTRODUCTION TO THE SYMPOSIUM ON INTERACTIONS
BETWEEN MAN AND MOLLUSCS

GEORGE M. DAVIS

Department of Malacology, Academy of Natural Sciences of Philadelphia, 1900 Benjamin Franklin Parkway, Philadelphia, Pennsylvania 19103, USA; davis@acnatsci.org

The 1998 World Malacological Congress symposium *Interactions Between Molluscs and Humans* was one of three symposia offered during the five-day congress. As molluscs have an enormous impact on man, it is not surprising that many persons from around the world wished to participate in the symposium and that the symposium was well attended. Thirty-seven papers were presented, of which 14 were invited and the others submitted for presentation. The papers may be grouped into four broad categories: advanced biotechnology (4 papers); medical malacology and disease transmission (15 papers); economics, including diseases of molluscs, impact of introduced species, and molluscan food production (8 papers); conservation and mariculture (10 papers).

Twelve of the papers are published here, including the elegant opening plenary address given by Dr. Dan Alkon of the United States National Institutes of Health, who reviewed the use of an opisthobranch snail for working out the molecular basis of learning and memory, and presented the implications of the findings to date for understanding Alzheimer's disease. The papers presented by Alkon and Yoshino are in the biotechnology category, but Yoshino's work also groups with papers by Davis et al., Kristensen & Brown, Brackenbery & Appleton, and Abd Allah in the medical malacology category. The papers by Anderson, Carlton, Pointier and Robinson involved economics and introduced species, although Pointier's paper also had implications for control of schistosomiasis (medical malacology category). Finally, Neves and Mansur presented papers that involve conservation. The Kristensen & Brown paper has implications for conservation of freshwater snails in Africa.

I would make special comment on Robinson's paper involving commercial transport of molluscs all over the world in ever increasing numbers and involving an incredible spectrum of species. It was an eye-opener to learn that over 4,900 interceptions of molluscs aboard cargo inbound to the United States from throughout the world had been intercepted by the United States Department of Agriculture. These interceptions, made in a five-year period up to 1998, involved 71 families, 197 genera and 369 species. In reading both Robinson's and Carlton's papers, one becomes immediately concerned about the impact of introduced species into all environments on ecology, survival of endemic species, and implications for plant pathogens and human disease.

MOLECULAR PRINCIPLES OF ASSOCIATIVE MEMORY THAT ARE CONSERVED DURING THE EVOLUTION OF SPECIES

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ABSTRACT

Time domains of homeostatic adaptation encompass: 1.) the lifetime of the species (an evolutionary domain) in which predictive information with survival value is incorporated into the genome, and 2.) the lifetime of organisms in which predictive information is stored within brain networks. Experiments over many years in our NIH lab have confirmed that behavioral, biophysical, and molecular mechanisms for storing learned information have been conserved in species as diverse as the nudibranch mollusc *Hermisenda* and mammals such as rat and rabbit. Further studies now indicate that these conserved molecular memory steps (e.g. K^+ channel inactivation, PKC and calcitonin activation, and intracellular calcium release) are also consistent targets of the human syndrome known as Alzheimer's disease. These findings highlight the elegant economy of natural selection and the utility of animal models for investigating complex brain disorders such as Alzheimer's disease.

Key words: molecular mechanisms, *Hermisenda*, memory, Alzheimer's disease

Adaptation of earth's organisms may be considered to occur over at least two distinct time domains. The first, one that may be called the "evolutionary" time domain, often occurs over many thousands, even millions of years. During this evolutionary time domain, a species survives by incorporating genetic programs that encode for adaptive structures and functions. The species acquires adaptive information that is then stored within the genome.

A second time domain for organisms' adaptation occurs during the lifetime of individual members of the species. Environmental stimuli in real time impact on an animal's development during critical periods of the animal's early life cycle. Environmental stimuli, particularly when they occur in temporal patterns that reoccur repeatedly within a brief time interval, also impact on mature, fully differentiated animals, and thus impact on an animal's subsequent behaviors. For the latter, information is acquired by the mature species member during a learning experience and stored within brain networks as "long-term" memory.

Given these two distinct time domains of adaptation, "evolutionary" and "learning", the cellular mechanisms responsible for information acquisition and storage are also quite distinct. Mutation-induced variations of genomic

information provide for the "evolutionary" time domain of adaptation. Second-messenger induced signaling cascades within neurons and their synaptic specializations provide for the time domains of learning adaptation.

A third, even longer time domain concerns the evolution of progressively more adaptive species. Thus, not only do individual species members adapt, and the species itself adapts, but progressively different successive species adapt. In this longest time domain, "life" on earth itself adapts to the environment by assuming different forms as distinct but still related species.

All of this adaptation and transformation notwithstanding, certain biological entities and their underlying functional principles may remain essentially unchanged, even over the course of many millions of years of evolution. One clear example of such conservation involves membrane channels or "pores" that gate the flux of ions across cell walls. One such channel, called I_C , is a calcium-dependent pore for potassium flux. When calcium rises inside a cell to a critical threshold level, it triggers the opening of channels that allow K^+ ions to flow down their chemical electrical gradient. This almost invariably means that K^+ ions flow from the inside to the outside of the cell, thereby making the electric potential

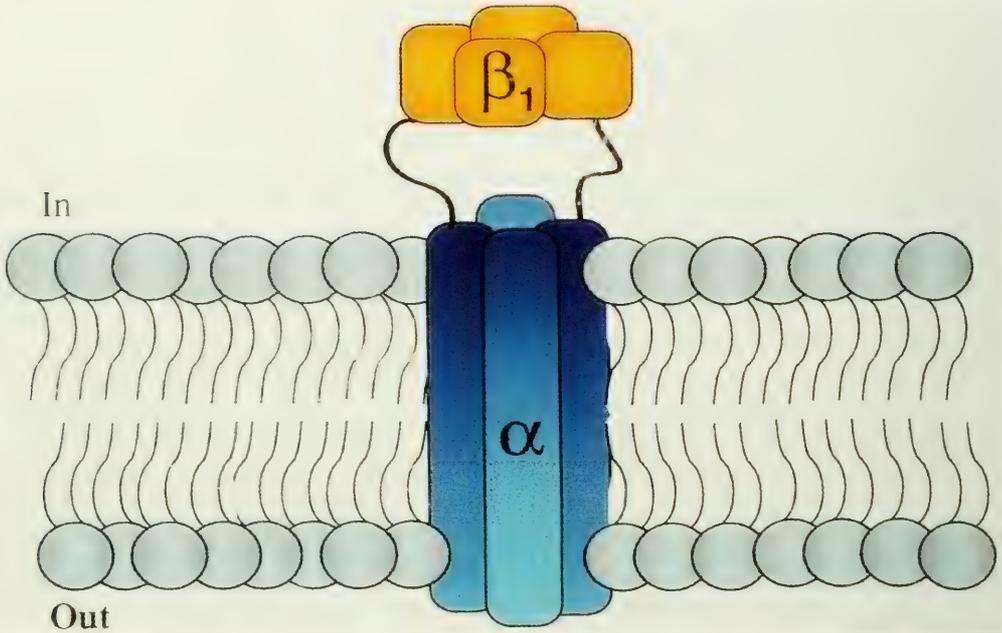


FIG. 1. A molecular model of a generic K⁺ channel formed from an octomeric protein (after Scott et al., 1994, provided courtesy of Noam Meiri).

across the cell wall negative inside the cell with respect to the outside environment. Such calcium-dependent K⁺ channels (of I_C channels) occur within the walls of single-cell organisms, such as *Paramecium*. Remarkably, very similar I_C channels also occur within the walls of large pyramidal-shaped cells in the human brain area known as the hippocampus. Once nature designed (through evolutionary time domains) these marvels of molecular configurations in single cell organisms it essentially maintained the channel design (Scott et al., 1994), even within our own, human species (Fig. 1).

Some years ago, I wondered if such examples of biological conservation—that is, across evolution—might also subserve adaptation itself in the time domain of individual human lifetimes. Specifically, were there mechanisms of cellular and molecular adaptation within the “learning” time domain that were conserved across evolutionary time?

To press this question of conserved cellular mechanisms of learning, I set out in search of what I thought of as a possible “evolutionary compromise”. To survive, a species would have evolved by making a compromise be-

tween a central nervous system that was not very complex, but just complex enough to achieve the capacity to learn crucial stimulus relationships. The nudibranch mollusc *Hermisenda crassicornis* can, after being subjected to many years of experiments, now be considered as such a compromise (Alkon, 1983). A similar sea snail, the gastropod mollusc *Aplysia californica*, had previously been found to be a favorable electrophysiologic preparation as well as to show behavioral changes, such as habituation and sensitization (Bailey & Kandel, 1993).

Most psychologists agree that human memory is diverse and requires diverse brain structures. Memories can be explicit or declarative, procedural or motoric, sensory or emotional. Despite its diversity, however, human memory invariably involves relationships of stimuli in time and/or space. This seemingly universal relational aspect, often called associational, can even be encountered in the context of introspective reporting, including that of therapeutic settings. No behavioral model of associative learning has been more precisely formulated or more precisely controlled than Pavlovian conditioning.

When a neutral stimulus, such as the sound of a bell, precedes a well-valued or reflexive stimulus, such as the smell of meat, by a precise temporal interval 350–450 msec, a dog will learn to salivate in response to the bell alone. The meaning (or response) of the smell is associated in the dog's brain with a previously undefined or "neutral" sound of the bell.

From the point of view of evolutionary conservation, it is most remarkable that virtually the same quantifiable characteristics of Pavlovian conditioning have been identified for dogs, rabbits, rats, and humans (Figs. 2, 3), but also for *Hermisenda* (Fig. 4) as well (Gormezano, 1966; Gormezano & Kehoe, 1981; Gormezano et al., 1983; Gormezano et al., 1962; Schreurs, 1993). These characteristics (e.g., CS-UCS transfer, temporal specificity, pairing specificity, extinction, savings) are themselves conserved across evolution. This remarkable behavioral conservation would seem to require that there be considerable conservation of underlying principles of neuronal network functions. Conserved be-



FIG. 2. Human conditioned eyeblink training. A puff of air (from the white outlet) on the subject's corneal surface is preceded by a discrete tone received through the earphone system. Courtesy of Bernard G. Schreurs.

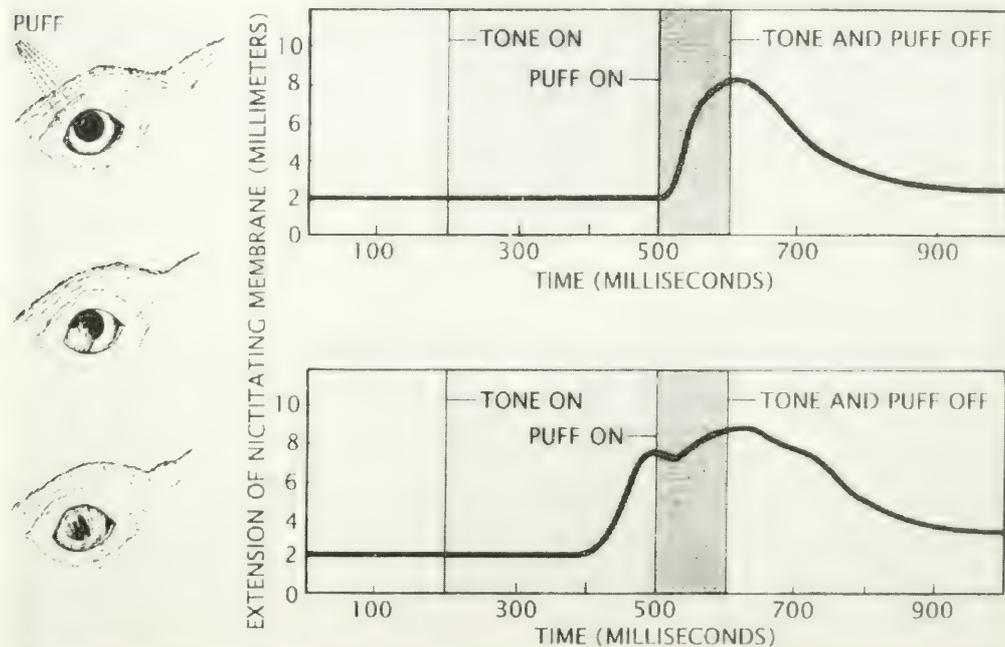


FIG. 3. Transfer of behavioral response in the rabbit occurs as a result of associative, or Pavlovian, conditioning. In this case the animal is taught to associate an auditory tone with a puff of air to its eye. The behavioral response — extension of the nictating membrane (left panel)— is transferred from the unconditioned stimulus (the puff of air) to the conditioned stimulus (the tone). The graphs show that before conditioning the membrane extends after the puff of air (top right); about 70 trials later, the animal learns to extend the membrane when it hears the tone (bottom right). Bernard G. Scheurs supplied the data for this figure.

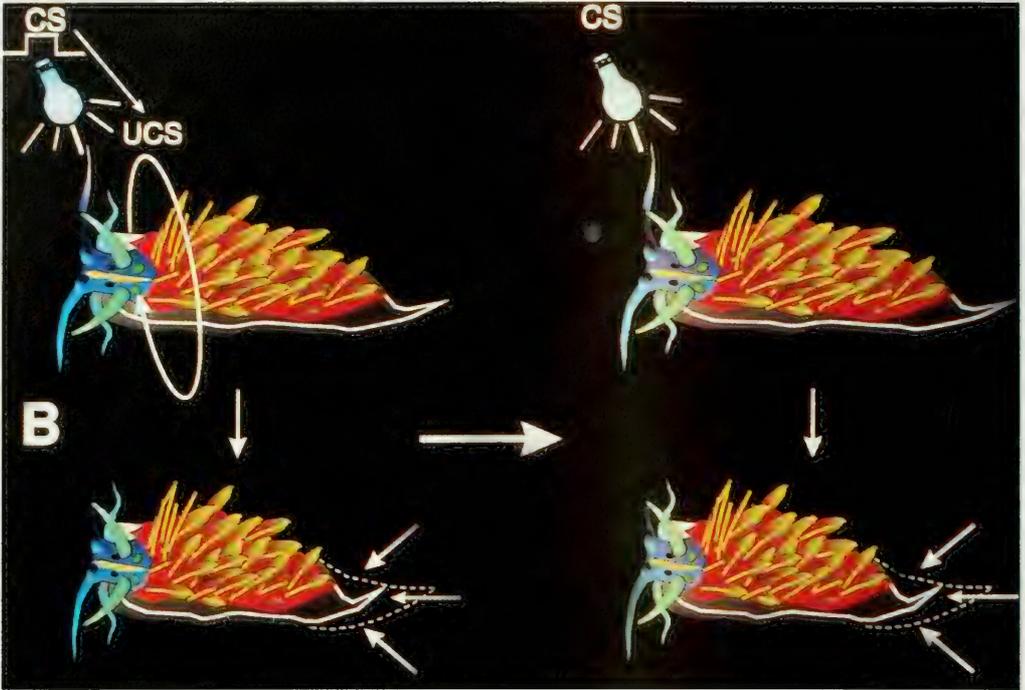


FIG. 4. Classical conditioning in the sea slug *Hermissenda crassicornis* leads to simple visual-vestibular associations. Excitation of sensory hair cells in the paired vestibular organs (statocysts) during rotation of this slug leads reflexively to contraction of the foot. In contrast, stimulation of photoreceptors in the paired eyes of the slug by flash of light does not cause the foot to contract but to weakly orient toward the light gradient (left). However, after a series of paired rotation and light flash stimuli, in which the rotation stimulus quickly follows a light stimulus, subsequent light stimulation of photoreceptors alone now causes the foot to contract (right).

havioral principles of association employ conserved network mechanisms that, in turn, imply crucial conserved biophysical and molecular mechanisms.

To test these conservation(s), we first turned to our evolutionary compromise. Applying a variety of electrophysiologic and biophysical recording techniques (e.g., current clamp, two microelectrode voltage clamp, patch clamp) to the neural pathways that mediate the Pavlovian training stimuli, we were able to construct a wiring diagram (Alkon, 1983) for the visual and vestibular pathways and their synaptic interactions (Fig. 5). With this circuit diagram, we traced precisely how the associated stimuli (CS: light, UCS: rotation) traveled throughout the visual-vestibular circuits to produce a learned conditioned response. Using this "blueprint" of synaptically connected neurons, we then reconstructed how synaptic strengths were changed during

learning by long-term alterations of voltage-dependent K^+ currents within post-synaptic membranes. Subsequently, it was possible to implicate an elaborate molecular cascade that regulates these channels and possibly structural aspects of the synaptic apparatus itself during learning and memory. The critical molecular cascade showed clear analogy to the molecular cascade (Fig. 6) responsible for muscle contraction and long-term changes of muscle structure (Alkon, 1989; Alkon et al., 1998). Among the crucial events in these cascades are enhanced mobilization of intracellular calcium, activation of the signaling enzyme protein kinase C (PKC), activation of the PKC substrate, the signaling protein calnexin, activation of the calcium releasing channel, the ryanodine receptor, and calcium-dependent regulation of m-RNA turnover for specific proteins (Fig. 7).

Many years of further studies on mam-

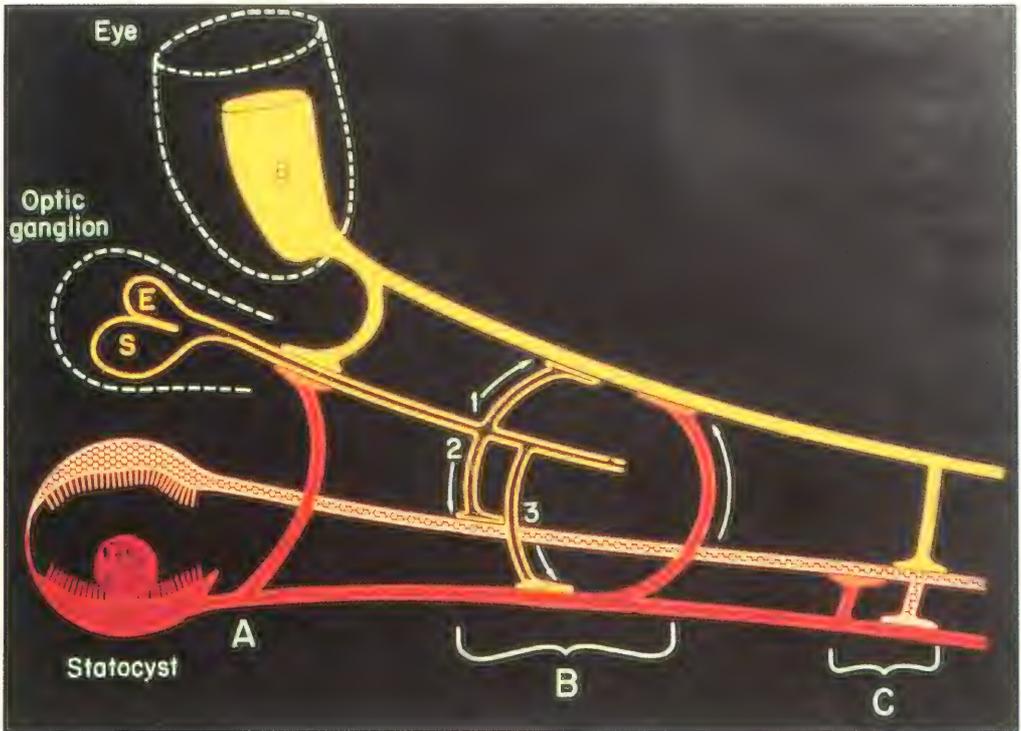


FIG. 5. Neural responses to stimulus pairing. Neural system (schematic and partial diagram) responsive to light and rotation. Each eye has two Type A and three Type B photoreceptors; each optic ganglion has 13 second-order visual neurons; each statocyst has 12 hair cells. The neural interactions (intersection of vertical and horizontal processes) identified to be reproducible from preparation to preparation are based on intracellular recordings from hundreds of pre- and post-synaptic neuron pairs.

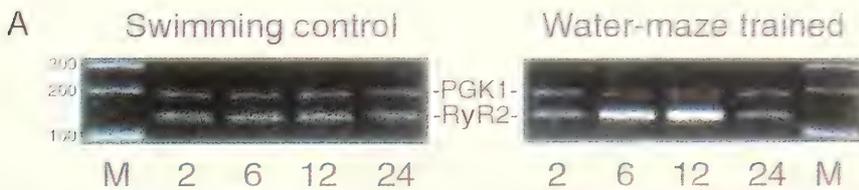


FIG. 6. Hippocampal changes in RYR2-mRNA levels after water-maze training. (A) RT-PCR analysis of RYR2-mRNA in control swimming and water-maze-trained rats at 2, 6, 12 and 24 h after training. No change in RYR2-mRNA levels was observed between control swimming and naïve animals (data not shown). (Figure continues.)

malian learning models, such as rat maze learning, rabbit eyeblink conditioning, and olfactory discrimination learning, provided strong confirmation that the molecular cascades found in *Hermisenda* were also critically involved in mammalian learning and memory. Thus, conserved associative learn-

ing behaviors from mollusc to mammal were correlated with conserved cellular and subcellular mechanisms responsible for those learning and memory behaviors. This conservation of learning behavior and its molecular basis received still further confirmation through studies of Alzheimer's disease and its molec-

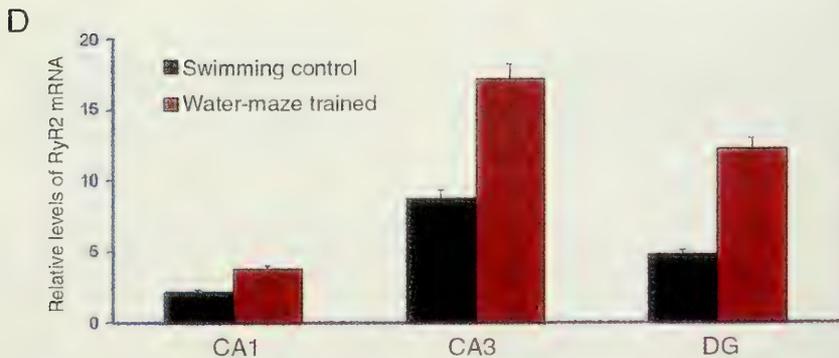
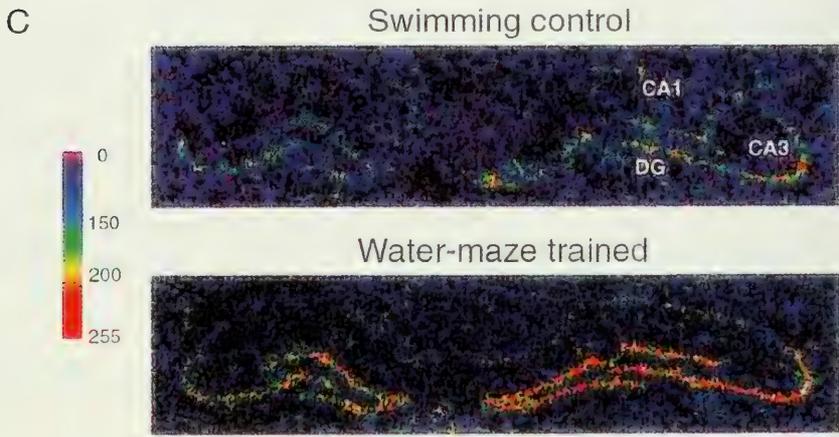
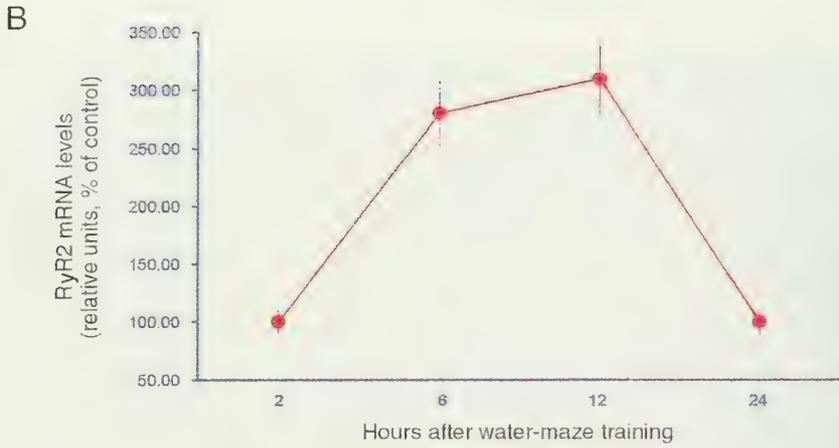


FIG. 6. (Continued) (B) Relative RYR2-mRNA levels in water-maze-trained rats at 2, 6, 12 and 24 h after training. To control for the integrity of RNA and for differences attributable to errors in experimental manipulation from tube to tube, primers for rat phosphoglycerate kinase 1 (PGK1) were included in the RT-PCR reactions. (C) Localization by in situ hybridization of RYR2-mRNAs in hippocampal subfields of control, swimming, and water-maze-trained rats 6 h after training. The color spectrum on the right side of the figure represents the pixel value of gray levels. (D) Relative RYR2-mRNA levels in different hippocampal subfields of control swimming and water trained-rats. Quantification of induction increase is achieved by comparison of pixel values of an area of interest in four sections from each of four pairs of rats. Changes in mRNA levels are expressed as density ratio of trained to control animals.

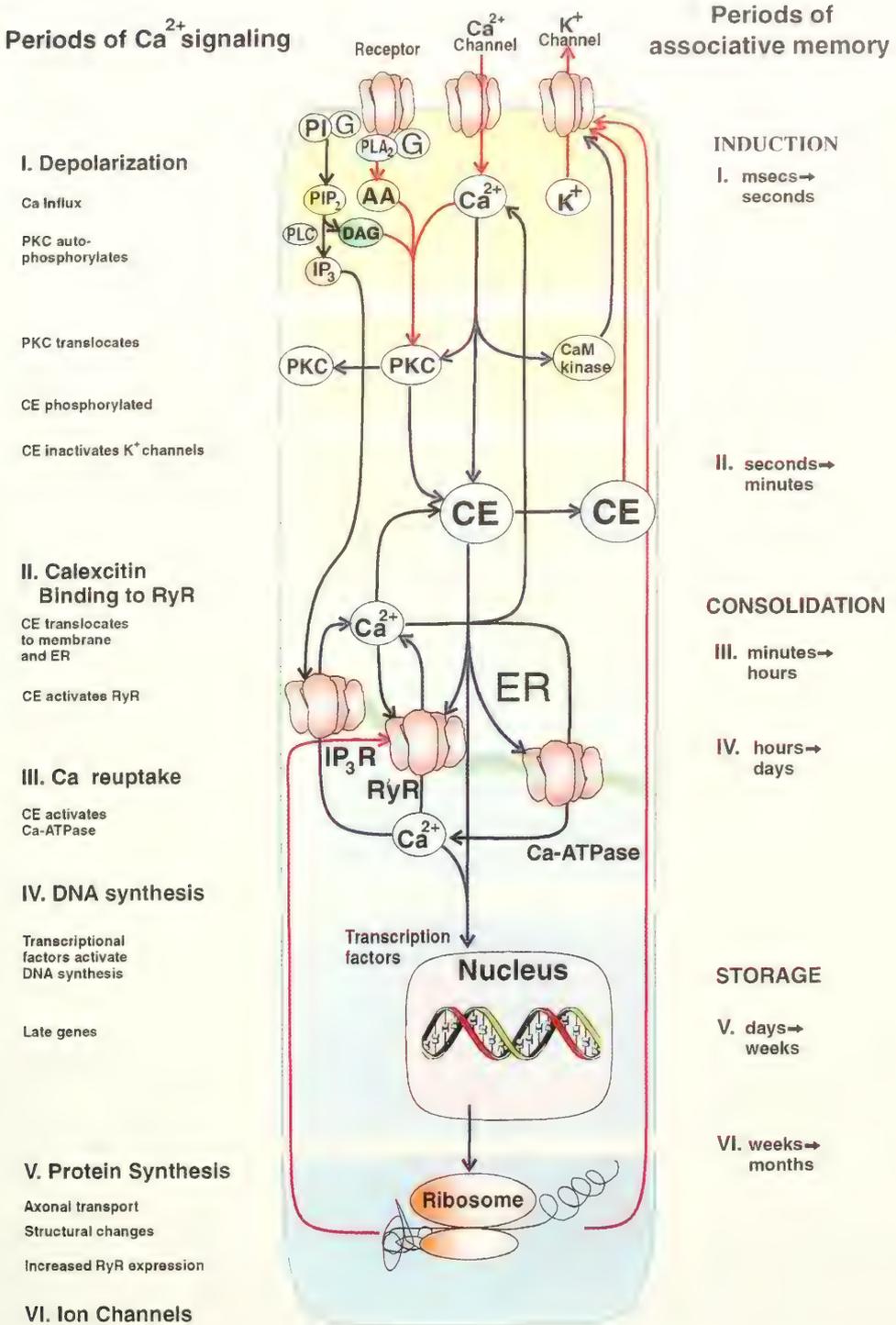


FIG. 7. Schematic diagram illustrating time domains of calcium signaling and associative memory. (Legend continues.)

FIG. 7. (Continued) Stage I: The neuron depolarizes as a result of a convergence of synaptic inputs, which activates G-protein coupled receptors (i.e., for acetylcholine, GABA, glutamate). Membrane depolarization also opens Ca^{2+} channels, causing an influx of Ca^{2+} . Diacylglycerol (DAG), arachidonic acid (AA), and inositol triphosphate (IP_3) are released by phospholipases A2 and C (PLA_2 and PLC) and, along with Ca^{2+} , activate protein kinase C (PKC), which is thereby translocated to the plasma membrane. Ca^{2+} also activates CaM kinase. The kinases undergo autophosphorylation which renders their activity independent of Ca^{2+} . PKC and CaM kinase may also inhibit K^+ and other channels by direct phosphorylation.

Stage II: Elevated $[\text{Ca}^{2+}]_i$ activates the Ca^{2+} -binding protein calyculin (CE). Phosphorylation of CE by PKC promotes its translocation to membrane compartments, where it inhibits K^+ channels, making the membrane more excitable to further depolarizing stimuli. CE also elicits Ca^{2+} release from ryanodine receptors on the membrane of the endoplasmic reticulum (ER) and possibly synaptic membranes, resulting in amplification of Ca^{2+} signals. IP_3 also releases Ca^{2+} by activating the IP_3 receptor (IP_3R).

Stage III: CE, after phosphorylation by PKC, no longer activates the RyR, and no longer inhibits Ca^{2+} -AT-Pase at the ER membrane, facilitating the removal of excess Ca^{2+} .

Stage IV: CE and/or Ca^{2+} , probably acting indirectly through transcriptional activators, induce new DNA transcription. CE may also indirectly increase mRNA turnover.

Stage V: Late genes are transcribed, resulting in increased synthesis of at least 21 different proteins, including RyR. At this stage, through an as-yet undetermined mechanism, retrograde axonal transport is also inhibited by CE; this may underlie the structural changes in branch morphology that were observed after *Hermissenda* associative learning.

Stage VI: New RyR receptors and ion channels are synthesized and transported to their respective membranes. These may be related to enhanced Purkinje cell dendritic excitability with rabbit conditioning.

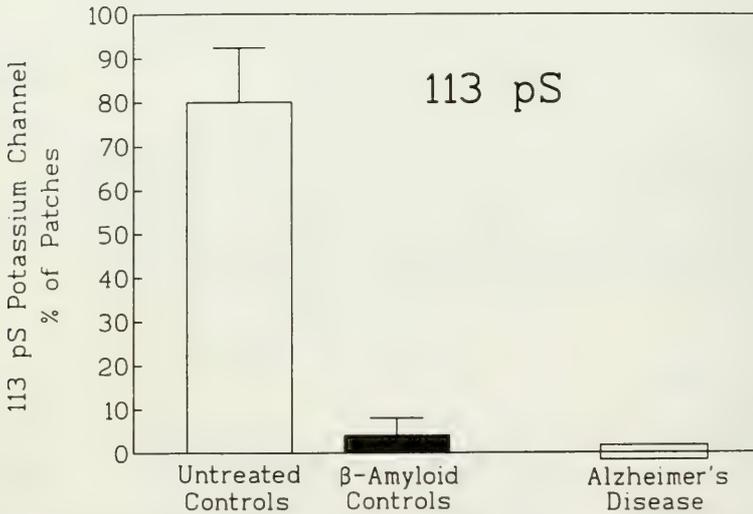


FIG. 8. Bar graph of the percentage change (from resting Ca^{2+} concentrations) shows the virtual elimination of the TEA response in treated control cells (eight cell lines, 194 cells) similar to observations for AD fibroblasts (four cell lines, 285 cells measured). Preparations of the various cell lines were tested for response to TEA in the absence (left) or presence (center) of βAP . The responses of AD fibroblasts shown here are in agreement with our previous report showing no responses in 13 different AD lines (> 700 cells measured).

ular correlates. To test the possibility of conserved molecular events responsible for human memory, we turned to that clinical entity most specific for human memory loss: Alzheimer's disease. This disease early in its progression rather specifically impairs human memory. Beginning with the hypothesis that

Alzheimer's disease is systemic (i.e., involving multiple tissue types through out the body) but causes clinical symptoms only through effects on the brain, we analyzed peripheral cell types, such as human skin fibroblasts. After many studies, we could conclude that, indeed, a succession of molecular steps in the con-

served memory cascade were in fact consistently targets of dysfunction in Alzheimer's disease. Diagnostic changes of K^+ channels (Fig. 8), PKC intracellular calcium release, and calyculin were consistently found in the skin fibroblasts (Etcheberrigaray et al., 1994) of Alzheimer's patients, but not for a variety of age-matched controls. Furthermore, low levels of soluble β -amyloid (nanomolar-comparable to endogenous levels found throughout the body) induced these diagnostic phenotypic abnormalities in the normal fibroblasts. Here, then, was evidence that early Alzheimer's disease involves several of the same molecular steps implicated as mechanism for animal learning.

Thus, based on these studies of species that range from the nudibranch *Hermisenda* to humans, we may infer that adaptation in the "learning" time domain uses functional principles that have been conserved across the "evolutionary" time domain. This conservation deserves our future attention not only because it illustrates and elucidates the elegant economy of natural selection. It also provides a strategy whereby we can use molecular cascades in primitive species as accessible models to guide our investigations of dauntingly complex and often inaccessible mysteries of human physiology and disease.

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THE *BIOMPHALARIA GLABRATA* EMBRYONIC (BGE) MOLLUSCAN CELL LINE:
ESTABLISHMENT OF AN IN VITRO CELLULAR MODEL FOR THE STUDY OF
SNAIL HOST-PARASITE INTERACTIONS

Timothy P. Yoshino^{1,2}, Christine Coustau³, Sylvain Modat³ & Maria G. Castillo¹

ABSTRACT

Cell lines of invertebrates, especially those of arthropod origin, have played crucial roles in addressing fundamental questions related to cell signaling and differentiation, gene expression, cell-pathogen interactions, and the like. They also have been instrumental in the development of genetic transformation systems and the development and testing of microbial insecticides. Recently, we have utilized a cell line originally derived from embryos of the freshwater snail *Biomphalaria glabrata* (Bge cell line; Hansen, 1976a) to investigate the complex cellular, biochemical and molecular interactions between snails and their trematode parasites. Because this cell line was derived from *B. glabrata*, possesses a fibroblast-like appearance similar to circulating hemocytes, and shares in common several hemocyte functions (substrate adhesion, phagocytosis, encapsulation, enzyme content), the Bge cell line is proposed as a cellular model for *B. glabrata* hemocyte structure and function. In support of this proposal, a hemocyte β -integrin cell adhesion receptor homologue recently was identified and cloned based on information from a previously acquired Bge cell β integrin subunit cDNA sequence. As a general approach, it is anticipated that Bge cells can be evaluated for genes associated with immune recognition/adhesion, and subsequently employed to generate molecular or immunological probes for use in hemocyte studies. Other applications of Bge cells to the study of parasite-snail host interactions include their use in the in vitro cultivation of intramolluscan stages of diverse trematode species, and in the development of genetic transformation systems for molluscan cells. Research in this latter area has focused on the identification of suitable Bge cell promoters and testing their abilities to drive expression of reporter gene constructs. It is concluded that the Bge cell line offers a diversity of valuable experimental approaches when applied to the study of molluscan cellular immune mechanisms or snail-trematode physiological interactions.

Key words: *Biomphalaria glabrata*, Mollusca, embryonic cell line, Bge cell line, hemocyte, *Schistosoma mansoni*, in vitro culture, transgenic.

INTRODUCTION

The use of cell lines as tools for addressing fundamental questions regarding molecular structure-function relationships is well established in those animal species for which such lines are available. For coelomate invertebrates, the vast majority of cell lines are of insect or arachnid origin (Bayne, 1998) and have been extensively employed in studies of gene regulation and protein expression (Berger & Morganelli, 1984; Jones et al., 1996), cellular shape-change and motility (Kosik & McConlogue, 1994), adhesion (Bieber, 1994), induction of immune peptides (Hultmark, 1994; Hoffmann & Reichart, 1997), and pathogen-host cell interactions (Stollar, 1993; Kopecky & Stankova, 1998; Lawrence,

1997). Moreover, there has been a long-standing interest in the use of insect cell lines in the production of recombinant proteins through baculovirus expression systems (Thomsen et al., 1993; McCarroll & King, 1997; Merrington et al., 1997), the selection and testing of microbial pesticides (Maramorosch & Mitsuhashi, 1997), and the development of new genetic transformation technologies (Fallon, 1991; Cherbas et al., 1994; O'Brachta & Atkinson, 1996). However, for reasons that are not yet understood, there exist very few cell lines outside of the arthropod classes Insecta and Arachnida, despite a critical need and strong interest in cell lines of diverse invertebrate organisms (Bayne, 1998).

Of the non-arthropod cell lines currently available, only one, the *Biomphalaria glabrata*

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embryonic (Bge) cell line, is of molluscan origin (ATCC# CL1494; American Type Culture Collection, Rockville, Maryland). It was derived from embryos of the freshwater pulmonate snail *B. glabrata* by Hansen (1976a), and has been stored/maintained in various laboratories over the past 20 years. The significance of the Bge cell line, in addition to its unique molluscan origin, also lies in the fact that the snail species from which this line was derived represents a primary intermediate host for transmission of the blood fluke, *Schistosoma mansoni* (Platyhelminth; Digenea), causative agent of human intestinal schistosomiasis in the New World and Africa (Basch, 1991). *Schistosoma* spp. are estimated to infect 200 million people in over 74 endemic countries, and, among parasitic diseases, is considered second in importance only to mosquito-transmitted malaria in its public health impact (World Health Organization, 1998).

Because molluscan and arthropod intermediate hosts of infectious diseases (broadly referred to as "vectors") are essential to human transmission, they have long been recognized as potentially vulnerable targets in disease control programs. However, because traditional methods of vector control, including pesticide usage and habitat destruction, have not provided sustainable solutions to disease control or prevention, new approaches aimed at disrupting parasite development within the host through genetic and/or molecular manipulation of vector competence are now being explored. This field has advanced most rapidly in insects where inducible antimicrobial and antiparasitic peptides (Faye & Hultmark, 1993; Hoffman & Reichhart, 1997; Lowenberger et al., 1999) and enzymes (Ashida & Brey, 1998) have been identified, their genes cloned, and the molecular pathways/factors regulating antimicrobial responses elucidated. In addition, methods for introducing and expressing immune peptides or function-disrupting antisense sequences into whole organisms through genetic transformation technologies are being developed (Carlson et al., 1997; O'Brachta & Atkinson, 1996). It is envisioned that the basic knowledge of anti-pathogen immune mechanisms and parasite developmental pathways will lead to molecularly-based strategies for interrupting pathogen survival in the vector host. Significantly, insect cell lines have played important roles in advancing the general area of host-pathogen relationships.

In contrast to the insects, little progress has

been made in the development of molecular vector control approaches for molluscs of medical and veterinary importance. For example, in the freshwater gastropods, few genes have been cloned and/or identified as being of potential significance in regulating vector competence (Dissous et al., 1990; Hoek et al., 1996; Adema et al., 1998; Knight et al., 1998), and there are still no genetic or physical maps available for vector snails (Knight et al. 1998). Recently, however, application of the *B. glabrata* embryonic (Bge) cell line to the in vitro cultivation of larval schistosomes (Yoshino & Laursen, 1995; Coustau et al., 1997) has provided significant opportunities to investigate larval blood fluke-host cell interactions at the molecular level. Work utilizing this cell line has already led to the identification of molecules potentially involved in host cell signal transduction (Lardans et al., 1998), immune adhesion (Davids et al., 1999), trematode development (Laursen & Yoshino, 1999) and to the establishment of protocols for DNA-mediated gene transfer into molluscan cells (Lardans et al., 1996; Yoshino et al., 1998). The present review focuses on these recent advances in the use of the Bge cell line as a tool for studying chemical communication mechanisms between trematode parasites and cells of their molluscan host.

Bge Cells as a *B. glabrata* Hemocyte Model

Two important criteria for establishing a comparative cellular model system include the demonstration that the cells being compared have a similar origin, both in terms of species and ontogeny, and that they share structural and functional similarities under in vitro conditions. In terms of species origin, it is fortunate that the Bge cell line was derived from *B. glabrata* (Hansen, 1976a), a major intermediate host of *S. mansoni* and an important experimental snail host for the study of schistosome-snail immune interactions (Fryer & Bayne, 1996a; Yoshino & Vasta, 1996; Adema & Loker, 1997). The precise ontological origin of the Bge cell line is not known since it was originally obtained from developing five-day old *B. glabrata* embryos (Hansen, 1976a). However, it has been speculated, based on morphology (Fig. 1) and Bge cell's ability to synthesize collagen-like molecules (Hansen, 1976b; Stein & Basch, 1977), that these cells probably are of fibroblast or fibroblast-like origin. A similar ontological origin

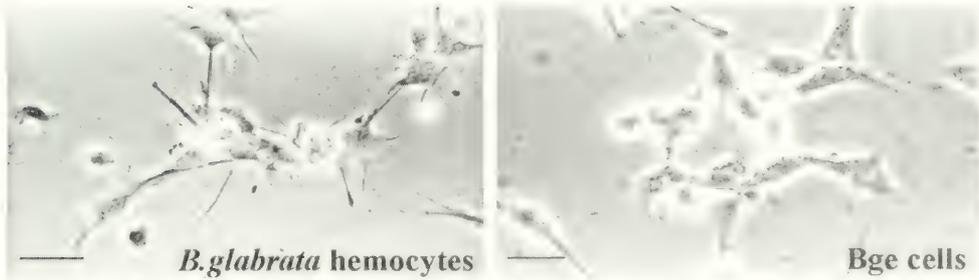


FIG. 1. Phase-contrast photomicrographs of *Biomphalaria glabrata* hemocytes (left) and cells of the *B. glabrata* embryonic (Bge) cell line (right) as they appear attached to and spread on a glass slide substrate. It has been noted that, as depicted here, a subset of Bge cells assume a more hemocyte-like appearance when they are washed of medium and permitted to reattach under serum-free conditions. This differs from more typical spindle-shaped morphology of Bge cells as they appear under normal culture conditions (Lardans & Dis-sous, 1998). Whether these differences in cell morphology reflect the possible presence of differentiating Bge cell subpopulations or a behavioral response to manipulation is presently unknown. Scale bar = 10 μ m.

also has been suggested for circulating hemocytes (Pan, 1958), although their exact embryological source in *B. glabrata* is still controversial (Jeong et al., 1983).

The second criterion for Bge cells to serve as an effective model for *B. glabrata* hemocytes is that the two cell-types must share important structural and functional characteristics in their interactions with foreign materials. Here we assume that shared function is based on similarities in biochemical and/or molecular mechanisms being used by these snail cell-types. Therefore, recently we have initiated experiments to begin evaluating the "immune" functions of phagocytosis and encapsulation in the Bge cell line. Phagocytic and encapsulation responses represent "hallmark" behaviors for hemocytes since these activities, in large part, define their immune effector capabilities (Ratcliffe et al., 1985; Bayne, 1990).

Phagocytosis: It is well documented that *B. glabrata* hemocytes are capable of phagocytosing a variety of foreign particles including bacteria (Cheng et al., 1978), mammalian erythrocytes (Abdul-Salam & Michelson, 1980; Noda & Loker, 1989; Zelck & Becker, 1992), yeast/zymosan (Fryer & Bayne, 1989; Connors & Yoshino, 1990), and latex beads (Uchikawa & Loker, 1992; Fryer & Bayne, 1996b). Moreover, excretory-secretory products (ESP) released by developing *S. mansoni* sporocysts exert inhibitory effects on phagocytosis by host hemocytes (Connors & Yoshino, 1990; Fryer & Bayne, 1990), suggesting the presence of ES factors capable of

binding to hemocytes and interfering with hemocyte-particle interactions.

However, to date, only anecdotal evidence is available that Bge cells possess similar phagocytic activity. Therefore, this function was tested more rigorously using an adhesion/phagocytosis assay modified from Uchikawa & Loker (1992). In this assay, uncharged latex beads (6.4 μ m in diameter; Sigma Chemical Co., St. Louis, Missouri) were rinsed 2x in Chernin's balanced salt solution (CBSS; Chernin, 1963) and resuspended in CBSS to a final concentration of 0.1%. To determine the effect of *S. mansoni* ESP (Connors & Yoshino, 1990) on phagocytosis, beads were treated with ESP (10 μ L beads in 1mL ESP) for 4 h at 22°C, followed by two washes in CBSS and resuspension in CBSS at a concentration of 0.1%. Beads were then presented to monolayered Bge cells in a ratio of 2 beads/cell. Because of the difficulty in discerning between surface membrane adherent and endocytosed beads (Fig. 2) (Uchikawa & Loker, 1992), an adhesion index (percentage of cells with adherent/phagocytosed beads) was determined for each treatment and control group at 1, 3, 6 and 24 h post-bead inoculation. As shown in Figure 3, untreated latex beads readily attached to Bge cells by 1 h (45%), and by 6 h had reached near maximum association (adherence/phagocytosis) with cells (approx. 80%). This rate of bead association was considerably higher when compared to *B. glabrata* hemocytes, which attained between 10–15% bead adherence (Uchikawa & Loker, 1992) at 1 h of incubation. In a similar study (Fryer & Bayne,

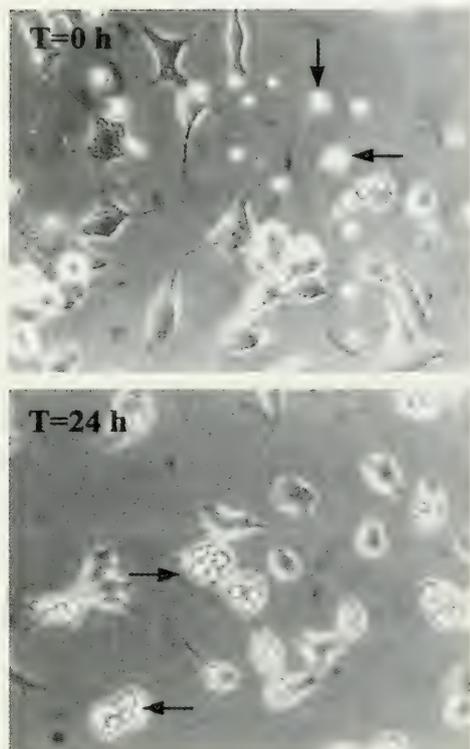


FIG. 2. Photomicrographs of *B. glabrata* embryonic (Bge) cells demonstrating surface attached and phagocytosed latex beads following 0 and 24 h incubations in snail saline (CBSS).

1996b), however, it is noted that hemocyte attained a phagocytic rate of 70% using smaller (2.5 μm) charged latex beads. Regardless of discrepancies in association rates, clearly Bge cells, like hemocytes, possess the capacity to phagocytose foreign particles.

Pretreatment of beads with ESP prior to incubation with Bge cells resulted in a significant increase in bead adhesion at all time points (Fig. 3), suggesting that ESP contains molecule(s) that mediate enhanced bead adhesion through interaction with putative ESP receptors on the Bge cell surface. The fact that untreated beads did not associate with cells above control levels when incubated in the presence of ESP supports this hypothesis; in this case, free ESP molecules saturate Bge cell receptors, thereby blocking enhanced adhesion due to bead-bound ESP. Recent flow cytometric analyses using fluorescein-labeled ESP (fESP) as a "probe" confirm that polypeptide(s) contained in ESP di-

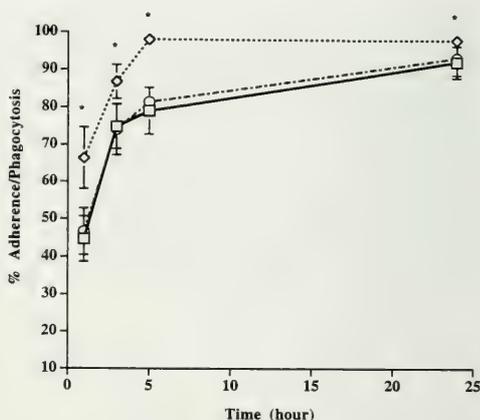


FIG. 3. Percentage of *B. glabrata* embryonic (Bge) cells with attached/phagocytosed beads acquired during 24 h of incubation in snail saline. Control: beads pretreated with CBSS prior to addition to Bge cells in CBSS (□); ESP treatment #1: beads pretreated with *S. mansoni* sporocyst excretory-secretory products (ESP) before addition to Bge cells in CBSS (◇); ESP treatment #2: beads pretreated with CBSS prior to addition to Bge cells in CBSS containing ESP (○); ESP treatment #3: beads pretreated with CBSS containing ESP (○·). *designates values that differ significantly ($p < 0.05$) from the control.

rectly bind to Bge cells, and that such binding is inhibited by the sulfated poly-fucose, fucoidan. Of particular relevance to this review, *B. glabrata* hemocytes also have been found to bind fESP via a fucoidan-inhibitable receptor (Johnston et al., in preparation), and it is hypothesized that these, or related, receptors are responsible for the ESP-mediated modulation of hemocyte function (e.g., Yoshino & Lodes, 1988; Lodes & Yoshino, 1990; Connors & Yoshino, 1990; Loker et al., 1992; Adema & Loker, 1997). In this case, Bge cells may serve as a unique model for investigating parasite ESP-binding hemocyte receptors.

Encapsulation: Encapsulation, or the multiple layering of cells around large foreign objects, is another function shared in common between *B. glabrata* hemocytes and Bge cells. Capsule formation represents the first line of cellular defense against larval helminths infecting molluscs, and is typified in the *B. glabrata*/*S. mansoni* system where hemocytes of resistant snail strains encapsulate and kill schistosome sporocysts under both in vivo (Sullivan & Richards, 1981; Loker et al., 1982) and in vitro (Bayne et al., 1980) conditions. At present, however, little is known

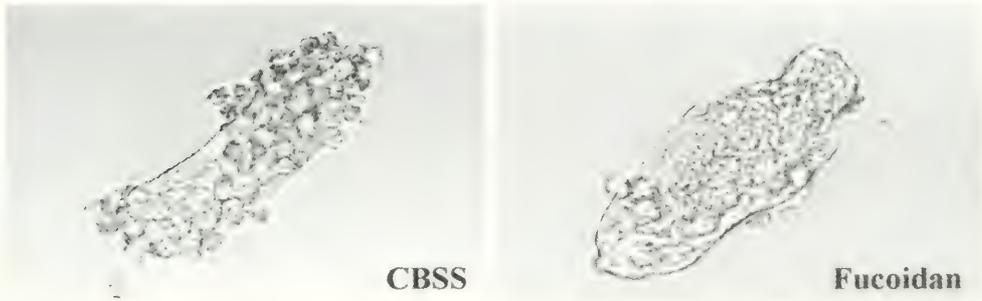


FIG. 5. Bright-field photomicrographs of *B. glabrata* embryonic (Bge) cells binding to the surface of *S. mansoni* mother sporocysts in the absence (left; CBSS only) and presence of an inhibiting polysaccharide, fucoidan (right; 1 mg/ml CBSS).

about the hemocyte molecules mediating parasite adhesion/recognition or how the encapsulation process is regulated. In order to address these questions, it may also be possible to use Bge cells as an encapsulation model. Bge cells co-cultured with *S. mansoni* sporocysts avidly bind to the larval surface forming multicellular capsules (Fig. 4) (Hansen, 1976b; Yoshino & Laursen, 1995), reminiscent of encapsulations by resistant snail hemocytes (Boehmler et al., 1996). In the case of Bge cells, however, encapsulation does not lead to parasite destruction, but instead are more comparable to in vitro capsules formed around sporocysts by hemocytes of susceptible snail strains (Bayne et al., 1980; Fryer & Bayne, 1995).

Due to this shared sporocyst adhesive behavior, Bge cells are currently being used to identify and characterize the surface receptor(s) responsible for initiating parasite adherence and for mediating subsequent cell-to-cell binding required for capsule formation. For example, using an in vitro cell adhesion assay, in which *S. mansoni* sporocysts were placed into 1.5-mL microcentrifuge tubes (200 larvae/tube) followed by addition of 2×10^6 Bge cells in a final volume of 200 μ L of CBSS, we have determined that Bge cells in suspension were capable of binding to the sporocyst tegument after incubation for 24 h at 26°C (Fig. 5). Qualitative and quantitative assessment of cell adherence in the presence or absence of various chemical inhibitors further demonstrated that Bge cell binding was mediated through a carbohydrate-inhibitable mechanism, possibly involving a lectin-like receptor(s) expressed on the Bge cell surface (Fig. 5). Whether or not *B. glabrata* hemocytes possess similar sporocyst binding sites

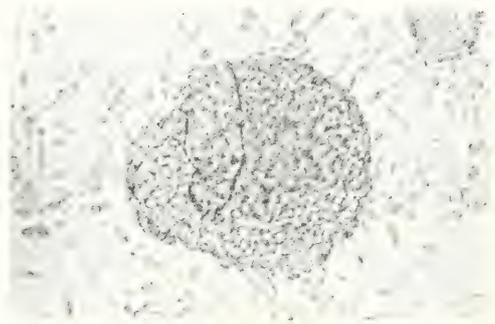


FIG. 4. Bright-field photomicrograph of an in vitro cultured *S. mansoni* mother sporocyst encapsulated by numerous *B. glabrata* embryonic (Bge) cells. Within Bge cell capsules sporocysts continue to develop, eventually giving rise to daughter sporocyst stages under in vitro conditions.

is at present unknown. However, it is anticipated that in-depth biochemical/molecular studies on the parasite-reactive receptors of Bge cell will lead to the development of valuable molecular and/or chemical probes for investigating similar membrane receptors associated with snail hemocytes.

Lysosomal Enzyme Content: Finally, since *B. glabrata* hemocytes previously have been shown to possess a variety of lysosomal enzymes (mainly hydrolases), we sought to determine whether or not Bge cells exhibited a similar enzyme repertoire. Acid and alkaline phosphatase, lysozyme, β -glucuronidase, lipase, nonspecific esterase, peroxidase, and aminopeptidase activities (Rodrick & Cheng, 1974; Cheng et al., 1978; Granath & Yoshino, 1983a; McKerrow et al., 1985; Cheng, 1985;

TABLE 1. Hydrolytic enzyme activity observed in Bge cell lysates (cell) or 2 hr-cultured supernatants (sn). C: Bge cells incubated for 2 hr in CBSS. B: Bge cells in the process of phagocytosing latex beads. ES: Bge cells in the presence of ES products. ES total: Control for enzymatic activity contributed by ES products alone. Semi-quantitative scores according to enzyme kit standards (APIzym™ Galery; bioMérieux): —, 0 nanomole of enzyme; +, 5 nanomoles; ++, 10 nanomoles; +++ 20 nanomoles; ++++, 30 nanomoles.

Enzymes tested	C cell	C sn	B cell	B sn	ES cell	ES sn	ES total
alkaline phosphatase	+	—	+	—	++	+++	—
esterase	++	+++	+++	+++	+++	+++	+
esterase lipase	++	+	+++	+	++	+	—
leucine arylamidase	++++	+	++++	+	++++	+	—
valine arylamidase	+	—	+	—	+	—	—
cystine arylamidase	+	—	+	—	+	—	—
acid phosphatase	++++	+	++++	+	++++	+	+
naphthol-AS-BI-phosphohydrolyase	+++	+++	+++	+++	+++	+++	++
β galactosidase	++++	++	++++	++	++++	++	—
N-acetyl-β glucosaminidase	++	—	++	—	++	—	—

and others) have been detected in *B. glabrata* hemocytes using various enzyme cytochemical and biochemical assays. Our assumption, as previously suggested, was that similarities in enzyme profiles may reflect shared functional and biochemical "backgrounds". Determinations of hydrolase activities in Bge cells were carried out using the APIzym™ Galery semi-quantitative enzyme assay kit (bioMérieux Vittek, Hazelwood, Missouri). In these experiments, Bge cell monolayers initially were washed with CBSS and cultured for 24 h in CBSS at 26°C prior to assay. Cells were then hypotonically lysed, cellular debris pelleted by centrifugation, and enzyme activity measured in the supernatants.

As shown in Table 1, the general profile of Bge cell hydrolase activities is comparable to that of snail hemocytes. Specifically, cells contained significant amounts (≥ 10 nanomoles) of nonspecific (simple) esterase, lipase, leucine arylamidase, carbohydrases, phosphohydrolyase and the "classical" lysosomal marker, acid phosphatase. Several enzymes (e.g., esterase, phosphohydrolyase and β galactosidase) appeared to be secreted into the medium during a 2 h incubation in CBSS. Also of interest was the observation that phagocytic stimulation or treatment of Bge cells with *S. mansoni* ESP had little effect on the hydrolase activity profile (Table 1). Exceptions included alkaline phosphatase, in which exposure to ESP induced an increase in both cellular and secreted enzyme activity, and simple esterase and lipase, in which phagocytosis appeared to increase their cellular enzyme content. Although selective, these types of inductive responses are similar to those re-

ported in snail hemocytes exposed to bacteria in vitro or from hosts with larval infection (Cheng, 1983, 1985; Granath & Yoshino, 1983b). Overall, the above functional and biochemical similarities exhibited by Bge cells and hemocytes reinforce the notion that Bge cells may serve as a legitimate model for *B. glabrata* hemocytes in investigations of parasite-snail immune interactions. In the following section, several examples are described as to how Bge cells are being used as tools to investigate schistosomus-snail relationships under in vitro conditions.

Applications of Bge Cells to the Study of Snail Host-Parasite Interactions

Structure and Function of Hemocyte Adhesion Molecules: Since both Bge cells and hemocytes are capable of adhering to and spreading on glass or plastic substrates via pseudopodial extensions of the cytoplasm (Fig. 1), it was hypothesized that these cells employ similar adhesion molecules to mediate substrate adherence or spreading. In a test of this hypothesis, we recently found that cell spreading on glass surfaces by both hemocytes (Davids & Yoshino, 1998) and Bge cells (Davids et al., 1999) was inhibited by the tetrapeptide, arg-gly-asp-ser (RGDS), but not the glu-substituted peptide, RGES (Fig. 6). Because the RGD peptide sequence is well known as a specific ligand for cellular receptors of the integrin family (Sonnenberg, 1993), our findings suggested that hemocytes and Bge cells share a common cell adhesion/spreading mechanism mediated by integrin-like receptors. In order to further investigate

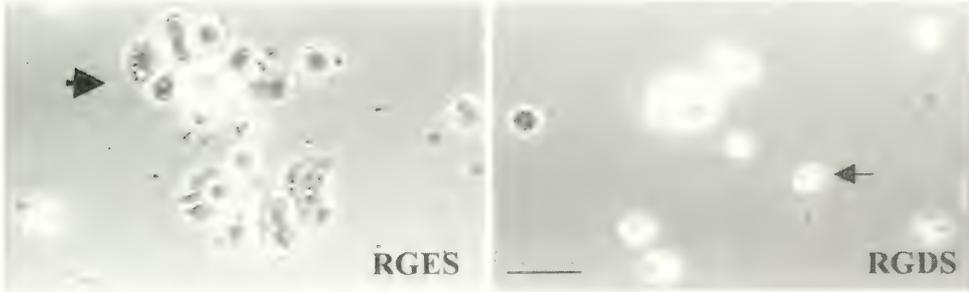


FIG. 6. Phase-contrast photomicrographs of *B. glabrata* embryonic (Bge) cells incubated in presence of the tetrapeptides RGES (nonspecific control peptide) or RGDS (specific test peptide). Note that RGDS strongly inhibits cell spreading on the glass substrate (arrow), whereas no such inhibition is seen in the presence of RGES (arrow). Scale bar = 10 μ m. Reprinted with modification from Gene, Davids, B. J., X. J. Wu & T. P. Yoshino, Cloning of a β integrin subunit cDNA from an embryonic cell line derived from the freshwater mollusc, *Biomphalaria glabrata*, 1999, with permission from Elsevier Science.

this possibility, we took advantage of the ready availability and ease of producing large numbers of Bge cells to identify and clone a molluscan integrin homologue that may be serving as a putative RGD-binding receptor. This was accomplished by PCR amplification of a 137 base pair Bge cell cDNA sequence corresponding to the highly conserved β integrin ligand binding domain (LBD) using degenerate primers to the LBD of several known β integrin subunits (Gettner et al., 1995), followed by PCR amplification of the 3' region of the Bge β integrin cDNA using an exact 5' primer contained within the 137 bp Bge LBD and an oligo-dT 3' primer. Sequence of the 5' end of the integrin cDNA was obtained by 5' RACE (random-amplification of cDNA ends) methods. Based on its predicted molecular mass of 87.6 kDa and characteristic domain structure (Davids et al., 1999), this molecule was identified as an authentic β integrin subunit homologue (designated β BGE) and the first to be cloned from the phylum Mollusca.

We then asked the question, do *B. glabrata* hemocytes also express a β integrin subunit cDNA similar to that of Bge cells? Based on Bge cell sequence data, RT-PCR was performed on snail hemocyte cDNA using exact primers synthesized from the β BGE integrin LBD. Amplified products from snail hemocytes shared 99% nucleic acid similarity and 100% amino acid identity to the Bge cell β integrin subunit, and from 49–71% amino acid identity with LBDs of other known invertebrate and human β subunits (Fig. 7). Cloning and sequencing of the complete *B. glabrata* hemocyte cDNA currently is in progress, but to

date, comparison of a 1900-nucleotide hemocyte sequence with β BGE has revealed 97% and 96% identities at the nucleic acid and amino acid levels, respectively (unpublished data). Thus, it appears that hemocytes also express a β integrin homologue, although like the β BGE subunit, it still remains to be determined whether this integrin subunit, in conjunction with an as yet unidentified *alpha* subunit, is responsible for the observed RGD-dependent cell spreading response (Davis & Yoshino, 1998). Moreover, it is important to recognize that the integrin gene sequence information acquired from Bge cells greatly facilitated our ability to generate comparable hemocyte data, and it is envisioned that this research strategy will continue to contribute critical molecular tools for investigating a variety of molecules and their functions in hemocytes.

In Vitro Cultivation of Larval Trematodes: A major technical hurdle in investigating the molecular mechanisms underlying intramolluscan schistosome development has been the lack of an in vitro culture system capable of supporting continuous growth and differentiation through all larval stages. Soon after the successful isolation of the Bge cell line from *B. glabrata* embryonic tissues (Hansen, 1976a), attempts were made to develop a Bge cell-based in vitro culture system capable of supporting the larval development of *S. mansoni*. Co-cultivation of Bge cells with in vivo-derived daughter sporocysts resulted in the production of a second daughter sporocyst generation (Hansen, 1976b), while in more recent experiments, *S. mansoni* mother

B. glabrata Bge	ENYPVDLYYFMDLNSMEDDKEKLALLGNKIAEQMSAITKNFRLGFGSFVDKVVSPYV. STVPQK
<i>B. glabrata</i> Hemocyte DLYYFMDLNSMEDDKEKLALLGNKIAEQMSAITKNFRLGFGSFVDKVVSPYV. STVPQK
<i>D. melanogaster</i> BPS	-G-----L----K-----A--ST--D-LS-T-KR--N-H-----LM---. --I-K-
<i>S. purpuratus</i> Bg	-D-----V----K-----LS--MD--DIL-SE-KN--S-----T-M---. ----E-
<i>C. elegans</i> Bpat-3	VD-----L----Y--K--Q--SE--DLL--R-RTV-----I--KLM-FI. DPRIE-
<i>P. leniusculus</i> B	RD-----L-----S--KQ--A--SEL-KL-KGL-SQ-T-----LM--A. D-S---
<i>A. millepora</i> Bcn1	-----L--M--S--K--LGN-RS-AGQM-TT-KE--S--K-----A---F-RT...E
<i>D. melanogaster</i> Bv	R-N-L---VL---TWT-R---KT-EE--AQLSQT LKN--G-Y-----A--PTL-MIL...-H
<i>O. tenuis</i> BPol	--F-I---LL---Y--R--LDN-KQ--ADL-ASIVGLST---I-----E---A-FTTLD-RFQ
<i>H. sapiens</i> B1	-D--I---L---Y--K--L-NVKS--TDIMNE-RR--SD--I-----E-T-M--I, --T-A-
<i>H. sapiens</i> B3	-D---I---L---Y--K--LWSIQN--T-L-T--RK--S-L-I---A---P---MYISP-EA
Consensus	EDYPVDLYYLMDLNSYSMKDD--KL--LGDKLAETMK--ITSNFRLGFGSFVDKVVMPYV-STVP-K

FIG. 7. Multiple amino acid (AA) alignment and consensus sequence of β integrin ligand binding domains (LBD). The sequence from *B. glabrata* embryonic (Bge) cells and hemocytes are aligned with the LBD of all known invertebrate species and two human LBD GenBank Accession Nos.: *B. glabrata* Bge cell [AF060203], *D. melanogaster* BPS [J03251], *S. purpuratus* [U77584], *C. elegans* [U19744], *P. leniusculus* [X98852], *A. millepora* [AF005356], *D. melanogaster* Bv [L13305], *O. tenuis* [AF005357], *H. sapiens* β 1 [X07979], and *H. sapiens* β 3 [J02703]. The LBD consensus sequence was derived from the nine LBD listed in the figure and is shown in the bottom line. The periods (.) represent gaps in the sequence introduced by PILEUP (GCG) to align each sequence. AA residues identical to those of the Bge/hemocyte sequence are indicated by dashes. Reprinted with modification from *Gene*, Davids, B. J., X. J. Wu & T. P. Yoshino, Cloning of a β integrin subunit cDNA from an embryonic cell line derived from the freshwater mollusc, *Biomphalaria glabrata*, 1999, with permission from Elsevier Science.

sporocysts, derived from in vitro transformed miracidia, were found to produce daughter sporocysts (Yoshino & Laursen, 1995). This latter study represented the first direct linkage of miracidium-through-daughter sporocyst development under in vitro conditions. In an important breakthrough, Barnes, Bayne and colleagues have now succeeded in completing the entire intramolluscan cycle of *S. mansoni* development (miracidium-to-cercaria) under in vitro conditions by co-cultivation of larvae in the presence of Bge cells and employing a combination of several media formulations (Ivanchenko et al., 1999). These studies now provide an established in vitro culture system that can be used to identify and characterize host (Bge) cell factors critical to the regulation of larval differentiation, or that can be chemically modulated to screen various agents (e.g., growth factors, cytokines, snail plasma factors, etc.) for their effects on parasite development (Lardans & Dissous, 1998).

In followup experiments, we were surprised to find that, like *S. mansoni*, Bge cells also supported significant parasite growth and differentiation when other trematode species were used in this culture system. For example, miracidia of *S. japonicum* (Coustau et al., 1997) and the deer liver fluke, *Fascioloides magna* (Laursen & Yoshino, 1999), transformed to mother sporocysts, which in turn produced daughter sporocysts and mother rediae, respectively. In these studies, of particu-

lar interest was the observation that Bge cells did not form large cellular encapsulations around mother sporocysts of either species as was the case with *S. mansoni*. In order to confirm this observation, a direct comparison of *S. mansoni* and *S. japonicum* sporocysts was made using the Bge cell adhesion assay described previously. In contrast to 94% of *S. mansoni* sporocysts exhibiting Bge cell binding, only 51% of *S. japonicum* sporocysts possessed adherent cells. Moreover, using a semi-quantitative measure of adhesion intensity (1 = no binding, 2 = <10 cells/sporocyst, 3 = >10 cells but <50% of sporocyst surface with cell binding, and 4 = >50% of sporocyst surface with bound cells), a cell adhesion index (CAI) was calculated according to the following formula: total adhesion intensity score/# sporocysts evaluated. Preliminary results of this analysis indicate that Bge cell adherence to *S. japonicum* sporocysts was significantly less (CAI = 1.51) than their ability to adhere to sporocysts of *S. mansoni* (CAI = 2.48). Two possible explanations for this outcome may be (1) the tegumental surface ligands responsible for Bge cell adhesion differ, either quantitatively or qualitatively, between *Schistosoma* spp. or (2) *S. japonicum* larvae may be secreting a factor(s) that interferes with Bge cell-sporocyst binding interaction (Adema & Loker, 1997). However, regardless of the mechanisms involved, these molecular differences exhibited between Bge cells and different schistosome species may be related

to the snail host specificity normally expressed by these parasites. It is anticipated that continued efforts to identify and characterize these Bge cell adhesion receptors will facilitate ongoing molecular studies on comparable hemocyte receptors, and eventually shed light on fundamental questions related to host-parasite compatibility.

Bge Cell Genetic Transformation—a Tool for the Future: The ability to transfect Bge cells with foreign DNA and effect its stable in vitro expression represents another important step in fully developing the research potential of this cell line. Successful application of this technology to Bge cells would provide a number of extremely useful tools: (1) a homologous genetic expression system in which recombinant snail proteins can be produced in "native" form; (2) in conjunction with *S. mansoni* (or other schistosome species), an in vitro culture system for evaluating the effects of snail host products (e.g., hemocyte cytotoxic peptides, growth factors, etc.) on larval survival; and (3) a system for testing gene transfer methodologies or approaches in advance of attempts to transfect whole organisms. However, as alluded to in the introduction of this paper, in comparison to the arthropods, the field of molluscan transgenic technology is only in its infancy.

The first gene transfer attempts into molluscan cells were reported in 1996 in which transient expression of luciferase reporter gene constructs driven by heterologous *Drosophila* heat-shock protein (HSP70) or human cytomegalovirus (CMV, early) promoters was achieved in the Bge cell line (Lardans et al., 1996) and oyster heart primary culture cells (Boulo et al., 1996). In efforts to develop a homologous promoter system for DNA transfers into Bge cells, Laursen et al. (1997) cloned a Bge cell HSP70 cDNA, which was then used as a probe to isolate and characterize the entire HSP70 gene, including a putative promoter region (Yoshino et al., 1998). The promoter function of this region was confirmed by demonstrating the heat-inducible expression of luciferase activity (reporter enzyme) in Bge cells transfected with HSP70 promoter-luciferase reporter constructs (Fig. 8) (Yoshino et al. 1998). These results, although preliminary in nature, demonstrate the feasibility of employing the Bge cell line as model molluscan system for developing new or adapting previously successful approaches to the eventual establishment of efficient, stable

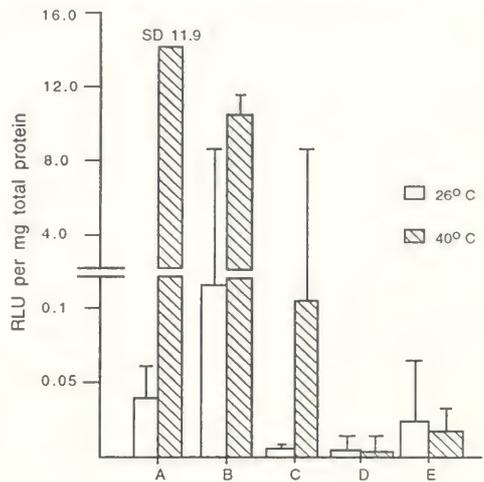


FIG. 8. Mean \pm SD of specific luciferase activity (in relative light units/mg protein) for *B. glabrata* embryonic (Bge) cells transfected with various test and control vector constructs, and subjected to heat-shock (40°C) or no heat-shock (26°C). A: Bge HSP_{0.5k} promoter-*Luc* construct; B: Bge HSP_{1.0k} promoter-*Luc* construct; C: Bge HSP_{0.5k} promoter only (no *Luc* control); D: *Luc* only (no promoter control); E: DOTAP only (lipofectin control). n = 3 independent replicates.

DNA gene transfers into snail cells and whole organisms. Whether or not advancement of this technology in molluscs will lead to practical applications in the control or prevention of human schistosomiasis is presently unknown. However, it is anticipated that in continuing to strive towards this goal, a great deal of valuable information on various molluscan genes, their regulation and the consequences of their expression on snail host-parasite interactions will be generated.

SUMMARY

Biomphalaria glabrata embryonic (Bge) cells, currently the only available molluscan cell line, is proposed as cellular model for circulating hemocytes of *B. glabrata*, a major snail intermediate host of the human blood fluke, *Schistosoma mansoni*. In addition to originating from the same snail species and possibly sharing a similar ontological origin, Bge cells and *B. glabrata* hemocytes also share important functional and biochemical

characteristics including substrate adhesive properties, phagocytic activities, encapsulation responses, and similar lysosomal enzyme content. Investigations of these properties in Bge cells should lead to development of useful molecular tools (e.g., DNA probes, antibodies) that, in turn, will facilitate similar studies on snail hemocytes. Such an approach currently is being applied to the identification and characterization of cellular adhesion proteins in *B. glabrata* hemocytes. In addition, Bge cells are further being exploited in the in vitro cultivation of larval trematodes of medical and veterinary importance and in the development of molluscan genetic transformation systems. To date the Bge cell line has proved to be an invaluable tool in its application to molluscan biotechnology, and will play an increasingly critical role in future studies on the molecular basis of snail-trematode compatibility.

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BIOMPHALARIA GLABRATA: A LABORATORY MODEL ILLUSTRATING THE POTENTIAL OF PULMONATE GASTROPODS AS FRESHWATER BIOMONITORS OF HEAVY METAL POLLUTANTS

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ABSTRACT

The potential value of pulmonate gastropod snails as biomonitors of pollution in freshwater environments is discussed, with the laboratory M line strain of *Biomphalaria glabrata* used to illustrate bioaccumulation of heavy metals. Adult *B. glabrata* exposed at 28°C to 0.25 µM chloride salts of lead, cadmium or mercury accumulated these heavy metals in the soft tissues within four weeks exposure. The mean tissue lead concentration increased approximately three fold, cadmium ten fold, and mercury 25 fold over the levels of these metals in snails not exposed to the dissolved chloride salts. Exposure to any of the three metal salts caused snail mortality. The mean LC₂₅ values for lead, cadmium and mercury at two weeks exposure were 82, 0.22 and 0.94 µM, respectively. Although survival was reduced in exposed snails, surviving individuals were viable as indicated by the relative levels of high energy phosphorus metabolites in the *in vivo* ³¹P NMR spectrum. The results suggest that pulmonate gastropods snails display potential for bio-monitoring heavy metal pollution in freshwater environments. Surveys of the natural molluscan populations in waterways of lower Egypt are currently underway in an effort to identify potential molluscs, including pulmonates, as biomonitors in polluted areas.

Key words: *Biomphalaria*, freshwater, biomonitor, heavy metals.

INTRODUCTION

Direct methods of chemical analysis have long been employed for identifying and quantifying environmental pollutants in air, water and soil. Although improvements in the sensitivity of such analytical methods will ensure their continued role as a means for monitoring pollution, the low concentration range of many environmental contaminants is often a serious obstacle, with analyses requiring special-purpose ultraclean laboratories and lengthily pre-concentration techniques. For many years, laboratory and field studies have indicated that analysis of pollutants in tissues of biological organisms can prove highly beneficial as an adjunct to traditional approaches of sampling and examination (Martin & Coughtrey, 1982). In aquatic environments, many plants and animals absorb and accumulate trace organic and inorganic pollutants, frequently concentrating these pollutants many-fold over the levels occurring in the natural abiotic environment.

Biomonitoring can provide significant advantages over direct chemical analysis. Be-

cause the pollutants are concentrated by the biomonitor, additional concentration prior to analysis is seldom required, thus simplifying the analytical procedure. In addition, biomonitors provide information on the "bioavailability" of a pollutant rather than the total abundance of a pollutant in the aquatic environment at large (Phillips & Segar, 1986). Exhaustive analytical studies to identify all chemical species of a pollutant are often unnecessary and investigations on the biological effects of each and every form may be avoided. Among the principal characteristics of a potential biomonitor are: (1) tolerance to the pollutant, (2) distinctive morphological changes associated with exposure to the pollutant, (3) accumulation of the pollutant within the organism, and (4) accumulation of the pollutant in a manner dependent on its concentration in the environment (Butler et al., 1971).

Although biomonitors have seldom been used as the sole means for routine detection and monitoring of aquatic pollutants, they have been employed to some degree for about three decades (Phillips & Rainbow, 1993). The "Mussel Watch Program" employ-

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ing *Mytilis* spp. for monitoring organic and heavy metal pollution is a well-known example demonstrating the considerable success achieved in monitoring contaminated marine and estuarine ecosystems (Goldberg et al., 1983; Cossa, 1989). While numerous biomonitors have also been identified for various pollutants in freshwater habitats, only a few studies have been conducted on molluscs (Phillips & Rainbow, 1993). Most investigation has focused on fish, plants and algae, although a few investigations have appeared on bivalves (Graney et al., 1984; Bias & Karbe, 1985), and Fantin et al. (1982) reported the accumulation of lead in *Viviparus viviparus*, a freshwater gastropod.

The present report summarizes the results of several investigations on a laboratory strain of *Biomphalaria glabrata*, a pulmonate gastropod, suggesting the potential of freshwater pulmonates as biomonitors of three heavy metals, cadmium and mercury, two List 1 substances (European Economic Community, 1976), and lead, a List 2 contaminant. The study was conducted to demonstrate the accumulation of heavy metals in the soft tissues and to examine the effects of metal exposure on snail viability and mortality. The results suggest that the metals do accumulate in snails and exposure to concentrations in excess of those generally encountered in contaminated environments a limited effect on snail survival. Thus, this pulmonate gastropod and related species may prove useful as biomonitors. A preliminary observation demonstrating that lead is accumulated within the digestive gland is also described.

MATERIAL AND METHODS

Snail Culture

The laboratory M line strain (Newton, 1955) of *B. glabrata*, the New World vector of *Schistosoma mansoni*, was continuously cultured from the egg stage at $24 \pm 1^\circ\text{C}$ in a commercial spring water (Arrowhead^R, Monterey Park, California, U.S.A.). Stock colonies were housed in 2 gallon aquaria. Snails were fed fresh Romaine lettuce supplemented with Aquarian^R (Mardel Laboratories, Waltham, U.K.) fish food, and were occasionally provided chalk as an additional source of calcium.

Metal Treatments and Mortality

Groups of 10 adult snails measuring 11.5 to 12.5 mm were placed in glass beakers containing 500 ml of an artificial spring water (MacInnis & Voge, 1970) prepared in pure water (< 15 mega-ohm/cm²). A population density of 1 snail/50 ml water replaced every three days was previously reported as that resulting in optimal growth (Thomas & Benjamin, 1974). Water was purified with a Millipore Milli-Q^R water system. "Ultra-pure" ($> 99.99\%$) reagent grade chloride salts of cadmium (Cd), lead (Pb), and mercury (Hg 2⁺), purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA), were dissolved to various concentrations. Preliminary trials were conducted to establish ranges of salt concentrations that resulted in mortality within a four-week exposure period. Thereafter, lead was tested at 0.25, 25 and 100 μM ; cadmium at 0.075, 0.1, 0.25 μM ; and mercury at 0.25, 0.5, 1.0 μM . In addition, however, a single short-term NMR experiment with mercury was conducted at selected higher concentrations (see below).

Experiments on the effects of metal exposure on mortality were conducted in incubators maintained at $27 \pm 1^\circ\text{C}$ (the approximate mean summer temperatures in the water-courses of lower Egypt). Each replicate experiment included one control without the heavy metals, together with three experimental treatments of each metal at the concentrations listed above. Mortality of the laboratory strain of *B. glabrata* at 28°C greatly exceeded that observed at the normal rearing temperature, 24°C , even after attempts were made to acclimatize snails to the higher experimental temperature before exposure to the metals. Snails were provided small portions of fresh lettuce at regular intervals such that food was always available. The entire experiment was replicated three times at each temperature.

Tissue Preparation for Metal Analyses

Levels of cadmium, lead and mercury were determined in the whole body of snails maintained at 0.25 μM , the approximate LC₂₅ for cadmium, the most toxic of the heavy metals (see below). Snails were maintained as described above and analyses were conducted after four weeks exposure. Snails were harvested in groups of four to six and their shells gently crushed between two petri plates. Shell fragments were completely removed and the

snail bodies were thoroughly rinsed in ultra-pure water. The shells were not analyzed during the present investigations.

Shelled snails were individually lyophilized and immediately digested with warm concentrated nitric acid (HNO_3) (25 mg/0.5 ml). The temperature was increased to near boiling. After cooling to room temperature an additional 0.25 ml of nitric acid was added. The solution was heated until it began to turn brown. After a third addition of 0.1 ml nitric acid, the volume was reduced to approximately 0.5 ml and 0.5 ml of 30% hydrogen peroxide (H_2O_2) added. The volume was reduced by heating and additional aliquots of hydrogen peroxide were added until the solution was clear. Again, the volume was reduced to 0.5 ml and 0.1 ml concentrated hydrochloric acid (HCl) was added. The volume was finally reduced by heating to 0.25 ml and was made up to 2 ml with water in a volumetric flask.

Analyses of Heavy Metals

Levels of cadmium and lead were determined by stabilized temperature graphite furnace atomic absorption spectroscopy at 228.8 and 283.3 nm, respectively, employing a Perkin-Elmer Zeeman /3030 spectrometer with metal specific-hollow cathode lamps and autosampler. Combustion was achieved in an Argon atmosphere. Standards were used to establish internal calibration curves for quantitation. Mercury was measured by cold vapor atomic absorption spectrometry at 253.6 nm employing a Perkin-Elmer 5000 spectrometer and argon as the carrier gas. Metal concentrations were established by comparison with external standards.

Preparation of Snails for *In Vivo* ^{31}P Nuclear Magnetic Resonance Spectroscopy (NMR)

Snail shells were removed as described above and approximately 20 shelled snails were placed in a 12 mm glass NMR tube. The snails were isolated in the observation region by placing a glass spacer, approximately 1 cm in length, in the bottom of the tube underneath the snails and securing the snails with a wad of glass wool added from above. Teflon tubing was inserted through a silicone stopper and into the snail mass. The tubing was then connected through a perfusion pump to a bubble trap and a fluid reservoir containing spring water, which served as the superfusate. The

latter was gassed with air through tubing attached to a small air pump. Spring water was continuously pumped through the snail bed at a rate of approximately 1.5 to 2 ml/min, and exited the NMR tube through tubing leading into a waste container. The apparatus employed was similar to that illustrated by Thompson & Lee (1985). Prior to analysis individual groups of snails were exposed to one of each of the heavy metals for four weeks at the concentrations listed above for each metal.

^{31}P NMR Analysis

Pulsed Fourier transformed ^{31}P NMR spectra of *B. glabrata* were generated at 121.5 MHz and 22°C in a wide-bore Nicolet 300 spectrometer interfaced with a Nicolet 1280 computer. A 45° (25 μsec) radiofrequency pulse, 409.5 msec acquisition time and 1 sec delay ensured maximum signal to noise ratio under non-saturating conditions. Individual spectra were generated from 4800 data acquisitions. A deuterium oxide field-frequency lock was employed prior to superfusion.

Assignments for the individual signals observed in the ^{31}P NMR spectra were based on chemical shift and a variety of analytical and NMR methods previously outlined (Thompson & Lee, 1987). To assess and compare the compositions of the *in vivo* ^{31}P NMR spectra between trials, curve fitting by computer simulations of Lorentzian line shapes were conducted to eliminate the contributions of overlapping spectral components. In all cases, residuals were less than 5% of the integrated area of the entire spectrum which is well within normal variation between experiments. Although the signals of biological NMR spectra seldom display exactly Lorentzian line shape, the total integrated intensities of the computer simulations for all samples were > 99% of the actual integrated intensities of each individual *in vivo* spectrum. The relative levels of ATP, phosphoarginine (PA) and inorganic phosphate (Pi) of metal exposed snails were compared with those levels in unexposed control snail preparations.

In addition to analyses of snails exposed to heavy metals for four weeks, a single experiment was conducted on snails exposed during the ^{31}P NMR analysis to high levels of mercury and changes in ATP, PA and Pi were continuously monitored, and a phosphorus index, $[\text{ATP}/\text{Pi}] / [\text{PA}/\text{Pi}]$, was calculated as an

indicator of the overall energy status of the snail preparation (Aunaas et al., 1991). Time course data were presented as the percentage change from the initial value. During these experiments spectra were generated each hour from 2400 data acquisitions.

Histological Examination of the Digestive Gland in Snails Exposed to Lead

The presence of lead in the digestive gland of *B. glabrata* was examined histologically by sectioning the tissue of snails exposed to 100 μM lead for eight weeks. Snails were shelled as described above and the digestive gland carefully excised from the head-foot and other tissues. The digestive gland was fixed in 10% formalin, dehydrated in ethyl alcohol and embedded in paraffin (Bancroft & Stephens, 1990; Pearse, 1985). Transverse sections, 5 μ thick, were prepared with a rotary microtome. Sections were then dried at 60°C, cooled and stained with 0.5% sodium rhodizonate for one h (Molnar, 1952). The stained sections were then differentiated in 1% light green for 1 min, and mounted in glycerin aqueous mounting media. Lead appeared scarlet red against a green background.

RESULTS

Metal Treatments and Mortality

The effects of four-weeks exposure to dissolved chloride salts of cadmium, lead and mercury on snail mortality were variable (Fig. 1). Generally, mortality increased with increasing concentration. Two-way ANOVA demonstrated a highly significant effect of metal treatment on mortality: lead, $P < 0.01$; cadmium, $P < 0.01$; mercury, $P < 0.05$. In all cases, a significant effect was also evident between mortality and time ($P < 0.01$), but no statistically significant interaction between treatment and time was evident for any metal. Snails were observed eating or moving about on the glass during most of the exposure period. Shortly before death, snails stopped feeding and withdrew into their shell. The same behavior was observed in trials with snails exposed to much higher levels of metals that resulted in rapid death.

To compare the toxicity of the three metals, the LC_{25} was estimated from the mortality data following two weeks exposure. Metal toxicity was cadmium > mercury > lead (Table 1). The differences between the metals were statistically significant at $P < 0.05$ as determined

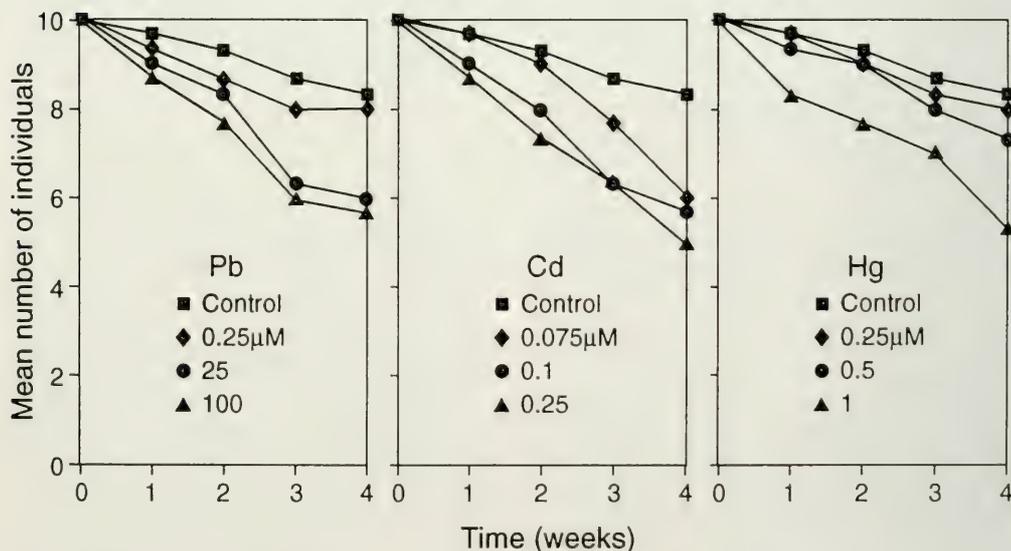


FIG. 1. Cumulative effects of cadmium, lead and mercury on mortality of *Biomphalaria glabrata* exposed to dissolved chloride salts for four weeks at 28°C. Data shows mean values for three replicates of 10 snails each. Statistical analyses are described in the Results section.

TABLE 1. LC₂₅ values and levels of cadmium (Cd), lead (Pb) and mercury (Hg) in the soft tissues of *Biomphalaria glabrata* exposed to dissolved chloride salts for two weeks at 28°C. Data shown as mean \pm standard error. See text for statistical analyses

Heavy Metal	LC ₂₅ (μ m)	Heavy metal level (μ g/g dry wt.)	
		Control	Treated
Cd	0.22 \pm 0.04	10 \pm 1	91 \pm 8
Pd	82 \pm 19	12 \pm 3	35 \pm 3
Hg	0.94 \pm 0.13	19 \pm 2	531 \pm 45

by Tukey's multiple comparison of means test.

Metal Content of Snail Tissue

Snails exposed to heavy metals for four weeks at 0.25 μ M accumulated these in the soft tissues (Table 1). In all cases, the increased level of metal in the exposed snails was statistically significant at $P < 0.05$ as determined by Tukey's multiple comparison of means test.

In Vivo ³¹P NMR Spectrum

Numerous NMR signals were observed in the ³¹P NMR spectrum of *B. glabrata* and the resonances for the phosphorus atoms of ATP and PA were clearly evident (Fig. 2A). Moreover, the β phosphorus signal of ATP was completely resolved. Spectral assignments are as follows: (1) β ATP; (2) glucose phosphate of uridine diphosphoglucose (UDPG); (3) nicotinamide adenine dinucleotide together with uridine phosphate of UDPG; (4) ∞ ADP + ∞ ATP; (5) β ADP + γ ATP; (6) PA; (7) free phosphatides, principally phosphatidylcholine; (8) glycerophosphorylcholine and diphosphatidylglycerol; (9) Pi; (10) phosphomonoesters, principally sugar phosphates; and (11) ceramide-aminoethylphosphonate.

The relative level of PA in *B. glabrata* is low (Fig. 2A) compared with the levels previously reported in other molluscs. This, however, was not due to handling or shell removal. Analyses of intact snails demonstrated the same level of PA relative to ATP (Fig. 2A, inset). Relatively low PA levels are typical of such snail species as *B. glabrata*, which employ cilia for locomotion (Barrow et al., 1980; Thompson et al., 1993). Shelled snails were employed for the present studies, because more tissue mass can be placed in the observation region than is possible with whole

snails with the shells intact, and NMR spectra with suitable signal/noise ratios can generated in much less time.

Effects of Metal Exposure on the *In Vivo* ³¹P NMR Spectrum

The ³¹P NMR spectrum was unaffected by four weeks exposure to cadmium, lead or mercury at any of the concentrations tested (results not shown). Direct comparison of peak heights and areas between control and exposed snails demonstrated that exposed snails had similar relative ATP and PA levels to those components observed in control unexposed snails. Acute exposure of previously unexposed snails to high levels of mercury, however, did result in observable spectral changes (Fig. 2B). At 10, 15 or 20 μ M, mercury significant decreases in signal intensities for PA and ATP were evident. Accompanying these effects was an increase in the relative intensity of Pi, as reflected by the phosphorus index. The above changes were generally progressive over the 6 hr exposure period. The relative level of ATP in snails exposed to 15 μ M mercury chloride approached 50% of its initial level after 6 hr exposure.

Histological Examination of Digestive Gland

Lead deposits were clearly evident in the tubule cells of the digestive gland in snails exposed to dissolved lead chloride (Fig. 3). No granules were observed in any sections from the digestive gland of unexposed snails.

DISCUSSION

The results summarized here demonstrate the potential for using *B. glabrata* and perhaps other related pulmonate gastropods as biomonitors of heavy metal pollutants, as indi-

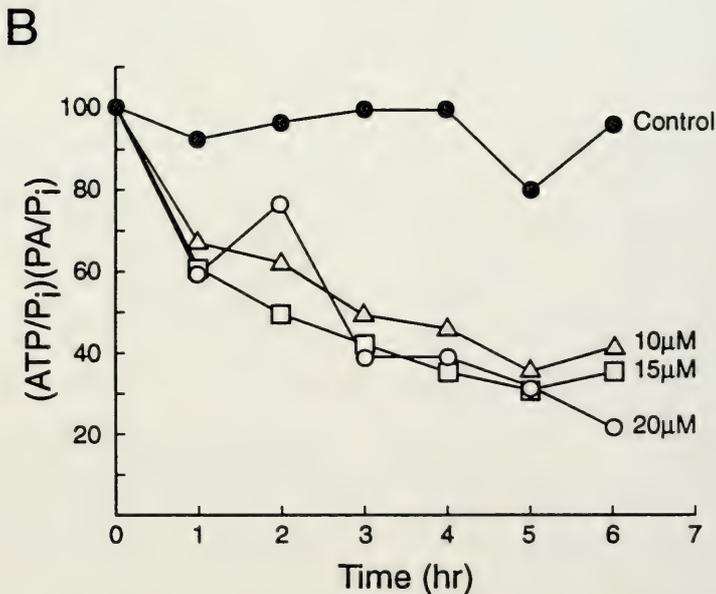
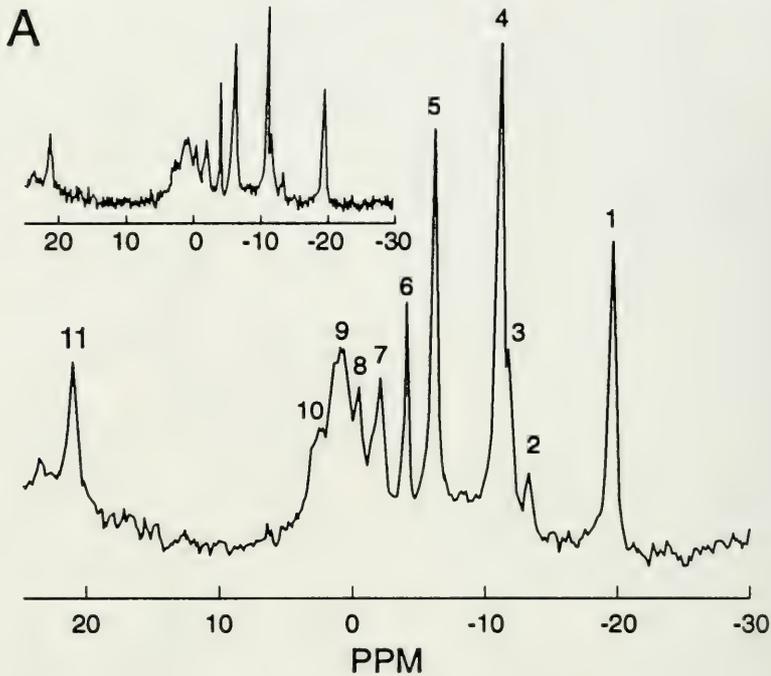


FIG. 2. *In vivo* ^{31}P NMR spectrum of shelled *Biomphalaria glabrata* and effects of acute exposure to very high mercury levels on the phosphorus index [(ATP/Pi)/(PA/Pi)]. A. Spectrum of shelled snails. Spectral assignments are as follows: (1) β ATP, (2) glucose phosphate of uridine diphosphoglucose (UDPG), (3) nicotinamide adenine dinucleotide and uridine phosphate of UDPG, (4) ∞ ADP + ∞ ATP, (5) β ADP + γ ATP, (6) phosphoarginine, (7) free phosphatides, (8) glycerophosphorylcholine and diphosphatidylglycerol, (9) inorganic phosphate, (10) phosphomonoesters, (11) ceramide-aminoethylphosphonate. Inset figure shows the spectrum of whole snails with the shell intact. Spectra required 4800 data acquisitions. B. Effects of 10, 15 and 20 μM Hg on the phosphorus index.

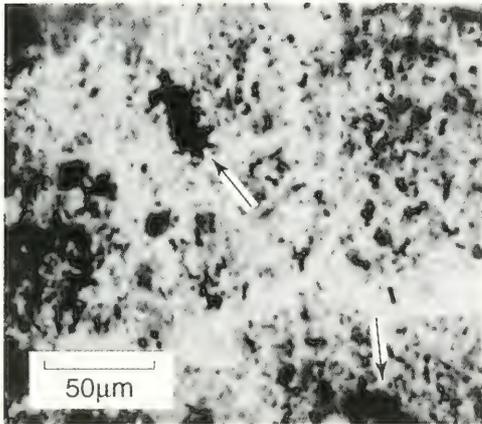


FIG. 3. Transverse section through the digestive gland excised from *Biomphalaria glabrata* exposed to 100 μM lead for eight weeks and stained with sodium rhodizonate (see Materials and Methods). Arrows indicate lead deposits.

cated for cadmium, lead and mercury. Snail mortality increased with increasing levels of each metal salt, but the LC_{25} levels for two weeks exposure were in every case two or more orders of magnitude higher than the levels presently observed in heavily contaminated freshwater environments (Forstner, 1984). At concentrations significantly less than the LC_{25} , snails accumulated significant amounts of lead and mercury in their soft tissues. Cadmium also accumulated in snails, but the lowest level tested was approximately equivalent to the LC_{25} . Results of previous studies with snails exposed to much higher levels of these metals indicated that metal accumulation is concentration dependent as well as temperature dependent (Abd Allah et al., 1998a). Moreover, metal exposure had no significant effects on egg laying, and eggs of exposed snails were equally viable as those of unexposed snails. Analyses, however, have not been conducted to determine if heavy metals accumulate within eggs with possible long-term consequences for the viability of progeny.

The results of the ^{31}P NMR analyses demonstrate that snails surviving heavy metal exposure are completely viable, as indicated by the effects on the phosphorus index. Recent studies demonstrated that acute exposure for 42 h to levels nearly an order of magnitude greater than the LC_{25} also had no effect on the ^{31}P NMR spectrum (Abd Allah, et al.,

1998b). In this regard, *B. glabrata* appears more tolerant to heavy metal exposure than is *M. edulis*. A similar ^{31}P NMR investigation by Aunaas et al. (1991) with the latter species demonstrated that exposure to 2.5 μM mercury or 16.5 μM cadmium for 96 h, concentrations equivalent to approximately three and five times respectively the LC_{50} for the same exposure time, resulted in a dramatic decline in the phosphorus index. With both metals, ATP and phosphoarginine were reduced and inorganic phosphate increased. In snails exposed to mercury, the phosphorus index decreased approximately 100-fold. In order to compare the effect of mercury exposure on *M. edulis* with *B. glabrata*, a single experiment was conducted during the present investigations by acutely exposing *B. glabrata* to extremely high levels of mercury. The levels tested, 10, 15 and 20 μM , were approximately equivalent to the same increase over the LC_{25} for mercury at two weeks exposure. The results were recalculated to show the phosphorus index (Fig 2B). At all concentrations, the phosphorus index decreased to less than half its initial value within 6 h. It should be emphasized that in both studies the levels of mercury tested are far beyond the levels reported in contaminated waters, and the results, therefore, have limited significance to the natural environment.

Numerous investigations have been conducted on the kinetics of heavy metal accumulation in molluscs and other biomonitors (George, 1982; George & Viarengo, 1985). Although such studies are essential for understanding the dynamics of uptake, depuration and detoxification (Simkiss & Mason, 1983), we have only begun such investigations. The present results demonstrate the accumulation of lead, histologically detected as distinct granules in the digestive gland of exposed snails, supporting the results of similar studies on other freshwater molluscan species exposed to lead (Fantin et al., 1982). Studies are being conducted to examine other tissues and to determine the accumulation sites for cadmium and mercury.

Pollution of the waterways of Egypt has recently become cause for serious concern by Egyptian authorities. Heavy metal contamination of the River Nile due to industrial activities surrounding Cairo (Lasheen, 1987), for example, has exacerbated the need to develop methods for monitoring pollutant levels (Olade, 1987; Peterle, 1991). Egypt has a rich fauna of freshwater snails (Brown, 1994),

many of which may prove to be of significance as bioindicators or biomonitors. Surveys are now being conducted in polluted waterways to determine heavy metal levels in native species and to evaluate their potential for monitoring pollution. In this regard, our interest in pulmonates also relates to the fact that Egypt is hyperendemic for schistosomiasis, and two pulmonate species, *Biomphalaria alexandrina* and *Bulinus truncatus*, are the major disease vectors. Studies are being conducted to establish how the effects of pollution and bio-accumulation of heavy metals affects schistosome survival and transmission.

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SNAIL-SCHISTOSOMA, PARAGONIMUS INTERACTIONS IN CHINA: POPULATION ECOLOGY, GENETIC DIVERSITY, COEVOLUTION AND EMERGING DISEASES

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ABSTRACT

This paper focuses on two snail-borne helminths in China infecting man, *Schistosoma* and *Paragonimus*, but primarily on *Schistosoma* (Asian caenogastropod-transmitted). Of concern are 1) the direction and timing of the evolution of the snail family Pomatiopsidae and the pattern of coevolution of *Schistosoma* and *Paragonimus* with defined clades within the Pomatiopsidae, 2) the question of monophyly of the Pomatiopsidae and its two subfamilies Pomatiopsinae and Triculinae, 3) the amount of genetic diversity within *Oncomelania* throughout China as revealed by allozymes and DNA sequences, 4) *Oncomelania* ecology and genetics with regard to different modes of transmission of *Schistosoma japonicum*, 5) the implications of the Three Gorges dam across the Yangtze River on emerging diseases.

Allozyme and COI gene sequence data confirm the monophyly of the Pomatiopsidae with its two subfamilies. The timing and direction of evolution of the Pomatiopsidae in Asia are congruent with area cladograms based on geological-paleontological events and evolving river systems (Yangtze, Mekong, Red) from about the end of the Miocene. The two subfamilies are highly divergent on the basis of morphology and ecology but less so on the basis of molecular genetics. The Pomatiopsidae are in a clade distinctly divergent from the Hydrobiidae, a family that has been used in China to classify the Pomatiopsinae and Triculinae. Figs. 2 and 3 show the relationships of the two parasite genera with the morphology-based clades of those caenogastropod families transmitting *Schistosoma* and *Paragonimus* throughout the world (excluding Africa for which too little is known). Two caenogastropod superfamilies are involved (Cerithacea and Rissoacea); Only *Paragonimus* has evolved with taxa of the Thiaridae, Pleuroceridae, Hydrobiidae; both parasite genera have evolved with various pomatiopsid taxa of both subfamilies. While all data available (reviewed in Davis, 1980, 1992) show that *Schistosoma* is tightly linked genetically in a coevolved system with its snail host, such a close linked genetic coevolved system operating at the population to genus level in *Paragonimus* is in doubt. *Erhaia* (Pomatiopsinae) and *Tricula* (Triculinae) were found in sympatry in Fujian Province, both reported transmitting putative *Paragonimus skrjabini*. *P. skrjabini* has been reported from at least 22 species of pomatiopsine and triculine snails in China. The evidence suggests that species of *Paragonimus* can switch hosts between different snail subfamilies and genera. The higher classification of *Erhaia* is in doubt. Should *Erhaia* be found not to be a pomatiopsid snail, then host switching can occur between some families (at least of the Rissoacea).

Based on COI and allozyme data there are three geographically isolated subspecies of *Oncomelania* on the mainland of China: *O. h. robertsoni* in Yunnan and Sichuan, *O. h. tangi* in Fujian Province, *O. h. hupensis* throughout the Yangtze drainage below the Three Gorges of the Yangtze River as well as parts of Guangxi and Zhejiang Provinces. Within *O. h. hupensis* there is considerable genetic diversity. Nei's minimum genetic distance among populations is 0.204 ± 0.085 . We conclude, on the basis of allozyme data from Miao River populations of *O. h. hupensis* in Hubei Province, that ribbed and smooth-shelled populations (shell with varix; shell growth with the same allometry) are the same species. Ribbing is found in populations affected by annual floods, especially the annual flooding of the Yangtze River. Smooth-shelled populations are upstream, above the effects of flooding. Thus, smooth-shelled *O. fausti* and *O. h. guangxiensis*, nominal taxa used by some authors, are synonyms of *O. h. hupensis*.

Using allozyme population genetics and COI gene sequence data we have found that there is considerable genetic instability in what one would initially presume to be a population. During

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flooding, snails are swept from flood plains and islands, float down the Yangtze, and are deposited in diverse locations or are swept into canals and become deposited along canals. These aggregates of snails derived from various places do not exhibit Hardy-Weinberg equilibrium for polymorphic loci. Haplotypes of the COI gene are shown to be most useful for demonstrating genetic instability. Data are presented from six "populations" from around Dong Ting Lake of Hunan Province. Sequence data were obtained from 10 individuals taken from each site. In a stable population, one expects 0 to 0.5% nucleotide differences within a population (0–3 nucleotide differences), or one to two haplotypes per 10 individuals. One such population was found at low elevation beyond effects of flooding. The other sites were around the edge of the lake and are flooded annually. Snails from these lowland localities had 6 to 10 haplotypes per 10 individuals (3.5 to 3.9% polymorphic sites), i.e. they were genetically unstable aggregates. The diversity of haplotypes enables us to map gene flow and patterns of intermixing of snails among localities.

Four different modes of transmission of *Schistosoma japonicum* are discussed. Differences are based on genetically differentiated subspecies, whether or not the populations are affected by annual flooding, life expectancy, population genetic stability, biogeography, and whether or not cattle play a dominant role in transmission of the disease. The implications of the Three Gorges Dam across the Yangtze River is discussed both in terms of the potential for snail transport into the vast reservoir but also of the impact on Poyang Lake, the largest lake in China and a major endemic area for schistosomiasis. A remote sensing image is used in conjunction with GIS technology to discuss snail-schistosome interactions and epidemiology on Poyang Lake marshlands.

Key words: *Oncomelania*, *Schistosoma*, schistosomiasis, *Paragonimus*, China, allozymes, DNA sequences, COI gene, population genetics, genetic diversity, coevolution, remote sensing, GIS, Three Gorges Dam, Yangtze River, Poyang Lake, evolution, ecology, disease transmission, emerging diseases.

INTRODUCTION

The Tropical Medical Research Center (TMRC) was established in 1996 at the Institute of Parasitic Diseases of the Chinese Academy of Preventive Medicine, Shanghai, P. R. China. Funded by the United States National Institutes of Health, there are four objectives: (1) to study the genetic diversity of three helminths infecting man; *Schistosoma*, *Paragonimus*, and hookworms; (2) to attempt to uncover emerging helminthic diseases by the study of genetic diversity throughout the southern provinces of China with emphasis on the riparian provinces of the Yangtze River; (3) to assess the impact of genetic diversity on human pathogenicity as well as on vaccine and drug research; (4) to assess the impact of the Three Gorges Dam on known and potentially emerging diseases.

By establishing a modern biotechnology laboratory in the TMRC and having open access to field sites throughout southern China, it became possible to address a number of questions that are fundamental to objectives of the TMRC. This paper focuses on the two snail-borne helminths, *Schistosoma* and *Paragonimus*, but primarily on *Schistosoma*. Studies are based (1) on basic principles of population genetics, and evolution; (2) on the patterns and processes of evolution and coevolution; (3) on the timing of evolution; and (4) on the direction of evolution. Additionally, we have focused on the ecological factors af-

fecting population genetics, evolution/coevolution, disease transmission and emerging diseases. A fundamental hypothesis continues to be: as snail populations diverge genetically in time and space, so must *Schistosoma* transmitted by these populations. This has proven to be the case in the Mekong River, Malaysia, and northern Thailand (Davis, 1980, 1992; Davis & Greer, 1980; Davis et al., 1976; Greer et al., 1997; Voge et al. 1978). What about *Schistosoma* in China? Does the same hold true for *Paragonimus*?

Major questions are: (1) How much genetic diversity is there among populations of *Oncomelania* (transmitting *Schistosoma japonicum*) throughout China? (2) If there are substantial differences, would one find parallel genetic differences among the schistosomes they transmit as called for in the hypothesis above? (3) Is *S. japonicum* the only schistosome infecting man in China? (4) Is there a triculine-borne schistosome infecting man in China? (5) What are the evolved relationships between triculine and pomatiopsine snails? Are they monophyletic? This is an important question as species of both lineages transmit schistosomes infecting man and other mammals. (*Schistosoma mekongi* in the Mekong River of Laos infects man and is transmitted by a triculine species.) (6) Are there substantial ecological differences among populations of Chinese *Oncomelania* and if so, are these reflected in genetic differences and differences in mode of transmission of *S. japonicum* such

that the differences must be taken into consideration in any epidemiological model for control of schistosomiasis? (7) What effect will the Three Gorges Dam have on the genetic diversity of *Oncomelania*, *Schistosoma* and the modes of disease transmission? (8) How many triculine and pomatiopsine species in China transmit *Paragonimus*? As numerous snail species of these subfamilies are implicated in the transmission of *Paragonimus* (Davis et al., 1994a), are there likewise numerous species of *Paragonimus* in China?

ANALYSIS AND SYNTHESIS

Direction and Timing of Evolution and Co-evolution: Phylogeny and Relevant Vectors

Figure 1 is derived from Davis (1979, 1992); it shows the area cladogram of evolving river drainage systems, the morphology-based phylogeny of the relevant taxa, and the timing of evolution. The direction of evolution is down evolving river systems from northern Burma and western Yunnan, China. *Oncomelania* (Pomatiopsinae) evolved as amphibious snails, while the Triculinae radiate as aquatic snails. However, *Erhaia*, currently classified in

the Pomatiopsinae, is found in an arc from northern India (Davis & Rao, 1997) into southern China (Davis & Kang, 1995); it is an aquatic genus. Note that *Schistosoma* infecting man is transmitted by genera of the two subfamilies, and that the triculine genus *Neotricula* is found not only in the Mekong River but also in southern China.

Figures 2 and 3 show the phylogeny of the superfamilies and families involved in the transmission of *Paragonimus* (human lung fluke) and *Schistosoma* (human blood fluke) infecting man in Asia. Our studies of *Schistosoma* are restricted to caenogastropod snails (snails with gill and operculum; separate sexes), thus excluding Asian schistosomes transmitted by pulmonate snails (snails with lung and no operculum; hermaphroditic), which do not infect man (also derived from the Indian Plate). On each branch of the trees a S or P is placed indicating the ability of taxa pertaining to those sections of the clades to transmit *Schistosoma* (S) or *Paragonimus* (P).

The result is an historical map of the evolution and coevolution of these genera with relevant snail lineages. We learn from these trees the following:

(1) *Paragonimus* evolved with two snail lineages, the Ceritheacea and Rissoacea, while

MONOPHYLETIC

DIRECTION

TIMING

TAXA

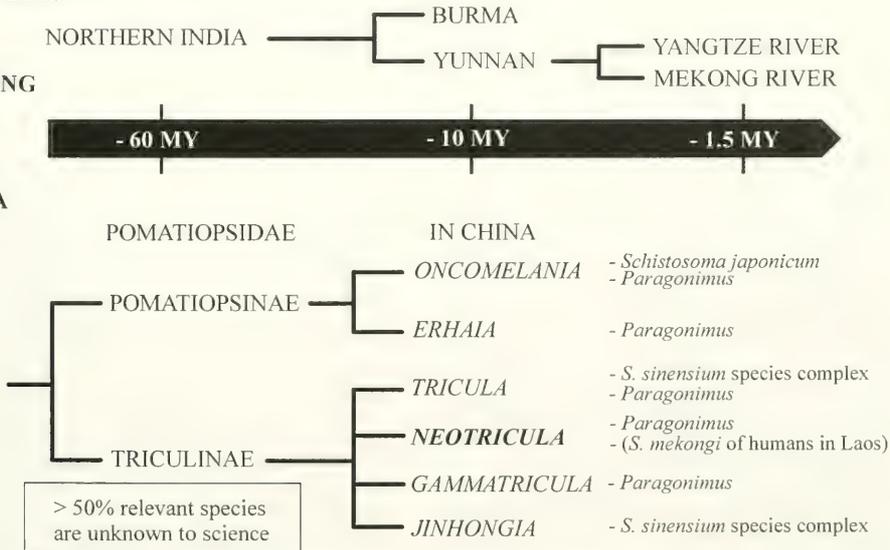


FIG. 1. Direction and timing of evolution of Asian Pomatiopsidae and relevant Asian river systems. The phylogeny of the relevant Pomatiopsidae is shown.

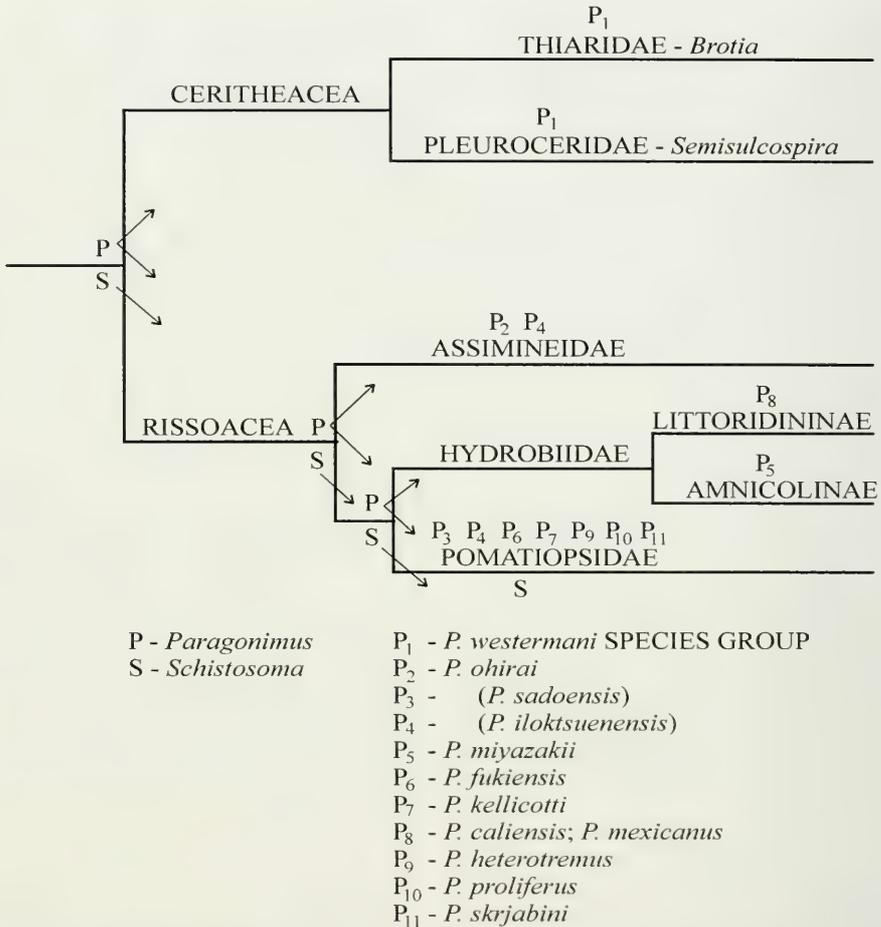


FIG. 2. Phylogeny of those caenogastropod superfamilies and families involved in the transmission of both *Paragonimus* and *Schistosoma*, and the pattern of co-evolution within those clades.

Schistosoma carried by caenogastropods was restricted to the Rissoacea. The Ceritheacea and Rissoacea are recognized as separate clades in the late Paleozoic Era, over 240 million years ago, long before the breakup of Gondwanaland.

(2) *Paragonimus* is transmitted by three families of rissoacean snails: Assimineidae, Hydrobiidae *s.l.*, and Pomatiopsidae. There may be one possible exception in that Blair et al. (1999) list *P. siamensis* as transmitted by the viviparid *Filopaludina martensi*. This is the only case where the Viviparidae have been implicated, and we see a need to confirm this possibility. *Schistosoma* is restricted to the Pomatiopsidae.

(3) Considering *Paragonimus*, the *P. westermani* species complex is restricted to the

ceritheacean families Thiaridae and Pleuroceridae. *Brotia* of the Thiaridae is of Gondwanian origin (distributed from India into S.E. Asia—southwestern China, Burma, Thailand, Cambodia, Indonesia, Malaysia, Philippines). *Semisulcospira* of the Pleuroceridae is derived from the east, related to N. American *Goniobasis*, *Pleurocera* and related taxa, diversifying in Japan and Korea and spreading to eastern and southeastern China including Taiwan. Based on this biogeographic pattern of origin and dispersal, *Paragonimus*, transmitted by these two clades of ceritheaceans, most likely is comprised of different species.

(4) The world's greatest species complexity involving *Paragonimus* is found in China and is reviewed by Davis et al. (1994a), and most recently by Blair et al. (1999). There are pos-

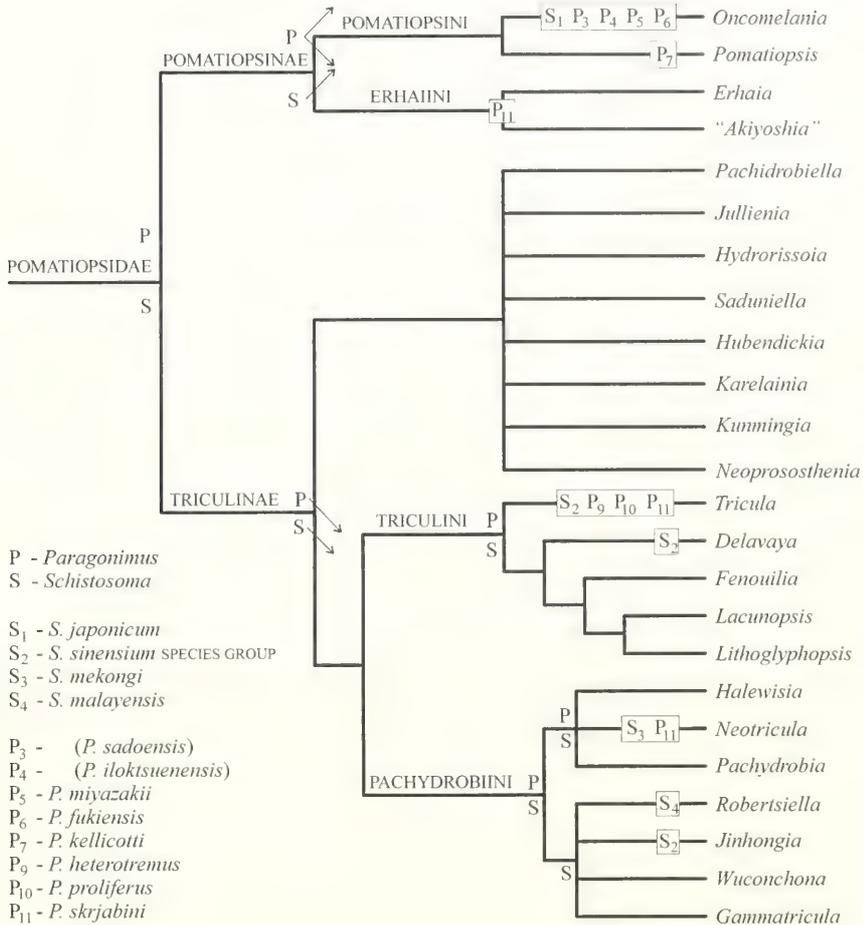


FIG. 3. Continuation of Fig. 2 with emphasis on the family Pomatiopsidae.

sibly 18 species (excluding numerous synonyms), but the exact number of species has yet to be determined on the basis of combining sound morphological and molecular data. This is an objective of the TMRC. One "species", *P. skrjabini*, deserves particular attention as it has been reported to be transmitted by some 22 species belonging to four genera of two families, and three tribes of the Pomatiopsidae (Davis et al., 1994a).

(5) *Oncomelania* transmits *Paragonimus*! There are two species of *Oncomelania* (Davis, 1979, 1980, 1992; Davis et al., 1994a). *Oncomelania minima* of Japan, a fully aquatic species, transmits *P. ohirai*; it does not transmit *Schistosoma*. *Oncomelania hupensis chiui* of Taiwan, more aquatic than amphibious, also transmits *P. ohirai*, but not *Schistosoma* in na-

ture. However, this subspecies can transmit all known geographic strains of *S. japonicum* in laboratory challenges. *Oncomelania h. tangi* from Fujian Province, China, is (or was, parasite now presumed extinct) the snail host for *P. fukiensis*. The sister genus to *Oncomelania*, *Pomatiopsis* in eastern U.S.A. transmits *P. kellicotti*.

Considering *Schistosoma* coevolution: (6) There are two species complexes of *Schistosoma* transmitted by the Pomatiopsidae that infect mammals. The complexes are recognized by adult worm morphology and, especially, egg morphology. Davis & Greer (1980) described the *S. japonicum* complex of three species, all infecting man with *S. japonicum* transmitted by *Oncomelania* of the Pomatiopsinae. *Schistosoma mekongi* (Mekong

River, Laos) and *S. malayensis* (Malaysia), are transmitted by two different genera of two different tribes of the Triculinae. Davis (1992) proposed a *S. sinensium* species complex in China and Thailand of at least three species, none of which, at this time, infect man. They are transmitted by three genera of two tribes of the Triculinae.

Specificity of Coevolution: All evidence to date indicates that schistosomes have coevolved through time with ever increasing specificity, so that today the snail-parasite interaction is species, regional and often population specific (Davis, 1992). While this is apparently true of schistosomes coevolving with pomatiopsine snails, the situation is not at all clear for *Paragonimus*. But then, there is much that is unknown about *Paragonimus* species boundaries, definitions, phylogenetic relationships, and parasite-snail interactions.

While there does seem to be a clear coevolved association between the *P. westermani* species complex and cerithacean snails, there appears to be board spectrum association between some species of *Paragonimus* and snails of different families of the Rissoacea. For example, *P. ohirai* is transmitted by both *Oncomelania* of the Pomatiopsidae (*O. minima* of Sado Island, Japan; and *Assimineia* of the Assimineidae in Japan and China). *Paragonimus fukiensis*, considered by some as *P. iloktsuensis* (a synonym of *P. ohirai*; Blair et al., 1999) of Fujian Province, China, is transmitted by *O. hupensis tangi*. *Paragonimus heterotremus* of China, Thailand, Laos, and Vietnam has been found in nature in *Tricula* (Pomatiopsidae) and reported from *Assimineia* sp. (Assimineidae) (Blair et al., 1999); it has been passed experimentally in various subspecies of *Oncomelania hupensis*, and *Neotricula aperta* (different subfamilies of Pomatiopsidae).

Finally, the taxonomic diversity and numbers of snail hosts of *P. skrjabini* suggest that, while there may indeed be more than one species of *Paragonimus* involved, this species may readily parasitize diverse, if not all, triculine taxa. On a recent field trip to Fujian Province, we collected, along with the parasitologists of the Fujian Institute of Parasitic Diseases, two species of minute rissoacean snails close to the headwaters of a small mountain stream. At the very top of the stream we collected a species of *Erhaia*. Some 100 m down stream we collected a species of *Tricula*. The Fujian IPD parasitologists said that they had screened thousands of these two

snails in the past and obtained the same species of *Paragonimus* from both species of snail, that is, *P. skrjabini*. As the snails are essentially sympatric, the implication is that species of *Paragonimus* can switch hosts between different subfamilies of the Pomatiopsidae.

There is a problem concerning the classification of *Erhaia*. Davis et al. (1985), Davis & Kang (1995), and Davis (1992) classified the genus in the Pomatiopsidae on the basis of overall anatomy. Species now classified as *Erhaia* had previously been considered by Chinese authors as *Bythinella* or *Pseudobythinella* of the Hydrobiidae. The above authors pointed out that the Chinese snails lacked the male reproductive system anatomy of *Bythinella*, a European genus belonging to the Hydrobiidae: Amnicolinae. The genus name *Pseudobythinella*, as applied to Chinese taxa, was preoccupied by the same generic name for a fossil Hydrobiidae of England. The problem of classification involves three morphological characters and character-states found in *Erhaia* that do not conform to overall Pomatiopsidae character-states: (1) the shape of the central tooth of *Erhaia* is trapezoidal while that of all other pomatiopsids is rectangular; (2) the shell has the characteristic shape of European *Bythinella*, a shape not found in all other pomatiopsids; (3) the spermathecal duct is so closely fused to the pallial oviduct as to seem indistinguishable from the pallial oviduct, while in all other pomatiopsids the spermathecal duct is seen to be clearly distinct from the pallial oviduct and separated from the latter. As *Erhaia* exists in an arc from northeastern India through Yunnan, China, down along the Yangtze River drainage into Hunan and Hubei with a species in Fujian Province, and as the genus is clearly not *Bythinella*, Davis et al. (1985), Davis (1992), and Davis & Kang (1995) considered the above three morphological character-states to be convergent on the Hydrobiidae: Amnicolinae.

To settle issue of familial status, the TMRC has initiated molecular genetic studies of *Erhaia*. Does *Erhaia* belong in the Pomatiopsidae, in the Amnicolinae, or is it unrelated to either?

The question arises: Is the important factor for *Paragonimus* transmission one of ecology rather than phylogeny? *Paragonimus ohirai* invades different rissoacean snail species in two different environments; *Oncomelania* in freshwater, *Assimineia* in brackish water. A second question then arises: if both a thiarid

or pleurocerid snail were sympatric with a rissocean snail along with appropriate crabs, would both transmit *Paragonimus* in that system? Does such sympatry occur in Asia, South and Central America? Davis (1982), in a review of historic and biogeographic factors involved in the evolution and radiation of all freshwater snail groups, noted that cerithacean and rissocean snail radiations in freshwater are generally biogeographically exclusive of each other. Where one finds populations of cerithacean snails, one generally does not find populations of rissocean snails in sympatry. This phenomenon requires close examination through field work and verification. For example, is the pleurocerid *Semisulcospira libertina*, host for *P. westermani* in Japan, sympatric with *Oncomelania minima* on Sado island? (4) It is noteworthy that rissocean families Stenothyridae and Bithyniidae, commonly found from the Ryuku Islands through southern China, are not implicated in the transmission of *Paragonimus*. We have found *Stenothyra* sympatric with triculine taxa. One would like to determine if *Stenothyra* can be found in sympatry with either a

cerithacean or rissocean in a habitat where *Paragonimus* is transmitted. The ecological requirements of the Bithyniidae perhaps remove them from any potential taxon hopping by *Paragonimus*.

The Question of Hydrobiidae in China and Monophyly of the Pomatiopsidae

Chinese workers have long considered *Oncomelania* to belong in the family Hydrobiidae (Liu et al., 1997; Liu, 1979; Kang, 1981, 1998a, b). On the basis of comparative anatomy of rissocean snails from China, there is no evidence for the family Hydrobiidae in China (unless *Erhaia* is found to belong to the Amnicolinae, and molecular data indicate the Amnicolinae belongs within the family Hydrobiidae). Based on sequencing data for the mitochondrial gene cytochrome c oxidase subunit I (COI), it is clear that the Pomatiopsidae, with *Oncomelania* and *Tricula*, belong to a family apart from the Hydrobiidae *s.l.* (Fig. 4, adopted from Davis et al., 1998).

There has been the question of the monophyly of the Pomatiopsidae with the Triculinae

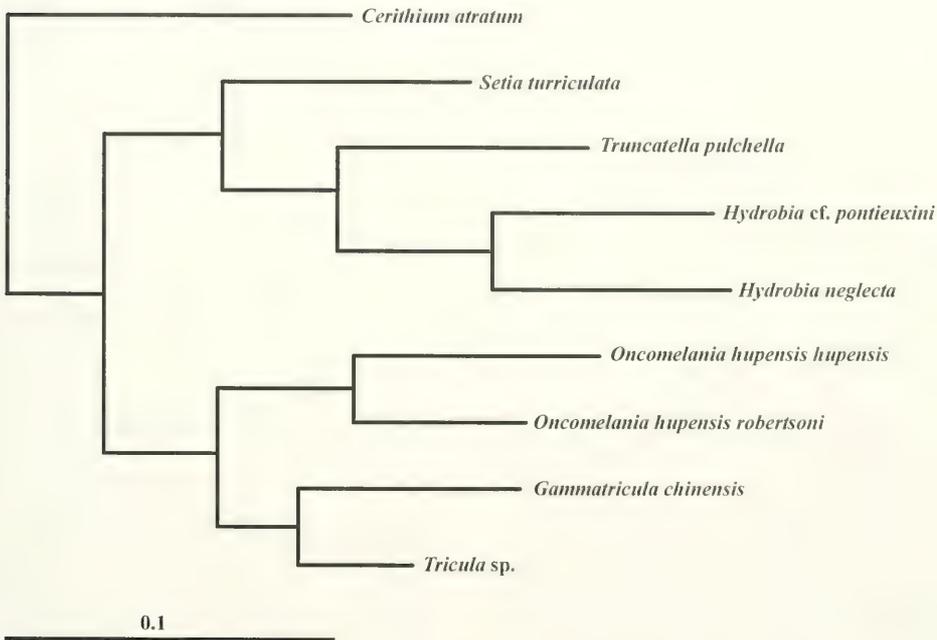


FIG. 4. Maximum likelihood tree based on partial sequences of the COI gene. *Cerithium atratum*, the outgroup, is a cerithacean snail; all others belong to the superfamily Rissoacea. *Setia* belongs to the Rissoidae; *Truncatella*, the Truncatellidae; *Hydrobia*, the Hydrobiidae. The lower cluster, greatly divergent from the Hydrobiidae, belongs to the Pomatiopsidae, with *Oncomelania* in the Pomatiopsinae and *Tricula*, *Gammatricula* in the Triculinae (adapted from Davis et al., 1998).

and Pomatiopsinae sister taxa. The question was raised because there are significant differences in the female reproductive anatomy between the subfamilies, and the inclusion of both in the Pomatiopsidae required considering the differences in how sperm enter the female reproductive system to have evolved from a common ancestor. In the Pomatiopsidae, sperm entry is via the spermathecal duct that extends from the bursa copulatrix to the anterior end of the mantle cavity. In the Triculinae, sperm enter into a spermathecal duct that ends at the posterior end of the mantle cavity, either beside the pericardium, or from passage through the pericardium.

Allozyme data (Davis et al., 1994b, 1995) and the COI data are concordant in demonstrating the monophyly of the Pomatiopsidae with the two sister subfamilies. Both data sets perhaps give insight into the evolution of the Chinese Pomatiopsidae. The COI data show *Tricula* sp. from Sichuan weakly supported in the triculine clade with some alternative trees placing it basal to the *Oncomelania* clade (Davis et al., 1998; fig. 3), other trees have *Tricula* sp. basal in the triculine clade. This is because the genetic distance between *Tricula* and *Oncomelania hupensis robertsoni* from Yunnan is less than it is between *Tricula* and *O. hupensis hupensis* (0.144 vs. 0.188). The anatomically derived *Gammatricula* is more genetically distant (0.171 vs. 0.195). The allozyme data set showed that smooth-shelled *Oncomelania hupensis* from Zhejiang Province was much more similar to triculine (*Gammatricula songi*) than two triculines were to each other *Neotricula lilii* vs. *Gammatricula chinensis* (Nei's D of 0.100 vs. 1.236). Because of this, the UPGMA phenogram based on the Nei distances did not show a pronounced separation of *Oncomelania* from the triculines (Davis et al., 1994b; fig. 4). On the basis of allozyme data from two studies (Davis et al., 1994b, 1995) smooth-shelled *Oncomelania* from Zhejiang Province were greatly divergent from ribbed-shelled *Oncomelania*. In the 1994b study, two smooth-shelled vs. one ribbed shelled populations differed by an average D of 0.173 vs. 0.051 between the two smooth-shelled populations. (We here correct an error in the 1994 paper. In figure 2, shells D and E are reversed; the smooth shell shown as D comes from population 1, not 2, etc.) In the 1995 study, the least divergent among 14 populations was between the smooth-shelled population from Zhejiang Province and smooth-shelled *O. hupensis*

robertsoni from Dali, Yunnan Province (D = 0.007), some 2,000 km away.

The following is a synthesis of the foregoing: (1) The Triculinae and Pomatiopsinae are highly divergent on the basis of robust morphological data; there are considerable differences in the ground-plans of the female reproductive systems. They are ecologically distinct. Molecular data support the monophyly of the Pomatiopsidae with the two divergent subfamilies. The subfamilies are soundly based. (2) *Tricula* from Sichuan is morphologically very similar to species of *Tricula* from Yunnan, China, to northern India (the type species is *Tricula montana* Benson, 1843, from northern India). *Neotricula* and *Gammatricula* are derived triculine genera distributed, in China, below the Three Gorges of the Yangtze River. (3) The primitive shell condition is small, smooth and without varix. Species of *Tricula* and *O. h. robertsoni* are small, smooth and without varix (Davis, 1979). (4) The direction of evolution is from the northern Indian Plate into northern Burma and western Yunnan, China, and subsequently down evolving river systems (Davis, 1979, 1980, 1992). (5) Considering molecular genetics, *Tricula* is more closely related to *O. h. robertsoni* than to downstream, derived, ribbed-shell *O. h. hupensis*. (6) The comparatively low molecular divergence between *Tricula* and *O. h. robertsoni* and between some triculine taxa and smooth-shelled *Oncomelania* that have close genetic affinity with Yunnan and Sichuan *O. h. robertsoni*, may indicate the relative recent divergence (Miocene) of the ancestral taxon into *Tricula* and all subsequent triculine taxa, and a small smooth-shelled *Oncomelania* that gave rise to all other *Oncomelania* dispersing down the Yangtze River and subsequently evolving ribs and the varix.

Oncomelania Genetic Diversity in China: Allozyme and Sequencing Data

Davis (1992, 1994) has reviewed the taxonomy of *Oncomelania* throughout Asia; these papers provide an overview that includes a discussion of the anatomical uniformity among the subspecies, the *Oncomelania hupensis* polytypic complex, and breeding genetics. The 1994 paper provides a species definition uniformly used for all of our studies of the Pomatiopsidae. The question then is: how genetically diverse is *Oncomelania* throughout China? On the basis of allozymes

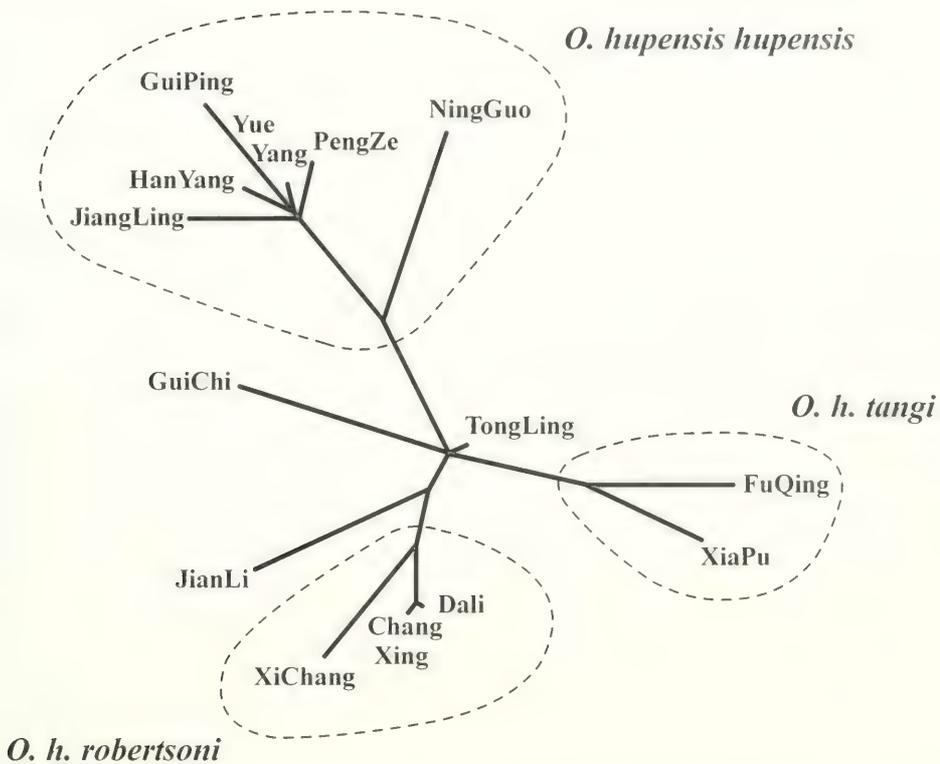


FIG. 5. FITCH tree based on allozyme data showing three clusters that are classified as subspecies. Three populations (Gui Chi, Jian Li, Tong Ling) are intermediate among the clusters and were considered "hybrids" by Davis et al. (1995). We now recognize these to be "unstable populations," aggregates of snails deposited from diverse locations by the annual floods of the Yangtze River.

(8 provinces, 14 populations, 30 loci), Davis et al. (1995) found that populations throughout China could be grouped into three subspecies (Figs. 5, 6). *Oncomelania hupensis robertsoni* occurs in Yunnan and Sichuan above the Three Gorges of the Yangtze River. It has a relatively small smooth shell without varix. *Oncomelania hupensis hupensis* occurs throughout the Yangtze River basin below the Three Gorges and in Guangxi Province. These populations have a relatively large shell of the same allometry as *robertsoni*, are primarily ribbed and with a strong varix. *Oncomelania hupensis tangi* is from Fujian Province, isolated from the Yangtze River drainage by tall mountains. This subspecies changes shell allometry; the shells are proportionally much wider than those of the other subspecies, are smooth, and have an exceptionally thick varix. Within *O. hupensis hupensis*, there is considerable genetic diversity;

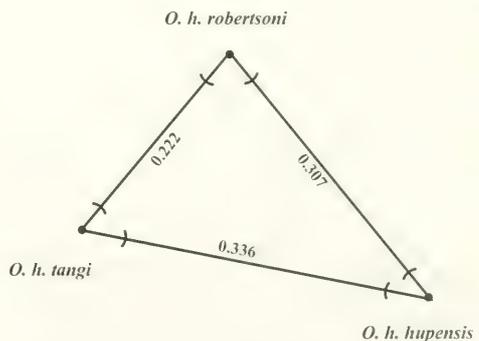


FIG. 6. Nei's minimum distances (in scale) among subspecies of *Oncomelania hupensis* based on allozyme data (standard deviations are marked as curved lines).

Nei's minimum $D = 0.204 \pm 0.085$ ($N = 21$). Sequence data from mitochondrial COI (Fig. 4) and *Cyb* genes (Spolsky et al., 1996) confirm the divergence between *O. h. hupensis* and *O. hupensis robertsoni*.

Smooth vs. Ribbed Snails: Systematic Implications: The question of the taxonomic status of smooth-shelled *Oncomelania* in China has been an ongoing debate for many years (Liu et al., 1981; Lou et al., 1982; Kang, 1998a). Above, we discussed the genetic relationships of two smooth-shelled populations from Zhejiang Province. Zhou et al. (1995) studied the allozymes of 34 populations from nine provinces. They used 16 loci of which five were esterase loci. In UPGMA clustering of Nei's (1978) "D", they also found that Sichuan and Yunnan snails clustered apart from *O. hupensis hupensis* (*sensu* Davis et al., 1995), as did the one population they had from Fujian (*O. hupensis tangi*). One population from Anhui with a smooth shell and varix, clustered with the ribbed-shelled populations. Using Fitch-Margoliash least-squared cluster analysis, all smooth-shelled *Oncomelania* clustered together, but the Anhui smooth-shelled population was basal and distinctly apart, rather intermediate between the smooth and ribbed-shelled populations. Zhou et al. (1995) concluded that there were two taxonomically distinct groups: ribbed and smooth-shelled types. Aside from the Sichuan, Yunnan and Fujian populations, they examined only three other smooth-shelled populations, one from Anhui and two from Jiangsu Province. They did not consider the shell morphological and biogeographical differences that separate *hupensis*, *robertsoni* and *tangi*. They did consider that smooth-shelled population groups might be separated into subspecies, for example, those from Fujian, Yunnan, Sichuan, and the hilly region of Jiangsu Province at the extreme eastern edge of China.

The Taxonomic Status of Smooth and Ribbed Shelled Oncomelania in China: We initiated work in 1994 in an attempt to resolve the smooth vs. ribbed-shell problem in the Yangtze River below the Three Gorges, having determined that *robertsoni* and *tangi* were distinct taxa. It has long been known that the ribbed-shelled *Oncomelania* occur only in China, in the marshes and flood plains of the Yangtze River and flood plains of the lower tributaries of the Yangtze River. Smooth-shelled *Oncomelania* were to be found in

"hilly" regions (Liu et al., 1981; Lou, et al., 1982). We used the Miao River of Hubei Province as a natural experiment (Davis et al., 1999). This small river, 25 km long, has ribbed-snail populations along the lower half and smooth-shelled populations in the upper half. The ribbed-shelled populations live on the flood plains of the Miao River, and are subjected to annual flooding. The smooth-shelled populations live above the effects of flooding. There is a bridge across the river, 13 km from the mouth of the river, just above the point reached by the annual floods. We studied the allozymes (starch gel electrophoresis, 35 loci) from four populations above the bridge and three populations below the bridge (example of shell types shown in Fig. 7). There were three control populations outside the Miao River drainage. The mean number of individuals studied from each population ranged from 44 to 122. The results were conclusive: ribbed-shelled and smooth-shelled populations did not assort into discrete clusters; one species is involved, *O. hupensis hupensis*. The mean overall Nei's (1978) D was (0.038 ± 0.035) . The mean D for populations above the bridge was (0.024 ± 0.016) ; for below the bridge, (0.045 ± 0.036) . A FITCH tree, based on Wright's modified Rogers' D , shows intermixing of ribbed and smooth-shelled populations (Fig. 8).

Based on this natural experiment, populations of *Oncomelania* below the Three Gorges of the Yangtze River, within the Yangtze River drainage, that are smooth (but with varix), and with the same allometry as snails of ribbed populations in the lower Yangtze drainage, are one subspecies, *O. hupensis hupensis*. This excludes the smooth-shelled populations in the hills of Jiangsu and Zhejiang Provinces. Ribbing is associated with annual flooding of the Yangtze River and its tributaries. Snails from any elevation, or a man-made situation that removes a population from the annual floods, attain a smooth shell but still retain the varix. Molecular genetic data do not support the concept of different taxonomic status for these two shell types. Accordingly, *Katayama fausti* Bartsch, 1925, is a synonym of *O. hupensis hupensis* Gredler, 1881. (*Katayama* used to be used as a genus to include all smooth forms of *Oncomelania hupensis*.) This same conclusion was reached by Lou et al. (1982), who noted that *fausti* and *hupensis* lived over the same geographic region and that the ribbing vs. smooth condition was strictly related to elevation above flooding.

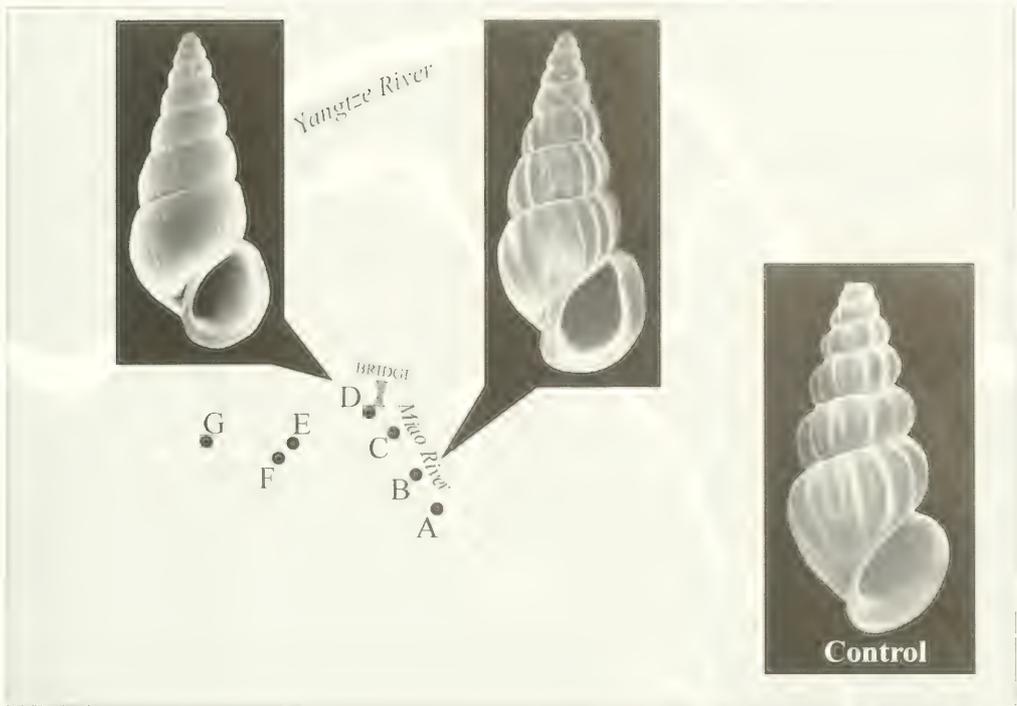


FIG. 7. Miao River localities (Hubei Province) of smooth-shelled and ribbed-shelled populations of *Oncomelania hupensis*. Populations with ribbed shells are below the bridge (A, B, C) and are inundated each year by the annual flooding of the Yangtze River. Populations above the bridge (D–G) have smooth shells; they are not flooded each year. The control snail is from the flood planes of the Yangtze River in Gui Chi County, Anhui Province.

Based on allozymes, smooth-shelled *O. hupensis guangxiensis* Liu et al., 1981, from Guangxi Province is also a synonym of *O. h. hupensis* (Davis et al., 1995).

It has yet to be determined how the eastern Chinese hill-dwelling populations of Jiangsu and Zhejiang relate to smooth-shelled snails studied here from the Miao River and Guangxi Province. These few special populations require intense study. Are they truly genetically divergent from *O. h. hupensis*? Are they part of the *robertsoni* complex, but have independently evolved a varix? Are they a distinct subspecies?

Ecology and Genetic Instability: Three populations studied by Davis et al. (1995) did not group with populations forming the *hupensis*, *tangi* or *robertsoni* clusters: GuiChi and Tong Ling from Anhui Province and Jian Li from Hubei Province (Fig. 5). They were called hybrids between the *robertsoni* and *hupensis* genomes. After revisiting and collecting snails

on Lao Zhou Island (an island in the Yangtze River in Tong Ling County, Anhui Province), we now understand why these populations did not cluster with one of the three subspecies clusters. Using COI sequence data, it is clear that these populations are indeed *O. h. hupensis*. What accounts for these results? These three localities are flooded, covered by water and swept during the annual floods. Snails found in these locations are not populations, really, but aggregates of snails imported from diverse areas and deposited with the receding floodwaters. Such populations have been called “genetically unstable” aggregates (Davis et al., 1999) in which true population structure does not attain and HWE is not attained in polymorphic loci.

Floatation during Yangtze River flooding is a major source of dispersion for *Oncomelania hupensis hupensis* and the schistosomes they transmit. This phenomenon is apparently not known outside China. During the floods, snails are lifted of the islands in the Yangtze

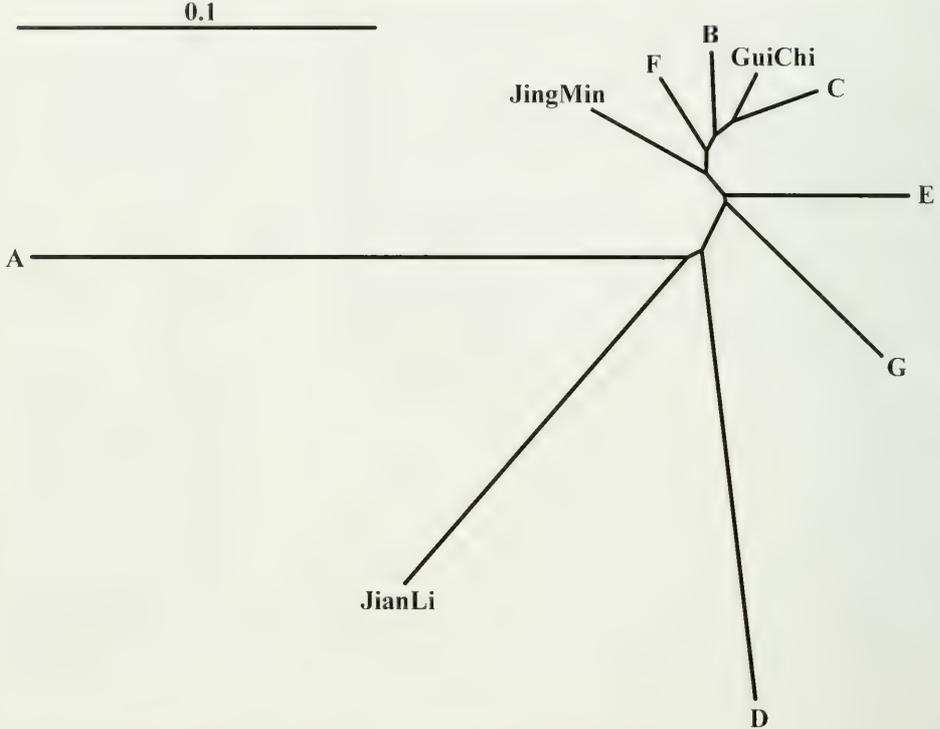


FIG. 8. FITCH tree based on Wright's modified Rogers' genetic distance (allozyme data) for ribbed (A–D) and smooth-shell populations (D–G) from the Miao River. Control populations are from Jian Li, Jing Min and Gui Chi.

and flood plains and floated by the millions down the river to be deposited on downstream flood plains or swept into canals when the flood gates are opened. The impact on importation into the canals of Hubei Province have been documented by Xu & Fang (1988), Xu et al. (1989, 1993), and Yang et al. (1992). While snails on the islands either float off or drown, snails on the flood plains often escape flooding by climbing tree trunks, often to heights of more than three meters.

As the vast flood plains of the Yangtze River below the Three Gorges are swept by floods each year, causing snails to be dislocated in vast numbers and carried over considerable distances, the TMRC is researching the effects of such dislocation and mixing on the genetics of both snails and the schistosomes these snails transmit. These aggregates of flood-deposited snails are some of the most highly infected snails in China. We have selected benchmark sites of stable populations and unstable aggregates of snails in different provinces. A stable population is one beyond

the effects of annual flooding and sufficiently removed in tertiary stream systems that there is the possibility that there has been no immigration or emigration for many years, and the population of snails has been out-breeding within the confines of the isolated population. Schistosomes in this stable populations would, theoretically, be genetically uniform, in contrast to the schistosomes isolated from unstable snail aggregates. What one discovers in analyzing schistosome genetics very much depends on schistosome breeding structure, how much one can truly sample of the schistosome population overall when one has to screen 6,000 snails to find four to six infected snails and one is limited to assessing the genetics of worms derived from the cercariae of only these few snails. Such low rates are the rule in endemic areas and in sites with stable populations. While coevolution of snails and schistosomes has been clearly demonstrated through breeding and infectivity studies (reviewed: Davis, 1980, 1992), it is not at all sure that studies of structural genes or mitochon-

drial genes will demonstrate the pathways of schistosome genetic diversification. Studies are in progress to attempt to answer some of these questions.

We have found a way of demonstrating genetic instability within and between aggregates of *Oncomelania hupensis hupensis* using haplotypes of the mitochondrial COI gene. We used six populations from Hunan Province as an example. Table 1 gives local-

ity data. Figure 9 provides a topographic map of China showing Dong Ting Lake in relationship to the Yangtze River. Figure 10 is a GIS map of the locality data with reference to Dong Ting Lake, the Yangtze River, and elevation contours. Table 2 gives the number of nucleotide differences among ten individuals from each population, the accumulative number of differences and the number of haplotypes (mitochondrial genes are maternally in-

TABLE 1. Locality data for six populations of *Oncomelania hupensis hupensis* from Hunan Province used for COI sequence analyses. Coordinates are in decimal degrees. These localities are mapped in Fig. 10.

Locality No.	Prefecture	County	Township	Administrative Village	Natural Village	Latitude	Longitude
MG96.16	Changde	Li Xian	Fang Shi	He Jia	He Jia Grp	29.79°N	111.44°E
MG96.17	Changde	Hanshou	Po Tou	San Xing	Huang Long Shan	28.92°N	112.13°E
MG96.19	Yue Yang	Yue Yang	Jun Shan	Mu Hu Pu	He Hua Zhou	29.37°N	113.00°E
MG96.20	Zi Yang	Yi Yang	Zi Hu Kou	Shi Ma	Huang Jia Hu	28.74°N	112.55°E
MG96.21	Yue Yang	Xiang Yin	Qing Tang	Shang Shan	Chen Jia Wan	28.86°N	112.89°E
MG96.22	Changde	Li Xian	Jiu Wan	Gan Jia	Meng Jiang Wai	29.56°N	111.92°E



FIG. 9. Topographic map of China showing the lowland basins of China, and the relationships of Dong Ting Lake and Poyang Lake to the Yangtze River. Dong Ting Lake of Hunan, China, is residual from a vast lake that filled the great basin of central China, i.e. Hubei Province north of the Yangtze River and Hunan Province south of the river. The great basin involves a vast area on either side of a line from Yichang to the east of Wuhan. This basin, full of marshes, lakes and canals is the most impacted by *Schistosoma japonicum* today. Poyang Lake, the largest lake in China, is likewise a vast area of marshlands heavily impacted by schistosomiasis.



FIG. 10. GIS-facilitated map of the Dong Ting Lake district.

TABLE 2. Genetic diversity, based on COI sequencing data, within and among populations of *Oncomelania hupensis hupensis* from Hunan Province. A–F represent these populations in Table 3 and Fig. 11.

	A	B	C	D	E	F	A–F
	MG96.16	MG96.17	MG96.19	MG96.20	MG96.21	MG96.22	TOTALS
No. individuals	10	10	10	10	10	10	60
No. and % polymorphic sites	8 (1.30%)	24 (3.80%)	25 (3.90%)	25 (3.90%)	23 (3.60%)	22 (3.50%)	75 (11.80%)
No. of nucleotide diff. (mean + stand. dev.)	2.84 ± 2.2	7.24 ± 11.0	6.82 ± 9.9	7.11 ± 10.7	7.18 ± 10.8	9.18 ± 17.0	9.75 ± 19.8
No. haplotypes	2	9	10	10	8	6	40

herited and thus only the female complement of DNA is involved). Table 3 provides the pairwise comparisons among these populations (total number and percentage of nucleotide differences). In Figure 11, a maximum likelihood tree shows the relationships among the haplotypes from these “populations.”

Most of these populations are from islands

or flood plains of Dong Ting Lake, areas that, unlike Hubei Province across the “punch bowl” of the Yangtze River, are unprotected by a continuous series of dikes that keep flood waters out. Note that at Yueyang, the Yangtze River connects to Dong Ting Lake, and here is the outlet for the four rivers of Hunan that flow into the lake. During flood season there is consid-

TABLE 3. Pairwise comparisons of six Hunan populations of *Oncomelania hupensis hupensis* for nucleotide differences (total number and percentage) in a 638 basepair fragment of the COI gene.

Populations	A	B	C	D	E	F
A	—					
B	11.5 (1.8%)	—				
C	13.2 (2.1%)	7.8 (1.2%)	—			
D	12.3 (1.9%)	7.1 (1.1%)	7.4 (1.2%)	—		
E	12.4 (1.9%)	7.8 (1.2%)	8.1 (1.3%)	7.8 (1.2%)	—	
F	11.5 (1.8%)	11.9 (1.9%)	12.8 (2.0%)	12.0 (1.9%)	11.0 (1.7%)	—

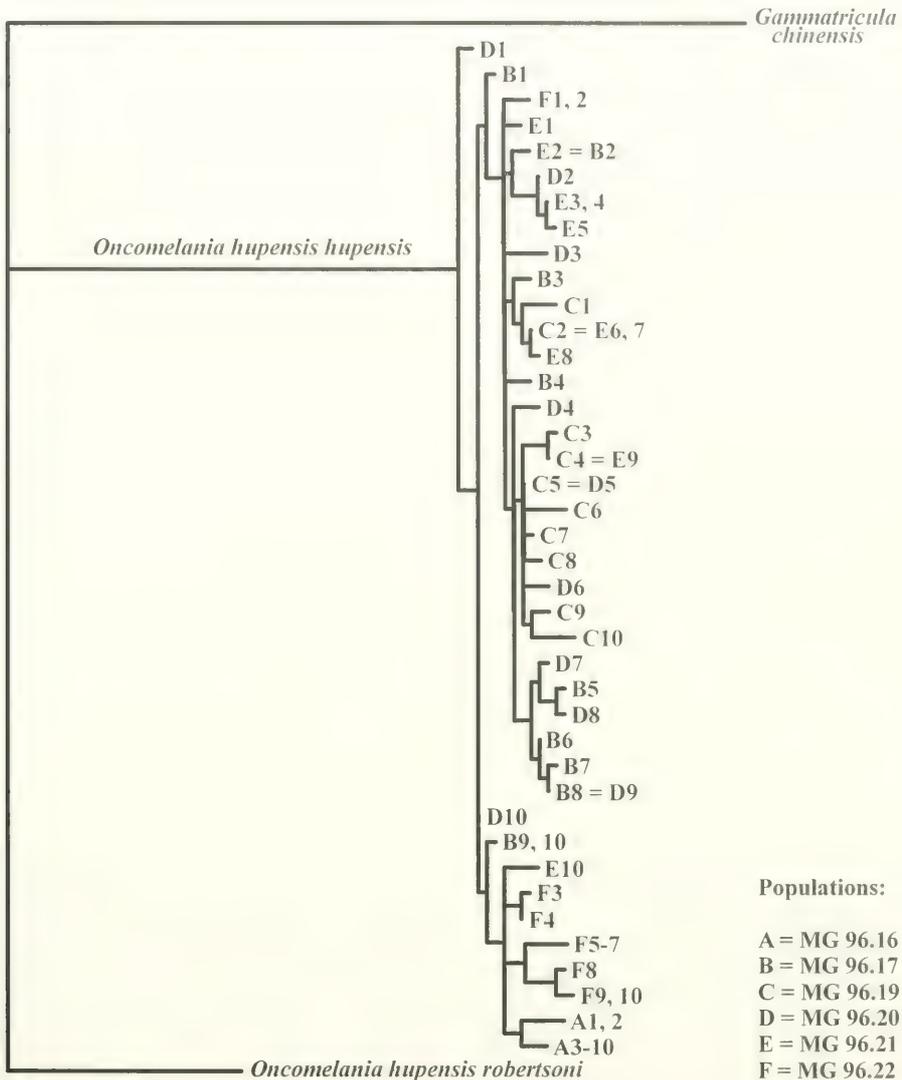


FIG. 11. Maximum likelihood tree based on partial sequences of the COI gene showing the interspersed haplotypes. Population A is stable. Populations B, C, D, E, and F are "unstable" populations, affected by flooding.

erable increase in the volume of the lake and inundation of grazing lands and marshes.

In a stable population, one would expect an average of 0 to 0.5% nucleotide differences within populations (0 to 3 nucleotide differences), or one to two haplotypes per ten individuals. As seen, only population A (MG 96.16) is stable with two haplotypes involving eight polymorphic sites; all individuals of this population are in a unique cluster apart from clusters with individuals from other localities. Its closest genetic relationship is with six individuals of population F (MG 96.22) and one individual from population B (MG 96.17). These two localities are the closest ones to A. Population A is far west of Dong Ting Lake and removed from the effects of the annual flood. It is in slightly elevated terrain, in the 100 to 500 meter contour lines on the map.

All the other so-called populations are "unstable," aggregates of individuals with a significant number of different haplotypes, indicating import from different areas of Hunan along Dong Ting Lake. For example, individuals from locality B (MG 96.17), at the west end of the lake, share haplotypes with individuals in localities C, D, E and F. Population C (MG 96.19), the closest locality to the Yangtze River, forms its own subcluster, except that the cluster includes an individual from D, and one C individual is found clustering with individuals from B and E. In two populations (C and D), there were ten different haplotypes for ten individuals; we had not reached the limit of numbers of haplotypes to be found. What we consider unstable aggregates of individuals had 60% to 100% different haplotypes per ten individuals.

These data clearly show that the flood-impacted areas around Dong Ting Lake host aggregates of snails swept together and de-

posited from diverse areas along the Yangtze and Dong Ting Lake, and that there is a surprising number of haplotypes to be found among these aggregates. These are not natural populations in the usual sense of the term. These findings have considerable implications for the genetics of schistosomes transported by these snails. Our data also indicate that with sequence data from four individuals of a population, when only one haplotype is found, the probabilities are very good that the populations are "stable", that is, a Yunnan population from Dali (Yunnan and Sichuan populations are in high plateaus or hilly regions unaffected by annual floods).

We will use haplotype diversity to map pathways of dispersals and introductions throughout China based on gene flow analyses (Hudson et al., 1992). Wilke & Davis (1999) have shown how this can be done with gastropod populations using European *Hydrobia ventrosa* and *Hydrobia ulvae* as a paradigm.

Ecology, Modes of Schistosome Transmission, and the Three Gorges Dam

Modes of Schistosme Transmission: Epidemiological models of schistosome transmission and control in China might be improved taking into consideration the fact that there are four distinctly different modes of transmission (Table 4). The fundamental differences are major. (1) There are genetically distinct subspecies of *Oncomelania hupensis* involved in transmission. (2) There are distinctly different snail ecologies associated with the genetic differences. (3) There are different snail life tables associated with the ecological differences. (4) Transmission to man is primarily caused by cattle infections (water buffalo and other cattle) in some areas, not in

TABLE 4. Modes of transmission of *Schistosoma japonicum* in China.

Mode of transmission	Snail subspecies	Annual floods	Environment	Short life expectancy	Stable population structure	Three Gorges	Cattle > 85% responsible for transmission
I	<i>hupensis</i>	yes	lake*	yes	yes	below	yes
II	<i>hupensis</i>	yes	river*	mixed	no	below	no
III	<i>hupensis</i>	no	canals* canal networks	no	yes	below	no
IV	<i>robertsoni</i>	no	high elevation*	no	yes	above	no

*High elevation = 500–2000m; below the Three Gorges along mid-to lower Yangtze River, elevations mostly 0–200 m; see text for details.

others. (5) Transmission is by snails of either genetically unstable aggregates, or by snails in stable populations.

The different modes may be better understood by discussing the biogeographical regions that set up different modes of transmission in China.

I: Poyang Lake. The subspecies of snail is *O. h. hupensis*. The lake, located in Jiangxi Province, is the largest lake in China (Figs. 12, 13). It is a major focus of transmission in China. We consider that over 85% of transmission is attributable to cattle (the hypothesis of the current TMRC epidemiological study (EPI) on Poyang Lake to assess the importance of cattle in maintaining the life cycle of *Schistosoma japonicum* relative to infecting man). The life expectancy of snails is considered to be about one year (Zhang et al., 1996).

All transmission to man occurs in the lake! All living accommodations for man and domestic animals are outside the dikes that

contain the lake. All snails are found on the flood plains and on the numerous islands inside the dikes. The annual dynamics of the lake dictate the life cycle of the snails. One may consider a "bathtub" model. With the annual flood of the Yangtze River, the lake fills like a bathtub, covering most of the islands with water contained by the dikes that have been built over centuries. Snails have no place to retreat to and adult snails presumably, for the most part, drown. Subspecies of *Oncomelania* are capable of living four to five years. In nature, their life expectancy exceeds two years. In Poyang Lake, life expectancy is about one year.

With reference to drowning, *Oncomelania hupensis* is an amphibious species. The young stay submerged during their early stages of development, often floating upside down, feeding on the surface of quiet water. As adults, the snails are found out of but near water, on the banks of irrigation ditches



FIG. 12. Topographic map of China showing the position of the dam across the Yangtze River and the reservoir that will eventually drown the Three Gorges. The dark tent-like bars focus ones attention to the mountain chains that serve as a funnel for the Yangtze River with the Three Gorges at the narrow end of the funnel. These mountains and the constraint of the Three Gorges have kept in isolation snail faunas and parasites in Yunnan and Sichuan provinces above the Three Gorges from faunas below the Three Gorges.



FIG. 13. GIS-facilitated map of Poyang Lake using ArcView 3.0a in connection with the Digital Map Database of China (ESRI Inc.) showing localities of the TMRC epidemiological study in progress. The lake is shown in full flood during the rainy season (late May to August or September).

and swamps, on shaded moist soil. During drought, the adults move down into the soil and aestivate. Adults cannot withstand continual submersion; they will drown. Because of this, drowning is a method used to control schistosomiasis in some areas of China.

The lake is connected to the Yangtze River by a very narrow outflow channel. It is probable that there is little immigration or emigration of snails in the lake during the annual floods. The flooding Yangtze backs up the flow of water from the lake to the river so that it is probable that there is little transport of snails in the lake. Accordingly, the snails on lake islands and flood plains are considered to have a "stable" population structure. However, after centuries of truncating the life history of the snails due to the annual floods, the question is: has selection altered the genetics of life history strategy? If it has, what is the effect on the dynamics of schistosome transmission?

In Poyang Lake, schistosomiasis is primarily a disease of cattle. People becoming infected are those who contact water during the low-water season within the lake basin, such as cattle herders, fisherman, and other boatmen.

II: Yangtze River Islands and Flood Plains. The subspecies of snail is *O. h. hupensis*. There is no stable population structure. Snails are aggregates of snails swept away during the annual floods and deposited with the lowering of water levels post flooding. Many aggregates of snails are old snails of varying age, from one to four or five years. Efforts to control snails in places such as the flood plains of Nanjing are thus far ineffective. Clearing out snails from a large section of flood plains here, close to the end of the Yangtze River in Jiangsu Province, is thwarted each year with the deposition of millions of heavily infected snails swept downstream with the floods.

III: Canals of Hubei Province; Canal Networks of Southern Yangtze River. The subspecies is *Oncomelania hupensis hupensis*. In the terminal ends of the greatly branched canal systems of this province, snail populations are presumably stable and with the normal life span. The influence of cattle on maintaining infections varies from place to place but does not approach the situation in Poyang Lake. At the Yangtze River, great flood gates keep the flooding Yangtze from the interior, contained by great dikes. When the flood gates are opened, snails from the Yangtze are swept into the lower canal systems (Xu & Fang, 1988; Xu et al. 1989, 1993; Yang et al., 1996). Migration along the lower canal sys-

tems would presumably cause unstable population genetics.

The Chinese literature discusses "hilly habitats" for some populations of *Oncomelania hupensis* and *O. h. robertsoni* (see Liu et al., 1981). The latter subspecies, discussed below, occurs at high altitude on the plateaus and hills, mountains of Yunnan and Sichuan Provinces, mostly at elevations ranging from 500 to 2000 m. A few populations live on lower plateaus or basins at 200–500 m. The snails live on more or less horizontal areas associated with agricultural practices such as rice farming (for example on terraces) although in Yunnan, *Oncomelania* has been found in small, trickling perennial flows of water flowing down hills with a slope of some 25 to 30 degrees at 1,000 m.

The former subspecies, as discussed above in the section on the taxonomic status of smooth and ribbed-shelled *Oncomelania*, primarily lives at low altitudes (0–200 m) and is affected by the annual floods of the Yangtze River and associated rivers; they have ribbed shells. When populations occur above the effects of the annual floods, the shells are smooth. Chinese workers have called the habitat hilly where the smooth-shelled populations are found, even if the altitude is slight (e.g., 90 m). We here limit the term "hilly" to populations dwelling at 500 m or greater.

There are a few populations of *O. h. hupensis* that live in mountain valleys such as found along the border of Zhejiang and Anhui provinces at an altitude of about 1,000 m. Populations are found in the northwestern parts of Guangxi Province, along the Yu Jiang and Hongshui He rivers at altitudes of 200 to 400 m, a few at about 1,000 m (Liu et al., 1981). These are hill-dwelling populations, few in number, and genetically all *O. h. hupensis*.

IV: Yunnan and Sichuan Provinces. The subspecies is *Oncomelania hupensis robertsoni*. The environment is in the hills and low mountains above the Three Gorges of the Yangtze. Populations are not affected by flooding and are presumed stable with a normal life expectancy. The contribution of horses, goats and cattle on maintaining infections varies from place to place but presumably does not reach the magnitude found in Poyang Lake. As the snails are highly divergent genetically from *O. hupensis hupensis*, presumably *S. japonicum* is likewise genetically divergent above and below the Three Gorges.

We do not place *O. hupensis tangi* in the discussion of modes of transmission because

this subspecies is now irrelevant to this issue. The subspecies, restricted to the coastal areas of Fujian Province, has been brought to the edge of extinction, with only one population maintained and guarded to save an example of this taxon.

Because *S. japonicum* has coevolved with its snail hosts (Davis, 1980, 1992), a number of questions arise. Has severe natural selection in Poyang Lake coupled with schistosome zoonosis in cattle modified *S. japonicum*'s genetics, pathogenicity and reactivity to drugs? Do flood-deposited aggregates of snails, demonstrably diverse genetically, host similarly genetically diverse aggregates of schistosomes? As *robertsoni* and *hupensis* are so diverse genetically, the schistosomes they transmit must also be diverse genetically. Is this true? A number of factors impact on this question. Much depends on the breeding structure of *S. japonicum* in wild populations. Are the worms normally outbreeding, or are they parthenogenetic? Are genetic factors involved with coevolution reflected in changes in structural genes or mitochondrial genes? Can one differentiate schistosomes from stable and unstable populations using standard genetic markers currently available?

These questions are under investigation within the Shanghai TMRC.

Implication of the Three Gorges Dam: The construction of the Three Gorges Dam across the mainstream of the Yangtze River constitutes man's largest alteration of the environment to date. The river is the third largest in the world, some 3,900 miles long. More than 400 million people live in the Yangtze River drainage system. The superdam, some 185 m high and 2.15 km wide, will back up a 600 km-long reservoir (Fig. 12). The huge reservoir will have an impact on the global environment as well as all of China. The Three Gorges is 250 km long, a churning caldron hemmed in by tall cliffs. Today, the cliffs and a barrier of mountains isolates Yunnan and Sichuan provinces from the rest of southern China to the east. These barriers have effectively kept the biodiversity of two vast regions separated; for example, *O. hupensis robertsoni* from *O. h. hupensis*.

With the advent of the dam, the immense reservoir, and climate changes, we make a number of predictions. Because of the locks and lateral canal systems enabling boats to traverse the dam and travel the reservoir, there will be movement of snails and para-

sites in both directions across this previously unassailable barrier. Snails and parasites will colonize suitable sections of the immense reservoir area. The considerable increase in the height of the water level behind the dam will cause significant backup of rivers that now flow into the future reservoir basin, creating new marshlands and bridges to *O. h. robertsoni* and *Tricula* habitats.

The vast changes in ecology will promote emerging diseases of a variety of types, snail-borne diseases and schistosomiasis in particular, among them. The stabilization of the Yangtze River, with the elimination of the great annual flooding, at a slightly higher level than today will mean stabilization of vast habitat areas for snails and considerably increased marshland habitats for snails. Poyang Lake should undergo a transformation with considerable land, formerly flooded annually, left above water all year. This means that prime snail habitat will be free from flooding all year. Should this materialize, there will be profound changes in selective pressure on snails in Poyang Lake and along the Yangtze River including its islands. There should be a massive shift from unstable aggregates of snails to stable population structure with concomitant shifts in parasite genetics. Snail population density should increase considerably.

The best way to monitor and track such sweeping changes is to combine remote sensing (RS) via satellite images and geographic information systems (GIS). The Shanghai TMRC has undertaken such studies with a special focus on the dynamics of Poyang Lake. Figure 13 shows a GIS-facilitated map of Poyang Lake during full flood and with TMRC epidemiological study sites marked. We are currently benchmarking selected sites for water levels: the lowest and highest water levels and the dates in relationship to rainfall patterns, temperature, humidity and incident radiation, as well as ecological factors supporting snail populations and schistosome infections.

We have placed special attention on four administrative villages where the TMRC is conducting the EPI study to assess the significance of water buffalo in the transmission of *S. japonicum*. Figure 14 is an enlargement of that section of Figure 13 showing the locations of the epidemiological study sites. Figure 15 is an overlay of a Landsat image on the study sites, showing the lake at lowest water in March. About 90% of the water during peak flood empties out leaving exposed countless



FIG. 14. GIS-facilitated map of an enlargement of that section of Poyang Lake of concern to the TMRC epidemiological study.

islands, floodplains suitable for cattle grazing, and vast mud and sand flats. Flooding covers all but the highest islands in the lake, and thus man and cattle are excluded from the lake basin. During low water, there is maximum use of the emergent grasslands for cattle grazing and availability to man. The RS image shows the extensive expanses of uncovered flood planes, marshes and mud-sand flats.

The focus of the TMRC epidemiological study is on the interaction between infected water buffalo and snail host populations throughout the buffalo's grazing range. GIS studies require a long-term view, because these grazing range environments are not static, and annual fluctuations may be considerable. On top of annual cycles, the Three Gorges Dam will cause permanent dramatic shifts in numbers of environmental variables. An example of annual dramatic environmental changes is the early spring flood in 1998 that devastated southern China, the worst flood of



FIG. 15. Landsat image of the epidemiological sites shown in Fig. 14. The image was taken during lowest water in March (courtesy of NASA). During low water only 10% of the water remains from full flood leaving exposed islands and vast flood planes as well as mud and sand flats.

some 40 to 50 years. In our EPI study, one administrative village's grazing land was dramatically changed. One section was swept clean of snails, and no living snails were found there in the fall of 1998 or the spring of 1999; in another section, an area that had no snails recorded in previous years was found to have snail populations; none were infected. This grazing land was the lowest of our study areas in altitude above mean low water, and was deeply impacted not only by the flooding but by the severe cold temperatures affecting reproduction. The factors of temperature, time and duration of rainfall and flooding had a negative impact on snail survival and reproduction. Increased depth of water brought on by the Three Gorges Dam will have a dramatic impact on this particular site; we predict that snails will not survive there.

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CONTROL OF INTERMEDIATE HOST SNAILS FOR PARASITIC DISEASES – A THREAT TO BIODIVERSITY IN AFRICAN FRESHWATERS?

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ABSTRACT

We address the question of whether there is conflict between the objectives of malacologists working to control transmission of snail-borne parasitic disease in Africa, and the aim of conservationists to preserve biodiversity.

Of the approximately 330 species of indigenous gastropods known from fresh and brackish waters in Africa, about two-thirds can be classified in the IUCN Red List category of "Threatened". Most are prosobranchs (especially Thiariidae, Bithyniidae and Ampullariidae), while only 23 are pulmonates, including some species of *Biomphalaria* and *Bulinus* that are intermediate hosts for schistosome parasites. A high proportion of the threatened fauna is concentrated in three geographical areas of endemism: the lakes region of eastern Africa, the Congo (Zaire) Basin, and West Africa.

From consideration of the distribution of the snail-borne diseases schistosomiasis, paragonimiasis and fascioliasis, and the past and potential use of snail control measures against them, it is concluded that endemic snails are in most danger from mollusciding in lakes in eastern Africa. Conservation of these lacustrine mollusc faunas and the riverine fauna in the Zaire (Congo) Basin is of high importance for the preservation of biodiversity.

Fortunately, the risk from mollusciciding has diminished due its high cost and the switch from area-wide to focal application. Possibly greater threats to biodiversity than snail control are pollution, hydrological engineering and degradation of habitat.

High biodiversity is a sign of the ecological health of an aquatic habitat. It is in the interests of medical malacologists and health workers, as well as conservationists, to do all they can to preserve biodiversity. Environmentally damaging dams and irrigation schemes can cause serious health problems as well as damaging natural ecosystems, while pollution and habitat degradation threaten water supplies as well as species.

There is good reason for conservation issues to be taken into account when snail control operations are planned. Medical malacologists can contribute to preserving biodiversity by minimising the use of molluscicide, by working with a knowledge of snails that should be protected, and planning operations in consultation with conservationists.

Keywords: Africa, biodiversity, conservation, fresh water, Mollusca, schistosomiasis, snail-borne disease, snail control

INTRODUCTION

For several decades, the means of controlling snail-borne parasitic diseases of humans and domestic livestock in Africa have included control of the intermediate host. This has been attempted mainly through the use of molluscicides, by environmental modification, and by the use of biological control agents.

The molluscan fauna of fresh water in Africa includes a high proportion of endemic species, of which many are known from only very small areas and are believed to be vulnerable to extinction (Baillie & Groombridge, 1996; Brown & Kristensen, 1998). The con-

servation of these organisms is desirable as part of the overall strategy to preserve the world's biodiversity. The occurrence of vulnerable species of snail is patchy, because to a great extent the endemic species are clustered in association with certain lakes and river systems. Some of the regions with snail faunas of high conservation value are also areas of snail-borne parasitic diseases of humans, while others are not. Controlling the intermediate hosts of schistosomiasis could bring medical malacologists into conflict with conservationists working to preserve biodiversity (Brown & Kristensen, 1998).

Snail control by chemical means has un-

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specific results, causing death of non-target organisms, including fish, amphibia and insects as well as harmless snails. Such indiscriminate killing is rightly regarded with concern by local people as well as professional conservationists. In such artificial habitats as reservoirs and irrigation systems, non-specific molluscicides may do little damage, since the snail faunas are usually low in diversity and composed of widely distributed species. But in rivers and lakes, snail control could do catastrophic harm to endemic snail faunas.

In a world with ever growing awareness of the importance of conserving biodiversity, snail control activities have to be questioned, and medical malacologists should take conservation issues seriously. By striving to minimise the damage done by snail control to non-target organisms, medical malacologists can play an active part in protecting the biodiversity on our planet.

This present paper summarises the conservation status of the freshwater snail fauna of the African continent, and assesses the degree of threat posed by measures to control the intermediate hosts for the major snail-borne parasitic diseases of humans and domestic livestock. Attention is drawn to the mutual interest of medical malacologists, health authorities and conservationists in working cooperatively. We also recommend practical ways in which medical malacologists can contribute to conservation.

SNAIL-BORNE PARASITIC DISEASES IN AFRICA

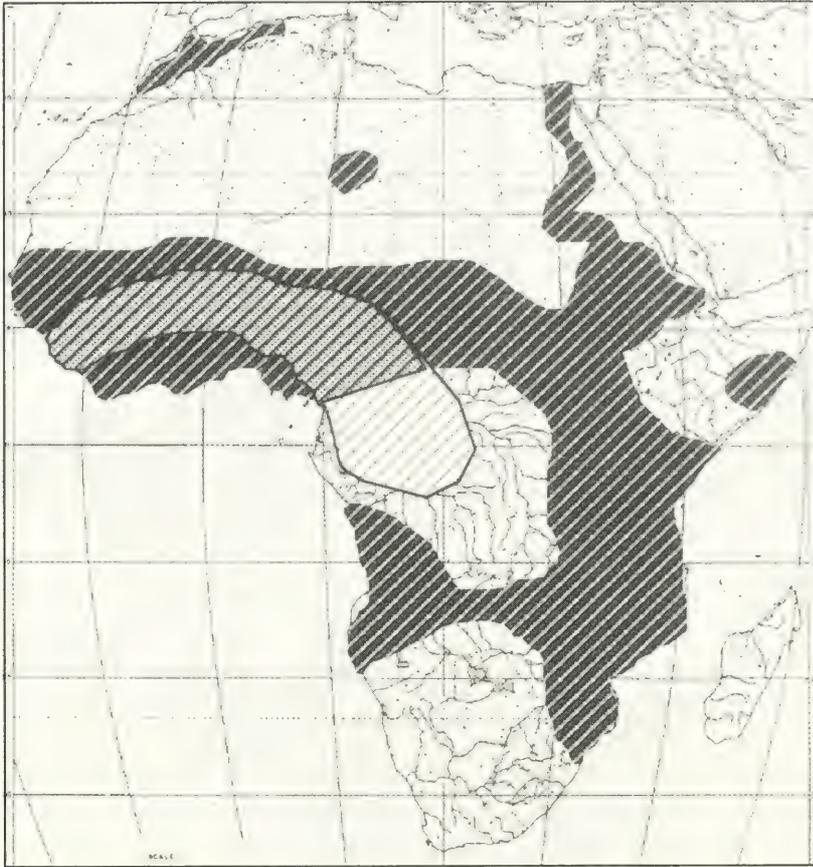
Schistosomiasis or bilharzia is by far the most important of the snail-borne diseases of humans in Africa. Over a period of several decades, snail control by molluscicides has played a part in controlling the disease, in limited areas and with widely varying success. Two species of *Schistosoma* are responsible for most cases of schistosomiasis in humans, *S. haematobium* causing the urinary form of the disease and *S. mansoni* the intestinal form. Their intermediate hosts belong, respectively, to the pulmonate genera *Bulinus* and *Biomphalaria*. Both these forms of schistosomiasis are transmitted in large areas of Africa (Fig. 1), parts of which have highly diverse faunas of aquatic snails that are of high priority for conservation and merit protection from snail control operations.

Paragonimiasis, the lung-fluke infection of humans, is reported from a comparatively small area of western Africa (Fig. 1), extending from Gambia to Zaire, and is found most commonly in Liberia, Nigeria and Cameroon (Brown, 1994). Apparently two species of *Paragonimus* are involved. Their metacercariae are found in freshwater crabs, which serve as the second intermediate host, but the identity of the molluscan first intermediate host is still not known with certainty. It probably is one or more of the species of *Potadoma* (Thiaridae) that occur in forest streams within the areas of disease transmission. No attempt has been made, to our knowledge, to control transmission of paragonimiasis by measures aimed against the first intermediate host. Any snail control activities that might be planned should take into account that the streams and rivers of West Africa are also inhabited by rare endemic prosobranch species (Brown & Kristensen, 1993; Brown, 1994).

Bovine schistosomiasis caused by *Schistosoma bovis*, *S. curassoni* and *S. mattheei* is common in cattle in Africa and severe infection can cause mortality and considerable economic loss. The intermediate hosts in most of Africa are species of *Bulinus*, while *Planorbarius* is possibly a host for *S. bovis* in northwest Africa. Snail control appears to have been used on only a small scale, as in a reservoir and associated drinking trough (Van Wyk et al., 1974: 46-47).

Paramphistomiasis is an infection of domestic livestock and wild grazing animals in Africa, caused by a variety of species of Paramphistomatidae and related trematode families, which can be of veterinary importance locally. Most of the recorded intermediate hosts are species of *Bulinus*, and some species of parasite develop in *Biomphalaria*, *Ceratophallus* and *Lymnaea* (Brown, 1994). As for bovine schistosomiasis, it appears that any snail control against paramphistomiasis has been only local. The development of acute infection in livestock depends on exceptional conditions, which lead to a dense population of infected snails in a limited site (Dinnik, 1964: 452). According to Dinnik, this takes at least two months, allowing time for the potential danger to be recognised and a decision to be taken on whether to apply molluscicide or to exclude livestock from the site.

Fascioliasis, or liver-fluke infection, is widespread in cattle and sheep in Africa, but human infection is rare in the tropical region



Intestinal and Urinary Schistosomiasis
 Paragonimiasis
 Intestinal and Urinary Schistosomiasis and Paragonimiasis

FIG. 1. Distribution of intestinal and urinary schistosomiasis, and paragonimiasis in Africa.

(World Health Organisation, 1990). Both *Fasciola gigantica* and *F. hepatica* are reported, the former widely distributed, the latter apparently restricted to highland areas in eastern and southern Africa. This pattern may reflect the preference of *F. hepatica* for the snail host *Lymnaea truncatula*, which is restricted to cool highland areas, whereas *F. gigantica* develops in *L. natalensis*, a snail found throughout tropical and subtropical Africa (Brown, 1994). Infection of domestic livestock can cause considerable economic loss, and trials to control the snail host with molluscicide have been carried out in Kenya (Preston &

Castelino, 1977) and Lesotho (Prinsloo & Van Eeden, 1977).

THREATENED FRESHWATER SNAILS
IN AFRICA

Assessing the Threat

World wide efforts to save species from extinction are focused in the International Union for Conservation of Nature and Natural Resources (IUCN). For nearly 30 years, IUCN has published Red Data Books and Red Lists

of species threatened by severe reduction in population and possible extinction. Information is collected by the Species Survival Commission of IUCN with the help of expert groups, one of which is the Mollusc Specialist Group. Many species of African freshwater snails are included in the most recent IUCN Red List of Threatened Animals (Baillie & Groombridge, 1996). Each species is classified in one of a number of categories according to its degree of rarity and a judgement of its vulnerability to extinction. The category for a mollusc is decided usually according to the number of localities where it has been found and the total area of its distribution. The system of classification is explained in detail in IUCN (1994) and discussed by Seddon (1998). At one end of the scale, an abundant and widely distributed species, such as *Biomphalaria pfeifferi*, would be classified as "Of Least Concern". At the other extreme, a species found in only a single locality or in a small area qualifies for inclusion in the Critically Endangered (CR). High conservation status is given to all species that occur in a single lake, no matter how large, because an incident of severe pollution could have a devastating effect on the fauna of even so big a waterbody as Lake Tanganyika.

Taxonomic Composition of the Threatened Snails

The total number of snail species in fresh and brackish water on the African continent is estimated at 400 (Brown, 1994). Uncertainty stems largely from ignorance of the exact number of species of endemic prosobranchs in Lake Tanganyika, of Hydrobiidae in North West Africa, and of the ancyliid genera *Burnupia* and *Ferrissia* throughout their African ranges. Of the 332 indigenous species listed by Brown (1994: 29–34), 207 are considered threatened—184 prosobranchs and 23 pulmonates (Table 1).

Among prosobranch families, the Thiaridae has the largest number of threatened species by far, due to the presence of endemic species in lakes Malawi and Tanganyika, and the restricted occurrence in western and central Africa of many species of *Cleopatra*, *Melanoides* and *Potadoma*, which live in rivers and streams.

Six other prosobranch families include high proportions of threatened species, as follows. Bithyniidae—31 threatened species, includ-

TABLE 1. Taxonomic composition of the snail fauna of fresh and brackish waters in Africa considered to be threatened (based on checklist in Brown, 1994: 29–34; IUCN Red List, 1996; Brown, 1994, and unpublished data). Notes: (1) A threatened species is one classified, or likely to be so classified, in one of the IUCN categories Critically Endangered (CR), Endangered (EN) or Vulnerable (VU); (2) Introduced species are excluded; (3) Hydrobiidae here include only the species found in tropical Africa; (4) In the Ancyliidae the species of *Burnupia* and *Ferrissia* are each treated as a single aggregate.

Family	No. of Species	No. Threatened
Prosobranch		
Neritidae	17	1
Viviparidae	19	17
Ampullariidae	27	13
Valvatidae	1	0
Hydrobiidae	13	12
Pomatiopsidae	10	10
Bithyniidae	35	31
Assimineidae	11	10
Thiaridae	109	90
Melanopsidae	1	0
Potamididae	4	0
Subtotal	247	184
Pulmonata		
Ellobiidae	7	1
Lymnaeidae	5	0
Ancyliidae	5	1
Planorbidae	68	21
Subtotal	85	23
Total number of species	332	207

ing about 20 *Gabbiella* found in Equatorial Africa in varied habitats from seasonal pools to large lakes, four *Sierraia* and one *Soapitia* known only from rivers in West Africa, three monotypic genera confined to the lower Congo River Basin (*Congodoma*, *Funduella* and *Liminitesta*) and two genera restricted to north east Africa (*Incertihydrobia* and *Jubaia*).

Viviparidae—17 threatened species of *Bellamyia* and *Neothauma*, mostly confined to large lakes.

Ampullariidae—13 threatened species, comprising a *Pila*, 10 *Lanistes* found in Equatorial Africa in lakes rivers and streams, and the monotypic genera *Afropomus* and *Saulea* known only from small areas in West Africa.

Hydrobiidae—12 threatened species in tropical Africa, comprising nine species of uncertain generic position, found in restricted localities scattered along the western coast and classified in *Hydrobia* and *Potamopyrgus*, and

TABLE 2. The species of *Biomphalaria* and *Bulinus* classified as Threatened or Near Threatened (category LR nt) in 1996 IUCN Red List (Baillie & Groombridge, 1996)

	Threatened	Near threatened	Distribution
<i>Biomphalaria angulosa</i>		x	Tanzania, Zambia, Malawi
<i>B. barthi</i>	x		Eastern Ethiopia
<i>B. salinarum</i>		x	Angola
<i>B. tchadiensis</i>	x		Lake Chad
<i>B. smithi</i> *	x		Lake Edward and Marambi Crater lake
<i>Bulinus hightoni</i> *	x		NE Kenya
<i>Bul. obtusus</i> *	x		Chad
<i>Bul. hexaploides</i>		x	Ethiopian highland
<i>Bul. nyassanus</i>	x		Lake Malawi
<i>Bul. octoploides</i>		x	Ethiopian Highland
<i>Bul. succinoides</i>	x		Lake Malwi
<i>Bul. transversalis</i>	x		Lake Victoria
<i>Bul. trigonus</i> *	x		Lake Victoria
<i>Bul. barthi</i>		x	Kenya, Tanzania
<i>Bul. browni</i>		x	Kenya
<i>Bul. camerunensis</i>	x		Cameroun, crater lake
<i>Bul. canescens</i>		x	Angola, Zambia
<i>Bul. crystallinus</i>		x	Angola, Gabon

*Not yet included in an IUCN List, but suitable for the vulnerable (vu) category.

three *Lobogenes* confined almost entirely to south east Congo (Democratic Republic). In this family, the total number of species and the number threatened will rise considerably when the fauna of North West Africa is better known.

Pomatiopsidae—10 threatened species of *Tomichia*, seven found in restricted localities near the southern coast and three in central Africa.

Assimineidae—10 threatened species, comprising an *Assimineia* from each of the east and west coasts, four *Eussoia* from eastern Africa and four species from the lower Congo River, below Kinshasa (*Pseudogibbula*, *Septariellina* and *Valvatorbis*).

Almost all the 23 threatened pulmonates belong to the Planorbidae (Table 1). The genera contributing most species are *Ceratophallus* with eight confined to lakes in eastern Africa, and *Biomphalaria* and *Bulinus* with a total of 10 (Table 2). *Biomphalaria barthi* is known only from two shell deposits in eastern Ethiopia and may be extinct, while *B. tchadiensis* is perhaps merely a conspecific form of *B. pfeifferi*, but taking into account also the eight species classifiable as "Near Threatened" (Table 2) it is clear that *Biomphalaria* and *Bulinus* are of interest to conservationists as well as medical malacologists.

Conservation status of *Biomphalaria* and *Bulinus*

Although none of the major intermediate hosts for schistosomes appears in Table 2, this list does include four species reported to be involved in transmission locally. *Biomphalaria angulosa* has been observed to transmit *S. mansoni* on the shore of Lake Malawi (Teesdale, 1982). *Biomphalaria smithi* is known only from Lake Edward and the nearby Mirambi crater lake in Uganda; it proved susceptible to infection with *S. mansoni* in the laboratory (Cridland, 1957) and therefore is likely to be an intermediate host in nature. *Bulinus camerunensis* is the intermediate host in a well-known focus of *S. haematobium* at Barombi Kotto crater lake in Cameroon (Duke & Moore, 1976). *Biomphalaria crystallinus* was implicated by Jelnes & Highton (1984) in transmission of *S. intercalatum* in Gabon.

It is their restricted distributions that give conservation interest to the snails listed in Table 2. *Bulinus camerunensis* is found in only one other lake besides Barombi Kotto, while others are each restricted to a single lake (*Biomphalaria tchadiensis*, *Bulinus nyassanus*, *B. succinoides*, *B. transversalis* and *B. trigonus*). The others are found in more small

waterbodies, but from only small geographical areas.

REGIONAL ENDEMIC SNAIL FAUNAS AND THE DISTRIBUTION OF SNAIL-BORNE DISEASE

The important contribution of lakes and rivers to the diversity of freshwater snails in Africa is seen when we consider the geographical distribution of the threatened species. Nearly 70% of them are concentrated within three geographical areas (Table 3), and they live mostly in rivers of West and central Africa and the lakes in the eastern rift valleys. The Congo River and lakes Tanganyika and Malawi each have an outstandingly rich assemblage of unique prosobranchs that deserve careful monitoring to ensure their protection.

These areas will be considered in more detail, after three other areas each with smaller numbers of threatened species.

Northwest Africa (Fig. 2)—The freshwater molluscs are mostly of palaeartic origin. This fauna is well described apart from the Hydrobiidae, which are represented by several genera, of which probably many species have yet to be described (Boeters, 1976; Kristensen, 1985; Ghamizi et al., 1999). The distribution of each species is apparently very limited, and many live in subterranean groundwaters and wells in the Mahgreb area of Morocco. Such habitats appear to be comparatively safe from operations to control *Bulinus truncatus*, the intermediate host for *S. haematobium*, which is widespread in northwest Africa. Yet it is important to be alert to the damage that could be done to this unique hydrobiid fauna by molluscicides seeping into ground waters.

Nile Basin, in Egypt and Sudan, and the upper Blue Nile catchment in Ethiopia (Fig. 2)—Within this basin are a wide variety of aquatic habitats, including the large lakes Albert and Victoria that are dealt with below in the section on East African lakes. The other areas considered here are for snails found in nonlacustrine waterbodies. In Egypt and Sudan, the comparatively few endemic species (Brown, 1994: table 12.16) are *Theodoxus niloticus*, *Valvata nilotica*, *Gabbiella schweinfurthi*, *Gyraulus ehrenbergi* and *Biomphalaria alexandrina*. This region is a major area of transmission for both *S. haematobium* and *S. mansoni*. As molluscicide has been ap-

TABLE 3. The geographical areas of Africa that support the major concentrations of threatened species of freshwater snails.

Area	No. Threatened Species
West Africa: Cameroun and westwards into Sierra Leone	29
Congo Basin and Lualaba river systems	40
Large lakes: Albert, Malawi, Tanganyika, Victoria and Mweru	72
Total	141 (69% of the total threatened species)

plied intensively for decades in irrigated areas, and there has been much human activity along the lower Nile for many centuries, it might be expected that some endemic molluscs have already been eliminated. No evidence can be seen for this, however, in deposits of shells of Neolithic age (Gardner, 1932) that are, apart from changes in nomenclature, no different from species still living in modern Egypt (Brown, 1994: 505). The most vulnerable of the endemic snails seem to be *Gabbiella schweinfurthi*, found in only a few localities fringing the Sudd swamp, and *Gyraulus ehrenbergi* known from a few canals and drains in the Delta Region of Egypt. It may be that snails indigenous to the lower Nile will suffer through competition from the introduced *Biomphalaria glabrata*, recently established in irrigation and drainage systems (Yousif et al., 1996).

The upper catchment of the Blue Nile in the Ethiopian highlands includes Lake Tana, which surprisingly seems to lack any endemic snails, as does the Blue Nile itself. In small tributary streams, however, live *Ancylus regularis*, *Bulinus hexaploides* and *B. octoploides*, all classified as Near Threatened (Baillie & Groombridge, 1996) because of their restricted distributions. *Schistosoma haematobium* is not known from this region, and although transmission of *S. mansoni* is widespread it is absent from above about 2200 m altitude. Fascioliasis is widespread in sheep (Goll & Scott, 1979). We have seen no report of snail control being used to restrict transmission of either liver-fluke or schistosomiasis. Widespread use of molluscicides would be cause for concern, as streams inhabited by *B.*

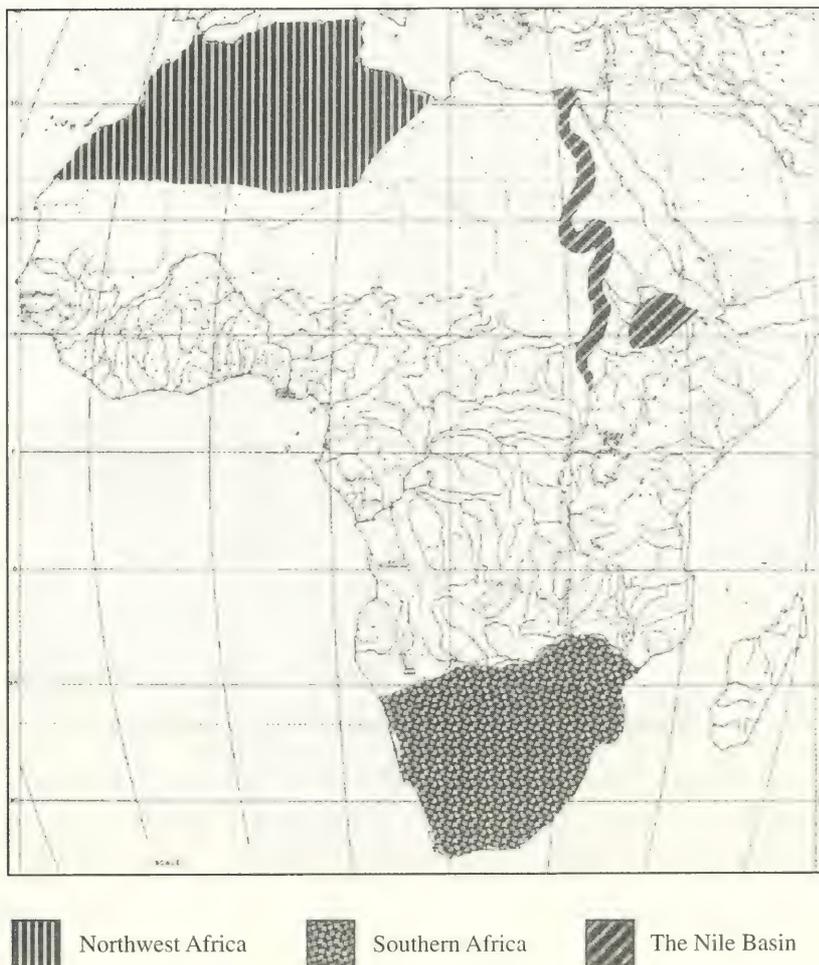


FIG. 2. Some areas of Africa with endemic snail faunas: Northwest Africa, Southern Africa, Nile Basin in Egypt and Sudan, and upper catchment of Blue Nile.

hexaploidus and *B. octoploidus* flow through the grazing pastures and close to villages.

Southern Africa (Fig. 2) — South of the Zambezi River endemic species are comparatively few, but of high conservation importance are the species of *Tomichia* (Pomatiopsidae) living in lagoons, streams and springs near the coast in the Republic of South Africa. All but one of these species occur in a climatic region too cool for transmission of schistosomiasis, and the main threat to their survival is destruction of habitat. Only *T. natalensis* occurs in the tropical region of South Africa, in a small area of the coastal plain of Zululand. Here it is vulnerable to operations to control

Bulinus globosus, the intermediate host for *S. haematobium* locally, though the main threat is probably sugar-cane cultivation.

Fascioliasis of domestic livestock is of sufficient economic importance in Lesotho for attempts to have been made to control the intermediate host *Lymnaea truncatula* by use of molluscicide. Fortunately, no snail species is known to be endemic to Lesotho and the whole snail fauna is poor, reflecting the high altitude and cool climate.

West Africa (Table 3, Fig. 3) — Most of the 29 threatened species are prosobranchs living in streams and rivers in highland forested areas. *Bulinus camerunensis* is known only

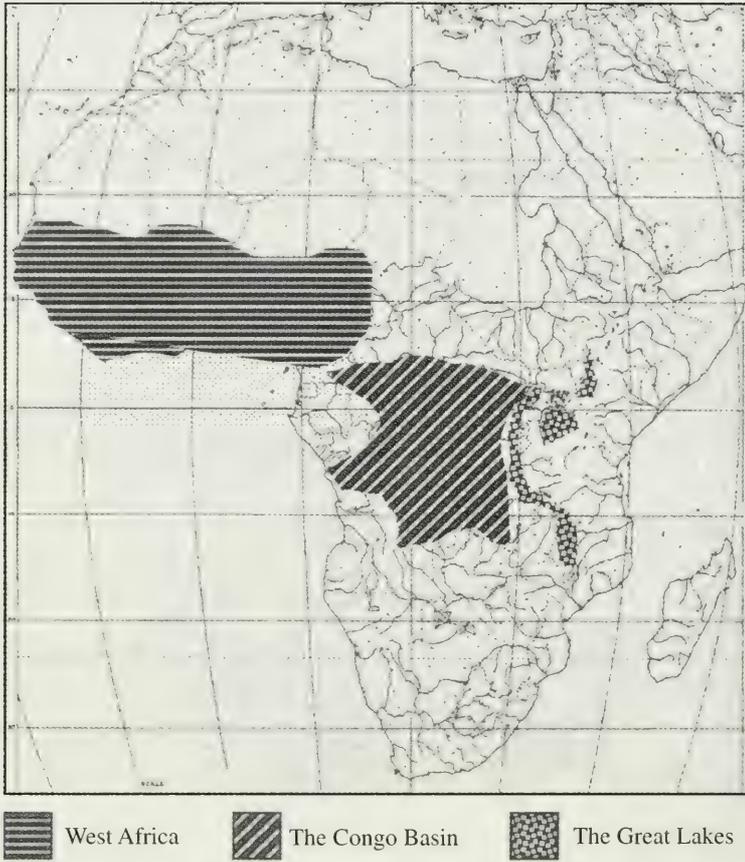


FIG. 3. Some areas of Africa with endemic snail faunas: West Africa, Congo Basin, East African lakes.

from two crater lakes in western Cameroon. Although both urinary and intestinal schistosomiasis are widespread in West Africa, the habitats of the endemic prosobranchs do not usually lie within the major areas of infection. Snail control by molluscicides in and near Lake Volta has probably not harmed the rare prosobranchs found only in the Volta Basin, as these (*Pseudocleopatra*) are known only from streams at higher altitude in the hills above the eastern side of the lake. A greater risk to threatened species in West Africa seems to lie in any attempt to reduce transmission of paragonimiasis through snail control, since this disease unlike schistosomiasis is associated with streams in highland forested areas.

Congo Basin (Fig. 3, Table 3)—Here occurs a major endemic snail fauna comparable in conservation importance to that of the Rift Valley lakes. About half of the 100 or so species

known are endemic (Brown, 1994: tables 12.14, 12.15). Of about 40 species believed to be threatened, few occur in standing waterbodies (e.g., *Gabbiella matadina* known only from a reservoir), and the majority are confined to the Congo/Lualaba river system. The riverine species can be divided into three groups: those in headwater streams (*Lobogenes*, *Potadoma*), those in the slower-flowing parts of rivers in the upper and middle basin (*Melanoides*, *Potadomoides*), and rheophilous species in rapidly flowing stretches of the lower Congo River (*Congodoma*, *Hydrobia*, *Limnitesa*, *Pseudocleopatra*, *Pseudogibbula*, *Septariellina*, and *Valvatorbis*).

Schistosomiasis is widespread in the Congo Basin, but most transmission takes place in small waterbodies rather than major rivers, which do not generally provide favourable habitats for pulmonates. Usually the volume of

water carried by the rivers is so large that the small amounts of molluscicide that might be received by drainage from treated sites near the river banks may cause little harm. However, extreme caution should be exercised in any application of molluscicide in the lower Zaire region, especially near Matadi where rocky rapids are the habitat for specialised rheophilous prosobranchs, including assimineids with remarkably modified shells (*Sep-tariellina* and *Valvatorbis*).

East African lakes (Fig. 3, Table 3) — Lakes in the rift valleys of eastern Africa support the largest group of endemic and threatened snails. Lakes Albert, Malawi, Mweru, Tanganyika and Victoria have about 87 endemic species (Brown, 1994; tables 12.6, 12.7, 12.8, 12.10, 12.11). Of about 70 classified as threatened, the biggest group is the more than 30 "thalassoid" prosobranchs of Lake Tanganyika. Also of high priority for conservation are the species of *Bellamya*, *Lanistes* and *Melanoides* endemic to Lake Malawi. The other lakes have varying numbers of endemic *Bellamya*, *Gabbiella*, *Melanoides* and *Cleopatra*. Endemic pulmonates are few, two species of *Bulinus* in each of lakes Malawi and Victoria and four species of *Ceratophallus* endemic to Lake Victoria. There may also be endemic lacustrine species of the ancyliids *Burnupia* and *Ferrissia*, but their validity needs confirmation.

These lakes lie within transmission areas for both urinary and intestinal schistosomiasis, and transmission sites of particular importance are known on or near the shores of lakes Malawi, Tanganyika and Victoria. Transmission of schistosomiasis has long been known in scattered marshy sites around Lake Malawi, but recently the incidence of urinary schistosomiasis has increased, possibly due to overfishing of the molluscivorous fish population and a resulting increase in the intermediate host *Bulinus globosus* (Msukwa & Ribbink, 1998, and references therein). People are apparently now acquiring infection on open shores, where endemic prosobranchs are found, as well as in marshy sites. At Lake Tanganyika there are important foci of schistosomiasis at Bujumbura and Kalieme, and the disease is widespread on the Ruzizi plains (Gryseels & Nkuliyinka, 1988). Increasing human population and developments in agriculture, industry and tourism around these lakes may lead to demand for more effective control of schistosomiasis. The risk of damage to the endemic snail faunas makes the use of molluscicide highly undesirable.

The rich endemic snail faunas in lakes and rivers contrast strongly with the poor assemblages of species found in artificial waterbodies such as dams and irrigation systems. In an irrigated area of the Niger Basin in Mali, only 13 species out of about 70 known from the West African region were found (Madsen et al., 1987), all of them widely distributed. In the Blue Nile Irrigation Project area in Sudan, only 11 species were recorded (Madsen et al., 1988).

THREATS TO THE FRESHWATER SNAIL FAUNA

Snail control is one of many threats to freshwater snails in Africa. Before assessing the danger from snail control we consider briefly some effects of environmental change (pollution, destruction of habitat) and the increasing possibility of competition from invasive species.

Environmental Changes

Besides sewage effluent, which the invasive *Physa acuta* tolerates remarkably well (Brown, 1994: 408, 431), African fresh waters are contaminated by industrial waste and agricultural chemicals (herbicides and pesticides), while the prospect of oil production poses further danger. Pollution in Lake Tanganyika already causes concern and extensive petroleum exploration has been conducted near the lake (Cohen, 1991). There is an urgent need for data to assess the impact of pollution on the ecosystem of Lake Tanganyika and other African fresh waters.

Destruction of habitat results from hydrological engineering, dam-building, construction of irrigation schemes, drainage and water extraction. Extensive parts of river basins have been submerged by large dams (e.g., Aswan, Kainji, Kariba and Volta), while large areas of flood plain and natural wetland have been replaced by major irrigation schemes (e.g., Gezira in Sudan, Office de Niger in Mali and South Chad in Nigeria). Such developments are not known to have caused the extinction of any species of aquatic mollusc, but they may reduce biodiversity locally, since in general dams and irrigation channels are colonised by a small number of molluscan species, of which a few may establish large populations. Fortunately, the Congo River system appears to be little affected by dam-

building. It cannot be too strongly urged that any plans for hydrological interventions in this basin should take into account the value of the endemic snail fauna.

A general threat to the habitats of molluscs in both rivers and lakes, and one of the most difficult to mitigate, is the high load of suspended sediment carried by inflowing streams, due to deforestation in the surrounding catchments.

Invasive Snails

African indigenous freshwater snails face competition from introduced species (Brown, 1994; Appleton & Brackenbury, 1998), of which some have become invasive and established extensive distributions (*Physa acuta* and *Lymnaea columella*). Others are found so far in only small areas, for example, *Amerianna carinata* and *Indoplanorbis exustus* in Nigeria (Brown, 1983; Kristensen & Ogunnowo, 1987) and *Gyraulus chinensis* in Guinea Bissau (Brown et al., 1998). *Helisoma duryi* was deliberately introduced into Tanzania for trial as a competitor and biological control agent of intermediate hosts for schistosomes, but although it appears in artificial habitats in many parts of Africa, presumably as an escapee from aquaria, it appears unable to establish itself in more natural habitats over any large area. Field trials with another snail considered to be a potential biological control agent, *Marisa cornuarietis*, were carried out in Egypt, Sudan and Tanzania (Brown, 1994: 406); it was concluded that this snail would be unlikely to establish populations in rice fields in the Nile Valley as flooded periods are too short (Haridi & Jobin, 1985). *Biomphalaria glabrata* is an apparently recent, accidental introduction of Egypt, where it is spreading (Yousif et al., 1996), possibly at the expense of *B. alexandrina*. The impact of introduced snails on the indigenous fauna has not been monitored, though laboratory experiments (Appleton & Brackenbury, 1998) indicate that *Physa acuta* is a successful competitor of both *Bulinus tropicus* and *Lymnaea natalensis*.

Snail Control

Snail control in Africa by means of molluscides has been attempted most ambitiously, and at great expense, in Egypt and Sudan. The earlier programmes were area-wide, applying molluscicide to entire water systems. Area-wide treatment did not produce the ex-

pected degree of disease control (Gilles et al., 1973; Fenwick, 1987; Webbe & El Hak, 1990), and today the consensus is that mollusciciding should be restricted to small focal sites. This change in thinking, combined with the high cost of mollusciciding and efforts to find substances and techniques to achieve selective killing of only target-snails, has considerably reduced the threat to other organisms. Yet while their threat to aquatic ecosystems should not be exaggerated, synthetic molluscicides are undoubtedly not selectively toxic to snails of medical and veterinary importance, and they are known to kill various other invertebrates, amphibians and fish (Appleton, 1985; Appleton & Madsen, 1998). Although rapid recovery of some invertebrates is reported, including unfortunately target snails, it does not necessarily follow that similar resilience would be shown by an isolated population of a snail species adapted to a specialised niche, since there might be no possibility of recolonisation.

Even at focal sites in lakes there are great technical problems in achieving adequate concentrations of molluscicide, because of the large volume of water and the presence of sheltered niches for snails among vegetation and rocks. Efforts to solve such problems in Lake Volta, Ghana, achieved only a short-term reduction in incidence of schistosomiasis (Chu et al., 1981). After intensive mollusciciding in the small crater-lake Barombi Kotto in Cameroon, *Bulinus camerunensis* rapidly reestablished its population and ten years later the prevalence of schistosomiasis was almost as high as before (Moyou et al., 1984).

These experiences should discourage health workers from attempting to control schistosomiasis by applying molluscicide on the open shores of such lakes as Malawi and Tanganyika. However, it might seem attractive to treat small pockets of marshy habitat, especially close to tourist resorts. Unfortunately, it would be difficult to ensure that this would cause no harm to non-target snails living nearby.

In order to make mollusciciding unnecessary, biological agents for the control of intermediate hosts of schistosomes have been sought for many years. The various candidate organisms tested include other snails, insect larvae, crustaceans and fish. Often the species have been indigenous to Africa, but some alien organisms have been introduced, as described above. Field trials have been few, and only limited success was achieved. As a general

principle, an alien mollusc should not be introduced into Africa, in view of the unpredictable effect it might have on the indigenous fauna.

RELATIONSHIPS BETWEEN CONTROL OF SNAIL-BORNE DISEASE AND CONSERVATION OF BIODIVERSITY

It is perhaps in lakes Malawi and Tanganyika that the endemic freshwater snails of Africa face the greatest potential threat from snail control operations. Schistosomiasis is not generally so much a problem in two other areas with important threatened snail faunas, the forested uplands of West Africa and the Congo Basin. The distribution of *Tomichia* in southern Africa lies almost entirely outside the range of intermediate hosts for schistosomiasis. Of other snail-borne trematode infections, paragonimiasis occurs in an extensive western area inhabited by rare species of prosobranch, but snail control is unlikely to be used against this disease. The only area of fascioliasis infection where snail control has been attempted is Lesotho, where no endemic freshwater mollusc is known.

From these considerations the potential threat to the endemic snail fauna and general biodiversity in African freshwaters from snail control seems no greater, and is perhaps less, than the dangers from pollution and habitat destruction. Providing those working for disease control behave responsibly, there should be no conflict with the interests of conservationists. On the contrary, we would stress that there are mutual interests to unite medical malacologists, health authorities and conservationists. All these specialists can advance their objectives by demanding that greater care be taken in the siting and planning of dams and irrigation schemes, since these not only destroy natural habitat but also can have bad consequences for public health (Hunter et al., 1993). A case in point is the Diama Dam across the lower Senegal River, completed in 1985. Since then, large populations of *Biomphalaria* have appeared in irrigated areas upstream, accompanied by a dramatic rise in incidence of intestinal schistosomiasis, which has reached prevalences among the highest recorded in Africa (Southgate, 1997).

Another area of concern to both health authorities and conservationists must be pollution and degradation of freshwater habitats, for these processes not only endanger threatened aquatic species, but also put at risk the

supply and quality of water essential to the health of human communities.

In this way it can be argued that a policy for conserving biodiversity in the fresh waters of Africa favours the objective of controlling snail-borne parasitic disease, and also contributes to the sustainable use of that indispensable natural resource, water.

CONCLUSIONS

Fortunately, only a few species of African freshwater snail are thought to have become recently extinct. But this is no reason for complacency, as there is no up to date information about many of the species that have been reported from a single site or only a few localities. Indeed, about two-thirds of all the species known can be classified as Threatened according to IUCN categories. Threatened species seem most exposed to potential danger from snail control in lakes in eastern Africa. Here, as in the Congo Basin, great care should be taken to avoid damage to endemic snail faunas that are of high importance for conservation. Yet in general the threat to biodiversity from snail control measures appears no more, perhaps less, than the threats from pollution, hydrological engineering and degradation of habitat. High biodiversity is a sign of the ecological health of a freshwater habitat. To preserve high biodiversity serves the interests of medical malacologists and health authorities as well as conservationists, since poorly planned dams and irrigation schemes can cause health problems, while pollution and degradation of habitat endanger water supplies that are essential for the health and prosperity of people in Africa. The diversity of the unique forms of gastropod that have evolved in the lakes of eastern Africa, and the radiations of species in western rivers, attract the interest of people worldwide who appreciate the wonderful variety of life on earth. Medical malacologists have an opportunity to help to ensure that people of future generations can see these species alive, not only as specimens in museums.

RECOMMENDATIONS

In order to avoid unnecessary damage to freshwater biodiversity from snail control programmes, the following code of practice is suggested for medical malacologists, who are

the people with direct responsibility for snail control measures.

(1) A medical malacologist who considers the use of molluscicide to be justified, having taken into account possible damage to biodiversity, should restrict the application to the smallest practicable area and minimise the dispersal of chemicals into the wider ecosystem.

(2) It should be the responsibility of medical malacologists working with health authorities to obtain knowledge of the conservation status of the molluscan fauna in their area.

(3) Before commencing a water resource development or mollusciciding programme, there should be consultation with national authorities and also non-governmental organisations concerned with conservation of aquatic life.

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STRUCTURAL DAMAGE TO THE FOOT-SOLE EPITHELIUM OF *BULINUS AFRICANUS* FOLLOWING EXPOSURE TO A PLANT MOLLUSCICIDE

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ABSTRACT

Adult *Bulinus africanus* were exposed to sublethal and lethal concentrations of the crude aqueous extract of the plant *Agave attenuata* for a 24-hour period. Sublethal toxic effects included retardation of mobility, swelling of the cephalopedal mass, and haemorrhagic blistering in the sub-epithelium of the foot sole, while lethal concentrations resulted in a cessation of mobility, severe swelling of the cephalopedal mass, increased mucous secretion, and haemorrhage. Light microscopy showed that the molluscicide had induced gross structural damage to the epithelium of the foot sole, especially at lethal concentrations. TEM revealed such cellular injuries as the reduction and degradation of cilia, and the breakdown of the connective tissue and blood sinuses, resulting in the accumulation of haemolymph below the epithelium. This caused the partial basal detachment and distortion of adjacent epithelial cells. Other molluscicide-induced effects included the accumulation of electron-dense vesicles in the apical region of epithelial cells, the discharge of glycogen, lateral compression of the nuclei, contraction of the nuclear envelope, swelling of the mitochondria and disruption of their cristae. Exposure to lethal concentrations caused the complete disintegration of the epithelium and accentuated the cellular damage observed at sublethal levels. These data imply that the active ingredients of *A. attenuata* alter the physiology of the epidermal tissue particularly that of osmoregulation.

Key words: plant molluscicide, histopathology, *Bulinus africanus*, epithelium, mode-of-action.

INTRODUCTION

As natural pesticides, plants have long held the interest of biologists wanting to control diseases such as schistosomiasis, fascioliasis, malaria, filariasis and dengue (Shoeb & El-Sayed, 1984; Dharmshaktu et al., 1987; Ferrer Lopez et al., 1993; Yong & Rodriguez, 1994; Brackenbury & Appleton, 1997a, b; Brackenbury et al., 1997a, b). A molluscicidal plant of particular interest in South Africa is the introduced Mexican species *Agave attenuata* (Family Agavaceae) which has been found to be highly toxic to *Bulinus africanus*, the snail host of the bloodfluke *Schistosoma haematobium* (Brackenbury & Appleton, 1997b). The identity of the active principle of *A. attenuata* is not known, but saponins are known to constitute an important group of chemicals isolated from *Agave* spp. (Wall et al., 1954; Kishor, 1990) and may, therefore, be responsible for the molluscicidal activity of *A. attenuata*. Wall et al. (1954) isolated

sarsasapogenin from *A. attenuata*, but its lethality to snails was not established.

There are several accounts of the histology of pulmonate tissues (Pan, 1958; Lufty & Demain, 1967; Rogers, 1971; Zylstra, 1972; Zylstra et al., 1978; Hernádi, 1981; Roldan & Garcia-Corrales, 1988), but only a few have investigated the histopathological effects of synthetic molluscicides. Of these, most have focused on the digestive tract of the terrestrial slug *Deroceras reticulatum* (Triebskorn, 1989, 1991a, b, 1995; Triebskorn & Künast, 1990; Triebskorn et al., 1990; Triebskorn & Florschütz, 1993; Triebskorn & Köhler, 1992) and the aquatic snail *Bulinus truncatus* (Banna, 1977). Most toxicological work on synthetic and plant molluscicides has centred on determining lethal doses, with some attempts to measure the physiological effects and elucidate the modes-of-action of the molluscicides on snail tissue (Sullivan & Cheng, 1975; Cheng & Sullivan, 1977; Banna, 1977; Adunmi & Ogbe, 1986; Bode et al., 1996). In

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South Africa, the toxicity of *A. attenuata* to *B. africanus* has been extensively researched (Brackenbury & Appleton, 1997b), and a quasi-field trial was conducted to determine field doses (Brackenbury, 1997). However, the damage it causes to the snail's tissues and its mode-of-action still need to be investigated. As *A. attenuata* is applied as a contact poison, the primary target is the snail's exposed epithelial tissue. The purpose of this baseline study was therefore to determine the structural damage to the epithelium of the foot sole after exposure to extracts of *A. attenuata* and to identify the likely mode-of-action of this molluscicide.

MATERIALS AND METHODS

Wild-caught, adult *B. africanus* (shell height 10–13 mm) were collected from an ornamental pond on a stream flowing through Randle Park, Durban, South Africa (29°49'S, 31°01'E). Uninfected specimens, namely those that did not show patent trematode infections, were acclimatized in pond water to laboratory conditions for seven days. Ten snails were then allocated to each of the three test groups and immersed in either untreated water (control), sublethal 0.020 g.l⁻¹ or lethal 0.027 g.l⁻¹ concentrations of a fresh, aqueous extract of *A. attenuata* leaves. Preparation of the extract and toxicity test protocols were described by Brackenbury & Appleton (1997b). After a 24-h exposure period, the cephalopodal mass was quickly excised from each snail. For light microscopy, tissue blocks (primary) were cut transversely from the central region of cephalopodal masses and fixed in fresh Zenker's fluid (Drury & Wallington, 1980) for 24 h. The material was dehydrated and embedded in paraffin wax. Serial sections were cut at a thickness of 5 µm and stained with Haematoxylin, counterstained with Eosin, and examined through a Nikon Biophot compound microscope. For electron microscopy, secondary tissue blocks were cut from the mid-ventral region of the primary blocks (of known orientation). The material was fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for two h, washed in the same buffer three times for five min each, and postfixed in 0.5% osmium tetroxide for a further two h. The tissue was washed again in buffer and dehydrated through a graded series of acetone and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and

lead citrate for 10 min each and examined with a Jeol 1010 TEM.

RESULTS

Benchside Observations

Snails in untreated water (controls) initially withdrew into their shells, but soon resumed normal activity, moving around the container with the foot extended. When a mechanical stimulus was applied to the foot sole, the snail immediately retracted into its shell. At sublethal concentrations of the *A. attenuata* extract, toxic effects became evident. These included the animals becoming torpid and eventually inactive after 24 h. This was accompanied by the visible swelling of the cephalopodal mass. Probing of the foot sole with a blunt needle elicited an incomplete withdrawal into the shell, often needing a greater stimulus for further responses. The partial withdrawal may have been due to the swelling of the foot. In an earlier trial (T. D. Brackenbury, unpublished data), it was noted that when snails were placed in extract-free water after sublethal exposure, they made a swift and full recovery. Another observed effect was the development of subepithelial haemorrhagic "blisters" of the foot sole itself. These "blisters" appeared approximately six h after exposure, forming transient red blebs beneath the epithelium in the central region covering about a quarter of the foot's surface area. In lethal solutions of *A. attenuata*, the same sequence of events occurred as at sublethal concentrations, but with the cephalopodal mass of each snail becoming severely swollen, turgid and failing to respond to mechanical stimulation. Mucus secretion was observed over most of the foot, but it was not excessive. After 15 h exposure, haemorrhagic "blisters" were observed over the entire ventral surface of the foot. Three h later hemolymph accumulation became apparent beneath the epithelium at the edge of the foot, lips and tentacles. After 21–24 h exposure, there was visible hemorrhage into the surrounding water and the snails had died, with their soft parts retracted into their shells.

Light Microscopy

Control: In the control animals the foot sole epithelium consisted of a single layer of tall, ciliated columnar epithelial cells (length

23–30 μm) (Fig. 1) interspersed with occasional goblet cells. Heterochromatic material and prominent nucleoli were visible in the spherical to oval nuclei of the former, which generally occupied the mid to basal half of the cell. The epithelial layer rested on a basal lamina that was not always easily discernible, but delineated the boundary between the epithelium and underlying connective tissue. Below this lamina was dense connective tissue permeated by an abundance of muscle cells, fibres, and other cellular elements embedded in a ground substance (Fig. 1). The muscle fibres were not arranged in a continuous sheet, but were scattered in the connective tissue. Single and clustered subepithelial gland cells, presumably foot-sole gland cells (Zylstra, 1972), were located in the connective tissue just below the basal lamina. When stained with Haematoxylin and Eosin, their cytoplasm appeared purple, with prominent, deep purple heterochromatic nuclei. The necks of these cells extended between the ciliated cells to the free surface. Muscle fibres were found to run through and between these groups of gland cells. Haemocoelic sinuses were found deeper ($>300 \mu\text{m}$) into the connective tissue and appeared as perforations surrounded by fibrous elements. Some sinuses were filled with haemolymph.

Sublethal Concentrations: At the light microscope level it was clearly demonstrated that sublethal concentrations of *A. attenuata* induced gross structural damage to the epithelial layer (Fig. 2). There was a marked reduction in the number of cilia on the cells. The basal half of the epithelial layer appeared to be intensely "vacuolated," which resulted in conspicuous spaces developing between the basal halves of many adjacent cells. The ciliate cells and some of their organelles, particularly nuclei, were laterally compressed. The cells remained joined at their apices, presumably due to the presence of septate cell junctions (Zylstra, 1972), so that the integrity of the epithelial sheet as a barrier to the external environment was maintained. Another notable effect was the accumulation of a homogenous substance, possibly haemolymph, between the basal lamina and the underlying connective tissue (Fig. 2). This resulted in the extensive corrugation of the lamina, which distorted the bases of the ciliate cells. This would correlate with the haemorrhagic "blisters" seen from benchside observations. The molluscicide also seemed to have caused a

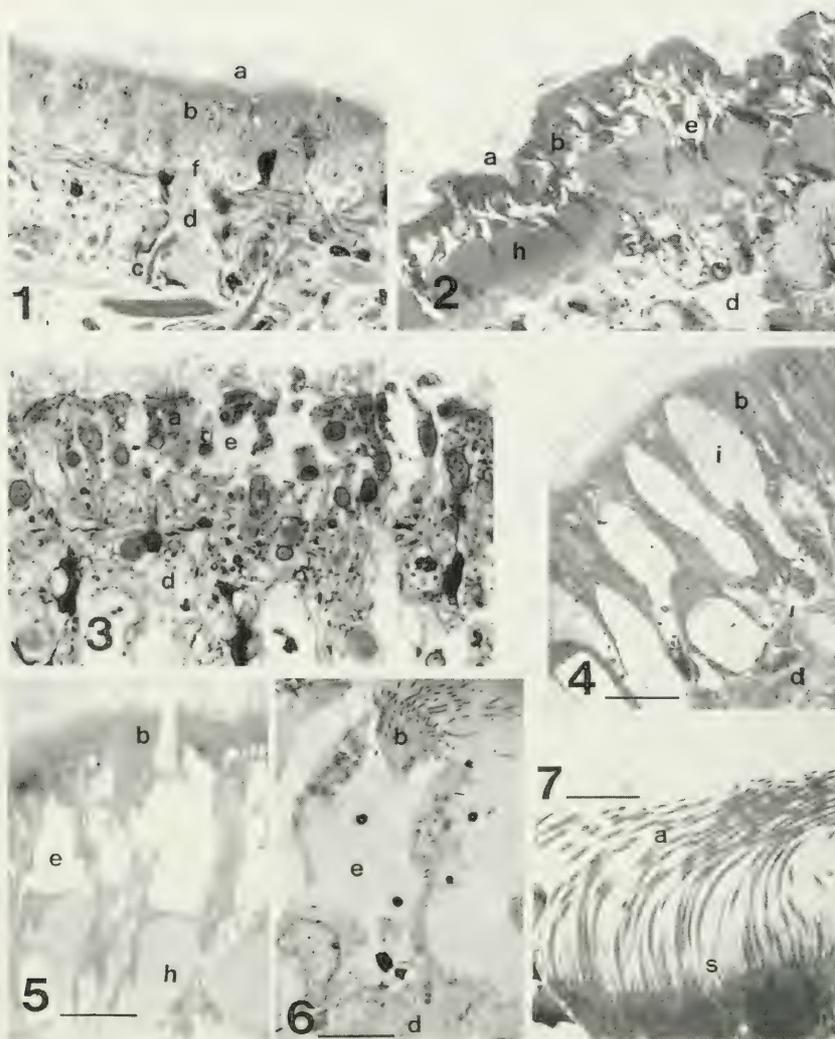
partial breakdown of the muscle fibres and ground substance of the connective tissue (Fig. 2). Haemocoelic sinuses were generally only evident up to 590 μm into the connective tissue. Most of the cellular damage was restricted to the area directly beneath the epithelium, extending to a maximum depth of 128 μm into the connective tissue.

Lethal Concentrations: Lethal concentrations of *A. attenuata* produced severe gross structural damage to the epithelium and the connective tissue immediately below this epithelium (Fig. 3). The epithelial layer had broken down entirely, with few ciliate cells remaining joined. The basal lamina could not be discerned so that the epithelial layer and connective tissue appeared continuous and the haemolymph "layer" lost. The connective tissue showed damage similar to that observed at sublethal concentrations, except that it was more intense and had penetrated deeper, up to 400 μm . Fewer haemocoelic sinuses were noted in the tissue.

Transmission Electron Microscopy

Cellular Profile: In untreated animals, the lateral plasma membranes of adjacent ciliate cells closely abutted one another, probably held together by cell junctions, as observed by Zylstra (1972) (Fig. 4). These opposing membranes were, however, separated by narrow intercellular spaces. The cells' basal plasma membranes were closely applied to the basal lamina (Fig. 4). At sublethal concentrations (Fig. 5), the molluscicide caused elongation and distortion of the profile of the ciliate cells. The "vacuoles" observed using light microscopy (Fig. 2) were caused by the expansion of the intercellular spaces between adjacent lateral plasma membranes. Electron light material was evident in many of these "vacuoles" (Fig. 5). The compression of the ciliate cells had a domino effect on the cells' organelles, especially the nuclei. The bases of the ciliate cells and basal lamina were further distorted by the accumulation of haemolymph (Fig. 5).

The most obvious cellular injury resulting from exposure to lethal concentrations of *A. attenuata* was the total disintegration of the epithelial layer. Contact between individual ciliate cells was generally lost, and the cells became detached from the underlying tissue. Not all cells were equally affected. No evidence of the basal lamina could be found re-



FIGS. 1-7. Ciliated foot-sole epithelium of untreated *Bulinus africanus* showing cilia (a) arising from a layer of columnar cells (b) resting on a basal lamina (f). Parts of the long ducts of sub-epithelial gland cells (c) are also visible in the connective tissue (d). Magnification 320 X.

FIG. 2. Ciliated foot-sole epithelium of *Bulinus africanus* exposed to sublethal concentrations of *A. attenuata*. Cilia (a) and columnar cells (b) are visible as are spaces between adjacent cells (e). Accumulations of a homogeneous substance (h), presumably haemolymph, intrude between the basal lamina and underlying connective tissue (d). Magnification 320 X.

FIG. 3. Ciliated foot-sole epithelium of *Bulinus africanus* exposed to a lethal concentration of *A. attenuata*. The ciliated epithelium (a) is severely disrupted by spaces between some of the cells (e) and the position of the basal lamina is no longer apparent. d = damaged connective tissue. Magnification 320 X.

FIG. 4. TEM micrograph of ciliated foot-sole epithelium of untreated *Bulinus africanus*. The ciliated columnar epithelium (b) is visible as are several mucous goblet cells (i) lying between the epithelial cells (these do not appear in the section in Fig. 1). D = connective tissue. Scale bar = 10 μ m.

FIG. 5. TEM micrograph of foot-sole epithelium of *Bulinus africanus* exposed to a sublethal concentration of *A. attenuata*. The ciliated epithelium (b) is largely intact but the cells are distorted and the spaces (e) between adjacent cells have enlarged. Intrusive accumulations (h), presumably haemolymph, are visible below the epithelial cells. Scale bar = 5 μ m.

FIG. 6. TEM micrograph of foot-sole epithelium of *Bulinus africanus* exposed to a lethal concentration of *A. attenuata*. The intercellular spaces (e) have enlarged to such an extent that the epithelium (b) has lost its integrity. d = sub-epithelial connective tissue. Scale bar = 5 μ m.

FIG. 7. TEM micrograph of foot-sole epithelium of untreated *Bulinus africanus* showing the cilia (a) and microvilli (s) on the apical surface of the columnar epithelial cells. Scale bar = 2 μ m.

sulting in the apparent fusion of the damaged connective tissue and epithelium (Fig. 6).

In the tissue of control snails, there was a profusion of cilia originating from the free surfaces of the ciliate cells with interspersed microvilli (Fig. 7). Immediately beneath the plasma membrane, each cilium was anchored to a basal body. These were regularly arranged and appeared to be linked to adjacent basal bodies by means of basal knobs (Fig. 8). Emanating from the basal bodies were well-developed, long striated rootlets extending for at least 12 μm into the finely granulated cytoplasm (Fig. 9). The striations had a periodicity of 65 nm. An obvious effect of sublethal doses of *A. attenuata* was the reduction in number of cilia, and this was accentuated at lethal concentrations, such that the physical aspect of the cilia also appeared to have deteriorated. Either the basal bodies protruded further from the surface plasma membrane or the membrane had retracted in response to intoxication (Fig. 10). The loss of cilia appeared to be the result of severance at the junction between the cilia and their basal bodies. The rootlets did not appear to be damaged by exposure to *A. attenuata*, but the microvilli had disappeared completely.

Mitochondria: In untreated tissue, the cells' apical cytoplasm just below the basal bodies, was characterized by a zone ($\pm 2.5 \mu\text{m}$ wide) of densely packed oblong to elongate mitochondria measuring $1.0\text{--}1.25 \times 0.16\text{--}0.3 \mu\text{m}$ with rounded or tapered ends (Fig. 11). The matrix of the mitochondria was homogenous with well-developed, regularly arranged cristae. In addition, the organelles tended to lie parallel to the long axis of the cell. They were less dense and scattered in the remaining cytoplasm. When the snails were exposed to increasing concentrations of the extract, the cytopathological damage became more evident. At lethal concentrations, the mitochondria became swollen, taking on an oval ($0.7 \times 0.53 \mu\text{m}$) to spherical shape (diameter $0.5 \mu\text{m}$), the matrix density decreased, and the cristae became more distended and disrupted, giving a transverse to erratic arrangement (Fig. 12). The outer membranes of several mitochondria were observed to have ruptured.

Nuclei: Normal nuclei were relatively rich in heterochromatin and had prominent nucleoli. The margins of the nuclear envelope were even and closely applied to the cytoplasm

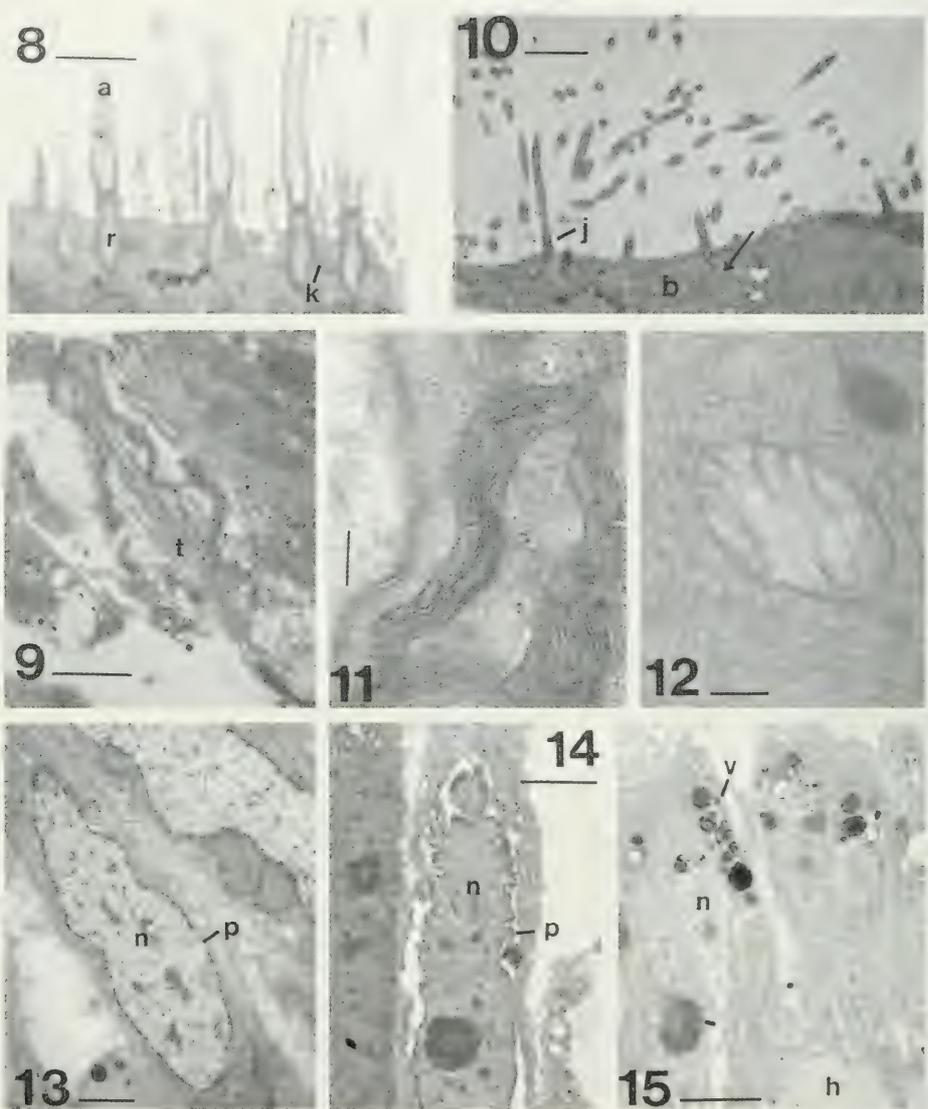
(Fig. 13). The amount of heterochromatin was observed to increase with increasing molluscicide concentration and was accompanied by a darkening of the karyoplasm. The nucleolus was also observed to have enlarged. Another effect was that the nuclear envelope, which had become corrugated, receded from the cytoplasm, leaving a space around most of the nucleus. This effect was intensified at lethal doses and is illustrated in Figures 13 and 14.

Golgi Complex and Endoplasmic Reticulum: Neither the golgi bodies nor the endoplasmic reticulum elicited any strong response to sublethal or lethal concentrations of *A. attenuata*, except for an occasional slight dilation of the cristae.

Secretory Products: A few small secretory bodies and lysosome-like vesicles were observed in the ciliate cells of untreated snails. These were situated mainly in the apical cytoplasm just below the mitochondrial zone, but were sporadic in the remaining cytoplasm. After exposure to sublethal and lethal concentrations of *A. attenuata*, these vesicles increased not only in number, but also in size with variable electron density (Fig. 15). Epithelial goblet cells, which produced a homogenous secretion, were stimulated to release most of their contents after exposure to *A. attenuata*. This would partly account for the benchside observation of vigorous mucus secretion. Distinctive groups of subepithelial gland cells were differentiated by their secretions, which varied from small electron-dense bodies to large electron-lucent bodies or vacuoles of variable density. In the absence of histochemistry, these cells could not be identified, and their secretory products are not illustrated. Nevertheless, they occupied much of the cytoplasm of these cells, which were noted to vary in abundance from one region of the foot sole to another. Their reaction to the molluscicide was similar to that of the goblet cells, that is, an increased production of secretion. The disintegration of the distal connective tissue and epithelium at lethal concentrations resulted in a loss of all secretory product from this subepithelial tissue.

DISCUSSION

After exposure to the extracts, *B. africanus* showed several of the behavioural responses indicative of intoxication, namely the distress



FIGS. 8–15. TEM micrograph of foot-sole epithelial cell of untreated *Bulinus africanus* showing the linkage between the basal bodies (r) of adjacent cilia (a) via a basal knob (k). Scale bar = 400 nm.

FIG. 9. TEM micrograph of the foot-sole epithelial cell of untreated *Bulinus africanus* showing the striated rootlet (t) of a cilium. Scale bar = 2 μ m.

FIG. 10. TEM micrograph of the apical surface of a foot-sole epithelial cell (b) of *Bulinus africanus* exposed to a lethal concentration of *A. attenuata*. Damaged cilia and the uneven surface of the cell are clearly shown. The junction between basal body and shaft of cilium (j) is the point at which cilia break. An unaffected rootlet is also visible (arrow). Scale bar = 2 μ m.

FIG. 11. TEM micrograph of a normal mitochondrion at the apical end of a foot-sole epithelial cell of untreated *Bulinus africanus*. Scale bar = 200 nm.

FIG. 12. TEM micrograph of a distended mitochondrion at the apical end of a foot-sole epithelial cell of *Bulinus africanus* exposed to a lethal concentration of *A. attenuata*. Scale bar = 200 nm.

FIG. 13. TEM micrograph of foot-sole epithelial cell of untreated *Bulinus africanus* showing a normal nucleus (n) with its smooth nuclear envelope (p). Scale bar = 2 μ m.

FIG. 14. TEM micrograph of the nucleus (n) of a foot-sole epithelial cell of *Bulinus africanus* exposed to a lethal dose of *A. attenuata*. The corrugated nuclear envelope (p) is clearly shown. Scale bar = 3 μ m.

FIG. 15. TEM micrograph of ciliated epithelium of *Bulinus africanus* exposed to sublethal concentrations of *A. attenuata*. Secretory and lysosome-like vesicles (v) are visible in the apical cytoplasm. n = nucleus, h = haemolymph. Scale bar = 5 μ m.

syndrome as described for other planorbid species by Harry & Aldrich (1963), Jurberg et al. (1995), Sullivan & Cheng (1975), and van Aardt & Coertze (1981). Swelling of the tissues was not restricted to the tentacles (van Aardt & Coertze, 1981; Wolmarans et al. 1986), but involved the whole cephalopedal mass. The inference of this observation was that the tissues of the cephalopedal mass had accumulated water, which caused haemorrhage at lethal concentrations. Being a pulmonate, the body tissues and haemolymph of *B. africanus* are continually subjected to water influx and ion efflux as they are hyperosmotic to the water in which they live. Active membrane regulation normally prevents uncontrolled water entry and allows for the uptake of ions (Cheng & Sullivan, 1977). However, the observations made in this study suggest that the toxins in *A. attenuata* had disrupted the permeability of the surface epithelium of the foot-sole by preventing its normal osmoregulatory functioning. The toxic effects were, however, reversible after sublethal exposure provided the snails were moved to toxin-free water for a recovery period. This was also noted for *Bulinus tropicus* and *Biomphalaria glabrata* (Harry & Aldrich, 1963; van Aardt & Coertze, 1981) after exposure to copper. Exposure to lethal concentrations of *A. attenuata*, caused irreversible cellular damage to *B. africanus*.

The assumption that the molluscicidal action of *A. attenuata* on *B. africanus* was due to the disruption of the osmoregulatory physiology of the foot-sole epithelium was supported by evidence of specific cellular injuries. One of the most conspicuous injuries after sublethal exposure was the accumulation of haemolymph beneath the basal lamina. If the molluscicide had altered the permeability of the surface membrane of the ciliate cells, the resultant water influx would have decreased the osmolality of the haemolymph, thereby increasing the retention of fluid in the tissue, as observed in *B. glabrata* when exposed to copper (Cheng & Sullivan, 1977). This would account for the swelling of the cephalopedal mass observed in *B. africanus*. Further evidence in support of increased water influx was the distention and empty appearance of the subepithelial connective tissue. The loss of haemocoelic sinuses was possibly due to the lysis of the cellular and fibrous components of the connective tissue as observed in *B. glabrata* by Sullivan & Cheng (1975).

The decrease in haemolymph osmolality,

the breakdown of connective tissue and haemocoelic sinuses could together account for the accumulation of haemolymph beneath the epithelial layer and which would have flowed along the path of least resistance, namely damaged tissue. Since the basal lamina remained intact, it had a damming effect on the haemolymph. The consequent swelling in this region caused the distension of the lamina, which in turn impinged on and distorted the ciliate cells. Higher concentrations of *A. attenuata* extract, further disrupted the osmoregulatory mechanisms, which made the tissue more permeable to water. This not only intensified the cellular damage observed at sublethal levels, but it extended deeper into the connective tissue. The excessive buildup of haemolymph stretched the basal lamina causing it to rupture and disintegrate. This would account for the absence of the lamina following lethal exposure. It is postulated from this that the haemorrhage observed at lethal concentrations was due to haemolymph passing between the weakened ciliate cells, breaking their apical junctions and escaping into the surrounding water. In doing this, it caused the destruction of the integrity of the epithelial layer and signalled the death of the snail.

The ultrastructural damage to the ciliate cells of *B. africanus* lent further support for the proposed mode-of-action of *A. attenuata*. The loss of cilia and microvilli from the surface epithelial cells was also observed in the epithelium of the digestive tract of the slug *Dero-ceras reticulatum* and rectal ridge of *B. glabrata* after exposure to synthetic molluscicides (Sullivan & Cheng, 1975; Triebkorn & Künast, 1990). This loss has been attributed to the toxins reacting with the plasma membrane, destabilizing the ionic regulation mechanism. An increase in membrane permeability would have resulted in cell death (Rez, 1986; Triebkorn & Künast, 1990).

Visible intracellular damage caused by *A. attenuata* was restricted to the plasma membranes of the ciliate cells and the mitochondrial membranes. These latter were the most severely affected organelles and showed swelling, disruption of the cristae, and rupture of the membrane. Similar observations were made on the epithelium of other snail species after exposure to Bayluscide[®], carbamate and metaldehyde (Goyer & Rhyne, 1975; Triebkorn & Künast, 1990; Triebkorn, 1989, 1995) and the molluscicidal plant, *Tetrapleura tetraptera* (Adewunmi & Ogbe, 1986; Bode et al., 1996). These injuries are described as cel-

lular stress symptoms and are considered non-specific, because they are basic changes that result from the physiological disturbance of the cell in response to the molluscicide, that is, it is probable that the injuries to these organelles are related to cell death rather than the direct action of the toxin. They have been attributed to the destabilization of mitochondrial membrane and the increase in permeability to ions (Triebkorn, 1989).

The increased production and release of secretory product in the various subepithelial gland cells in *B. africanus* may serve to form a protective covering over the epithelium as a response to the molluscicide (Sullivan & Cheng, 1975). The material present in the "spaces" between the bases of the ciliate cells observed after sublethal exposure was possibly secretory in nature. This might have arisen from increased production and secretion by sub-epithelial gland cells. The accumulation of this material and haemolymph culminated in the distortion of the ciliate cells. Damage to the nuclei was neither intense nor specific, but likely to be the cumulative effect of the disruption of the cell's physiology (Triebkorn, 1995).

The effect of the *A. attenuata* extract involves the disruption of the osmoregulatory physiology of the foot-sole epithelium. Osmolality and electrolytic studies are required to confirm this mode-of-action. There is the possibility that enzyme-mediated pathways are affected, but this can only be substantiated by enzymic-histochemical and biochemical studies. There is also a need to determine whether or not the activity of *A. attenuata* is target-specific. It is possible that if other tissues are affected, different mechanisms might be involved.

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INVADING FRESHWATER GASTROPODS: SOME CONFLICTING ASPECTS FOR PUBLIC HEALTH

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ABSTRACT

Invading molluscs have received special attention for many years because they often have a catastrophic ecological impact on local biodiversity. Considering their significance to public health, invading molluscs may have either negative or positive influence. The negative influence can be illustrated by two major parasitic diseases transmitted by freshwater gastropods – blood and liver flukes – for which recent studies carried out in the New World, have brought new and sometimes conflicting insights on the consequences of snail introductions. It is well established that the parasites responsible for these diseases were introduced into the neotropical area in the last centuries. It was formerly thought that the parasites had there encountered suitable snail intermediate hosts. However, recent genetic studies have revealed a non local origin of some of these hosts. Conversely, freshwater snail invasions may be beneficial to public health. For example, some thiarid and ampullariid species are good competitors of the pulmonate hosts of blood and liver flukes, and several biological control programs have demonstrated their usefulness in the Caribbean area. However, the relationships between invading molluscs and public health are more complex, because some of these “beneficial” species are also suspected to play the role of intermediate hosts for several animal or human parasites, such as the paragonimiasis.

Key words: (*Paragonimus westermani*), centrocestiasis (*Centrocestus formosanus*), or meningitis (*Angiostrongylus cantonensis*).

INTRODUCTION

Biological invasions are one of the serious consequences of human activities. The case of invading molluscs has been studied for many years because of their influence on local biodiversity, their economic damage and disturbance, or their consequences to human health. For example, the introduction of the land snail *Euglandina rosea* into the Society Archipelago, French Polynesia, is responsible for the extinction of a remarkable endemic land snail fauna (Tillier & Clarke, 1983; Tillier, 1992; Civeyrel & Simberloff, 1996). Regarding human health, freshwater gastropods have received special attention because they can play the role of intermediate hosts for numerous parasites infecting domestic animals and humans (Malek & Cheng, 1974; Malek, 1980). Recent studies carried out in the New World on two major parasitic diseases, schistosomiasis and fascioliasis, have brought new and sometimes conflicting light on the consequences of snail introductions on human health (Pointier et al. 1991a, b; Pointier & Guyard, 1992; Jabbour-Zahab et al., 1997;

Woodruff & Mulvey, 1997). This paper mainly deals with the medical consequences of freshwater snail invasions, distinguishing between those that may be considered as negative and positive from the human health point of view. Environmental impacts of introductions that also have serious implications have not been considered in this short review.

INVADING SNAILS AS A NUISANCE FOR PUBLIC HEALTH: THE EXAMPLE OF THE SNAIL INTERMEDIATE HOSTS OF PARASITES

It is well established that *Schistosoma mansoni*, the trematode responsible for intestinal schistosomiasis, was introduced repeatedly into South America and the Caribbean islands through African slaves (Combes, 1990). It was thought that the parasite had encountered there a suitable local host, the planorbid snail *Biomphalaria glabrata*. However, recent genetic studies carried out on several populations of *B. glabrata* from the Dominican Republic, Puerto Rico, St. Lucia

and Brazil revealed African affinities of this species (Bandoni et al., 1995; Woodruff & Mulvey, 1997). In their study, Woodruff & Mulvey (1997) compared *B. glabrata* with four other Neotropical and three African species of *Biomphalaria*. Their four main results were: (i) *B. glabrata* clustered with the African *B. sudanica*, *B. alexandrina* and *B. Pfeifferi* and not the neotropical *B. havanensis*, *B. straminea*, *B. tenagophila* and *B. occidentalis*, (ii) the African species plus *B. glabrata* form a monophyletic clade, whereas the neotropical species are paraphyletic, (iii) the lineages of the neotropical species diverged earlier than those of the African clade, (iv) *B. glabrata* appears to have diverged more recently than the other species studied. These results are in agreement with the fossil record establishing that the oldest recorded occurrence of *Biomphalaria*-like shells is in South America and not Africa. Woodruff & Mulvey (1997) suggested that *B. Pfeifferi*, *B. alexandrina*, *B. sudanica* and *B. glabrata* evolved in Africa in the Pliocene and Pleistocene as a result of earlier trans-Atlantic dispersal of their ancestor from the Americas, and that several semispecies currently referred to *B. glabrata* returned to America through the slave trade over the last 500 years. However, this hypothesis would imply the current presence of *B. glabrata* somewhere in West Africa, but it has never been recorded there in spite of extensive studies (Brown, 1994). If this snail was present in the past, it is difficult to imagine that it has now disappeared from this region. Nevertheless, very few molecular genetic studies have been conducted on *Biomphalaria* species in West Africa (Mimpfundi & Greer, 1990a, b); further such studies would help to clarify the taxonomic position and phylogenetic relationships of the West African *Biomphalaria*.

Another Neotropical species, *B. straminea*, has proven in the last decades its capacities for travelling and colonizing new territories (Madsen & Frandsen, 1989). This species is an important intermediate host for intestinal schistosomiasis in northeastern Brazil in spite of its low susceptibility to the parasite (Lucena, 1963; Barbosa & Coelho, 1956; Barbosa & Figueiredo, 1970). It was first reported outside the Neotropics in Hong Kong in 1973 (Meier-Brook, 1974), and it is currently invading the mainland of China (Dudgeon & Yipp, 1983; Woodruff et al., 1985a, b; Yipp, 1990). Intestinal schistosomiasis does not occur in Hong Kong and China, and the Asiatic schistosomes use local prosobranch snails as

obligatory intermediate hosts; moreover, parasitological surveys did not reveal the presence of trematodes infecting *B. straminea* in Hong Kong and China (Tang, 1990). Consequently, this species should be considered only as a potential nuisance from the human health point of view. However, its rapid spreading through the mainland of China actually constitutes a threat.

The introduction of *B. straminea* was also reported into several West Indian islands, including Martinique in the 1950s (Grétilat, 1967), Grenada in 1970 (Ferguson & Buckmire, 1974), Guadeloupe in 1985 (Pointier et al., 1993a) and St Lucia in 1992 (Pointier, 1993). Contrary to China, intestinal schistosomiasis was already present in most of these islands with *B. glabrata* as intermediate host. Several control programmes of the disease were undertaken during the last decades and the parasite transmission is now interrupted or at a low level. As in China, *B. straminea* was never found infected by *S. mansoni*, but its current presence represents a non-negligible risk of reactivation of foci. Other Neotropical countries were also invaded by *B. straminea*: Colombia in 1966 (Barbosa, 1968), Costa Rica in 1976 (Paraense et al., 1981), and Uruguay in 1987 (Paraense & Correa, 1989). However, intestinal schistosomiasis is, until now, absent from these countries.

The liver fluke, *Fasciola hepatica*, was introduced into the New World during the last 400 years through cattle importation (Mas-Coma et al., 1995). The main intermediate host for this parasite in the Old World is the lymnaeid snail *Lymnaea truncatula* (Malek, 1980). In the New World, several native lymnaeids, such as *L. cubensis*, *L. viatrix* or *L. columella*, were known to be responsible for the parasite transmission (Malek, 1985). However, recent studies carried out in the human fascioliasis focus of the northern Bolivian Altiplano have demonstrated the local lymnaeids to be European *L. truncatula*, strongly suggesting an introduction of this snail from the Iberian Peninsula during the Spanish colonization (Jabbour-Zahab et al., 1997). These authors conducted a multilocus analysis of allozyme variation in 16 Bolivian populations, six populations of *L. cubensis* from the Caribbean area and one population of *L. truncatula* from each of Morocco, Portugal and France. Two genetic clusters could be differentiated displaying different fixed alleles at 11 loci among the 18 studied and a large genetic divergence. One cluster included samples of *L. cubensis*, while the other cluster included all samples of *L. truncatula*

and the samples from Bolivia previously identified as *L. viatrix*. The Bolivian snails were identical to those from the Portugal and were only slightly different from those from Morocco and France. A subsequent study of lymnaeid snails from southern Chile, previously identified as *L. viatrix*, also showed them to be *L. truncatula* (P. Durand, unpublished data). This suggests that the European lymnaeids were repeatedly introduced into South America during the Spanish colonization. The introduction of the snail host, together with adult parasites within imported cattle into the Bolivian Altiplano, constituted the key factor explaining the current presence of very high prevalence and intensity of infection in humans (Esteban et al., 1997). In other South American countries where fascioliasis is endemic, the particular role of introduced *L. truncatula* is unknown because of the presence of morphologically very similar local species, such as *L. viatrix* or *L. cubensis*. It is thus impossible to evaluate the particular role of the introduced *L. truncatula* without more specific studies.

INVADING GASTROPODS AS A BENEFIT FOR PUBLIC HEALTH: THE EXAMPLE OF THE BIOLOGICAL CONTROL OF THE SNAIL HOSTS OF SCHISTOSOMES

Introduction of freshwater snails may present some more positive features regarding public health. The case of some species belonging to the families Thiaridae and Ampullariidae that were used in several biological control programmes in the Caribbean area is especially relevant. For example, *Melanooides tuberculata* and *Tarebia granifera* originating from East Africa and Southeast Asia respectively, both now have a worldwide distribution in the tropics. Their accidental introduction into the Caribbean area through aquatic plant trade and the invasion processes during the last few decades are well documented (Chaniotis et al., 1980; Pointier & McCullough, 1989; Pointier et al., 1993b). These two thiarid species are also known as competitors of pulmonates, and their impact on the snail hosts of schistosomiasis have been extensively studied in the Caribbean area following several biological control programmes (Butler et al., 1980; Prentice, 1983; Pointier et al., 1991a; Pointier & Guyard, 1992; Pointier et al., 1994).

Tarebia granifera was reported for the first time in Puerto Rico in 1954 (Harry & Aldrich, 1958), and it rapidly invaded numerous water-

bodies (Chaniotis et al., 1980). Between 1964 and 1969, a competitive displacement of a *B. glabrata* population by *T. granifera* was recorded along a stream (Butler et al., 1980). A recent malacological survey carried out by Giboda et al. (1997) in several types of habitats confirmed the colonization of the whole island and revealed the apparent absence of *B. glabrata*. The ampullarid *Marisa cornuarietis* was also used in Puerto Rico as a competitor/predator in some biological control experiments after its deliberate introduction as ornamental aquaria snails. Its efficiency was demonstrated in irrigation ponds, where it eliminated *B. glabrata* from 89 out of 97 ponds nine years after its introduction (Palmer et al., 1969). Similar results were achieved in artificial lakes (Jobin et al., 1977) and also in some streams near Monte Belo (Jobin & Laracunte, 1979).

In 1978, the thiarid snail *M. tuberculata* was introduced to seven sites on the island of St. Lucia. Two years later, it had eliminated *B. glabrata* from these sites (Prentice, 1983). More recently, in 1992, a malacological survey carried out in sites where *B. glabrata* occurred in large populations in the past, revealed its absence from seven sites, its presence in low or very low densities in 17 sites, and its presence in large populations in only two sites (where *M. tuberculata* was absent) (Pointier, 1993).

In Martinique, *M. tuberculata* was discovered for the first time in 1979, and the following years the species was found at several sites (Pointier et al., 1993b). The origin of this introduction is unknown, but it is probably a consequence of the strong increase of trade in aquarium fishes and plants that occurred in the 1980s on the island. A biological control programme was initiated in 1981 using *M. tuberculata* and focused on watercress cultures that constituted the last transmission sites on the island. The thiarid snail was introduced at the beginning of 1983 to several groups of watercress beds. A long-term survey showed a rapid colonization by the competitor, and by 1990, *B. glabrata* and *B. straminea* had totally disappeared from eight sites and only a few individuals could be recorded from the remaining sites (Pointier & Guyard, 1992).

The case of Guadeloupe is particularly relevant to the efficiency but also the limits of biological control. Several competitors of *B. glabrata* already present on the island have been tested and the results were variable according to the type of habitat. Two ampullariids, the native *Pomacea glauca* and the alien

Marisa cornuarietis, were successively introduced into a small lake, a sylvatic focus of schistosomiasis characterized by the presence of heavily infected populations of rats. Fourteen years after ampullariid introductions, *B. glabrata* had completely disappeared from the lake, and samplings carried out at the end of the experiment in 1990 and 1991 confirmed the eradication of both the snail host and its parasite (Pointier et al., 1991b).

Melanooides tuberculata was also tested in several Guadeloupean habitats some years after its first record on the island. As in Martinique, the origin of this introduction is unknown but probably linked to the aquatic plant trade (Starmühlner, 1983). Some good results were achieved in springs, canals and small streams (Pointier et al., 1991a), but an experiment carried out in a marshy forest zone located behind a mangrove swamp on Grande-Terre Island was a failure because of some particular ecological characteristics of the habitat (Pointier et al., 1993c).

The biological control of *B. glabrata* was also tested in a schistosomiasis area in Venezuela (Pointier et al., 1991c). *Melanooides tuberculata* was introduced into several types of habitats, including irrigation ponds, reservoirs, streams, rivers and canals. Results were very variable according to the type of site and revealed the importance of some ecological factors limiting the competition between snails. These factors include water level fluctuations in ponds, floodings in streams and rivers, presence of emerged or submerged macrophytes and sewage pollution (Pointier et al., 1991c). On the other hand, unassisted introductions of *T. granifera* and *M. tuberculata* into some rivers of the littoral central region of Venezuela occurred at the beginning of the 1979s. Several of these rivers were active transmission sites for schistosomiasis and *B. glabrata* was very common. Several surveys carried out before 1975, and between 1975 and 1983, showed a rapid spread of the thiarid snails in the whole hydrographic system along the coast. In 1990, almost all the rivers harboured dense populations of thiarid snails, whereas *B. glabrata* was not recorded (Pointier et al., 1994).

INVADING GASTROPODS AS A THREAT IN INTRODUCING NEW PARASITES

The relationships between invading snails and public health are more complex and may

sometimes be conflicting when snails that are used in biological control are suspected to play the role of intermediate hosts for several parasites of medical or veterinary importance. The examples of some flukes, such as the lung fluke, *Paragonimus westermanii*, food-borne trematodes *Metagonimus yokogawai*, *Haplorchis taichui*, *Centrocestus formosanus*, or nematodes such as *Angiostrongylus cantonensis*, are relevant to this problem.

It has long been asserted that *T. granifera* is an intermediate host of the lung fluke, *P. westermani* (Abbott, 1952; McMullen, 1973), thus curtailing the use of this species in biological control programmes. However, Michelson (1992) reviewed evidence incriminating this snail as a host for this parasite and showed that this reputation rests on an uncritical reading of the literature and the transfer and acceptance of this charge by authors and textbooks. This author wrote: "It is of further interest that the cercariae of originally described by Nakagawa (1917) as being those of *P. westermani* were, in fact, misidentified, and it is questionable if the snails collected at that time were truly infected with *P. westermani*. As noted by Yokogawa et al. (1960), Nakagawa first described the cercariae as a small virgulate xiphidiocercariae, an error which was soon corrected by himself (Nakagawa, 1919) as well by Yokogawa (1964)." Moreover, in the Neotropical area, *Paragonimus* spp. are transmitted by hydrobiid snails, not by thiarids (McCullough & Malek, 1984). Thus, the potential of *T. granifera* transmitting this parasite in the Neotropics appears questionable.

Melanooides tuberculata is the intermediate host of *C. formosanus*, the parasite responsible for a food-borne intestinal infection in humans in Asia. The reservoir hosts include rats, cats, dogs, chickens and ducks and the source of infection for humans is a freshwater fish (Yu & Mott, 1994). *Centrocestus formosanus* was introduced to a fish-farm in Mexico in 1979 together with five pairs of the black carp, *Mylopharyngodon piceus* (López-Jiménez, 1987). Its snail intermediate host, *M. tuberculata*, was also introduced to this country at the beginning of the 1970s (Abbott, 1973). Recent studies have confirmed the presence of both the parasite and its snail host in Oaxaca state, Mexico (Amaya-Huerta & Almeyda-Arigas, 1994). According to these authors, fish-eating migratory birds may play an essential role in the fluke's dispersal. However, the risk of infecting humans seems low,

because local people do not consume raw fish as is the case in Asia.

The nematode *A. cantonensis* is responsible for an eosinophilic meningitis in humans, and a number of molluscs are involved in its life cycle, including slugs, terrestrial pulmonate snails, and also freshwater snails (Malek, 1980). The ampullariid and thiarid snails introduced into the Caribbean area during the last few decades and used in biological control programmes may also serve as intermediate hosts for this parasite (Richards & Merritt, 1967). As for *C. formosanus* the risks of infection in humans seems low but not nil. For example, in some countries, local customs include eating some species of ampullariid snails, such as *Pomacea urceus*, in the southern part of Venezuela or *P. paludosa* in Cuba (Perera & Walls, 1996).

CONCLUSIONS

It is obviously difficult to assess the balance between the negative and positive aspects of the introduction and spreading of freshwater gastropods in relation to public health. Biological control using competitor snails may work well in some situations and in some habitats make a significant contribution to integrated control programmes of parasitosis. The Caribbean example is quite relevant to this aspect. In the Caribbean, many accidental introductions followed by successful invasions of numerous water-bodies occurred the last few decades. These introductions may have several origins. Some freshwater snails or egg masses may be dispersed by water birds or mammals, but the more important means of introduction seems linked to the trade in aquarium plants (Abbott, 1952; Madsen & Frandsen, 1989).

The case of Martinique island is particularly illustrative: eight new taxa were added to the 15 local species in the last 30 years (Tables 1, 2). These taxa include four planorbid species, one ampullariid and three thiarids (Pointier et al., 1993b; Pointier, 1996; Pointier et al., 1998). All these introductions were accidental. As previously mentioned, their origin is probably linked to the strong increase of the trade of aquarium fishes and plants that occurred in the 1980s on the island. For example, eight fish-shops have been recorded on the island in 1984 (A. Mosser, personal communication). Four species out eight remained restricted to very few sites, whereas *B. straminea*, and

TABLE 1. Native freshwater molluscs of Martinique (Bordaz, 1899; Deyfuss, 1953; Grétilat, 1967; Guyard & Pointier, 1979).

NATIVE SPECIES
PLANORBIDAE
<i>Biomphalaria glabrata</i> (Say, 1822)
<i>Drepanotrema depressissimum</i> (Moricand, 1839)
<i>Drepanotrema cimex</i> (Moricand, 1837)
<i>Drepanotrema lucidum</i> (Pfeiffer, 1830)
PHYSIDAE
<i>Physa cubensis</i> Pfeiffer, 1839
<i>Aplexa marmorata</i> (Guilding, 1828)
LYMNAEIDAE
<i>Lymnaea cubensis</i> Pfeiffer, 1839)
ANCYLIDAE
<i>Gundlachia radiata</i> (Guilding, 1828)
HYDROBIIDAE
<i>Potamopyrgus coronatus</i> (Pfeiffer, 1840)
NERITIDAE
<i>Neritilla succinea</i> (Récluz, 1841)
<i>Neritina virginea</i> (Linné, 1758)
<i>Neritina punctulata</i> Lamarck, 1816
AMPULLARIIDAE
<i>Pomacea glauca</i> (Linné, 1758)
SPHAERIDAE
<i>Pisidium punctiferum</i> (Guppy, 1867)
<i>Eupera viridans</i> (Prime, 1865)

subsequently, all thiarids showed strong invasive capacities (Table 2). The invasion by thiarids resulted in a strong reduction of populations of two species, *B. glabrata* and *B. straminea*. Regarding the rest of the malacological fauna, no quantitative data are available but no apparent extirpations have been detected so far and populations of native ampullariid (*Pomacea glauca*) or neritid snails (*Neritina punctulata*, *N. virginea* and *Neritilla succinea*) are commonly encountered together with introduced thiarid snails. In Guadeloupe, a survey was carried out in 1996 in 134 sites that had already been investigated in 1972. Introductions of four snails, including *M. tuberculata*, *M. cornuarietis*, *B. straminea*, and *H. duryi*, occurred between 1972 and 1996 (Pointier & Augustin, in press). In 1996, the number of sites housing *B. glabrata*, *Physa cubensis* and the bivalve *Eupera viridans* had strongly declined. No other environmental impacts have been observed, but more specific studies are needed.

In the Greater Antilles, the consequences of snail invasions on the native fauna seems much more serious because of the presence of several endemic species. For example, in

TABLE 2. Accidental introductions of freshwater snails into Martinique in the last 50 years.

INTRODUCED SPECIES	FIRST RECORD	DISTRIBUTION	ORIGIN	INTRODUCTION
PLANORBIDAE				
<i>Biomphalaria straminea</i> (Dunker, 1848)	Around 1950 (Grétilat, 1967)	Invasive	Venezuela	Birds or aquatic plant
<i>Helisoma duryi</i> (Wetherby, 1879)	Around 1970 (Guyard & Pointier, 1979)	Restricted (2 sites)	North America	Aquatic plant trade
<i>Amerianna carinata</i> (H. Adams, 1861)	1987 (Pointier, 1996)	Restricted (4 sites)	Australia	Aquatic plant trade
<i>Gyraulus</i> sp.	1982 (unpublished data)	Restricted (1 site)	?	Aquatic plant trade
AMPULLARIIDAE				
<i>Marisa cornuarietis</i> (Linné, 1758)	1987 (unpublished data)	Restricted (2 sites)	Venezuela	Unknown (aquarists?)
THIARIIDAE				
<i>Melanooides tuberculata</i> (Müller, 1758)			East Africa, Asia?	
FAL morph	1979 (Pointier, 1989)	Invasive		Aquatic plant trade
MAD morph	1979 (Pointier, 1989)	Invasive		Aquatic plant trade
PAP morph	1979 (Pointier, 1989)	Invasive		Aquatic plant trade
PDC morph	1990 (unpublished data)	Invasive		Aquatic plant trade
CPF morph (FAL × PAP hybrid)	1990 (Samadi et al., 1999)	Invasive		Local hybrid
DFD morph (FAL × PDC hybrid)	1994 (Samadi et al., 1999)	Invasive		Local hybrid
<i>Tarebia granifera</i> (Lamarck, 1822)	1991 (Pointier et al., 1998)	Invasive	East Asia	Aquatic plant trade
<i>Melanooides</i> sp.	1997 (unpublished data)	Invasive	Java	Aquatic plant trade

Cuba, invading thiarids have been found to have a significant impact on the native malacofauna. A recent survey carried out in the eastern part of the island (Yong, 1998) showed the disappearance of the endemic pleurocerid *Pachychilus violaceus* from several rivers of the Santiago area that have been invaded by *T. granifera*. This invading species was also recorded in the Pinar del Río province, where two endemic thiarids, *Hemisinus brevis* and *H. cubanius* are still present but seem already in danger to extinction.

The majority of freshwater gastropods introductions are accidental, and unfortunately it seems now difficult to stop or even to diminish this phenomena. Long-term field observations and rigorous investigations on the competitive interrelationships between introduced snails and intermediate hosts of parasites on the one hand, and endemic snail faunas on the other hand, seem highly desirable and deserve further support.

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ALIEN INVASIONS: THE EFFECTS OF THE GLOBAL ECONOMY ON NON-MARINE GASTROPOD INTRODUCTIONS INTO THE UNITED STATES

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ABSTRACT

With the expanding global economy, increasing trade volume and international trade agreements facilitating commodity movements worldwide, the risks of "alien" introductions are increasing. Of over 4,900 molluscan interceptions from almost 100 countries by USDAAPHIS PPQ inspectors over the last five years on commodities entering the United States, some 369 gastropod taxa, belonging to 197 genera in 71 families, have been identified. Although expected on agricultural and horticultural products, "hitch-hikers" also are found in and on shipping containers, household tiles, military hardware, aquarium supplies, and other items, as well as being deliberately introduced. Many of these mollusks belong to a lengthening list of traveller species, and the number of introduced species in each country is increasing inexorably despite efforts to slow their invasions. A listing of these travelling species is given, together with their distributions in the principal regions of the world with which the United States has commerce. This is drawn from a survey of the world malacological bibliography, which is provided. Most countries lack or have minimal quarantine restrictions, or where inspection agencies exist, they may be ineffective and/or underfunded. Local agriculture potentially will be negatively effected, native species adversely impacted, and the establishment of a non-indigenous species will create a reservoir for its further spread when that country exports its own products abroad. These invasive species may also be human and livestock disease vectors. As we lack specific ecological information for most of these taxa, there is an urgent need for the collection and pooling of any such data from concerned scientists and governments worldwide.

Key words: travelling species, invasive, terrestrial snails, aquatic snails, slugs, introduced, quarantine.

INTRODUCTION

The potential problems associated with the introduction of non-indigenous species into new habitats has long been known. The "alien" species are generally perceived as potential agricultural (or environmental) pests, as many such pests worldwide are seen to be taxa introduced from elsewhere. It would be more accurate, however, to understand these species as being typically associated with human activity, as the disturbed environments caused by such activity prove to be fertile ground for the establishment of such organisms. For example, many slug species that are pests to European agriculture are indeed native to that region, and have travelled with European colonists worldwide over the last few centuries.

With the protection of local agriculture in

mind, and having already had the experience of the establishment of foreign pests, many countries have established inspection and quarantine programs to prevent the further introduction of such taxa. Within the United States Department of Agriculture, this responsibility is undertaken by Plant Protection and Quarantine (PPQ), a division of the Animal and Plant Health Inspection Service (APHIS). PPQ is charged with the protection of U.S. agriculture and the environment, as well as facilitating international trade, elements that can come into conflict with one another. As the global economy expands, and such international trade organizations and treaties as GATT, NAFTA, WTO, play an increasingly significant role in promoting trade and lowering trade barriers between nations, the potential for new species introductions will increase in direct proportion to the volume of trade.

INTERCEPTION PROCEDURES

Shipments carrying mollusks and other organisms are intercepted at seaports, airports and border crossings into the United States every day. When a snail or slug is found, the shipment upon which the organism has been found is held, pending the identification of the mollusk. Normally the specimen is first sent to the Port Identifier, a taxonomist based at the port where the interception is made, who may be able to make the identification. If unable to do so, the specimen is shipped by overnight mail to me as the PPQ Mollusk Specialist, with the result that the shipment maybe held for up to one working day before an identification can be made. Once the mollusk is identified, a decision is then made as to whether the shipment is refused entry into the United States, treated to kill the organism (usually fumigated with methyl bromide), or allowed into the country without further treatment or delay. At ports with the appropriate technology, the Port Identifier takes digital images of the specimen(s) including multiple views of the shell, and dissection of the genitalia when possible, and transmitted electronically to the Mollusk Specialist. When species identification is possible by this means, a final decision on cargo disposition can be made sometimes within an hour or less.

It is important to understand that of all organisms, not only mollusks, but insects, plant diseases, noxious weeds, that are intercepted on incoming shipments, PPQ or any country's agricultural quarantine agency can only "take action," that is treat by fumigation or order re-exportation, when the organism is considered a "quarantine pest," and not simply any "foreign" or non-introduced species. A quarantine pest is defined as being "of potential economic importance to the area endangered thereby and not present there, or present but not widely distributed and being officially controlled" (FAO, 1995; NAPPO, 1995). Therefore, if an intercepted snail or slug cannot be proven to be of agricultural concern (based on the published literature), or if it is present somewhere in the country, and the agricultural department of the individual state is not taking active measures to control or eradicate the population of the species in question, then PPQ by international agreement is unable to prevent its entry. As a result, any species intercepted on any commodity that cannot be proven to be an uncontrolled agricultural pest, can pass unimpeded with its commodity un-

treated. For this reason, there is an urgent need to obtain feeding or other ecological information on any species that could potentially be of concern in order to justify "taking action," especially with those taxa determined to be travelling species. Without this information, many more travelling species will become introduced into the country and PPQ is essentially powerless to prevent it.

Since March 1993, when PPQ began recording all molluscan interceptions, including species deemed of agricultural significance or otherwise, as well as the frequency of each taxon, there have been over 4,900 recorded interceptions of gastropods, terrestrial and freshwater, representing 368 taxa in 197 genera belonging to 71 families (Appendix) (bivalves and marine species are excluded from this analysis). Of these, 93 species, approximately one quarter of the total, belong to a lengthening list of snail and slug taxa that repeatedly crop up in the molluscan literature worldwide as having a greater potential for becoming established in regions far from their native ranges due to several factors. These include being eclectic in their ecological requirements, displaying an unusually high adaptability to a wide range of environmental conditions, often wider than in their native ranges. They may be particularly unselective in their feeding requirements, and they may also have a very high reproductive capacity. Their dispersal into new habitats seems to be wholly or in part due to human activity, and as such they are called travelling species (Smith, 1989).

TRAVELLING SPECIES

These invasive taxa have been referred to as "tramp" species (Solem, 1964), and Harry (1964) referred to them as foreign or "exogenous" snails. Harry attempted to define his terms, using criteria that remain useful today. If a "detailed knowledge of the circumstances of introduction" were not known, then the following criteria were proposed in order to identify these introduced species.

1. Time of importation estimated from the species not being present during earlier faunal surveys.
2. No close relatives in the area where it is introduced.
3. Species remains in the habitat much affected by human activity.

4. Species remains localized in the new area, and has no vagility to invade new habitats there.
5. Species is known as a foreign snail elsewhere.
6. Species may develop enormous population densities in its new home, exceeding the population densities of native snails.

Later, referring to them as "tourist snails" (Harry, 1966), he noted that snails "of very large size are nearly always introduced deliberately, and usually for food," and "snails of intermediate size . . . are only introduced by man accidentally into new areas." "Tourist snails tend to remain in an environment highly modified by man, and do not through their own natural ability, invade more natural surrounding areas. . . . Thus, man not only is the agent of introduction, but also is responsible for maintaining the environment which allows them to become established in their new locality." Although Harry's criteria remain useful, it should be noted that some of these taxa may not necessarily remain in habitats affected by human activity in newly invaded regions, and may invade relatively undisturbed areas due to their ecological flexibility and lack of predators/parasites that would otherwise control their distribution and abundance in their native ranges. Such species include *Arion intermedius* in Australia (Smith, 1989), and *A. lusitanicus* moving into more natural areas in Sweden (Proschwitz, 1994a, 1996b). The invasion of introduced predatory snails into relatively unspoiled habitats on islands in the Indo-Pacific Basin highlights this propensity and raises the specter of the disastrous effects of other invasive species.

Smith (1989) coined the term "travelling snails" and was the first to analyze these species in a global context, identifying 59 taxa. Here the addition of more travelling taxa brings the total to 163, and the list will continue to grow as workers demonstrate that further species are spreading due to human activity. This list (Table 1), drawn from the available literature and by the use Harry's original six criteria, plus an additional one: species intercepted on shipments from "secondary" or intermediate geographic areas. PPQ has intercepted species from countries that have yet to report these species as part of their established molluscan fauna.

Some travelling species may be considerably more widespread than is currently reported in the literature. In some cases, they

may be described by workers as new taxa due to their unfamiliarity with groups from other parts of the world. To name just a few: *Lauria cylindracea* was described from Jamaica as *Pupa grevillei* Chitty, 1853 (Mienis, 1994a) and in South Africa as *Pupa tabularis* Melvill & Ponsonby, 1893 (van Bruggen, 1991); *Euconulus fulvus* was described in Australia as *Helix paramattensis* Cox, 1864 (Stanisic, 1981a), and *Zonitoides arboreus* as *Alienator lyndhurstoides* McLaughlin, 1954 (Bishop, 1978). Solem (1964) suggested that the New World *Pupisoma dioscoricola* is synonymous with the Old World *Pupisoma orcula*. If so, then Adam's name would have priority, and the taxon would have a known distribution spanning most tropical and subtropical parts of the world, and in temperate regions in such artificial microhabitats as greenhouses. In other cases, PPQ inspectors have repeatedly intercepted species from countries where they have yet to be reported in the literature. Examples of this include the Pacific Island species *Liardetia samoensis* from the Dominican Republic, and the European *Candidula intersepta* from Colombia and Chile. The Neotropical *Guppya gundlachi* is regularly being found in cut flower and live plant shipments from Thailand, indicating that the species is well established there, at least in orchid farms. These last species fulfill the seventh criterion of being travelling species, in addition to the other six. More unusual and unexpected interceptions include a Southeast Asian *Amphidromus* species on a banana shipment from Costa Rica, and the Caribbean Basin *Drymaeus multilineatus* (Say, 1825) on wood products from Guam. These are more likely to be "secondhand," resulting from the snail in question being shipped in containers from one port to another without being detected until it shows up as an anomalous interception. Only if these species continue to be found in cargoes from these countries would they be considered travelling taxa.

There are a number of very widespread snails that often span more than one region, such as *Quickia concisa*, that may be shown in the future to fulfill the criteria of being travelling species, but I have yet to find unequivocal evidence that they are being distributed by human activity, rather than simply natural range extensions in adjacent territory. Others, such as *Zootecus insularis* and *Pupoides coenopictus*, apparently do have a considerable native distribution, supported by paleontological evidence (Seddon, 1992), but that

TABLE 1. List of travelling species and their distributions worldwide according to region, with PPQ interceptions of each species expressed as a percentage of the total interceptions over the 1993–1998 time period.

	% of inter- ceptions	World Distribution*						Ocea.
		NAm.	LAm.	Eur.M.	Afr.	E.As.	ANZ	
HELICINIDAE								
<i>Alcudia striata</i> (Lamarck, 1822)	0.14	I	N					
AMPULLARIIDAE								
<i>Marisa cornuarietis</i> (Linné, 1758)	0	I	N		I			
<i>Pila conica</i> (Wood, 1828)	< 0.1					N		I
<i>Pomacea canaliculata</i> (Lamarck, 1804)	0	I	N			I		I
<i>Pomacea bridgesii</i> (Reeve, 1856)	< 0.1	I	N					I
<i>Pomacea cumingii</i> (King, 1851)	0		N					
<i>Pomacea paludosa</i> (Say, 1829)	0	N	N					I
VIVIPARIDAE								
<i>Bellamya heudei guangdongensis</i> (Kobelt, 1906)	0					N	I	
<i>Cipangopaludina chinensis</i> (Griffith & Pidgeon, 1834)	0	I				N		I
<i>Cipangopaludina japonicus</i> (von Martens, 1861)	0	I				N		
ASSIMINEIDAE								
<i>Assiminea nitida</i> (Pease, 1865)	< 0.1				I	I		N?
HYDROBIIDAE								
<i>Potamopyrgus antipodarum</i> (Gray, 1853)	0	I		I			N	
THIARIDAE								
<i>Melanoides tuberculata</i> (Müller, 1774)	0.12	I	I	I	I	N	I	I
<i>Tarebia granifera</i> (Lamarck, 1822)	< 0.1	I				N		I
<i>Tarebia scabra</i> (Müller, 1774)	< 0.1				I	N		I
PHYSIDAE								
<i>Physella acuta</i> (Draparnaud, 1805)**	0.22	I	I	N	I	I	I	
<i>Physella acuta</i> , auctt., non (Draparnaud, 1805)**	0	N	I		I			
<i>Physella ancillaria</i> (Say, 1825)	0	N	I?					
<i>Physella virgata</i> (Gould, 1855)	0	N						I
LYMNAEIDAE								
<i>Galba truncatula</i> (Müller, 1774)	< 0.1	I	I	N	I			
<i>Fossaria viridis</i> (Quoy & Gaimard, 1832)	< 0.1					N		I
<i>Lymnaea peregra</i> (Müller, 1774)	0			N			I	
<i>Lymnaea stagnalis</i> (Linné, 1758)	0	N		N	I		I	
<i>Pseudosuccinea columella</i> (Say, 1817)	0	N	I	I	I		I	I
<i>Radix auricularia</i> (Linné, 1758)	0.18	I		N		N		
<i>Radix auricularia rubiginosa</i> (Michelin, 1831)	< 0.1			I		N		
PLANORBIDAE								
<i>Amerianna carinata</i> (H. Adams, 1861)	0.2		I		I	I	N	
<i>Biomphalaria straminea</i> (Dunker, 1849)	0		N			I		
<i>Biomphalaria guadeloupensis</i> (G. B. Sowerby I, 1821)	0		N		I			
<i>Indoplanorbis exustus</i> (Deshayes, 1834)	0.1				I	N	I	
<i>Planorbella duryi</i> (Wetherby, 1879)	0	N		I	I			I
<i>Planorbarius corneus</i> (Linné, 1758)	< 0.1	I	N				I	
ANCYLIDAE								
<i>Ferrissia wautieri</i> (Mirolli, 1960)	0			N				
CARYCHIIDAE								
<i>Carychium minimum</i> (Müller, 1997)	< 0.1	I		N				
<i>Carychium tridentatum</i> (Risso, 1826)	0			N				
VERONICELLIDAE								
<i>Belocaulis angustipes</i> (Heynemann, 1885)	0	I	N					
<i>Diplosolenodes occidentalis</i> (Guilding, 1825)	0.14	I?	N					
<i>Laevicaulis alte</i> (Férussac, 1822)	< 0.1	I?			N	I	I	I
<i>Leidyula floridana</i> (Leidy & Binney, 1851)	0	N	I					
<i>Sarsinula plebeia</i> (P. Fischer, 1868)	0.45	I	N		I	I	I	I
SUCCINEIDAE								
<i>Succinea putris</i> (Linné, 1758)	0.82	I		N				

TABLE 1. (Continued)

	% of inter-ceptions	World Distribution*						
		NAm.	LAm.	Eur.M.	Afr.	E.As.	ANZ	Ocea.
CIONELLIDAE								
<i>Cionella lubrica</i> (Müller, 1774)	0.14	N		N	I		I	
<i>Cionella lubricella</i> (Porro, 1838)	0			N	I			
PUPILLIDAE								
<i>Lauria cylindracea</i> (Da Costa, 1778)	0.41	I	I?	N	I			
<i>Pupilla muscorum</i> (Linné, 1758)	0	N	N				I	
<i>Pupoides coenopictus</i> (Hutton, 1834)	< 0.1		I	N	I	N		
VERTIGINIDAE								
<i>Gastrocopta pediculus</i> (Shuttleworth, 1852)	0					?	N?	I
<i>Gastrocopta servilis</i> (Gould, 1843)	0		N				I	I
<i>Pupisoma dioscoricola</i> (C. B. Adams, 1845)**	0.18	I	N					
<i>Pupisoma orcula</i> (Benson, 1850)**	0				N	N	I	I
<i>Vertigo ovata</i> Say, 1822	0	N					I	
VALLONIIDAE								
<i>Vallonia costata</i> (Müller, 1774)	0.12	N		N	I		I	
<i>Vallonia excentrica</i> Sterki, 1892	< 0.1	N		N		I	I	
<i>Vallonia pulchella</i> (Müller, 1774)	< 0.1	N		N	I	I	I	
PLEURODISCIDAE								
<i>Pleurodiscus balmei</i> (Potiez & Michaud, 1838)	0			N			I	
ENIDAE								
<i>Ena montana</i> (Draparnaud, 1801)	0			N			I	
<i>Rachistia histrio</i> (L. Pfeiffer, 1855)	0			N	I	I	I	
BULIMULIDAE								
<i>Bulimulus guadalupensis</i> (Bruguière, 1789)	0.43	I	N					
<i>Bulimulus tenuissimus</i> (d'Orbigny, 1835)	0.27	I	N					
ACHATINIDAE								
<i>Achatina fulica</i> Bowdich, 1822	0.1	(I)	I	?	N	I	(I)	I
<i>Archachatina marginata</i> (Swainson, 1821)	0.71		(I)		N			
<i>Limicolaria aurora</i> Jay, 1839	< 0.1		I		N			
SUBULINIDAE								
<i>Allopeas clavulinum</i> (Potiez & Michaud, 1838)	< 0.1	I	I	I	N	I	I	I
<i>Allopeas gracile</i> (Hutton, 1834)	0.18	I	N		I	I	I	I
<i>Allopeas micra</i> (d'Orbigny, 1835)	0	I	N					I
<i>Beckianum beckianum</i> (L. Pfeiffer, 1846)	0		N					I
<i>Erelopeas tuckeri</i> (L. Pfeiffer, 1846)	0						N	I
<i>Leptinaria lamellata</i> (Potiez & Michaud, 1838)	< 0.1		N					
<i>Opeas hannense</i> (Rang, 1831)	< 0.1	I	I	I		N		I
<i>Opeas opella</i> (Pilsbry & Vanatta, 1906)	0					N		I
<i>Paropeas achatinaceum</i> (L. Pfeiffer, 1846)	0					N		I?
<i>Rumina decollata</i> (Linné, 1758)	0	I		N				
<i>Subulina octona</i> (Bruguière, 1789)	0.53	I	N	I	I	I	I	I
<i>Subulina striatella</i> (Rang, 1831)	< 0.1			I	N			
<i>Zootecus insularis</i> (Ehrenberg, 1831)	0			N	N	N		
FERUSSACIIDAE								
<i>Ceciliooides acicula</i> (Müller, 1774)	0	I	I	N			I	
<i>Ceciliooides aperta</i> (Swainson, 1840)	0	I	N					I
<i>Ferussacia follicula</i> (Gmelin, 1791)	0	I		N			I	
OLEACINIDAE								
<i>Varicella</i> sp. (spp. ?)	0		N	I?				I?
STREPTAXIDAE								
<i>Gonaxis kibweziensis</i> (E. A. Smith, 1894)	0				N			I
<i>Gonaxis quadrilateralis</i> (Preston, 1910)	0				N			I
<i>Gulella io</i> Verdcourt, 1974	0				N			
<i>Gulella wahlbergi</i> (Krauss, 1848)	0		I		N			I?
<i>Huttonella bicolor</i> (Hutton, 1834)	0	I	I		I?	N?	I	I
<i>Streptostele musaecola</i> (Morelet, 1860)	0		I		N			I?
SPIRAXIDAE								
<i>Euglandina rosea</i> (Férussac, 1821)	0	N						I

(continued)

TABLE 1. (Continued)

	% of inter- ceptions	World Distribution*						Ocea.
		NAm.	LAm.	Eur.M.	Afr.	E.As.	ANZ	
TESTACELLIDAE								
<i>Testacella haliotidea</i> (Draparnaud, 1801)	0	I		N			I	
<i>Testacella maugei</i> (Férussac, 1819)	0			N	I			
CHAROPIIDAE								
<i>Discocharopa aperta</i> (Möllendorff, 1888)	0			I			N	N
ENDODONTIDAE								
<i>Helicodiscus paralellus</i> (Say, 1821)	0	N		I				
<i>Helicodiscusingleyanus inermis</i> H. B. Baker, 1929	< 0.1	N		I				
DISCIDAE								
<i>Discus rotundatus</i> (Férussac, 1821)	1.08	I		N				
ARIONIDAE								
<i>Arion ater</i> (Linné, 1758)	< 0.1	I		N			I	
<i>Arion circumscriptus</i> Johnston, 1829	0			N				
<i>Arion fasciatus</i> (Nilsson, 1822)	0	I		N				
<i>Arion hortensis</i> Férussac, 1819	< 0.1	I		N	I		I	
<i>Arion intermedius</i> Normand, 1852	0			N	I		I	
<i>Arion lusitanicus</i> Mabile, 1868	0			N				
<i>Arion rufus</i> (Linné, 1758)	< 0.1	I		N				
<i>Arion subfuscus</i> (Draparnaud, 1805)	0.16	I		N				
<i>Arion sylvaticus</i> Lohmander, 1937	0	I		N				
PHILOMYCIDAE								
<i>Meghimatium striatum</i> van Hasselt, 1823	< 0.1					N		I
ARIOPHANTIDAE								
<i>Macrochlamys indica</i> Godwin-Austen, 1888	0				I	N		
CHRONIDAE								
<i>Ovachlamys fulgens</i> (Gude, 1900)	1.04		I			N		I
EUCONULIDAE								
<i>Euconulus fulvus</i> (Müller, 1774)	< 0.1			N			I	
<i>Guppya gundlachi</i> (L. Pfeiffer, 1840)	1.26		N					
<i>Liardetia doliolum</i> (L. Pfeiffer, 1846)	< 0.1	I?				I	I	N
<i>Liardetia samoensis</i> (Mousson, 1865)	< 0.1		I			I		N
GASTRODONTIDAE								
<i>Zonitoides arboreus</i> (Say, 1816)	1.67	N	I	I	I	I	I	I
OXYCHILIDAE								
<i>Oxychilus alliaris</i> (Miller, 1822)	< 0.1	I		N	I		I	I
<i>Oxychilus cellarius</i> (Müller, 1774)	< 0.1	I		N	I		I	I?
<i>Oxychilus draparnaudi</i> (Beck, 1837)	< 0.1	I		N	I		I	
<i>Oxychilus helveticus</i> (Blum, 1881)	0	I		N				
VITREIDAE								
<i>Hawaii minuscula</i> (Binney, 1840)	0	N	I	I			I	I
<i>Vitrea contracta</i> (Westerlund, 1871)	0			N			I	
<i>Vitrea crystallina</i> (Müller, 1774)	0			N	I		I	
DYAKIIDAE								
<i>Quantula striata</i> (Gray, 1834)	0					N		I
MILACIDAE								
<i>Milax gagates</i> (Draparnaud, 1801)	< 0.1	I	I	N	I		I	
<i>Tandonia budapestensis</i> (Hazay, 1881)	< 0.1			N			I	
<i>Tandonia sowerbyi</i> (Férussac, 1823)	0			N			I	
LIMACIDAE								
<i>Lehmannia marginata</i> (Müller, 1774)	0.37	I		N		I		
<i>Lehmannia nyctelia</i> (Bourguignat, 1861)	< 0.1		I	N	I		I	
<i>Lehmannia valentiana</i> (Férussac, 1821)	0.2	I	I	N	I		I	
<i>Limacus flavus</i> (Linné, 1758)	0	I		N	I	I	I	
<i>Limax maximus</i> Linné, 1758	0.14	I		N	I		I	
<i>Limax pseudoflavus</i> Evans, 1978	0	I		N				
BOETGERILLIDAE								
<i>Boettgerilla pallens</i> Simroth, 1912	0			N				

TABLE 1. (Continued)

	% of inter- ceptions	World Distribution*						
		NAm.	LAm.	Eur.M.	Afr.	E.As.	ANZ	Ocea.
AGRIOLIMACIDAE								
<i>Deroceas agrestis</i> (Linné, 1758)	< 0.1			N				
<i>Deroceas laeve</i> (Müller, 1774)	8.16	N	I	N	I	I		I
<i>Deroceas panormitanum</i> (Lessona & Pollonera, 1882)	0.22	I		N	I		I	
<i>Deroceas reticulatum</i> (Müller, 1774)	2.16	I	I	N	I	I	I	I?
SAGDIDAE								
<i>Lacteoluna selenina</i> (Gould, 1848)	0	I	N					I
POLYGYRIDAE								
<i>Polygyra cereolus</i> (von Mühlfeld, 1816)	0.16	N	I					I
<i>Praticolella griseola</i> (L. Pfeiffer, 1841)	0.2	N	I					
CAMAENIDAE								
<i>Caracolus marginellus</i> (Gmelin, 1791)	0	I	N					
<i>Zachrysia auricoma</i> (Férussac, 1821)	0.16		N					
<i>Zachrysia provisoria</i> (L. Pfeiffer, 1858)	< 0.1	I	N					
BRADYBAENIDAE								
<i>Bradybaena similis</i> (Rang, 1831)	1.7	I	I	?	I	N	I	I
HELICIDAE								
<i>Arianta arbustorum</i> (Linné, 1758)	< 0.1	I		N				
<i>Cepaea nemoralis</i> (Linné, 1758)	0.84	I		N				
<i>Cantareus apertus</i> (Born, 1778)	0.53	I		N			I	
<i>Cryptomphalus aspersus</i> (Müller, 1774)	5.98	I	I	N	I	I	I	I
<i>Eobania vermiculata</i> (Müller, 1774)	5.06	I		N			I	I
<i>Helix lucorum</i> Linné, 1758	0.16			N				
<i>Helix pomatia</i> Linné, 1758	< 0.1	I		N				
<i>Otala lactea</i> (Müller, 1774)	0.69	I	I	N			I	
<i>Otala punctata</i> (Müller, 1774)	0.24	I	I	N				
<i>Theba pisana</i> (Müller, 1774)	4.02	(I)	I	N	I		I	
HYGROMIIDAE								
<i>Candidula intersepta</i> (Poiret, 1801)	1.94		I	N				I
<i>Cernuella cisalpina</i> (Rossmässler, 1837)	1.98	I		N				
<i>Cernuella virgata</i> (Da Costa, 1778)	3.51	I		N			I	
<i>Cernuella neglecta</i> (Draparnaud, 1805)	0.24	I		N			I	
<i>Hygromia cinctella</i> (Draparnaud, 1801)	2.71			N				
<i>Microxeromagna armillata</i> (Lowe, 1852)	0.47			N				I
<i>Monacha cantiana</i> (Montagu, 1803)	0.33	I		N				
<i>Monacha cartusiana</i> (Müller, 1774)	1.92			N				
<i>Trichia hispida</i> (Linné, 1758)	0.53	I		N				
<i>Trichia striolata</i> (L. Pfeiffer, 1828)	< 0.1	I		N				
<i>Trochoidea elegans</i> (Gmelin, 1791)	< 0.1	I		N				
<i>Xerotracha conspurcata</i> (Draparnaud, 1801)	4.67	I		N				
COCHLICELLIDAE								
<i>Cochlicella acuta</i> (Müller, 1774)	0.63	I		N			I	
<i>Prietocella barbara</i> (Linné, 1758)	1.65	I		N	I		I	
TRISSEXODONTIDAE								
<i>Oestophora barbula</i> (Rossmässler, 1838)	< 0.1			N				

*World Distribution: NAm. = North America (U.S. & Canada); LAm. = Latin America (South and Central America, Caribbean Islands); Eur.M. = Europe and Mediterranean Basin, and Mid-Atlantic islands; Afr. = African (Subsaharan Africa); E.As. = eastern Asia (including India, China and Southeast Asia); ANZ = Australia and New Zealand; Ocea. = Oceania (Pacific Islands including the Hawaiian Islands); N = native; I = introduced, (I) = introduced, and eradicated/being eradicated. **Species determination unclear. Data for this table compiled from the available literature and PPQ interception records.

subsequently has been further extended by human activity to such isolated islands as the Seychelles and Mascarene Islands, in the case of the former (Connolly, 1925), and in the case of the latter to the West Indies and South Africa (Pilsbry, 1920–1921) and so are included here as travelling species. Clearly, the distinction between “travelling” and “non-travelling” species is an arbitrary one, and some discussion is yet needed to decide if such “borderline” taxa are to be included in the overall list.

Travelling species do not include those species that have become considerably more abundant in their native distributions due to human activity, even to the point of becoming agricultural pests, but have yet to spread to other regions of the world beyond range extension or invasion into neighboring territory. However, they do clearly show the greatest potential of becoming travelling taxa due to their association with humans, their adaptability to disturbed environmental conditions, and their relative abundance. Quarantine agencies worldwide should be familiarized with these species as well as those defined as travelling species, and alerted to their potential to becoming introduced into new countries.

Table 1 lists terrestrial and aquatic gastropods that are here considered to be travelling species and their distributions in the principal regions of the world based on a review of the literature using the criteria listed above. The regions listed are here loosely and informally defined as those areas from which the United States intercepts these molluscs via trade and human passenger movement. A more detailed analysis of any particular taxon requires distribution data of much higher resolution, and for this the bibliography provided can be used as a starting point. It is clear that the most important source of these mostly terrestrial travelling species, about half of the total number, are native to Europe and the Mediterranean Basin, and that many of these taxa became such during the colonial period of the European powers, as trade and human migrations unwittingly carried molluscan hitch-hikers to all parts of the globe. The fact that many of these travelling species are well established in North America, but now are rarely, if ever, intercepted by PPQ inspectors, indicates that the restrictions currently placed on the importation (e.g., no soil carried with horticultural plants to be used for propagation) are indeed effective in preventing the intro-

duction of new similar species or that the means of such introductions have changed (e.g., immigrants no longer bring plant cuttings, fruits, vegetables, due to their availability in the United States). Many of the first travelling species must have had the ability to survive for long periods under relatively hostile conditions in an estivating state, for example on long ocean voyages, in order to survive to reach new regions. In the modern world, however, that ability is no longer essential.

With the expanding global economy, however, it can safely be stated that the number of travelling species from other regions of the world will increase. The speed of travel, as well as a wider range of destinations is making the potential for future introductions much greater. Just as the European and Mediterranean Basin species, representing primarily temperate climate forms, will continue to spread to corresponding environments worldwide, including cooler higher-altitude climates in the tropical regions, the tropical species will spread to similarly tropical habitats worldwide, as well as warmer microhabitats in the cooler regions of the world. Such thermophile species living in areas otherwise too cold include those in naturally and artificially warmed microhabitats, such as *Melanoides tuberculata* in natural thermal waters in Provincia de Castellón, Spain (Gasull, 1974; Escobar et al., 1990), and in the cooling water discharge of greenhouses in the Netherlands (Bij de Vaate et al., 1994). Other species falling into this category include *Cryptomphalus aspersus* overwintering in warm compost areas in Norway (Andersen, 1996), and such greenhouse subulinids as *Allopeas clavulinus*, *Opeas han-nense*, and *Subulina octona* (Proschwitz, 1994b), as well as *Hawailia minuscula* and *Helicodiscus singleyanus inermis* in greenhouses in Sweden (Proschwitz, 1996a). The African streptaxid *Gulella io* was found in European greenhouses (Verdcourt, 1974) before its origin in Liberia could be pinpointed (Verdcourt, 1979; Kerney & Cameron, 1979).

Latin America and Southeast Asia are now the principal direct sources of travelling aquatic snails, both as home aquaria species and commestible species. Some other species are also being deliberately introduced as a means of biological control of snails that are considered intermediate hosts of various diseases, including schistosomiasis. Although the effectiveness of the latter in controlling disease carriers in some cases has been

shown to be promising (Perera et al., 1989; Pointier, 1989; Pointier et al., 1991; Vargas et al., 1991; Pointier et al., 1994, among others), their effects on native mollusks and the environment is sometimes unclear. The fact that some of these introduced species may also be potential disease vectors themselves (Noble & Damian, 1991) should also be further investigated. In view of the well-documented, disastrous effects associated with the biological control attempts of *Achatina fulica* on islands in the Pacific Basin, it is hoped that lessons will have been learned on the use of snails being used in the biological control of other snail species, and their release into new habitats without a full understanding of the risks involved.

Russia, Central and Southwestern Asia are not included in Table 1, as no travelling species can be shown to have originated from these areas. Nor have any gastropods, travelling or otherwise, other than a single crushed unidentifiable hygromiid from the Russian Federation, have been intercepted by PPQ on cargoes from these areas in the period being discussed here (1993–1998). This is not to mean that these areas lack any travelling taxa (Likhachev & Rammelmeier, 1952; Neubert, 1996; Muratov, 1998).

North American species are also being intercepted on cargoes from other regions, these native American species “returning” to their original home. These include *Zonitoides arboreus*, one of the most common species being intercepted from Brazil, as well as *Helicodiscus singleyanus inermis*. *Polygyra cereolus*, a native Florida species, has already spread around the Caribbean Basin, and is periodically intercepted on cargoes from Puerto Rico and the Dominican Republic. Interceptions of this species have been made from Europe on tiles from Spain and Italy, although possible contamination at the ports of entry has not been ruled out in these particular cases. Its introduction into the Persian Gulf region and into Hawaii has already been documented (Neubert, 1996; Cowie, 1996), and as a travelling species, it is expected to spread into warm climatic environments with calcium carbonate substrata.

Travelling species, specifically those deliberately introduced for biocontrol purposes, are often one of the major causes of extinctions of endemic species, a phenomenon that has been well documented in the cases of oceanic islands (Clarke et al., 1984; Murray et

al. 1988; Cowie, 1992a,b). Most notably “ill-conceived biological control programmes, targeted at the Giant African Snail, *Achatina fulica*, constitute currently the most serious threat” [to partulid snails] (Cowie, 1992a). Molluscan biocontrol species have already been implicated in the extinction of almost 100 others worldwide, not to mention an even greater number that have become endangered as a result of these introduced species (Howarth, 1992). However, in spite of the disastrous cases using predatory snails, which are rarely if ever “host-specific” and are likely to feed on any species that is available to them, the use of any kind of biocontrol in other disciplines should not be dismissed out-of-hand. In the case of insect biocontrol agents, claims have been made as to their efficacy and lack of deleterious effects to the environment (Messing, 1992); situations may exist where the lack of any kind of control of a pest also will result in severe agricultural and environmental damage. But, considerable study must be made before biocontrol can be sanctioned, and in the case of mollusks, adequate analysis has yet to be documented in the attempts made so far. As pointed out by Cowie (1992a), “rarely are adequate pre-release and post-release monitoring of the impacts of new biological control agents carried out.” An example of this in the United States would be the widespread use in California of *Rumina decollata* to “control” the Brown garden snail, *Cryptomphalus aspersus* (Fischer & Orth, 1985).

PATHWAYS INTO THE UNITED STATES

The means by which travelling gastropods can potentially enter the United States are varied. Some 60 ports, both air and maritime, receive an increasing volume of cargo (growing by 4 to 15% each year), as well as burgeoning human passenger travel. In addition to these ports, there are considerable numbers of overland entry points along the borders with Canada and Mexico, and these have become more porous, at least in terms of cargo, since the NAFTA treaty went into effect. Factor into this the vast intake of sealed packages entering by regular mail, as well as the various express mail services, and as a result the resources of a quarantine agency such as PPQ are stretched.

It can be seen from Table 2, during the

TABLE 2. Principal pathways of introduction for all gastropods species intercepted by PPQ inspectors 1993–1998.

	% of total interceptions
(1) Tiles	23.33
(2) Plants (horticultural)	17.19
(3) Containers	16.09
(4) Cut flowers	11.68
(5) Fresh fruit, vegetables, and herbs	6.73
(6) Plants for aquaria	4.1
(7) Personal baggage (by air)	3.69
(8) Military cargo	1.15
(9) Mail	< 1.0
(10) Importation for consumption (under permit)	< 1.0

1993–1998 period, the importation of household tiles (ceramic, marble, cement, etc.) is clearly the most important pathway, representing almost one quarter of all interceptions. Tiles often contain a high proportion of calcium carbonate, necessary for the production of shells by snails, and the manner by which they are shipped provides a dark, humid environment for the duration of ocean voyages, facilitating the transportation of large numbers, both in terms of individuals and of species. The main source of imported tile is from the Mediterranean Basin, in particular Italy, and to a lesser extent, Spain. Correspondingly, Italy accounts for 30% of all mollusk interceptions, and Spain, almost 7%, the bulk of which are found on tile shipments. Tile shipments represent an unregulated commodity, and as such no exact figures can be obtained as to the volumes imported into the United States each year. The Port of Miami, Florida, handles the greatest volume of tiles annually. It is estimated that one million metric tons of tiles passed through this port during 1997 from various Mediterranean Basin countries and South America. The effectiveness of PPQ inspections due to limited human resources is maximized by taking account the previous history of interceptions from different countries. For example, only 25% of the nearly 13,000 shipping containers of tiles from Brazil and 15% of the nearly 12,000 containers from Venezuela were inspected. However, due to the abundance of molluscan “hitchhikers” from the Mediterranean Basin, tiles from Italy (in excess of 20,000 containers), Spain

(over 13,000 containers), and Greece (150+ containers) receive 100% inspections, and 50% of the over 1,000 Turkish containers were inspected (W. Tang, pers. comm.). Other ports such as Houston, Texas, Long Beach, California, and Charleston, South Carolina, are also major gateways for the importation of tiles. In all cases, a container found to contain one or more quarantine pests will be fumigated with methyl bromide to kill any others that may still be hidden within. Nevertheless, due to the volume of this kind of commodity, there remains a considerable risk that travelling species will slip into the country undetected.

The exterior of containers also represents an important pathway, particularly as most cargoes are transported in such containers. Although they may be relatively inhospitable to mollusks, they often remain for long periods of time in a wide variety of locations in their countries of origin, and snails and slugs are attracted to cooler and more humid spots on and under these containers. When the container is ultimately filled with cargo and transferred aboard a cargo ship, the hidden gastropods are loaded as well, and apparently some can survive long enough in an estivating state to reach countries thousands of miles and several weeks away.

Cut flowers and plants imported for horticultural purposes together represent another quarter of the total interceptions. Despite the attempts of growers in countries of origin to control all quarantine pests, knowing that any contaminated shipments will be fumigated or re-exported, pests can easily hide on plants and pass undetected. Again, the volume of importation of such items can be surprising to those unfamiliar with the trade. Approximately 70% of the cut flowers consumed are imported through Miami International Airport, an average of 30–35,000 boxes of cut flowers per day, representing a dollar volume of approximately \$800,000,000 during fiscal year 1997 (A. Ferguson, pers. comm.). The cut-flower industry imports stocks from extensive flower farms in Central and South America, Southeast Asia, and the Netherlands, which is a major trans-shipment point of flowers from all over Europe and the Middle East. Again, as a result of limited human resources, less than 5% of these plants can be inspected by PPQ, which concentrates on those flower species and countries of origin that have a greater history of pest interceptions. Inevitably, there is a

great potential for travelling species to spread via this trade, between all countries of the world. Several *Liardetia* species appear to be spreading due to human activity (Baker, 1938; Verdcourt, 1992; Bauman, 1996), including *L. samoensis* mentioned above. *Euconulus gundlachi* is a species normally associated with the Neotropics, and one that is well established in Florida (Pilsbry, 1946), but is being intercepted by PPQ with increasing frequency on cut flowers and live plants from such Southeast Asian countries as Thailand. This would indicate that the species is well established there, at least in plant nurseries and flower farms. It is important to note, however, that few faunal surveys are made within commercial operations, in the United States or in other countries, and these represent a potential reservoir of travelling species of a scale that is largely unknown.

Not unexpectedly, the importation of fruit and vegetables represent an important pathway for travelling species. However, in the United States, these are carefully regulated as they have a long history of carrying pests, particularly insects, the Mediterranean fruit fly being a well-known example. Therefore, great care is taken by foreign growers to exclude quarantine pests, and their transportation in refrigerated containers or ships' holds and inspection of high-risk commodities reduces the percentage of snail and slug interceptions. Many fruits and vegetables as well as cut flowers and live plants are unable to withstand methyl bromide fumigation, and so if quarantine pests are found, the contaminated produce is re-exported to a country that is willing to receive the shipment. Such a country would be one already infested with the pest, or one that considers itself climatically unsuited or otherwise immune to the establishment of the pest. Fresh fruit (*Malus* spp., *Musa* spp., and in particular *Citrus* spp.) are among the more important species for travelling mollusks, and among vegetables, *Apium* sp., *Cucurbita* spp., *Allium* spp., *Lactuca* sp., and especially *Brassica* spp. are of particular concern. Fresh mushrooms are an especially important pathway for a wide variety of slug species from Europe and Asia. Fresh herbs (including *Ocimum* spp., *Rosmarinus* sp., *Eryngium* sp., *Thymus* sp., *Mentha* spp., *Origanum* spp., *Zingiber* sp.) from numerous countries all carry their molluscan "hitch-hikers."

Plants for aquaria represent a significant pathway for the introduction of travelling

species. As noted by Mienis (1994b), "Singapore serves as an international transit center for tropical freshwater flora and fauna from all around Southeast Asia as well as Australia, South America and Africa." Almost all aquatic plants for the aquarium trade, together with the associated gastropods, enter the United States directly from Singapore, with much smaller volumes from other Asian countries. There has been a general perception in agricultural circles that aquatic species represent little or no threat to local agriculture, and for the most part, at least in cooler climates, this may still hold true. For this reason, the trade has been largely unregulated in the United States. However, the severe problems in many countries due to ampullariid species, in particular *Pomacea canaliculata*, feeding on such crops as rice, taro (Cowie, 1995), have shown this to be a mistake, and the PPQ now considers most species of this family to be of quarantine significance. Unfortunately, there is little to no data to indicate which aquatic species are already present in the country, being sold in aquarium and pet shops across the country, and no state or federal government agency has yet undertaken to survey for them. The recent establishment of *P. canaliculata* in Lake Miramar, San Diego County (Cerutti, 1998), and in Florida (Thompson, 1997), together with *P. bridgesii* (Clench, 1966a), are testament to the ease of introduction of these potentially damaging species, most likely resulting from private individuals emptying out their aquarium tanks into the nearest body of water. *Pomacea canaliculata* may also have self-sustaining populations in southern Texas (Neck, 1986; Neck & Schultz, 1992). Of additional concern, many of these aquatic species are known to be intermediate vectors of various parasitic diseases, particularly liver flukes, of which *Schistosoma* spp. are among the most well known, and *Angiostrongylus cantonensis* and *A. costaricensis*, potentially very serious disease-causing nematodes. It should be noted that the monitoring of molluscan potential disease vectors in the aquarium trade in the United States is virtually nonexistent. Some of these species may also carry parasites that can affect livestock.

Personal baggage is a pathway of considerable importance. In the most cases, the snails are being deliberately smuggled into the country to be kept as a "pet," for personal consumption, or for release into gardens or

empty lots in order to establish colonies for future consumption. The potential for such snails to reach the environment is greater than that of "hitchhikers," as great care is taken that they reach their destinations alive. In fact, many if not most of the colonies of European edible helicids, such as *Otala lactea*, *Eobania vermiculata*, and *Helix pomatia*, were deliberately introduced into back yards, with subsequent escapes into the surrounding neighborhoods (Archer, 1937; van der Schalie, 1938; Grimm, 1964; Hanna, 1966; Mead, 1971). These helicid species, as well as *Theba pisana*, *Cepaea nemoralis* and *C. hortensis*, and *Cantareus apertus* and the large hygromiid, *Cernuella virgata*, are routinely found in airline passenger luggage from Europe, often in volumes weighing up to 10 kg, confiscated and destroyed. *Archachatina marginata* and *Achatina achatina* are also frequently intercepted in luggage from airline passengers arriving from West Africa. Not included in the totals in the Table 1 is the routine confiscation of live *Achatina fulica* from luggage of tourists leaving Hawaii for the U.S. mainland, with the express purpose of being maintained alive as pets when they return home. The infestation in Miami, Florida, of this voracious pest from 1966 to 1972 was as a result of such a deliberate introduction (Mead, 1979). Intentional releases of what are considered to be esthically pleasing snails, such as Cuban camaenids and orthalicids into Florida in the early 1900s (Clapp, 1919; Aufenberg & Stange, 1993a, b), are likely to be repeated by private individuals unaware of the consequences of such an act.

The interception of travelling gastropods on military cargo (vehicles, ordnance, as well as household goods of military personnel) returning to the United States is another identifiable means of potential introductions. From U.S. military bases abroad, for example from Mediterranean and Middle Eastern countries, many of the species generally associated with those regions are intercepted, but in addition, more unusual species have been intercepted from points of origin such as Saudi Arabia, Somalia and Socotra.

Mail packages containing such ornamental plants as orchids, cacti and other plants are frequently intercepted with tiny snails and slugs amongst roots or leaves. The deliberate smuggling of live snails through the mail is also an avenue for the introduction of travelling species, and for which few hard data can be obtained. Snails for human consumption

are sometimes found in airmail packages, or being imported mislabeled as "fresh seafood," or "native handicrafts." Shipments of edible freshwater snails, consisting primarily of various viviparid, ampullariid, and the larger potamidid species, are most likely to originate from eastern Asia. Illegal shipments of helicid snails also are periodically encountered from Europe. Despite the interception of such shipments, it is likely that more are entering the country undetected.

The remaining approximately 24% of miscellaneous pathways include various wood products, such as pallets and crating, live material for research purposes, flower pots and other earthenware, quarry products, such as granite and ornamental rocks, machinery and heavy equipment, aircraft parts, chemical containers, and household goods. Also subject to PPQ inspections are those products found in "ship's stores," such as food items not destined specifically for importation into the country, but which could be potentially be a pathway for travelling species.

It is evident from Table 3 that the most commonly intercepted species not unexpectedly are those of European origin, and these are for the most part associated with the importation of tiles and on the exterior of containers, the two most important pathways to the United States for terrestrial gastropods. Only five of the 17 most commonly intercepted species are from different regions, *Succinea costaricana* and *Guppya gundlachi* from Latin America, and *Zonitoides arboreus* of North American origin, that are associated for the most part with the flower trade. Two are of eastern Asian origin, *Ovachlamys fulgens* and *Bradybaena similaris*. Interestingly, almost all interceptions of *O. fulgens* are from Costa Rica, where it became established as a horticultural pest during the last 15 years (Barrientos, 1996, 1998). *Bradybaena similaris* is a pest of almost worldwide distribution. All but one are considered to be travelling species; *S. costaricana* has yet to be reported from regions outside of its native Central America, but this is hardly surprising as succineids are notoriously difficult to identify to the species level. An effort to further identify the various "*Succinea* spp." that are found in greenhouses, flower farms and open field agriculture in countries that are recipients of Central American cut flowers, horticultural plants and vegetables may provide the justification to add this species, as well as other succineids, to the travelling list.

TABLE 3. List of the species most commonly intercepted by PPQ during 1993–1998 (all those over 1% of total number of interceptions).

Species	% of interceptions	Region of natural occurrence	Travelling species
<i>Cryptomphalus aspersus</i> (Müller, 1774)	5.976	Europe/Medit. Basin	Yes
<i>Eobania vermiculata</i> (Müller, 1774)	5.058	Europe/Medit. Basin	Yes
<i>Xerotricha conspurcata</i> (Draparnaud, 1801)	4.67	Europe/Medit. Basin	Yes
<i>Theba pisana</i> (Müller, 1774)	4.018	Europe/Medit. Basin	Yes
<i>Cernuella virgata</i> (Müller, 1774)	3.508	Europe/Medit. Basin	Yes
<i>Hygromia cinctella</i> (Draparnaud, 1801)	2.71	Europe/Medit. Basin	Yes
<i>Succinea costaricana</i> (von Martens, 1898)	2.529	Central America	?
<i>Deroceras reticulatum</i> (Müller, 1774)	2.16	Europe/Medit. Basin	Yes
<i>Cernuella cisalpina</i> (Rossmässler, 1837)	1.978	Europe/Medit. Basin	Yes
<i>Monacha cartusiana</i> (Müller, 1774)	1.97	Europe/Medit. Basin	Yes
<i>Candidula intersepta</i> (Poiret, 1801)	1.938	Europe/Medit. Basin	Yes
<i>Zonitoides arboreus</i> (Say, 1816)	1.672	North America	Yes
<i>Prietocella barbara</i> (Linné, 1758)	1.652	Europe/Medit. Basin	Yes
<i>Guppya gundlachi</i> (L. Pfeiffer, 1840)	1.264	Central America	Yes
<i>Bradybaena similaris</i> (Rang, 1831)	1.16	Eastern Asia	Yes
<i>Discus rotundatus</i> (Férussac, 1821)	1.08	Europe/Medit. Basin	Yes
<i>Ovachlamys fulgens</i> (Gude, 1900)	1.04	Eastern Asia (Japan)	Yes

CONCLUSIONS

Due to the enormous volume of cargo and people moving between countries today, and the fact that this will greatly increase over time due to the expanding global economy, quarantine restrictions even at their most effective can only delay the introduction of new mollusks, increasing the number of travelling species overall and exacerbating their economic and ecological impacts. Although this paper focuses on the United States, the problems, whether they are recognized as such or not, are faced by all countries. In fact, many if not most countries in the world lack any kind of inspection and quarantine agencies whatsoever, and of those that do exist, they are largely underfunded and lack the necessary personnel. The ever-increasing list of travelling species are most likely moving from one region to another, largely undetected, and each area or country affected becomes a reservoir of these potentially dangerous taxa, which then move to the next destination as that country exports its own commodities abroad. As only a small percentage of what enters the United States through its various sea- and airports and border crossings can be inspected due to limited resources, and of those species intercepted only those that can be shown to be quarantine pests can be prevented from entering, there is a substantial risk of these invasive species becoming introduced into the country.

The impact of some travelling or alien

species on island ecosystems is already well documented, and as a result, some governments are now more sensitive to the issue of further introductions. However, the commonly asserted assumption that island species are somehow more vulnerable to these invaders than mainland taxa has been questioned by Simberloff (1995). Until more detailed studies can be made to thoroughly analyze the effects of introduced species in differing ecosystems, the relative complacency in some governmental circles and departments of agriculture worldwide that somehow "the mainland" is less vulnerable to travelling species should be questioned. The inevitable result of the introduction of many of these travelling taxa will be adverse effects on local agriculture, native species, and the environment, with little chance of controlling these new populations.

The need for determining which taxa fulfill the criteria of being travelling species based on sound data on their feeding and environmental requirements, and identifying those that can be stopped from being introduced while adhering to international treaty stipulations cannot be overstated. Concerned malacological workers are urged to initiate faunal surveys, not only in remote regions, but also in urban and suburban areas, and especially commercial growing operations, areas that are generally ignored by most malacologists and deemed of little to no interest. Freshwater species must also be included in such surveys, and public health authorities need to coordinate their activities with quarantine agen-

cies to prevent the spread of possible disease vectors. The species found need to be identified, potential parasites determined, and their feeding preferences noted, together with any visible damage to the vegetation or the environment. Quarantine agencies need to track the movements of these travelling species, and share this vitally needed information, as it will be to the benefit of all to slow the worldwide spread of invasive taxa.

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Oligyra orbiculata (Say, 1818)
Oligyra oweniana (L. Pfeiffer, 1849)
Sturyanella plicatilis (Mousson, 1865)
- CYCLOPHORIDAE
Cyclotus sp. [Vietnam]
- NEOCYCLOTIDAE
Neocyclotus dysoni (L. Pfeiffer, 1851)
- DIPLOMMATINIDAE
Cochlostoma septemspirale (Razoumowsky, 1789)
- AMPULLARIIDAE
Lanistes varicus (Müller, 1774)
Pila ampullacea (Linné, 1758)
Pila polita (Deshayes, 1830)
***Pomacea bridgesii* (Reeve, 1856)**
- VIVIPARIDAE
Bellamyia costulata (von Martens, 1892)
Filopaludina sp. [Singapore]
Idiopoma ingallsiana (I. Lea, 1856)
- BITHYNIIDAE
Gabbia pygmaea (Preston, 1908)
Gabbia wykoffi (Brandt, 1968)
Wattebledia siamensis Möllendorff, 1902
- ASSIMINEIDAE
***Assimineia nitida* (Pease, 1865)**
Cyclotropis bedaliensis (Rensch, 1934)
Cyclotropis carinata (I. Lea, 1856)
- PLEUROCERIDAE
Pachychilus laevisimus (G. B. Sowerby I, 1825)
- THIARIDAE
Adamietta housei (I. Lea, 1856)
Brotia asperata (Lamarck, 1822)
***Melanoides tuberculata* (Müller, 1774)**
***Tarebia granifera* (Lamarck, 1822)**
***Tarebia scabra* (Müller, 1774)**
- POTAMIDIDAE
Cerithidea obtusata (Lamarck, 1822)
Tympanotonos fuscatus (Linné, 1758)
- POMATIASIDAE
Revoilia guillaini (Petit, 1850)
Lithidion forbesianum (E. A. Smith, 1903)
Pomatias elegans (Müller, 1774)
- PHYSIDAE
Physella sp. [Mexico]
***Physella acuta* (Draparnaud, 1805)**
Stenophysa marmorata (Guilding, 1825)
- LYMNAEIDAE
Fossaria viridis (Quoy & Gaimard, 1832)
***Galba truncatula* (Müller, 1774)**
Lymnaea sp. [Italy]
Lymnaea sp. [Korea]
***Radix auricularia* (Linné, 1758)**
***Radix auricularia rubiginosa* (Michelin, 1831)**

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APPENDIX

Taxa intercepted over the 1993–1998 period. Species printed in **bold** are considered here as travelling species. Those intercepted gastropods that were identified to the genus level only are given with their country of origin in square brackets.

HELICINIDAE

- Alcudia* sp. [Dominica]
***Alcudia striata* (Lamarck, 1822)**
Helicina amoena L. Pfeiffer, 1849
Helicina chrysocheila Binney, 1851
Helicina funcki L. Pfeiffer, 1849

- Radix auricularia swinhoi* (H. Adams, 1861)
Radix luteola (Lamarck, 1822)
- PLANORBIDAE
***Amerianna carinata* (H. Adams, 1861)**
Bulinus sp. [Singapore]
Camptoceras jiraponi Hubendick, 1967
Gyraulus convexiusculus (Hutton, 1849)
Gyraulus siamensis (von Martens, 1867)
***Indoplanorbis exustus* (Deshayes, 1834)**
***Planorbarius corneus* (Linné, 1758)**
Planorbella trivolvris (Say, 1817)
- ANCYLIDAE
Ferrissia baconi (Bourguignat, 1858)
Ferrissia sp. [Sri Lanka]
- CARYCHIIDAE
***Carychium minimum* (Müller, 1774)**
- VERONICELLIDAE
Diplosolenodes occidentalis (Guilding, 1825)
Laevicaulis alte (Férussac, 1821)
Leidyula moreleti (P. Fischer, 1871)
Leidyula sp. [Jamaica]
Sarasinula plebeia (P. Fischer, 1868)
- SUCCINEIDAE
Omalonyx matheroni (Potiez & Michaud, 1838)
Oxyloma elegans (Risso, 1826)
Oxyloma sarsii (Esmark, 1886)
Quickia calcuttensis Patterson, 1970
Quickia concisa (Morelet, 1848)
Succinea africana Krauss, 1848
Succinea costaricana von Martens, 1898
Succinea dominicensis (L. Pfeiffer, 1853)
Succinea guatemalensis Morelet, 1849
Succinea horticola Reinhardt, 1877
Succinea hyalina Shuttleworth, 1854
Succinea lauta Gould, 1859
Succinea luteola (Gould, 1848)
Succinea lyrata Gould, 1859
Succinea manaosensis Pilsbry, 1926
Succinea orientalis Benson, 1851
Succinea panamensis Pilsbry, 1920
***Succinea putris* (Linné, 1758)**
Succinea recisa Morelet, 1851
Succinea undulata Say, 1829
Succinea sp. A. [Canada]
Succinea sp. B. [Colombia]
Succinea sp. C. [Dominica]
Succinea sp. D. [Dominican Republic & Haïti]
Succinea sp. E. [Egypt]
Succinea sp. F. [Jamaica]
Succinea sp. G. [Mexico]
Succinea sp. H. [Nigeria]
Succinea sp. I. [Peru]
Succinea sp. J. [Thailand]
- Succinea* sp. K. [Trinidad & Tobago]
Succinea sp. L. [Turkey]
Succinea sp. M. [Sri Lanka]
Succinea sp. N. [Vietnam]
- ACHATINELLIDAE
Elasmias sp. [American Samoa]
Tornatellides sp. [Hawaii]
- CIONELLIDAE
***Cionella lubrica* (Müller, 1774)**
- PUPILLIDAE
***Lauria cylindracea* (Da Costa, 1778)**
Leiostyla sp. [Madeira]
***Pupilla muscorum* (Linné, 1758)**
***Pupoides coenopictus* (Hutton, 1843)**
- VERTIGINIDAE
Columella sp. [France]
Nesopupa sp. A. [Thailand]
Nesopupa sp. B. [Philippines]
***Pupisoma dioscoricola* (C. B. Adams, 1845)**
Pupisoma everzardi (Blanford, 1880)
Pupisoma sp. A. [Philippines]
Pupisoma sp. B. [Vietnam]
Vertigo sp. A. [Japan]
Vertigo sp. B. [Thailand]
Vertigo sp. C. [Madeira]
Vertigo eogea Pilsbry, 1919
- VALLONIIDAE
***Vallonia costata* (Müller, 1774)**
***Vallonia excentrica* Sterki, 1892**
***Vallonia pulchella* (Müller, 1774)**
- STROBILOPSIDAE
Strobilops texana (Pilsbry & Ferriss, 1906)
Strobilops (*Discostrobilops*) sp. [Panama]
- CHONDRINIDAE
Granaria frumentum (Draparnaud, 1801)
Granaria illyrica (Rossmässler, 1837)
- ORCULIDAE
Fauxulus capensis (Küster, 1841)
- ENIDAE
Chondrula tridens (Müller, 1774)
Ena sp. [Somalia]
Euchondrus ledederi (L. Pfeiffer, 1868)
Mabielliella moussoniana (Petit de la Saussaye, 1851)
Merdigera obscura (Müller, 1774)
Rachis punctata (Anton, 1836)
Zebrina cylindrica (Menke, 1828)
Zebrinops sp. [Saudi Arabia]
- CLAUSILIDAE
Cochlodina comensis (L. Pfeiffer, 1848)
Cochlodina lamellata (Montagu, 1803)
Clausilia rugosa parvula Férussac, 1807
Clausilia pumila (C. Pfeiffer, 1828)
Clausilia bidentata (Ström, 1765)
Charpentieria itala (von Martens, 1824)

- Macrogaster densestriata* (Rossmässler, 1838)
Papillifera papillaris (Müller, 1774)
Phaedusa sp. [China]
- CERIONIDAE
Cerion glans (Küster, 1844)
Cerion regina Pilsbry & Vanatta, 1896
Cerion uva Linné, 1758
- BULIMULIDAE
Bulimulus corneus (G. B. Sowerby I, 1833)
Bulimulus diaphanus (L. Pfeiffer, 1855)
Bulimulus erectus (Reeve, 1849)
***Bulimulus guadalupensis* (Bruguière, 1789)**
Bulimulus sepulchralis (Poey, 1852)
***Bulimulus tenuissimus* (d'Orbigny, 1835)**
Bulimulus tenuissimus puellarius (G. B. Sowerby I, 1833)
Bulimulus sp. [Bolivia]
Drymaeus attenuatus costaricensis (L. Pfeiffer, 1862)
Drymaeus discrepans (G. B. Sowerby I, 1833)
Drymaeus emeus (Say, 1829)
Drymaeus multilineatus (Say, 1825)
Drymaeus sulphureus (L. Pfeiffer, 1857)
Drymaeus virgulatus (Férussac, 1822)
Drymaeus sp. A. [Brazil]
Drymaeus sp. B. [Costa Rica]
Drymaeus sp. C. [Dominican Republic]
Drymaeus sp. D. [Ecuador]
Drymaeus sp. E. [Guatemala]
Drymaeus sp. F. [Mexico]
Drymaeus sp. G. [Peru]
- ORTHALICIDAE
Orthalicus maracaibensis (L. Pfeiffer, 1856)
Orthalicus princeps (Broderip, 1833)
- AMPHIBULINIDAE
Amphibulina patula (Bruguière, 1789)
- UROCOPTIDAE
Microceramus bonairiensis (E. A. Smith, 1898)
- ACHATINIDAE
Achatina achatina (Linné, 1758)
***Achatina fulica* Bowdich, 1822**
***Archachatina marginata* (Swainson, 1821)**
***Limicolaria aurora* (Jay, 1839)**
- SUBULINIDAE
***Allopeas clavulinus* (Potiez & Michaud, 1838)**
***Allopeas gracile* (Hutton, 1834)**
***Allopeas micra* (d'Orbigny, 1835)**
***Leptinaria lamellata* (Potiez & Michaud, 1838)**
***Opeas hannense* (Rang, 1831)**
- Opeas hunanensis* (Gredler, 1884)
Opeas pyrgula (Schmacker & O. Boettger, 1891)
Opeas (Pseudopeas) sp. [Vietnam]
***Subulina octona* (Bruguière, 1792)**
***Subulina striatella* (Rang, 1831)**
- STREPTAXIDAE
Gulella sp. [South Africa]
Streptaxis sp. [Mexico]
- SPIRAXIDAE
Euglandina sp. A. [Mexico]
Euglandina sp. B. [Guatemala]
Euglandina sp. C. [Costa Rica]
- CHAROPIIDAE
Phortion sp. [South Africa]
Ptychodon sp. [New Zealand]
Radiodiscus sp. [Guatemala]
Trachycystis sp. A [South Africa]
Trachycystis sp. B [South Africa]
Trachycystis sabuletorum (Benson, 1851)
- ENDODONTIDAE
***Helicodiscus singleyanus inermis* H. B. Baker, 1929**
- DISCIDAE
***Discus rotundatus* (Müller, 1774)**
Discus ruderatus (Férussac, 1821)
- PUNCTIDAE
Punctum pygmaeum (Draparnaud, 1801)
Punctum sp. [Japan]
- ARIONIDAE
***Arion ater* (Linné, 1758)**
***Arion hortensis* Férussac, 1819**
***Arion subfuscus* (Draparnaud, 1805)**
Arion (Carinarion) sp. [France]
Prophysaon andersoni (Cooper, 1872)
- PHILOMYCIDAE
***Meghimatium striatum* van Hasselt, 1823**
Pallifera costaricensis (Mörch, 1858)
- MEGALOBULIMIDAE
Megalobulimus oblongus (Müller, 1774)
- RHYTIDAE
Rhytida sp. [New Zealand]
- HELICARIONIDAE
Helicarion sp. A [Australia]
Helicarion sp. B [Thailand]
- ARIOPHANTIDAE
Macrochlamys cincta (Möllendorff, 1897)
Microparmarion sp. A [Fiji]
Microparmarion sp. B [Vietnam]
- CHRONIDAE
***Ovachlamys fulgens* (Gude, 1900)**
Parakaliella satsumanus (Pilsbry & Hirase, 1908)
- EUCONULIDAE
Conuplecta turrita (Semper, 1873)
Euconulus alderi (Gray, 1840)

- Euconulus fulvus* (Müller, 1774)**
***Guppya gundlachi* (L. Pfeiffer, 1840)**
***Liardetia doliolum* (L. Pfeiffer, 1846)**
***Liardetia samoensis* (Mousson, 1865)**
Liardetia sculpta (Möllendorff, 1883)
Liardetia tenuisculpta (Möllendorff, 1893)
Lousia barclayi (Benson, 1850)
Wilhelminia mathildae (Preston, 1913)
- UROCYCLIDAE
Urocyclus flavescens (Keferstein, 1866)
- GASTRODONTIDAE
***Zonitoides arboreus* (Say, 1816)**
Zonitoides nitidus (Müller, 1774)
- OXYCHILIDAE
***Oxychilus allarius* (Müller, 1774)**
***Oxychilus cellarius* (Müller, 1774)**
***Oxychilus draparnaudi* (Beck, 1837)**
Oxychilus mortilleti (L. Pfeiffer, 1859)
- VITREIDAE
Vitrea diaphana (Studer, 1820)
- DAUDEBARDIDAE
Nesovitrea hammonis (Ström, 1765)
Nesovitrea sp. [Netherlands]
- MILACIDAE
***Milax gagates* (Draparnaud, 1801)**
Milax nigricans (Philippi, 1836)
***Tandonia sowerbii* (Férussac, 1823)**
- VITRINIDAE
Insulivitrina sp. [Canary Islands]
Semilimax sp. [China]
Vitrina sp. [Germany]
- LIMACIDAE
***Lehmannia marginata* (Müller, 1774)**
***Lehmannia nyctelia* (Bourguignat, 1861)**
***Lehmannia valentiana* (Férussac, 1821)**
***Limacus flavus* (Linné, 1758)**
***Limax maximus* Linné, 1758**
- AGRIOLIMACIDAE
***Deroceras agrestis* (Linné, 1758)**
***Deroceras laeve* (Müller, 1774)**
***Deroceras panormitanum* (Lessona & Pollonera, 1882)**
***Deroceras reticulatum* (Müller, 1774)**
Deroceras rodnae (Grossu & Lupu, 1965)
- SAGDIDAE
Hojeda sp. [Dominican Republic]
- POLYGYRIDAE
Ashmunella sp. [origin uncertain]
Daedalochila texasiana (Moricand, 1833)
***Polygyra cereolus* (von Mühlfeld, 1816)**
Praticolella berlandiana (Moricand, 1833)
***Praticolella griseola* (L. Pfeiffer, 1841)**
- THYSANOPHORIDAE
Thysanophora plagioptycha (Shuttleworth, 1854)
Thysanophora rhoadsi Pilsbry, 1920
Thysanophora sp. A. [Colombia]
- Thysanophora* sp. B. [Guatemala & Panama]
Thysanophora sp. C. [Jamaica]
- CAMAENIDAE
Amphidromus sp. [origin uncertain]
Camaena cicatricosa (Müller, 1774)
Caracolus insititia (Shuttleworth, 1854)
Ganesella sp. [China]
Grabauia sp. [India]
Pleurodonte guadeloupensis dominicana Pilsbry & Cockerell, 1937
Polydontes lima (Férussac, 1821)
Thelidomus asper (Férussac, 1821)
***Zachrysia auricoma* (Férussac, 1821)**
***Zachrysia provisoria* (L. Pfeiffer, 1858)**
- BRADYBAENIDAE
Acusta despecta (G. B. Sowerby I, 1833)
Aegista permellita (Heude, 1886)
Aegista (Plectotropis) sp. [China]
Bradybaena fruticum (Müller, 1774)
Bradybaena ravida (Benson, 1842)
***Bradybaena similaris* (Rang, 1831)**
Bradybaena sp. A [China]
Bradybaena sp. B [China]
Bradybaena sp. C [Thailand]
Bradybaena sp. D [Indonesia]
Bradybaena sp. E [Korea]
Cathaica fasciola (Draparnaud, 1801)
Euhadra sp. [Japan]
Trishoplita sp. [Japan]
- XANTHONYCHIDAE
Averellia coactiliata (Deshayes, 1839)
Hemitrochus graminicola (C. B. Adams, 1849)
Hemitrochus maynardi (Pilsbry, 1891)
Levicepolis monodonta (I. Lea, 1832)
- SPHINCTEROCHILIDAE
Sphincterochila candidissima (Draparnaud, 1801)
- HELICIDAE
Arianta arbustorum (Linné, 1758)
***Cantareus apertus* (Born, 1778)**
***Cepaea hortensis* (Müller, 1774)**
***Cepaea nemoralis* (Linné, 1758)**
Chilostoma cingulatum (Studer, 1820)
Chilostoma cingulatum carrarense (Strobel, 1852)
***Cryptomphalus aspersus* (Müller, 1774)**
Eobania constantinae (Forbes, 1837)
***Eobania vermiculata* (Müller, 1774)**
Helix cincta Müller, 1774
Helix engaddensis Bourguignat, 1852
***Helix lucorum* Linné, 1758**
***Helix pomatia* Linné, 1758**
Helix secernenda Rossmässler, 1847
Helix texta Mousson, 1861
Isognomostoma sp. [Italy]

Massylaea punica (Morelet, 1851)

***Otala lactea* (Müller, 1774)**

***Otala punctata* (Müller, 1774)**

***Theba pisana* (Müller, 1774)**

HYGROMIIDAE

Candidula gigaxii (L. Pfeiffer, 1850)

***Candidula intersecta* (Poiret, 1801)**

Candidula olisippensis (Servain, 1880)

Candidula subapicina (Mousson, 1873)

Candidula unifasciata (Poiret, 1801)

***Cernuella cisalpina* (Rossmässler, 1837)**

Cernuella depressula (Parreyss, 1839)

Cernuella neglecta (Draparnaud, 1801)

***Cernuella virgata* (Da Costa, 1778)**

Helicella itala (Linné, 1758)

Helicopsis sp. [Italy]

Helicopsis instabilis (Rossmässler, 1835)

***Hygromia cinctella* (Draparnaud, 1801)**

Hygromia limbata (Draparnaud, 1801)

***Microxeromagna armillata* (Lowe, 1852)**

Monacha bicinctae (Benoit, 1840)

***Monacha cantiana* (Montagu, 1803)**

***Monacha cartusiana* (Müller, 1774)**

Monacha ignorata (Morelet, 1845)

Monacha inchoata (Morelet, 1845)

Monacha obstructa (L. Pfeiffer, 1842)

Monacha olivieri (Férussac, 1821)

Monacha syriaca (Ehrenberg, 1831)

Monachoides incarnatus (Müller, 1774)

Ochthephila madeirensis (Wood, 1828)

Perforatella rubiginosa (A. Schmidt, 1853)

Platytheba sp. [origin uncertain]

Ponentina ponentina (Morelet, 1845)

Portugala inchoata (Morelet, 1845)

Stenomphalia ravergieri (Férussac, 1835)

***Trichia hispida* (Linné, 1758)**

Trichia leucozona (C. Pfeiffer, 1828)

Trichia plebeia (Draparnaud, 1805)

***Trichia striolata* (C. Pfeiffer, 1828)**

Trochoidea davidiana (Bourguignat, 1863)

***Trochoidea elegans* (Gmelin, 1791)**

Trochoidea meda (Porro, 1840)

Trochoidea pyramidata (Draparnaud, 1805)

Trochoidea simulata (Ehrenberg, 1831)

Trochoidea trochoides (Poiret, 1789)

Xerolenta obvia (Menke, 1828)

Xeropicta derbentina (Krynicky, 1833)

Xeropicta krynickii (Krynicky, 1833)

Xeropicta millepunctata (O. Boettger, 1889)

Xeropicta protea (Ziegler, 1838)

Xeropicta vestalis joppensis (A. Schmidt, 1855)

Xerosecta cespitum arigonis (A. Schmidt, 1853)

Xerotricha apicina (Lamarck, 1822)

***Xerotricha conspurcata* (Draparnaud, 1801)**

Zenobiella sp. [Italy]

COCHLICELLIDAE

***Cochlicella acuta* (Müller, 1774)**

Cochlicella conoidea (Draparnaud, 1801)

***Prietocella barbara* (Linné, 1758)**

TRISSEXODONTIDAE

Caracollina lenticula (Férussac, 1821)

***Oestophora barbula* (Rossmässler, 1838)**

HELICODONTIDAE

Helicodonta obvoluta (Müller, 1774)

Helicodonta sp. [Greece]

Lindholmiola lens (Férussac, 1832)

MOLLUSCAN INVASIONS IN MARINE AND ESTUARINE COMMUNITIES

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ABSTRACT

The distributions of many species of marine and estuarine mollusks have been altered dramatically by human movements over the past 2,000 and more years. Vectors have included vessels, mariculture, the aquarium trade, intentional or accidental releases into the wild, and canals. Most marine mollusk distributions are held to be "natural" prior to the 19th century, whereas mollusk distributions during or since the 19th century are held to be potentially subject to human modification. However, that pre-19th century invasions occurred is clear, suggesting that the antiquity of human-mediated mollusk introductions has been extensively underestimated. The Asian oyster *Crassostrea gigas* was introduced to Europe by the 1500s, the Northern Hemisphere mussel *Mytilus* may have arrived in the Southern Hemisphere by the early 1500s, and shipworms have similarly been widespread by shipping. A subset of 38 Northern Hemisphere introduced mollusks reveals distinct geographic patterns: 63% originate in the North Atlantic Ocean/Mediterranean area, while 37% originate in the North Pacific Ocean. Within the Atlantic Ocean, the western Atlantic is a significantly stronger donor area, accounting for 75% of those North Atlantic taxa that have dispersed globally. Similarly, the western Pacific Ocean is also a strong donor region, exporting 93% of all those originating in the Pacific. Ecologically, in San Francisco Bay, California, the introduced infaunal or near-surface bivalves *Mya*, *Gemma*, *Venerupis*, *Musculista* and *Potamocorbula* may be sufficiently abundant as to control water column productivity. The European snail *Littorina littorea* (Linnaeus, 1758) has had vast and complex impacts on intertidal hard and soft bottom communities from Canada to the mid-Atlantic America. In general, far more attention must be paid to experimentally demonstrating the impacts of invasive species.

Key words: invasion, bioinvasion, exotic, alien, nonindigenous, introduced.

INTRODUCTION

The natural structure of most shallow-water marine and estuarine communities has been altered dramatically by human activities over the past 2,000 and more years (Carlton, 1996c; Ruiz et al., 1997) Protist, invertebrate, vertebrate, algal, and seagrass populations have been either completely extirpated (removing entire populations) or decimated (severely reducing population size, often to a point of functional extinction). In contrast, numerous species have been accidentally or intentionally introduced by human activities to communities. The result is that both the diversity and relative abundance of species within many marine communities have been fundamentally changed.

Marine, including estuarine, mollusks provide an excellent platform to examine the scale of change in shallow-water ecosystems. No group of marine invertebrates is better known, thanks to a combination of a fossil,

archeological, and long historical record. Best understood are changes in population size (examples are the global demise of most shallow-water edible shellfish populations) and the introduction of nonindigenous species. Poorly understood is the scale of global extinction of marine mollusks in historical time (Carlton, 1993), in part because the subject has received little attention.

Biological invasions consist of species moved through human activities (introductions) and by natural means (range expansions) (Carlton, 1989). In understanding the importance of the human-mediated movement of mollusks, it is critical to note that such dispersal is not simply a matter of accelerating "normal" processes that "have always been happening" or that "would happen anyway." Natural movements of species are almost always along predictable corridors, whether these corridors are continental margins, transoceanic currents, or routes that open or are created over geological time. In

contrast, human-mediated movements of species often involve the unpredictable and instantaneous global translocation of species independent of space or time barriers. Thus, there is no natural biotic flow between, for example, the temperate estuaries of southern Australia and the temperate estuaries of Western Europe. However, either by intent (such as a commercial species moved in the hold of an airplane) or by accident (such as a species living on the hull or in the seachest or in the ballast of a ship) a marine mollusk can be transported between Australia and Europe in a matter of hours to days.

I review here aspects of the global introductions of marine mollusks. A particular focus is placed on the potential scale of underestimation of the number of introductions that may have occurred.

HUMAN-MEDIATED DISPERSAL: MECHANISMS AND BIODIVERSITY OF TRANSPORTED ORGANISMS

The mechanisms by which marine organisms have moved or are now moved around the world by other-than-natural means is well documented, although the quantitative and qualitative aspects of these movements, relative to the diversity and abundance of species, are often poorly known. Potential vectors include vessels (ships and in the 20th century semisubmersible exploratory drilling platforms), aquaculture (mariculture), including the movement of commercial oysters, the aquarium and ornamental organism trade, intentional or accidental releases into the wild, plant (seagrass and marsh plant) transplantations, and canals (Carlton, 1985, 1987, 1992a, 1994; Carlton & Geller, 1993; Carlton et al., 1995; Cohen & Carlton, 1998; Minchin, 1996). Vessels in particular are now recognized as one of the major long-term homogenizers of coastal biotas (Carlton, 1985, 1996a; Carlton & Hodder, 1995; Zibrowius, 1992).

Historically, wooden vessels were floating biological islands, with extensive fouling communities on their hulls (that is, intertidal and sublittoral epibenthic assemblages), boring communities in their hulls, and additional suites of organisms in the sand ballast and rock ballast typically carried by pre-20th century ships. Intertidal organisms can occur at the ships' water line, and may include algae, limpets, and barnacles. Sublittoral fouling congregations may include a rich assemblage

of algae, sponges, hydroids, sea anemones, sedentary polychaete annelids, sessile mollusks, barnacles, bryozoans, and ascidians. Vessels with interstices, holes, abandoned shipworm and gribble burrows, empty barnacles, empty but articulated bivalves (such as deeply cupped oysters), and other hollows and cavities, could further support such mobile organisms as flatworms, free-living polychaetes, crabs and other crustaceans, and fish. Boring communities included shipworms (teredinid bivalves) and gribbles (limnoriid isopod crustaceans) and associated commensal or symbiotic species.

While it is possible to derive an overall hypothetical picture of the general composition of fouling and boring mollusk assemblages (discussed below), we know little about the mollusks that may have been carried in sand ballast or rock ballast inside a vessel. Survival in this solid ballast in a ship's hold may have been dependant on how wet the ballast remained over a given length of time and, in the case of sand in particular, how anoxic the sediment became. Given relatively humid if not aqueous conditions, interstitial and small sand-dwelling mollusks and rock-dwelling mollusks (especially in deeper rock crevices) may have been transported frequently.

Over the centuries, hundreds or thousands of species of mollusks must have been transported in hundreds of thousands of ship voyages. Carlton (1999) has suggested that on and in pre-18th century vessels the molluscan assemblages may have included smaller gastropods such as periwinkles (Littorinidae) carried in rock ballast, sealugs (nudibranch opisthobranchs) associated with hydroid and bryozoan fouling, and such bivalves as chamids (Chamidae), mussels (Mytilidae) and oysters (Ostreidae), and other gastropods such as limpets (Lottiidae), associated with hull fouling. Shipworms (Teredinidae) were presumably often common to abundant in wooden vessels; how many different species of shipworms a single vessel could support appears not to have been reported in the literature. In turn, shipworm burrows may have supported a secondary assemblage of nestling and other boring bivalves, such as hiatellids (Hiatellidae), venerids (Veneridae), petricolids (Petricolidae), and piddocks, including *Martesia* (Pholadidae).

In addition to these taxa, hull fouling in temperate waters likely also supported populations of the jingle shells *Anomia* and *Pododesmus* (Anomiidae), *Hiatella* (Hiatellidae),

commensal and crevicolous clams in the family Lasaeidae (*Kellia* and *Lasaea*), and *Entodesma* (Lyonsiidae), and in tropical waters the wing oysters *Pinctada* and *Pteria* (Pteriidae), *Isognomon* (Isognomonidae), and the pen shells *Atrina*, *Streptopinna* and *Pinna* (Pinnidae). Benthic infaunal, soft-bottom epifaunal, and even salt marsh species may also occur in the interstices of ships' fouling, and this phenomenon may have been far more common in earlier times. For example, small (<1 cm) infaunal clams, such as *Mya arenaria* Linnaeus, 1758, and mussels, such as *Geukensia demissa* (Dillwyn, 1817), can occur embedded in hull-fouling communities.

Carlton (1999) further noted that chitons (Polyplacophora) may have occurred on ship anchors. I observed the attachment of a 1 cm-long individual of the chiton *Chaetopleura apiculata* (Say, 1834) on the anchor of a 40-meter vessel after the anchor had been in 10 meters of water near Woods Hole, Massachusetts, USA, overnight for about 14 hours. In earlier maritime history, with longer coastal residencies, anchors would have been available for colonization on and in bottom sediments, including mixed rubble and rock bottoms, for long periods of time. In turn, anchors may remain wet over considerable distances due to wave splash.

Marine organisms continue to move on and in ships in modern times. Wood hull boring communities are vastly reduced in number (except in local wooden vessels, especially in tropical waters), and few if any such ships regularly move around the world anymore. However, fouling communities, while not as vast as they once were, are still transported. Antifouling paints, increased ship speeds, and reduced port residencies have presumably changed the quality and quantity of such assemblages. In modern ships, water ballast has replaced dry and hard ballast and has received a good deal of attention as a dispersal mechanism (Carlton, 1985; Williams et al., 1988; Baldwin, 1992; Kelly, 1993; Carlton & Geller, 1993). It is probable that hundreds of species of mollusks are in motion in ship ballast water on an hourly basis around the world at the beginning of the 21st century. Ironically, these may include the larvae of some shipworms, and thus shipworms may still in motion around the world, despite the demise of ocean-going wooden ships.

Mollusks may also move in modern ships in seachests, a mechanism that requires far more study than it has received. Seachests

(also known as sea inlet boxes or suction bays) are spaces in a ship's hull into which water is drawn in order to then be pumped into a ship's ballast system. The sea chest can provide a settlement area for both attached and mobile species. Richards (1990) found a population of the tropical muricid snail *Thais blandfordi* Melville, 1893, living on the walls of the seachest of a cargo vessel having served in the New Guinea archipelagoes. The cruise track of the vessel had included Saudi Arabia, Kenya, Malaysia, Singapore, and Papua New Guinea, and then via Hong Kong to Hull, England. The population structure of the snails suggested that they had reproduced in the sea chests. The snails had also survived British winter water temperatures before returning to the tropics and being found in the harbor of Kimbe, Papua New Guinea, where Richards sampled the vessel. The snails had become sufficiently abundant to the point that they had blocked the pipes and filters of the water cooling system. Muricid snails have crawl-away young that emerge from deposited egg capsule. Young snails may thus have been drawn into the seachests on floating seaweed or debris, had survived feeding on barnacles, and had grown to adults in the sea chest.

Three interesting conclusions may be drawn from Richards' observation. First, seachests may be the modern day manifestation of the deep, sheltered galleries of empty shipworm burrows in pre-20th century (wooden) vessels, in terms of offering a protected microhabitat in the hull of the vessel for organisms not normally associated with external hull fouling. Second, the interpretation of the natural distribution of species with crawl-away young is thus further complicated by the advent of the seachest in the evolution of the ship. Three, taxa not normally associated with shipping may clearly be entrained and moved by ships. *Thais blandfordi* is a species that lives in exposed reef habitats. Richards speculated that the vessel may have entrained these snails in the Indian Ocean near the barrier reef off Mombasa, Kenya. As this snail was carried into the vessel by some unknown means, so it presumably could be carried out (unless the snails had grown too large to escape through the grate holes), and thus *Thais* could potentially be introduced to a new region.

In summary, a wide diversity of gastropods and bivalves were or are thus susceptible to transport on ships. The taxa noted above, and

TABLE 1. Conjectural examples (not necessarily actual records) of marine molluscan taxa whose global distribution has been susceptible to alteration by ship transport, arranged by ship "habitat."

	Hull fouling	In shipworm burrows	Fouling in seachests	Rock ballast	Water ballast ³
Polyplacophora	x	x	x ¹		x
Gastropoda					
Lottiidae	x	x	x	x	x
Fissurellidae	x	x	x		x
Assimineidae	x ²	x		x	x
Pomatiopsidae				x	x
Littorinidae		x	x	x	x
Calyptraeidae	x	x	x		x
Vermetidae	x	x	x		x
Muricidae		x	x		x
Ellobiidae	x ²	x		x	x
Siphonariidae	x	x	x	x	x
Opisthobranchia	x	x	x		x
Bivalvia					
Mytilidae	x	x	x		x
Arcidae	x	x	x		x
Pteriidae	x	x	x		x
Isognomonidae	x	x	x		x
Malleidae	x	x	x		x
Pinnidae	x	x	x		x
Pectinidae	x	x	x		x
Spondylidae	x	x	x		x
Anomiidae	x	x	x		x
Ostreidae	x	x	x		x
Chamidae	x	x	x		x
Lasaeidae	x	x	x		x
Veneridae	x	x	x		x
Petricolidae	x	x	x		x
Myidae	x	x	x		x
Hiatellidae	x	x	x		x
Pholadidae		x			x
Teredinidae	x	x			x
Lyonsiidae	x	x	x		x

¹Also potentially on anchors.

²Referred to by Carlton (1999) as "hull nestlers", that is, species living in cracks in the wood hull at or above the waterline.

³Those taxa with non-planktonic larvae could be taken into the ship with ballast water on floating seaweeds, sea-grasses, or debris.

additional taxa, are assembled in Table 1 by ship "habitat"—hull fouling, in shipworm burrows, as fouling in seachests, in rock ballast, and in water ballast. With few exceptions, external transport (on hull fouling) and internal transport (in seachests, in shipworm burrows, and in ballast water) serve as potentially viable mechanisms for most taxa. Rock ballast is necessarily—and *a priori*—more selective, and is here scored conservatively as involving primarily supralittoral or high intertidal species of gastropods. While the associated fauna of rock ballast was not recorded at the time of its use, as noted above, experimental studies recreating historical conditions would be of

great value in evaluating this mechanism and in refining the nature of the transportable taxa.

THE POTENTIAL ANTIQUITY OF MARINE MOLLUSCAN INTRODUCTIONS

How long have human activities moved marine mollusks around the world? The evidence from maritime history indicates that mollusks have been moved for millennia *within* ocean basins and for at least centuries *between* ocean basins (Carlton, 1999). And yet, despite the potentially diverse array of species

that may have been associated with shipping over these long lengths of time, few introductions are recognized that are linked to global shipping prior to the 19th century. This relatively late recognition is not surprisingly related to when the first reliable distributional records of mollusks become available. It is thus not surprising to note that most marine mollusk distributions have long been held to be "natural" prior to the 19th century, whereas mollusk distributions during or since the 19th century are held to be potentially subject to human modification.

That this dichotomy is an artificial one is illustrated by the following three examples:

(1) The Japanese oyster *Crassostrea gigas* (Thunberg, 1793) was transported to southern Europe by Portuguese explorers by the 1500s (Edwards, 1976). It was described from Europe as a different and presumptive native Atlantic species, *Crassostrea angulata* (Lamarck, 1819). Despite suggestions since the 1940s that the two species were the same, based upon morphological, behavioral, physiological and reproductive evidence, and despite the absence of a fossil or early archeological record in Europe (Ranson, 1967; Edwards, 1976), oyster biologists continued to use the junior synonym *C. angulata*, with the stated or implied view that it is native (Arakawa, 1990; Heral, 1990; Michinina & Rebordinos, 1997). In part this usage was no doubt reinforced by the intentional introduction of *C. gigas* into Europe commencing in the 1960s (Ribera & Boudouresque 1995; Zibrowius, 1992), and the concomitant desire to be able to refer to the pre-existing stocks by a separate name. Ó Foighil et al. (1998) have again clearly demonstrated that *C. angulata* and *C. gigas* are the same species based upon molecular genetic studies (mitochondrial cytochrome oxidase I gene sequences).

(2) It was recognized as early as the 1940s that the northwestern Atlantic Ocean clam *Mya arenaria* did not occur in modern Europe until the 1500s. Petersen et al. (1992) have pushed the date of arrival back another 200 years or more, discovering Danish midden shells dated to the 1200s–1300s. *Mya* may thus have been brought to Europe from America by Vikings (perhaps intentionally as a new food), and may have been reintroduced by later explorers and colonists returning from North America as well. Alternatively, as *Mya* occurs in ship fouling (as noted above) it may also have been transported in the once-richer

vessel fouling communities of earlier centuries.

(3) The northern hemisphere mussels in the genus *Mytilus* may have arrived in the Southern Hemisphere, on the east coast of South America and in the South Pacific Ocean, by the early 1500s, when these regions were first explored by European vessels (Haws, 1975). The sibling species of mussels *Mytilus trossulus* Gould, 1850 (of the North Pacific Ocean), *Mytilus edulis* Linnaeus, 1758 (of the North Atlantic Ocean), and *Mytilus galloprovincialis* Lamarck, 1819 (of the Mediterranean Sea), are all common ship fouling organisms and have been transported globally for centuries. As is true with many introductions (Carlton, 1979b), introduced populations of Northern Hemisphere *Mytilus* carried to the Southern Hemisphere were given a host of new names (Table 2). Thus, *Mytilus galloprovincialis* arrived sometime before 1819 in Australia (where it was renamed as *Mytilus planulatus* Lamarck, 1819), and *Mytilus edulis* arrived by the 1840s in eastern South America (where it was renamed as *Mytilus platensis* Orbigny, 1846). Other invasions by this species continued throughout the 19th and 20th centuries, and both *M. edulis* and *M. galloprovincialis* continued to be redescribed around the world until the 1970s (Table 2).

Curiously, the primarily northeastern Pacific Ocean *Mytilus trossulus* appears to have failed as a colonizer in the Southern Hemisphere (although it may owe its presence in the North Atlantic Ocean to ship dispersal), either because of competition with already-present introduced populations of *M. edulis* and *M. galloprovincialis*, or with native southern mytilids, or because of other factors. This lack of invasion success is, however, in concert with other elements of the temperate northeastern Pacific Ocean biota which, with few exceptions, have failed to depart the eastern Pacific and colonize other parts of the world, as discussed further below.

SHIPWORMS AS A MODEL GUILD FOR EARLY HISTORIC INTRODUCTIONS

Shipworms provide a particularly compelling guild of mollusks through which to analyze the long-term role of global shipping in altering aboriginal marine invertebrate distributions. While the very name "shipworm" suggests an intimate association with vessels,

TABLE 2. The introduction and redescription of *Mytilus* species around the world.

Introduced to	Redescribed as/by (*)		= <i>Mytilus</i>	Reference
Southern Hemisphere				
Pacific Ocean				
New Zealand	<i>aoteanus</i>	Powell, 1958	<i>galloprovincialis</i>	Koehn, 1991
Australia	<i>planulatus</i>	Lamarck, 1819	<i>galloprovincialis</i>	Koehn, 1991
Argentina	<i>platensis</i>	Orbigny, 1846	<i>edulis</i>	Seed, 1992
Chile	<i>chilensis</i>	Hupé, 1854	<i>edulis</i>	Seed, 1992
Indian Ocean				
Kerguelen Islands	<i>desolationis</i>	Lamy, 1936	<i>edulis</i>	Koehn, 1991
	<i>kerguelensis</i>	Fletcher, 1938	<i>edulis</i>	Koehn, 1991
Atlantic Ocean				
South Africa	—	—	<i>galloprovincialis</i>	Grant & Cherry, 1985; Griffiths et al., 1992
Northern Hemisphere				
Pacific Ocean				
California	<i>diegensis</i>	Coe, 1945	<i>galloprovincialis</i>	McDonald & Koehn, 1988
Russia (Pacific)	<i>zhirmunksii</i>	Scarlato & Starobogatov, 1979	<i>galloprovincialis</i>	Seed, 1992
Japan, China	—	—	<i>galloprovincialis</i>	Wilkins et al., 1983

*Literature references to original descriptions are given in Soot-Ryen (1955) and Seed (1992).

the possibility that many shipworm species — particularly those in marine bays and estuaries that were converted to ports and harbors — could in fact owe a good deal of their modern distribution to shipping appears, curiously, to have never been extensively explored on a global basis. While shipworm workers have consistently referred to the possible role of ships (Edmondson, 1942, 1962; Turner, 1966), drifting wood has almost always been offered as an equally viable alternative to explain now-cosmopolitan distributions. Indeed, a leading paradigm of shipworm biogeography may be taken to be as follows, here rendered as a modified syllogism:

Shipworms live in wood.
Wood floats.
Wood floats in ocean currents.
Thus, shipworm distributions are natural.

A potential flaw in this deductive argument is the assumption that shallow-water dwelling species of shipworms can survive while floating in wood across the high seas during the weeks or months involved in a transoceanic or interoceanic voyage, and then arrive alive to make a "landfall" on a distant shore in a condition sufficient to reproduce. Neither detailed quantitative observations nor experimental evidence appear to be available to support this assumption. In contrast, Edmondson (1962) noted that when open-ocean wood is examined, such wood is almost al-

ways occupied by shipworm species that are not found in harbors — that is (and somewhat predictably!) oceanic wood is colonized by a pelagic shipworm guild. In the Central and North Pacific Ocean these include *Teredo princesae* Sivickis, 1928 (= *Teredo gregoryi* Dall, Bartsch & Rehder, 1938) and *T. triangularis* Edmondson, 1942. Edmondson (1962) further considered *Uperotus clavus* (Gmelin, 1791) and *Teredo palauensis* Edmondson, 1959, to be species that have adopted a "typically oceanic existence." Of course, it is not impossible that these species, too, may have been subjected to ship-mediated dispersal. *Teredo palauensis*, which Turner (1966) considered as possibly conspecific with *Teredothyra excavata* (Jeffreys, 1860), was first described from the hull of a wrecked ship in the Caroline Islands that may have picked it up while at sea.

Vessels with shipworms would have made direct harbor-to-harbor transits in a matter of days and weeks. Carlton (1999) has further suggested a "port renewal" hypothesis, wherein ocean-going ships periodically return to coastal waters, permitting the fouling community to be revitalized under the trophic, salinity, temperature, and other conditions in which they evolved. Such port-returns may have further led to intensive periods of larval release, triggered by more eutrophic and warmer coastal waters (while inhibited by cold, high salinity, oligotrophic ocean waters).

TABLE 3. Examples of introduced and cryptogenic shipworms (Teredinidae) around the world

Species	Type Locality ¹	Reproductive Mode
<i>Teredo bartschi</i> Clapp, 1923	Florida	LLT
<i>Teredo clappi</i> Clapp, 1923	Florida ²	LLT
<i>Teredo furcifera</i> von Martens, 1894	Molucca Islands	LLT
<i>Teredo navalis</i> Linnaeus, 1758	Netherlands	LST
<i>Bankia bipalmulata</i> (Lamarck, 1801)	India	O
<i>Bankia carinata</i> (Gray, 1827)	Sumatra ³	O
<i>Bankia fimbriatula</i> Moll & Roch, 1931	Scotland ⁴	O
<i>Lyrodus affinis</i> (Deshayes, 1863)	Reunion	LLT
<i>Lyrodus bipartitus</i> (Jeffreys, 1860)	England ⁵	LLT
<i>Lyrodus massa</i> (Lamy, 1923)	Yemen and Arabia	LLT
<i>Lyrodus medilobatus</i> (Edmondson, 1942)	Hawaiian Islands	LLT
<i>Lyrodus pedicellatus</i> (Quatrefages, 1849)	Spain	LLT

O = oviparous (free-swimming larvae, in water column for days to weeks); LST = larviparous, held short-term (free-swimming larvae, in water column for days); LLT = larviparous, held long-term (larvae in water column for hours)

¹The type locality may not be where the species is native; this caveat especially pertains to the seven taxa first described from the Northern Hemisphere.

²Described from specimens collected from the keel of ship.

³The material reported by Turner (1966) as *Bankia carinata* at a great depth (7,488 meters) in the Banda Sea (eastern Indonesia) may bear reexamination.

⁴Described from "teak wood" (drifting from tropical waters in the Gulf Stream to Scotland, or discarded imported tropical wood).

⁵Described from shells in wood that came ashore in England from the Gulf Stream.

None of this argument contradicts the potential for shipworms to be naturally transported for short or even longer distances within the tropical and subtropical Atlantic, Pacific, and Indian Oceans in drifting wood, coconut husks, and mangrove roots. In these regions, sorting out aboriginal distributions from ship-created ones may now be impossible.

Turner (1966) recorded the remarkable case of the dispersal of the warm-water shipworm *Teredo furcifera* von Martens, 1894, by a vessel travelling largely in the Northern Hemisphere. The *Bounty II* left Tahiti for the Hawaiian Islands in September 1961. It was drydocked (length of time unknown) in Honolulu "where some but not complete work was done to repair damage by shipworms," and then proceeded to the Los Angeles area (San Pedro and Long Beach), where it remained in the water until spring 1962, at which time it was drydocked again (and again, length of time unknown). The vessel then proceeded north for stops in British Columbia, Washington, and California (all in marine or brackish waters), before proceeding through the fresh-water Panama Canal. It then visited marine or brackish ports in Louisiana and Florida, proceeded north to the cold water marine port of Boston, crossed the ocean to France and England, then south to the Canary Islands, and crossed the ocean westbound again to New York State. It proceeded to a shipyard in

November 1962, where it remained in the water until October 1963. The winter of 1962–1963 was characterized by "severe freezing" conditions at the site where the vessel was docked. When plankton was removed from the *Bounty II* in October 1963, *T. furcifera* was found to be alive and to have produced larvae, presumably in the summer of 1963 in New York.

It thus perhaps not surprising that the same suite of species of *Teredo*, *Bankia* and *Lyrodus* are today found in temperate or tropical ports and harbors around the world (Table 3). I suggest that all of these common harbor shipworm species have had their natural distributions broadly and deeply altered by shipping. For any given location, consideration must be given to whether the species and populations in question should be regarded as native, introduced, or cryptogenic. In some regions of the world, the historical record is clear and the arrival of certain species can be more easily detected. For many regions this is not the case. Noting that certain shipworms can survive for long periods with their pallets closed (Turner, 1966), a compelling test of the hypothesis that neritic shipworms do not survive long transoceanic or interoceanic voyages would be to experimentally subject neritic species to oceanic conditions for the length of time that it would take to transit selected high seas routes. It would be of further interest to

examine in greater detail the biogeography of species with an oviparous life cycle, such as in the genus *Bankia*, compared to those with both long- and short-term incubated larvae, such as in the genera *Teredo* and *Lyrodus* (Table 3). Species in the genus *Bankia*, with long-term planktrophic larvae, may represent more panmictic stocks, whereas those with shorter-term larvae—and thus possibly more subject to the establishment of isolated populations via dispersal as adults—may represent more genetically unique populations, either through founder effects or later genetic changes.

INTRODUCTIONS OF MARINE MOLLUSKS: SYSTEMATIC, BIOGEOGRAPHIC, AND ECOLOGICAL OVERVIEWS

No global review of the total number of marine mollusks transported by human activity is available. I estimate the total number of introduced marine bivalves, gastropods, and chitons to be about 100 species, but many cryptogenic taxa could increase that number significantly. This number includes invasions through the Suez Canal (Por, 1978; Spanier & Galil, 1991).

To illustrate the systematic, geographic, and ecological breadth of these invasions, I examine here a subset of 38 introduced species the evolutionary origins of which are known to be in the Northern Hemisphere (Table 4, arranged by source region). Omitted from this table are opisthobranch (nudi-branchs and sacoglossans) and pyramidellid gastropods, invasions through the Suez Canal, species of uncertain establishment, certain intracontinental introductions, primarily such freshwater taxa as the bivalves *Corbicula* and *Dreissena* and the snail *Potamopyrgus*, and tereidinids, discussed above, the geographic origins of introduced or cryptogenic species being as yet uncertain.

A wide phylogenetic and ecological breadth of taxa are subject to human-mediated geographic alteration. These include salt-marsh and high intertidal taxa (the ellobiid *Myosotella*, the pomatiopsid *Cecina*, the periwinkle *Littorina saxatilis* (Olivi, 1792), the mussel *Geukensia*), intertidal and sublittoral soft bottom species (Batillariidae, Nassariidae, Melongenidae, Corbulidae, Myidae, Pharidae, Petricolidae, Veneridae, Pectinidae, Semelidae) and intertidal and sublittoral hard bottom

(including fouling) species (Littorinidae, Callyptaeidae, Vermetidae, Muricidae, Mytilidae, Ostreidae, Dreissenidae, Trapezidae, Laterulidae). Similarly, transport is not selective for any feeding mode, and includes omnivores, herbivores, carnivores, grazers, and suspension feeders.

Geographic patterns are distinct (Table 5). In terms of donor regions, 24 taxa (63%) originate in the North Atlantic Ocean/Mediterranean area, while 14 (37%) originate in the North Pacific Ocean. Within the Atlantic Ocean, the western Atlantic is a significantly stronger donor area, with 18 taxa or 75% (versus 6 from the North Atlantic (*Mytilus edulis*) and Eastern Atlantic/Mediterranean) having dispersed globally from Atlantic North America. Similarly, the western Pacific Ocean is a strong donor region, with 13 taxa (versus one from the Eastern Pacific), or 93% of those originating in the Pacific, having been exported (Table 5).

In terms of receiver regions, Eastern North Pacific shores are comparatively strongly invaded, with 28 species (Table 5) more than twice the total of 13 species that have invaded the entire North Atlantic/Mediterranean/Black Sea region (Table 4).

Carlton (1992b, 1996a) and Cohen & Carlton (1995) have briefly reviewed some of the ecological impacts of molluscan invasions. In many regions, introduced mollusks are now the most abundant infaunal or epifaunal species present. In the northeastern Pacific Ocean, in San Francisco Bay, the infaunal or near-surface bivalves *Mya*, *Gemma*, *Venerupis*, *Musculista* and *Potamocorbula* may be sufficiently abundant as to control water column productivity (Cloern, 1982; Officer et al., 1982; Alpine & Cloern, 1992; Cohen & Carlton, 1995). The introduced mussels *Mytilus galloprovincialis* and *Geukensia demissa* likely add significantly to this role in San Francisco Bay but remain unstudied in this regard. In Pacific Northwest embayments, *Mya arenaria* may be the only common large clam at the upland end of many estuaries (Carlton, 1979a). Griffiths et al. (1992) conclude that the introduced *Mytilus galloprovincialis* is the dominant mussel throughout the Western Cape region of South Africa, largely displacing the native mussel *Aulacomya ater* (Molina, 1782). In New Zealand, the introduced *Mytilus galloprovincialis* forms aggregations up to several hundred square meters in area intertidally and subtidally in New Zealand, where it overgrows serpulid polychaetes, bryozoans, hydroids,

TABLE 4. Examples of introductions of marine mollusks originating in the Northern Hemisphere (*) = see footnote

Geographic Origin/Species	Invading	References
Eastern Atlantic — Mediterranean Species		
Gastropoda		
Littorinidae		
<i>Littorina littorea</i> (Linnaeus, 1758) (periwinkle) (*)	Atlantic North America	Carlton, 1992b; Vadas & Elner, 1992; Zibrowius, 1992
Ellobiidae		
<i>Myosotella myosotis</i> (Draparnaud, 1801) (marsh snail)	Atlantic and Pacific North America	Carlton, 1992b
Bivalvia		
Mytilidae		
<i>Mytilus galloprovincialis</i> Lamarck, 1819 (mussel)	Pacific Ocean (New Zealand, Australia; Japan; Russia; California); South Africa	Carlton, 1992b; Geller et al., 1994; MacKenzie et al., 1997
Ostreidae		
<i>Ostrea edulis</i> Linnaeus, 1758 (oyster) (*)	Atlantic North America	Barber, 1997; MacKenzie et al., 1997
Corbulidae		
<i>Corbula gibba</i> (Olivi, 1792) (corbula) (*)	Australia	Healy & Lamprell, 1996
Western Atlantic Species		
Gastropoda		
Littorinidae		
<i>Littorina saxatilis</i> (Olivi, 1792) (periwinkle) (*)	Pacific North America	Carlton & Cohen, 1998
Vermetidae		
<i>Vermetus alii</i> Hadfield & Kay, 1972, in Hadfield et al. 1972 (wormsnail) (*)	Hawaiian Islands	Hadfield et al., 1972; Kay, 1979
Calyptraeidae		
<i>Crepidula fornicata</i> (Linnaeus, 1758) (slipper snail)	Europe; Pacific North America; Mediterranean	Carlton, 1992b; Zibrowius, 1992; Blanchard, 1995; Minchin et al., 1995; Eno et al., 1997
<i>Crepidula convexa</i> Say, 1822 (slipper snail)	Pacific North America	Carlton, 1992b
<i>Crepidula plana</i> Say, 1822 (slipper snail)	Pacific North America	Carlton, 1992b
Melongenidae		
<i>Busycotypus canaliculatus</i> (Linnaeus, 1758) (whelk) (*)	Pacific North America	Carlton, 1992b
Nassariidae		
<i>Ilyanassa obsoleta</i> (Say, 1822) (mudsnail) (*)	Pacific North America	Carlton, 1992b
Muricidae		
<i>Urosalpinx cinerea</i> (Say, 1822) (oyster drill)	Europe; Pacific North America	Carlton, 1992b; Eno et al., 1997
Bivalvia		
Mytilidae		
<i>Geukensia demissa</i> (Dillwyn, 1817) (mussel)	Pacific North America	Carlton, 1992b
Dreissenidae		
<i>Mytilopsis leucophaeata</i> (Conrad, 1831) (false mussel)	Europe	Oliver et al., 1998
Ostreidae		
<i>Crassostrea virginica</i> (Gmelin, 1791) (oyster)	British Columbia (Boundary Bay); Hawaiian Islands (Oahu: Pearl Harbor)	Carlton, 1992b; Carlton & Mann, 1996; Barber, 1997; MacKenzie et al., 1997
Pectinidae		
<i>Argopecten irradians</i> (Lamarck, 1819) (scallop)	Western Pacific Ocean (China)	Chew, 1990; Wang, 1995; MacKenzie et al., 1997
Myidae		
<i>Mya arenaria</i> Linnaeus, 1758 (softshell clam)	Europe; Black Sea; Pacific North America	Carlton, 1992b; Zolotarev, 1996; Eno et al., 1997; MacKenzie et al., 1997
Pharidae		
<i>Ensis directus</i> Conrad, 1843 (razor clam)	Europe	Rasmussen, 1996; Eno et al., 1997

(continued)

TABLE 4. (Continued)

Geographic Origin/Species	Invading	References
Western Atlantic Species		
Bivalvia (continued)		
Tellinidae		
<i>Macoma "balthica"</i> (Linnaeus, 1758)* (*)	Pacific North America	Meehan et al., 1989
Petricolidae		
<i>Petricolaria pholadiformis</i> (Lamarck, 1818) (angel wing)	Europe; Mediterranean and Black Sea; Pacific North America	Zibrowius, 1992; Carlton, 1992b; Eno et al., 1997
Veneridae		
<i>Mercenaria mercenaria</i> (Linnaeus, 1758) (quahog) (*)	Europe; Mediterranean; Pacific North America	Zibrowius, 1992; Eno et al., 1997; MacKenzie et al., 1997
<i>Gemma gemma</i> (Totten, 1834) (gem clam)	Pacific North America	Carlton, 1992b
North Atlantic Species		
Bivalvia		
<i>Mytilus edulis</i> Linnaeus, 1758 (mussel) (*)	Indian Ocean (Kerguelen Islands); Southwest Atlantic Ocean (Argentina); Southeast Pacific Ocean (Chile)	Koehn, 1991; Seed, 1992
Eastern Pacific Species		
Gastropoda		
Calyptraeidae		
<i>Crucibulum spinosum</i> (G. B. Sowerby I, 1824) (cup-and-saucer shell)	Hawaiian Islands	Kay, 1979
Western Pacific Species		
Gastropoda		
Pomatiopsidae		
<i>Cecina manchurica</i> A. Adams, 1861 (supralittoral snail)	Pacific North America	Carlton, 1992b
Muricidae		
<i>Rapana venosa</i> Valenciennes, 1846 (whelk) (*)	Black Sea, Mediterranean	Zibrowius, 1992; Zolotarev, 1996
<i>Ocenebrellus inornatus</i> (Récluz, 1851) (oyster drill) (*)	Pacific North America	Carlton, 1992b
Batillariidae		
<i>Batillaria attramentaria</i> (G. B. Sowerby II, 1855) (cerith)	Pacific North America	Carlton, 1992b
Nassariidae		
<i>Nassarius fraterculus</i> (Dunker, 1860) (basket snail; nassa)	Pacific North America	Carlton, 1992b
Bivalvia		
Mytilidae		
<i>Musculista senhousia</i> (Benson, 1842) (mussel)	Pacific North America; New Zealand; Australia; Mediterranean	Carlton, 1992b; Zibrowius, 1992; Reusch & Williams, 1998
Ostreidae		
<i>Crassostrea gigas</i> (Thunberg, 1793) (oyster)	Europe; Mediterranean; Pacific Ocean (widespread)	Grizel & Heral, 1991; Carlton, 1992b; Zibrowius, 1992; Gouilletquer & Heral, 1997; Eno et al., 1997; MacKenzie et al., 1997; Kunz-Jacques, 1997; Barber, 1997
Corbulidae		
<i>Potamocorbula amurensis</i> (Schrenck, 1861) (corbula) (*)	Pacific North America	Carlton et al., 1990; Alpine & Cloern, 1992; Duda, 1994; Kimmerer et al., 1994
Psammobiidae		
<i>Nuttallia obscurata</i> (Reeve, 1857) (*)	Pacific North America	Forsyth, 1993; Gillespie, 1995; Merilees & Gillespie, 1995
Semelidae		
<i>Theora lubrica</i> Gould, 1861 (semele)	Pacific North America; New Zealand	Climo, 1976; Carlton, 1992b
Veneridae		
<i>Venerupis philippinarum</i> (A. Adams & Reeve, 1850) (little-neck clam) (*)	Pacific North America; Europe, Mediterranean Hawaiian Islands	Carlton, 1992b; Zibrowius, 1992; Laruelle et al., 1994; Gouilletquer & Heral, 1997; MacKenzie et al., 1997; Gouilletquer, 1997

TABLE 4. (Continued)

Geographic Origin/Species	Invading	References
Western Pacific Species		
Bivalvia (continued)		
Trapezidae		
<i>Neotrapezium liratum</i> (Reeve, 1843) (trapezium) (*)	Pacific North America	Carlton, 1992b
Laternulidae		
<i>Laternula marilina</i> (Reeve, 1860) (lantern shell) (*)	Pacific North America	Miller et al., 1999

Notes:

Bivalvia

Ostrea edulis is widely held in aquaculture (mariculture) systems around the world, and while adventitious specimens can be found occasionally in the wild near those systems, permanently reproducing wild populations appear to be established outside its native Europe only in eastern North America from Maine to Rhode Island.

Potamocorbula amurensis occurs in Pacific North America in San Francisco Bay, California, only. Bernard et al. (1993) placed *P. amurensis* in synonymy with the earlier *P. ustulata* (Reeve, 1844). I examined (1997) the syntypes of *P. ustulata* (ex Cuming coll., type locality Singapore) in The Natural History Museum, London, and compared them to a population of *Potamocorbula* collected in San Francisco Bay in November 1996. The latter closely match the *P. amurensis* of Asian literature (Carlton et al., 1990). The syntypes of *P. ustulata* of the same size as *P. amurensis* from San Francisco Bay are heavy, thick, well-calcified shells, the umbones of which are thus not subject to being easily broken; they further have a deeply incised pedal retractor scar above the anterior adductor muscle scar, and the pallial sinus is a distinct albeit shallow indentation. *Potamocorbula amurensis* is a comparatively thin, fragile shell, whereby the inner curve of the umbo is subject to breakage when the internal resilium plug in the right valve under the umbo breaks away; the pedal retractor scar is not incised, and the pallial sinus is only a very minor undulation of the pallial line. I tentatively regard the two as separate species, pending further consideration on to what extent some of these characteristics may be phenotypic.

Corbula gibba has been found in Australia in Port Phillip Bay, Victoria, but is likely to spread along the mainland and into Tasmania.

Nuttallia obscurata occurs from British Columbia to Oregon (C. Mills & J. Chapman, pers. comm., 1998).

Macoma "balthica": The population of this Atlantic *Macoma* in San Francisco Bay is genetically similar to Western Atlantic Ocean stocks. If the *Macoma* of Atlantic North America is distinct from European *M. balthica*, then the name *Macoma petalum* (Valenciennes, 1821) may apply to the Atlantic American and San Francisco Bay populations (Meehan et al., 1989; Cohen & Carlton, 1995).

Mercenaria mercenaria occurs in Pacific North America in Alamitos Bay, in southern California, only.

Venerupis philippinarum goes by perhaps a greater variety of names than any other species on this list. The name *Venerupis philippinarum* is used following Carlton (1992b) and Coan & Scott (1997) and the arguments set forth therein. Other specific names in use are *japonica* Deshayes, 1853, and *semidecussata* Reeve, 1864, with these and *philippinarum* variously placed in the genera *Tapes*, *Venerupis*, and *Ruditapes*.

Neotrapezium liratum is known from Ladysmith Harbor, British Columbia (Carlton, 1992b), but may be more widespread but overlooked in the Pacific Northwest.

Laternula marilina is known only from Humboldt Bay, California (Miller et al., 1999) and may be more widespread but overlooked in the Pacific Northwest.

Gastropoda

Littorina saxatilis is cryptogenic in both the Mediterranean Sea (Zibrowius, 1992) and in at least two locations in South Africa (Reid, 1996). Curiously, it was first described from Venice in 1792, making it perhaps one of the earlier introductions of a marine mollusk. It occurs in Pacific North America in San Francisco Bay, California.

Ocenebrellus inornatus is known in most literature as *Tritonalia japonica* (Dunker, 1860), *Ocenebra japonica* or *Cerastostoma inornatum*; see Amano & Vermeij, 1998.

Rapana venosa is a senior synonym of *Rapana thomasiana* Crosse, 1861.

Busycotypus canaliculatus occurs in Pacific North America in San Francisco Bay, California, only.

Ilyanassa obsoleta occurs in Pacific North America only in three locations: San Francisco Bay, California, Willapa Bay, Washington, and Boundary Bay, British Columbia.

Vermetus alii is here tentatively regarded as originating from the subtropical western Atlantic Ocean, although alternatively it may originate from the subtropical eastern Pacific (R. Bieler, pers. comm., 1996); it is here regarded as introduced to the Hawaiian Islands.

and coralline algae, and is often locally the most abundant and competitively dominant epifaunal invertebrate (Witman & Grange, 1998). In Europe, Pacific North America, and elsewhere, large intertidal and shallow sublittoral reefs of the Pacific oyster *Crassostrea gigas* (Heral, 1990; Barber, 1997), may play similar ecological roles in terms of altering water column phytoplankton densities and in terms of benthic community structure.

In other regions, introduced gastropods have become both aspect dominants and community structural engineers. The European snail *Littorina littorea* (Linnaeus, 1758) has had vast and complex impacts on intertidal hard- and soft-bottom communities from Canada to the mid-Atlantic American coast (Carlton, 1992b; Vadas & Elner, 1992; Bertness, 1999), altering the abundance and distribution of numerous other plants and ani-

TABLE 5. Donor and receiver regions of introduced mollusks originating in the Northern Hemisphere

	DONOR REGION							TOTAL ALL
	North Atlantic-Mediterranean				North Pacific			
	NA	EA/M	WA	TOTAL	EP	WP	TOTAL	
Gastropoda	0	2	8	10	1	5	6	16
Bivalvia	1	3	10	14	0	8	8	22
TOTAL	1	5	18	24	1	13	14	38

	RECEIVER REGION: NORTHERN HEMISPHERE					
	North Atlantic-M-BS			North Pacific		
	M/BS	EA	WA	EP	WP	HAWAII
Gastropoda	2	2	2	12	0	2
Bivalvia	6	6	1	16	3	3

	RECEIVER REGION: SOUTHERN HEMISPHERE				
	WSP	ESP	WSA	SA	IO
	Gastropoda	0	0	0	0
Bivalvia	5	1	1	1	1

Abbreviations: BS, Black Sea; EA, Eastern Atlantic Ocean; EP, Eastern Pacific Ocean; ESP, Eastern South Pacific Ocean (Chile); IO, Indian Ocean; M, Mediterranean Sea; NA, North Atlantic Ocean; SA, South Africa; WA, Western Atlantic Ocean; WP, Western Pacific Ocean; WSA, Western South Atlantic Ocean (Argentina); WSP, Western South Pacific Ocean (Australia, New Zealand).

mals. The Atlantic mudsnail *Ilyanassa obsoleta* (Say 1822) may have had a similar range of impacts on soft-bottom communities in San Francisco Bay, but, while its negative impact on a native mudsnail has been demonstrated (Race, 1982), much of its ecosystem-level interactions in California remain to be studied. The Atlantic slipper limpet *Crepidula fornicata* (Linnaeus, 1758), is locally very abundant in western Europe, although little appears to be available on its ecological impact, although it is regarded as a nuisance and competitor in the oyster industries (Eno et al., 1997). A range of carnivorous molluscivores, including the Atlantic drill *Urosalpinx* and whelk *Busycotypus* and the Asian drill *Ocenebrellus* and whelk *Rapana*, have been transported by a variety of means around the world, and may have local significant impacts, although quantitative, experimental data are lacking for all introduced populations.

CONCLUDING REMARKS

Three general conclusions or observations arise from the current data and knowledge

about marine bioinvasions, with particular reference to the patterns of molluscan introductions under discussion here.

First, human-mediated dispersal—potentially ancient—offers a set of alternative hypotheses to the presumptions of “naturalness” embedded in many if not most classical interpretations of the historical biogeography of a vast number of species. The fact that a given species *could* disperse naturally does not prove that it did so, nor does the fact that a given species occurred or occurs in or on the bottom of a ship (or with commercial oysters, or with other vectors) prove that it was dispersed by that means either. Rather, a great many species populations must be regarded today as cryptogenic—neither clearly native or introduced—until further data are gathered (Carlton, 1996b). There can be little doubt, however, that a staggering number of species were *subjected* to broad and repeated dispersal by human activities centuries before the first biological surveys commenced. Carlton (1999) estimated that between 900 and 1,500 coastal species of marine organisms now regarded as naturally distributed may in fact have been dispersed by ships between the years 1500 and 1800 alone. It is predictable

that work would reveal the pre-18th or pre-19th century absence of certain now-common species in selected areas of the world where careful comparative analyses of the fossil, archeological, and modern faunas had not yet been undertaken.

Second, biogeographic patterns that emerge from the molluscan invasion data in hand now bear further consideration at broader systematic and geographic levels. These include the apparent predominance of the western sides of oceans (the eastern sides of continents) as donor areas, and possible explanations for this predominance, in terms of the complex interplay of the evolutionary histories of these biotas, the scale of regional productivity, and the probabilities over time and space of interfacing with constantly changing patterns of human-dispersal vectors. These patterns further include more detailed consideration of the northeastern Pacific Ocean as a region particularly remarkable for its role as a receiver (versus donor) area. Only one molluscan species, the slipper limpet *Crucibulum spinosum* (G. B. Sowerby I, 1824), appears to have departed the Pacific coast of North America, a pattern in concert with the low export diversity among other taxa (Carlton, 1979a, who noted that only a few Pacific American crustaceans have colonized other regions of the world). The depth and breadth of this phenomenon remain to be adequately explored.

Third and last, we may appeal to the need for far greater quantitative and experimental data on the ecological impacts of marine invasions, including those by mollusks. While invasive species often become the aspect dominant members of many communities—thus leading to the expectation or prediction that a given invasive species is having a significant ecological impact—more attention must be paid to experimentally demonstrating the actual mechanisms of such impacts. Without such data, both the predictive level of invasion ecology, and a full understanding of the importance of invasions in altering community and ecosystem structure, will remain limited.

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DETERMINATION OF THE DIET OF *OCTOPUS RUBESCENS* BERRY, 1953
(CEPHALOPODA: OCTOPODIDAE), THROUGH EXAMINATION OF ITS
BEER BOTTLE DENS IN PUGET SOUND

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ABSTRACT

Den middens have been used to determine the diet of several species of octopuses, but are not available for the red octopus (*Octopus rubescens* Berry, 1953). As a result, its diet in the wild is poorly known. To determine their diet, *O. rubescens* were collected in beer bottle dens, evicted from the bottles for measurement, and released. The shell contents of the bottles were then sieved, identified and compared to those from bottles not containing octopuses. The shell contents of bottles containing octopuses had significantly more species and significantly more shells than bottles without octopuses. In this mud/sand area, the octopuses were consuming predominantly the gastropod *Olivella baetica*. Beer bottle trash on the sea floor is a non-polluting den resource for *O. rubescens*, and is shown here to be a valuable tool for diet analysis.

Key words: Octopus, diet, habitat, Puget Sound, beer bottles.

INTRODUCTION

The small red octopus, *Octopus rubescens* Berry, 1953, is the most common octopus in the nearshore area on the west coast of the United States (Hochberg & Fields, 1980), but its habits are not very well known. Several indications of prey preference have been given for *O. rubescens*, mostly from laboratory studies. For example, Warren et al. (1974) delineated its color changes while attacking prey, and Dorsey (1976) described its natural history and social behavior. Anderson (1997) and Boyle (1991) described methods for its aquarium husbandry and laboratory maintenance. Hochberg (1997) listed the diets observed in the laboratory, which included a variety of species of molluscs and crustaceans. There is little information on the feeding of *O. rubescens* in the wild, although Hochberg & Fields (1980) stated that *O. rubescens* prefers small crabs, and Laidig et al. (1995) observed them eating small euphausiids. This limited information on the natural diet of *O. rubescens* inspired the present study.

Many species of octopuses take refuge in a den over a period of days or weeks (Mather, 1982, 1991; Ambrose, 1983; Hartwick et al., 1984; Voight, 1988; Cigliano, 1993). The dens of octopuses are a conspicuous aspect of their natural history, and are a source of infor-

mation about feeding, because many octopuses leave middens of prey remains in front of their dens (Mather, 1991; Hanlon & Messenger, 1996). The dens of *O. rubescens* have only been inferred from their being trawled up in empty giant barnacle shells, beer bottles, and other hard containers of appropriate size (Dorsey, 1976). A female guarding her eggs was even found in a cast-off shoe (Anderson, 1994). Other than beer bottles (Anderson, 1994, 1997), natural dens have not been described for *O. rubescens* from observations in the wild. Octopuses have used discarded human trash as dens for millennia (Lane, 1957; Cousteau & Dirole, 1973). Fishermen have taken advantage of the octopuses' propensity to inhabit shelters by lowering strings of jars; the octopuses crawl into them and can be harvested when the jars are retrieved. This behavior has been used to assess octopus populations by Voight (1988), who placed 325 ml beer bottles on intertidal sand flats as shelters. Such collection succeeded because octopuses prefer shelter that is dark and has a small entrance (Mather, 1982; Aronson, 1986). A routine collection of *O. rubescens* in bottles revealed the shell remains of likely prey items and suggested beer bottles could serve a further purpose, as a tool for analyzing the previously unknown prey choice of *O. rubescens*.

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MATERIALS AND METHODS

Sixteen *Octopus rubescens* were found in "aged" 355 ml beer bottles with a mouth opening size of 18.0 mm inside diameter by scuba divers during the fall and winter of 1997–1998 in 20–25 m deep water at Federal Way, 30 km south of Seattle, Washington State, USA. Long duration in the sea was indicated by a dense covering of barnacles (*Balanus crenatus*) and/or sea anemones (*Metridium giganteum*). Sixteen aged bottles unoccupied by octopuses were also collected. Octopuses in their bottles were placed into zippered plastic bags and transferred to the Seattle Aquarium using the methods of Anderson (1997). Octopuses were removed from the bottles by holding the bottles mouth downward over a pail of water. Remaining bottle contents were rinsed out thoroughly into the pail. The octopuses were sexed, weighed, measured for mantle length (ML), and released into the water near the Aquarium, a known habitat for *O. rubescens* (Jeff Christiansen, pers. comm.). The contents of the bottles were then strained through a sieve with a 2.0 mm openings, and the shells collected were kept and identified.

The labels were removed from 30 new bottles (short, brown 355 ml Budweiser® bottles, with a mouth opening size 18.0 mm inside diameter) and they were painted black, because octopuses prefer dark dens (Mather, 1982; Voight, 1988). The bottoms of the bottles were left unpainted so octopuses inside could be seen with the aid of a dive light. They were laid out on the bottom about 100 m from where the previous octopuses were collected. After 66 days, all bottles were retrieved and any octopuses inside were measured, weighed, and released. Any shells inside were sieved, retrieved and identified. The taxonomy of some of the small gastropods examined is in a state of flux (Ronald L. Shimek, pers. comm.), so they were not identified beyond genus, using the guides of Abbott (1974), Kozloff (1987), and Rice (1971).

An *O. rubescens* found in a bottle at the same site was taken to the Seattle Aquarium, where the interior of its bottle was cleaned out and the octopus allowed to re-occupy the bottle. It was placed in an aquarium with 39 live *Alia* sp., collected from the same area from eelgrass. The octopus was ejected from the bottle after 10 days and the bottle's contents examined.

Comparisons of the number of likely prey species between occupied and unoccupied bottles were made for the five most common

prey species. We also compared the number of shell species in bottles with octopuses to those without, using a niche breadth index developed by Cardona (1991). This index looks at frequencies of occurrence of the different prey species to calculate a niche breadth index, and we considered unoccupied bottles and occupied ones as representatives of two predator "species".

RESULTS

All octopuses found at the site during the day were within bottles, possibly because no suitable dens other than *Polinices* shells, always occupied by hermit crabs (see Jensen, 1995), were available. Significantly more octopuses were found in aged brown bottles (16 occupied, 7 unoccupied) than in clear ones (0 occupied, 9 unoccupied) when tested with a two-way Chi-square test ($X^2 = 16.358$; $p < 0.001$). The octopuses' mean weight was 8.26 g, and their mean mantle length was 19 mm.

Possible prey remains consisted of molluscan shells and barnacle fragments and were found in nearly all bottles. They came from a large number of species, but predominantly from the molluscs *Olivella baetica*, *Alia* sp., *Kurtziella* sp., and *Nassarius mendicus*, and the barnacle *Balanus crenatus* (Table 1). Comparisons of number of shells between occupied and unoccupied bottles could only be made for these five species because of the small numbers found of the other species. Chi-square analyses revealed that each of these five species were found significantly more often in bottles that had been occupied by an octopus (Table 2).

The niche breadth index developed by Cardona (1991) applied to the shells found in the bottles (Table 3) uses occurrence frequencies, that is, in how many individuals collected of each species of shell a certain food type was found. Cardona's index is an improvement/extension of the Gladfelter-Johnson Index (Gladfelter & Johnson, 1983) in that it looks at numbers of occurrence rather than relative percentage of total volume or weight of the collected food items. By using these niche-breadth calculations, we found a niche breadth for the occupied bottles to be $B' = 0.2417$ and for the unoccupied bottles $B' = 0.1275$. The occupied bottles had about twice the niche breadth of the unoccupied bottles, which is a clear indication of octopus activity.

After 10 days, the octopus in captivity had consumed 14 of the 39 *Alia* sp. available in its

TABLE 1. Mean number of molluscan and crustacean prey remains per bottle found on mud/sand habitat in Puget Sound.

Octopus-occupied old bottles (N = 16)	Unoccupied old bottles (N = 16)	Octopus-occupied new bottles (N = 2)	Unoccupied new bottles (N = 28)
<i>Olivella baetica</i> 27.75	<i>Olivella baetica</i> 5.625	<i>Olivella baetica</i> 6	<i>Olivella baetica</i> 0.5
barnacle fragments 7.06	Barnacle fragments 3.562	<i>Nassarius mendicicus</i> 0.5	barnacle fragments 0.143
<i>Alia</i> sp 4.125	<i>Alia</i> sp 0.9375	<i>Alia</i> sp. 0.5	<i>Alia</i> sp. 0.428
<i>Kurtziella</i> sp 0.875	<i>Nassarius mendicicus</i> 0.125		<i>Eulima</i> sp. 0.036
<i>Nassarius mendicicus</i> 0.625	<i>Clinocardium nuttallii</i> 0.0625		<i>Mopalia muscosa</i> 0.036
<i>Pododesmus macrochisma</i> 0.31	<i>Eulima</i> sp 0.0625		
<i>Clinocardium nuttallii</i> 0.25	<i>Kurtziella</i> sp. 0.0625		
<i>Chlamys hastata</i> 0.1875	<i>Lucinoma annulatum</i> 0.0625		
<i>Protothaca staminea</i> 0.1875	<i>Macoma nasuta</i> 0.0625		
<i>Cancer oregonensis</i> 0.125	<i>Pododesmus macrochisma</i> 0.0625		
<i>Crepidatella lingulata</i> 0.0625	<i>Polinices lewisii</i> 0.0625		
<i>Eulima</i> sp 0.0625	<i>Protothaca staminea</i> 0.0625		
<i>Lucinoma annulata</i> 0.0625			
<i>Macoma nasuta</i> 0.0625			
<i>Simomactra falcata</i> 0.0625			
<i>Natica clausa</i> 0.0625			
<i>Ocostomia</i> sp 0.0625			
<i>Polinices lewisii</i> 0.0625			

TABLE 2. Results of Chi-square tests (χ^2), with Yates' correction, on the five most-common shell remains in red octopus occupied and unoccupied beer bottles.

Shell item	Number in occupied bottles	Number in unoccupied bottles	χ^2	$\alpha <$
<i>Olivella baetica</i>	444	90	233.35	0.001
Barnacle fragments	113	57	17.79	0.001
<i>Alia</i> sp.	66	15	30.86	0.001
<i>Kurtizella</i> sp.	14	1	9.60	0.005
<i>Nassarius mendicus</i>	10	2	4.08	0.05

TABLE 3. Niche breadth indices (B') of red octopus occupied and unoccupied beer bottles.

Shell item	Occupied Bottles		Unoccupied Bottles	
	*Frequency of occurrence	%	*Frequency of occurrence	%
<i>Olivella baetica</i>	13	81.25	11	68.75
Barnacle fragments	15	93.75	12	75.00
<i>Alia</i> sp.	11	68.75	8	50.00
<i>Kurtizella</i> sp.	7	43.75	1	6.25
<i>Nassarius mendicus</i>	6	37.50	2	12.50
<i>Pododesmus macrochisma</i>	2	18.75	1	6.25
<i>Clinocardium nuttalli</i>	4	25.00	1	6.25
<i>Protothaca staminea</i>	3	18.75	1	6.25
<i>Eulima</i> sp.	1	6.25	1	6.25
<i>Lucinoma annulata</i>	1	6.25	1	6.25
<i>Macoma nasuta</i>	1	6.25	1	6.25
<i>Polinices lewisii</i>	1	6.25	1	6.25
<i>Chlamys hastata</i>	3	18.75	0	0.00
<i>Cancer oregonensis</i>	1	6.25	0	0.00
<i>Crepidatella lingulata</i>	1	6.25	0	0.00
<i>Simomactra falcata</i>	1	6.25	0	0.00
<i>Natica clausa</i>	1	6.25	0	0.00
<i>Odostomia</i> sp.	1	6.25	0	0.00
Niche breadth indices:		$B' = 0.2417$		$B' = 0.1275$

*Frequency of occurrence: In how many of the 16 bottles in each category a species was found.

aquarium and had left all the empty shells inside its bottle den. None of the shells were drilled.

DISCUSSION

It is not surprising that a small octopus species like *Octopus rubescens* occupies beer bottles, because a number of other *Octopus* species utilize man-made items for their dens. In fact, beer bottles may allow *O. rubescens* to utilize the sand/mud habitat, as cockle shells do for *O. joubini* in the sea-grass beds of Florida (Mather, 1982). Few other den sites were available. There were only the occasional clam shells *Tresus capax*, *Panopea abrupta* and *Clinocardium nuttalli*, and the large moon snail *Polinices lewisii*, which was usually occupied by the large hermit crab

Pagurus armatus. Natural den space was limited and may be competed for by other animals (Mather, 1982), such as *P. armatus*, which is invariably found in *Polinices* shells in this area (Jensen, 1995). It was obvious the octopuses preferred darker brown bottles and those obscured with marine growth. Thus, the octopus-occupied bottles were preferred dens and contained more shells than unoccupied bottles, which may have been occupied by octopuses on a transitory basis and hence had fewer shells in them. The experiment in the aquarium confirmed *O. rubescens* keeps its prey remains in its bottle dens.

It is perhaps a bit surprising to find an octopus that leaves its food remains in its den, since *O. vulgaris*, *O. dofleini*, and *O. cyanea* all eject prey remains (studied by Mather, 1991; Hartwick et al., 1978; and Yarnall, 1969, respectively). There are possible advantages

and disadvantages to this behavior. The possible disadvantage is that food wastes may foul the den. The advantage is that the den site will not be "advertised." Scuba divers have long used den middens to spot an octopus (High, 1976) and likewise a hungry fish or seal might use a midden to detect its potential octopus prey, as suggested by Ambrose (1983).

This population of small *O. rubescens* appears to be feeding mostly on gastropod molluscs. The diet of *O. rubescens* may indicate habitat-specific prey selection similar to that of *O. bimaculatus*, which prefers crabs but consumes predominantly the molluscan prey widely available in its habitat, such as snails, chitons or bivalves (Ambrose, 1986). *Olivella baetica* is very common in Puget Sound (Shimek, 1992), and its presence in the prey remains indicates the octopuses were taking advantage of its wide availability in the sand. Although crabs may be a preferred food, octopuses have been described as "generalist predators" (Ambrose, 1986) and flexible prey choice is indicated here. The number of *Alia* sp. in the bottles may also indicate octopuses can go a considerable distance to forage, as these *Alia* sp. live in the eelgrass beds in shallow water and they were approximately 100 m inshore of the octopus collection location (pers. obs.); this is similar to the results found by Hartwick et al. (1981) for *O. dofleini*.

The octopuses inhabiting bottles at the time of this study (November 1997–January 1998) were juveniles. *Octopus rubescens* can grow to 400 g (Hochberg, 1998), although females can spawn at just 23 g (Osborn, 1995). An octopus of a mean weight of 8.26 g is occupying just 2.3% of a 355 ml beer bottle. The small size of these octopuses represent one stage of the life cycle and hence these prey items may only be eaten by juveniles. *Octopus rubescens* up to 99 g have been found in beer bottles (Seattle Aquarium, unpubl. data), and larger animals may be eating different prey or may not be leaving their food remains in the bottles.

More than one explanation is possible for the presence of the barnacle fragments in the bottles. Octopuses have been known to eat barnacles (Nixon & Maconnachie, 1988), but we cannot be sure they are doing so in this case. The barnacles on many of the bottles examined were dead, possibly eaten by sea stars, a major barnacle predator (Mauzey et al., 1968). The barnacle shells might then have been held by the octopuses as barriers at the mouths of their beer bottle dens (Hochberg, 1997). On the other hand, they

could have consumed the barnacles. If so, the barnacle-covered bottles would have provided both homes and a food source.

Octopuses consume hermit crabs in mollusc shells (Iribarne et al., 1991), and it is possible that *O. rubescens* were eating hermit crabs in *Alia* sp. shells found in the shallow eelgrass beds. Few hermit crabs were seen at the depths where the octopus-occupied bottles were found, and no hermit crab remains were found in the bottles. It is possible that crab remains might pollute the interior of the bottles more than molluscan remains and hence be ejected by the octopus, but the most common shell found inside the bottles, *Olivella baetica*, has a narrow aperture unsuitable for occupation by local hermit crabs. Thus, the *O. baetica* were probably directly consumed by the octopuses.

This unusual kind of animal/human interaction, with octopuses using our discarded trash, has offered us an interesting research opportunity. If an octopus species does not discard its food remains in front of its den, prey choice assessment is difficult. The shape and color of beer bottles have made them an appropriate shelter for small octopus species (Mather, 1982; Voight, 1988). Food remains of octopuses in different climates may pollute the interior of their bottle dens and hence the remains may be ejected, but in this case the bottles have allowed us to "capture" prey remains of *O. rubescens* that would otherwise be scattered by scavengers or removed by water currents. We encourage other researchers to examine the bottle dens of other species of octopus to look for what Dodge & Scheel (1997) called "the remains of the prey."

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CONSERVATION AND COMMERCE: MANAGEMENT OF FRESHWATER MUSSEL (BIVALVIA: UNIONOIDEA) RESOURCES IN THE UNITED STATES

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ABSTRACT

The United States is blessed with the world's greatest diversity of freshwater mussels (Unionoidea), providing numerous ecological, scientific, and economic benefits to the nation. However, as a result of intense economic development in the 19th and 20th centuries, this fauna was subjected to habitat destruction, water pollution, and benign neglect that resulted in the loss of populations and species. Presently, about 35 mussel species are presumed extinct, 69 species are federally protected as endangered or threatened, and numerous other species are candidates for protection. Propagation of these endangered species is underway to expedite their recovery. In contrast to this sizable group of rare species, a small assemblage of ubiquitous species occurring in large rivers and reservoirs within the Mississippi River drainage supports a multi-million dollar commercial shell industry. Most shells are shipped to the Far East to provide beads for a thriving cultured pearl industry; however, exports in this decade peaked in 1995 and face an uncertain future. Harvest and management regulations are being unified in the Mississippi River in 1998 to conserve mussel resources from overexploitation, to resolve law enforcement problems among states, and to consider the exotic zebra mussel, *Dreissena polymorpha*, which now infests most commercially exploited unionid populations. In the next decade, the amount of attention given to conservation will decide the fate of this world-class mussel fauna.

Key words: unionoidea, conservation, freshwater, United States.

INTRODUCTION

The decline of species within the superfamily Unionoidea is of international scope, driven by habitat degradation and destruction in river systems that once teemed with an abundance of aquatic mollusks. To address this and other global-scale losses in biological diversity, the International Union for the Conservation of Nature (IUCN) began formalizing lists of endangered species in the early 1960s, to focus attention on the plight of rare species (Mace, 1995). The publication of Red Lists and Red Books by IUCN has promoted the formation of similar national lists of plants and animals in need of protection (Burton, 1984; Thomas & Morris, 1995). Although the focus on species only in such lists will not achieve the desired maintenance of biodiversity and ecological integrity, primarily because habitat loss is not addressed, species continue to be the units of extinction. Therefore, the development of practical strategies to achieve conservation goals and preserve biological diversity must

make use of the flagship role played by species, so that public and legislative support can be mobilized for tangible benefits to both the species and their supporting ecosystems.

In a recent review of the global status of mollusks, Kay (1995) highlighted the current unprecedented rate of extinction of mollusks. From an admittedly incomplete data base of 1,130 taxa considered to be endangered, threatened, rare, or recently extinct, three possible explanations for the propensity of mollusks to become endangered were postulated. First, most of these species were essentially restricted to North America (United States), Australia and New Zealand, and Europe. These countries emerge seemingly as trouble spots only because of their more thorough biological inventories and monitoring programs. Thus, this apparent continental or national problem likely reflects a bias in available information. Second, most (98%) of the species are freshwater and terrestrial mollusks, with 61% from only nine families. Again, a possible reason for this phenomenon is the

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available data base on these taxonomic groups. Finally, life history traits shared by these designated families include k-selection, restricted distribution, and specialized habits and habitats. This commonality of biological traits is definitely correlated with endangerment, and describes the reason for declining status of most aquatic and terrestrial biota.

Of the 1,130 species identified as in trouble, 197 (17%) are bivalves and 158 (14%) of those species belong to the superfamily Unionoidea in the United States (Rosenberg, 1995). Because this taxon is recognized as the most endangered group of mollusks in the world, this paper will provide an assessment of the nearly 300 species and subspecies of freshwater mussels in the United States and discuss both conservation measures for the rare species and regulatory management for harvest of the abundant species. The management of this world-class resource has been a challenge for federal and state regulatory agencies; namely, to completely protect some species while allowing the exploitation of other species, often within the same river.

My goal is to provide readers with an appreciation of the complexity of management issues, and to describe the conservation measures being implemented to recover rare species and to ensure the viability of all species.

CONSERVATION OF MUSSELS

Threatened and Endangered Species

An assessment of the fauna, flora, and ecosystems of the United States was recently completed to evaluate the state of the nation's environment (LaRoe et al., 1995). Of the roughly 300 species and subspecies of freshwater mussels in the United States, 69 (23%) are included on the federal Endangered Species List (Table 1). The Endangered Species Act (ESA) of 1973 was promulgated to protect a species in danger of extinction or endangerment throughout all or a significant portion of its range. The ESA also has a stated goal to protect the ecosystems that sustain those species considered to be endangered or

TABLE 1. Endangered and threatened species of freshwater mussels in the United States in 1998.

<i>Alasmidonta atropurpurea</i>	Cumberland elktoe	<i>Lampsilis subangulata</i>	shinyrayed pocketbook
<i>Alasmidonta heterodon</i>	dwarf wedgemussel	<i>Lampsilis virescens</i>	Alabama lampmussel
<i>Alasmidonta raveneliana</i>	Appalachian elktoe	<i>Lasmigona decorata</i>	Carolina heelsplitter
<i>Amblema neislerii</i>	fat threeridge	<i>Lemiox rimosus</i>	birdwing pearlymussel
<i>Arkansia wheeleri</i>	Ouachita rock pocketbook	<i>Margaritifera hembeli</i>	Louisiana pearlshell
<i>Cyprogenia stegaria</i>	fanshell	<i>Medionidus acutissimus</i>	Alabama moccasinshell
<i>Dromus dromas</i>	dromedary pearlymussel	<i>Medionidus parvulus</i>	Coosa moccasinshell
<i>Elliptio chipolaensis</i>	Chipola slabshell	<i>Medionidus penicillatus</i>	gulf moccasinshell
<i>Elliptio steinstansana</i>	Tar River spiny mussel	<i>Medionidus simpsonianus</i>	Ochlockonee moccasinshell
<i>Elliptioideus sloatianus</i>	purple bankclimber		
<i>Epioblasma brevidens</i>	Cumberlandian combshell	<i>Obovaria retusa</i>	ring pink
<i>Epioblasma capsaeformis</i>	oyster mussel	<i>Pegias fabula</i>	littlewing pearlymussel
<i>Epioblasma florentina curtisii</i>	Curtis pearlymussel	<i>Plethobasus cicatricosus</i>	white wartyback
<i>Epioblasma florentina florentina</i>	yellow blossom	<i>Plethobasus cooperianus</i>	orangefoot pimpleback
<i>Epioblasma florentina walkeri</i>	tan riffleshell	<i>Pleurobema clava</i>	clubshell
<i>Epioblasma metastrata</i>	upland combshell	<i>Pleurobema collina</i>	James spiny mussel
<i>Epioblasma obliquata obliquata</i>	cat paw	<i>Pleurobema curtum</i>	black clubshell
<i>Epioblasma obliquata perobliqua</i>	white cat paw	<i>Pleurobema decisum</i>	southern clubshell
<i>Epioblasma othcaloogensis</i>	southern acornshell	<i>Pleurobema furvum</i>	dark pigtoe
<i>Epioblasma penita</i>	southern combshell	<i>Pleurobema georgianum</i>	southern pigtoe
<i>Epioblasma torulosa gubernaculum</i>	green blossom	<i>Pleurobema gibberum</i>	Cumberland pigtoe
<i>Epioblasma torulosa rangiana</i>	northern riffleshell	<i>Pleurobema marshalli</i>	flat pigtoe
<i>Epioblasma torulosa torulosa</i>	tuberled blossom	<i>Pleurobema perovatum</i>	ovate clubshell
<i>Epioblasma turgidula</i>	turgid blossom	<i>Pleurobema plenum</i>	rough pigtoe
<i>Fusconaia cor</i>	shiny pigtoe	<i>Pleurobema pyriforme</i>	oval pigtoe
<i>Fusconaia cuneolus</i>	finerayed pigtoe	<i>Pleurobema taitianum</i>	heavy pigtoe
<i>Hemistena lata</i>	cracking pearlymussel	<i>Potamilus capax</i>	fat pocketbook
<i>Lampsilis abrupta</i>	pink mucket	<i>Potamilus inflatus</i>	Alabama heelsplitter
<i>Lampsilis atilis</i>	finelined pocketbook	<i>Ptychobranchus greenii</i>	triangular kidneyshell
<i>Lampsilis higginsii</i>	Higgins eye	<i>Quadrula cylindrica strigillata</i>	rough rabbitsfoot
<i>Lampsilis perovalis</i>	orangenacre mucket	<i>Quadrula fragosa</i>	winged mapleleaf
<i>Lampsilis powelli</i>	Arkansas fatmucket	<i>Quadrula intermedia</i>	Cumberland monkeyface
<i>Lampsilis streckeri</i>	speckled pocketbook	<i>Quadrula sparsa</i>	Appalachian monkeyface
		<i>Quadrula stapes</i>	stirrupshell
		<i>Toxolasma cylindrellus</i>	pale lilliput
		<i>Villosa perpurpurea</i>	purple bean
		<i>Villosa trabalis</i>	Cumberland bean

threatened in the United States. Scientists postulate that more than 500 species of plants and animals became extinct in the United States, primarily due to habitat loss and degradation (U. S. Fish and Wildlife Service, 1995). Unfortunately, some of the mussel species that are on the list are presumed extinct (Table 2). Soon after the law was implemented, 23 species of unionids were added to the list in 1976, in response to a petition to list all animals on the Appendix I list of the Conservation on International Trade in Endangered Species (CITES). Subsequent listing of mussel species has occurred sporadically, reflecting federal priorities, political climate, delays in the acquisition of sufficient data, and other requirements specified in the listing process (Fig. 1). The chronology of mussel species listed for federal protection, beginning in 1987, represents recognition of a backlog of species in need of protection, group listings of species within the same river, and the tenacity of the staff biologists responsible for preparing the documents needed to qualify particular species for protection.

Each mussel species on the endangered species list has a recovery plan prepared, which identifies the problems threatening the species and the actions needed to correct them. The plan summarizes our knowledge of the status, biology, and threats, and focuses on recovery actions essential to maintain existing populations and to re-establish sufficient historic populations so that the species can eventually be downlisted to threatened status or delisted. Because of the highly clustered, geographic distribution of federally listed species (Fig. 2), states such as Alabama and Tennessee are much more involved with recovery than other states. Recovery can involve natural increases in the abundance and range of a species due to improvements in habitat quality or availability, as well as human-assisted increases through habitat restoration, amelioration of threats, or artificial propagation. The goal is to implement those courses of action that will ultimately lead to the species' recovery. In the case of freshwater mussels, some species are in such critical condition that the only realistic goal at this time is to prevent extinction. These species in the "basket case" category are usually given highest priority so that the likelihood of near-term extinction may be reduced. Recovery of the remaining species is actively being pursued through improvements in physical habitat and water quality, as well as propagation through cooperative efforts between the U. S. Fish and Wildlife Ser-

TABLE 2. Species of freshwater mussels presumed extinct in the United States in 1998.

<i>Alasmidonta mccordi</i>	Coosa elktoe
<i>Alasmidonta robusta</i>	Carolina elktoe
<i>Alasmidonta wrightiana</i>	Ochlockonee arc mussel
<i>Elliptio nigella</i>	winged spike
<i>Epioblasma arcaeiformis</i>	sugarspoon
<i>Epioblasma biemarginata</i>	angled riffleshell
<i>Epioblasma flexuosa</i>	leafshell
<i>Epioblasma florentina florentina</i>	yellow blossom
<i>Epioblasma haysiana</i>	acornshell
<i>Epioblasma lenior</i>	narrow catspaw
<i>Epioblasma lewisii</i>	forkshell
<i>Epioblasma obliquata obliquata</i>	catspaw
<i>Epioblasma personata</i>	round combshell
<i>Epioblasma propinqua</i>	Tennessee riffleshell
<i>Epioblasma sampsonii</i>	Wabash riffleshell
<i>Epioblasma stewardsonii</i>	Cumberland leafshell
<i>Epioblasma torulosa gubernaculum</i>	Green blossom
<i>Epioblasma torulosa torulosa</i>	tubercled blossom
<i>Epioblasma turgidula</i>	turgid blossom
<i>Lampsilis binominata</i>	lined pocketbook
<i>Medionidus mcglameriae</i>	Tombigbee moccasin-shell
<i>Pleurobema altum</i>	highnut
<i>Pleurobema avellanum</i>	hazel pigtoe
<i>Pleurobema bournianum</i>	Scioto pigtoe
<i>Pleurobema chatanoogaense</i>	painted clubshell
<i>Pleurobema flavidulum</i>	yellow pigtoe
<i>Pleurobema hagleri</i>	brown pigtoe
<i>Pleurobema hanleyianum</i>	Georgia pigtoe
<i>Pleurobema johannis</i>	Alabama pigtoe
<i>Pleurobema murrayense</i>	Coosa pigtoe
<i>Pleurobema nucleopsis</i>	longnut
<i>Pleurobema rubellum</i>	Warrior pigtoe
<i>Pleurobema troschelianum</i>	Alabama clubshell
<i>Pleurobema verum</i>	true pigtoe
<i>Quadrula couchiana</i>	Rio Grande monkey-face
<i>Quadrula tuberosa</i>	rough rockshell

vice and the states where those species reside. Implementation of a recovery plan is a lengthy and difficult process, dependent less on biology than the human dimension to resource management. Without the support of numerous federal and state regulatory agencies, community leaders responsible for land management, and myriad other stakeholders, recovery would not be possible.

An evaluation of the number and distribution of protected species provides an effective means to identify watersheds in greatest need

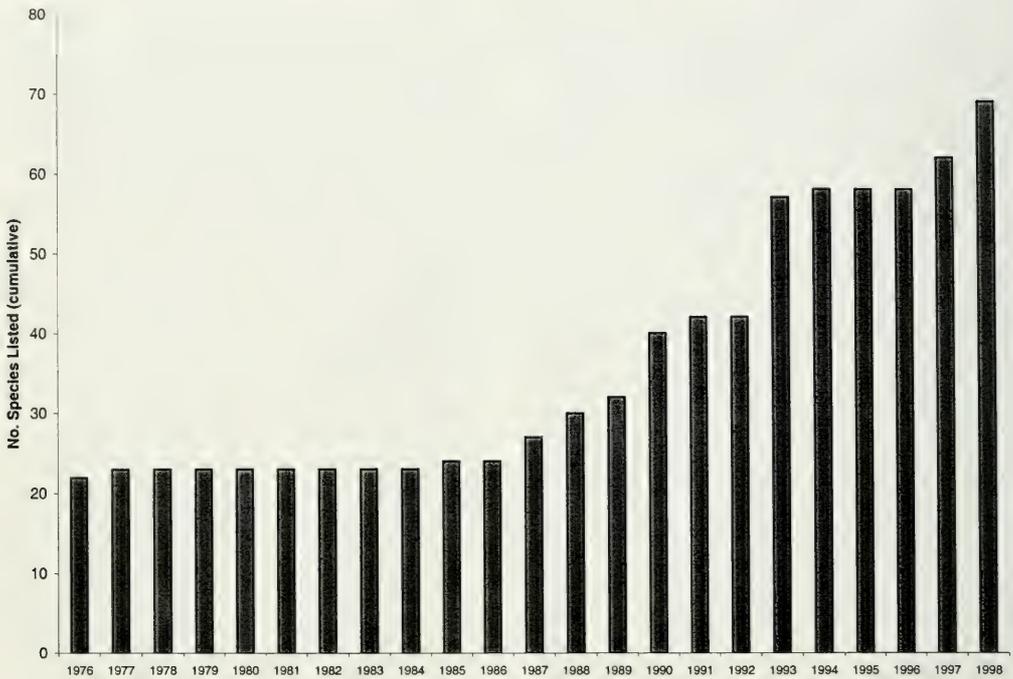


FIG. 1. Chronology of freshwater mussel species listed under the federal Endangered Species Act, 1976–1998.



FIG. 2. Numbers of federally endangered and threatened species of freshwater mussels in the various states.

of conservation efforts. As with most endangered or threatened species, the principle cause for their decline and endangerment is cumulative habitat alteration and destruction. The Tennessee River system provides an excellent case study and one of the best-documented descriptions of molluscan changes from anthropogenic manipulations of a large river. Therefore, the next section will describe the effects of habitat alteration over the last 60 years.

Tennessee River Case Study

The Tennessee River drainage in the southeastern U.S. includes watersheds in seven states: Tennessee, Virginia, North Carolina, Georgia, Alabama, Mississippi, and Kentucky. Within this river system of roughly 105,000 km² is the richest fish and mussel fauna for its size of any temperate river system in the world (Starnes & Ethnier, 1986). The 224 taxa of native fishes and 91 taxa of mussels were widespread in habitats ranging from headwater streams to small natural lakes and the main-stream river. Because the species richness of mussels often is correlated with the diversity and abundance of their host fishes, environmental alterations could directly affect mussel populations and indirectly affect long-term viability through effects on host fishes. Thus, the ecosystem's biotic and ecological integrity was vulnerable to large-scale changes from human intervention.

Documented watershed-scale changes began in the 1930s when the Tennessee Valley Authority (TVA) was established by Congress in 1933 to produce electricity, control flooding, and improve navigation in the Tennessee River Valley. With these goals in mind, the TVA proceed to build 36 multi-purpose dams, with nine of these on the mainstem Tennessee River. The effects of these dams on aquatic fauna, both in the impoundments and in downstream tailwaters were immediate (Yeager, 1993). The chain of reservoirs on the Tennessee River essentially eliminated free-flowing river reaches, causing much more dramatic changes in the mussel fauna than in the fish fauna (Starnes & Ethnier, 1986). Obligatory riverine species began to decline and disappear within the impoundments. Along with the gradual loss of many unionids (Isom, 1969), there was a concurrent increase in resident lentic-tolerant species and a few species that invaded the new reservoirs. These invasive species, either by host fish immigration or through the initiative of commercial har-

vesters, eventually formed large populations that became the focus of a commercial shell industry in Tennessee and Kentucky. It appears that only one fish species became extinct in the Tennessee River (Starnes & Ethnier, 1986), whereas 30 species of mussels became extinct or were extirpated from the river (Neves et al., 1997). Perhaps the refugia for fishes were the lower portions of large, free-flowing tributaries that remained unimpounded and allowed populations of so-called "big river" fishes to survive. The sedentary and more vulnerable mussel species were unable to escape as a population, during the relatively brief period of inundation. The current status of the 91 native unionoid species of the Tennessee River is as follows: 10 extinct, 20 extirpated, 24 endangered, 9 relic, and 28 stable at this time. Thus, a diverse riverine fauna was replaced by a depauperate, lentic-tolerant assemblage of species as a result of increased sedimentation, minimal flow, loss of host fishes, reduced dissolved oxygen, and other hydrologic changes typical of newly constructed reservoirs.

Downstream of these dams, similar biotic changes were underway. The TVA dams were constructed with water intake structures low on the dam to increase hydropower efficiency (Krenkel et al., 1979). From the hypolimnion, the cold water discharges drastically altered the seasonal temperature regime in tailwaters. Because short-term brooders (subfamily Ambleminae) require warm summer temperatures to initiate gametogenesis, spawning, glochidia release, and the presence of warm-water fishes to serve as hosts, unionid populations experienced reproductive failures (Heinricher & Layzer, 1999). Long-term brooders (subfamily Lampsilinae) also were negatively affected by the low water temperature's effects on reproductive biology and host fishes, such that declines in mussel populations occurred over several decades. Senescent mussels still reside below many of these dams as reminders of the biological cost to improve standards of living for residents of the valley. The richest fauna of freshwater mussels became, in roughly five decades, a shell of its former self.

Water Quality Management

Water pollution in rivers became a major problem to mussels in U.S. rivers since the late 1800s. Lewis (1868), Smith (1899), and Ortman (1918) recorded the damage to mussel beds resulting from industrial wastes, sewage,

and myriad chemicals dumped into public waterways. Nearly every major river in the U.S. experienced acute or chronic pollution events during the twentieth century, resulting in precipitous declines in its mussel fauna.

Much of the improvement in water quality over the last 25 years has been derived principally from improvements in municipal wastewater discharges (Smith et al., 1987). The investment of roughly \$300 billion in the construction and operation of wastewater treatment plants has produced noticeable improvements in some rivers and only slight improvements in others (USEPA, 1984, 1994; Smith et al., 1987). The environmental stress and altered characteristics and functions of streams caused by exploitative users and modifications are obviously reflected in the status of freshwater fisheries (Judy et al., 1984). Undoubtedly, what causes stress to the fish community also has an adverse effect on the mussel community through host fish availability and their similar physiological requirements for survival. More than 50% of the nation's rivers have fish communities adversely affected by turbidity, high temperature, toxins, and low dissolved oxygen (USEPA, 1994). Roughly 40% of perennial streams are affected by low flow, siltation, bank erosion, and channelization. Annual sediment loads in major rivers range from 111 million to 1.6 trillion metric tons, with roughly 75% deposited in river beds, reservoirs, or flood plains. Although improvements in water quality have occurred, there is still the need for an integrated policy to protect and enhance water quality to sustain societal benefits (Water Environment Federation, 1992).

Although the mussel fauna in many rivers continues to decline from marginal to unsuitable habitat conditions, the restoration of rivers that serve as historic habitat for federally protected and other mussel species has been underway since about 1970. The U.S. is home to more than 5.1 million kilometers of streams and rivers. Early laws, such as the River and Harbors Act of 1899, were promulgated to prevent further degradation of rivers from refuse disposal, while others, such as the Water Pollution Control Act of 1948, attempted to abate water pollution. However, effective environmental legislation began with the National Environmental Policy Act of 1970. Its goal was to prevent or eliminate damage to the environment and biosphere, to enrich our understanding of ecological systems and natural resources of national impor-

tance, and to bring society into harmony with nature. This was a tall order to fill, in a nation bent on sustaining its gross national product and stature in the global economy. Environmental protection became a national priority, which led to further legislation to improve the quality of our land and water resources. A flurry of environmental legislation followed to address various national concerns, but the Clean Water Act of 1972 became the most significant law to maintain and restore rivers and streams long abused by effluent discharges. The goal of this Act is "to restore and maintain the chemical, physical, and biological integrity of the Nation's water". One of its specific objectives is to allow for the protection and enhancement of fish, shellfish, and wildlife through the elimination of harmful pollutants discharged into national waterways. National standards and regulations to promote clean water were established. As a result, there are numerous success stories over the last 25 years of rejuvenated rivers with recoveries of native fauna, due largely to this single piece of legislation (National Research Council, 1992; Becker & Neitzel, 1992).

Current degradation in water quality of our national waterways stems mostly from deleterious management practices on the landscape. In 1970, the major problem was point-source discharges of effluents from a variety of sources. Today, nonpoint source pollution is the leading cause of impairment (USEPA, 1994). Rivers continue to be plagued by elevated levels of bacteria and silt, derived primarily from agriculture and municipal wastewater treatment plants. Nitrate concentrations continue to be high in surface waters downstream of agricultural areas, whereas elevated levels of ammonia and phosphorus occur primarily downstream of urban areas (Mueller et al., 1995). Because half of all citizens receive their drinking water from surface water supplies (Mueller et al., 1995), public health is also at issue and a motivating force to continue the national focus on improving water quality, particularly in rivers degraded by sediment and nutrients derived from agriculture. If agricultural pollution can be reduced, then nearly every river of present or historic significance for unionids will become suitable for restoration of its native fauna.

A determination of existing water quality problems in each river is critical to prioritizing recovery actions in watersheds with endangered species. Using this knowledge and the known effects of degrading physical and

chemical factors on survival of unionid populations, and the life history and ecology of each species, a team of natural resource specialists can plan an effective recovery of the fauna to accommodate current conditions that limit natural recovery. As judged by current trends in ambient water quality at the state and national levels, and the level of effort and expenditures to achieve adequate water quality in most river systems, I believe that sufficient suitable habitat soon will be available to recover many federally endangered mussel species. The availability and suitability of habitat in rivers for endangered mussel species is sufficient to initiate recovery actions now for most species. Thus, the bottleneck to recovery of endangered and threatened mussels is less an issue of habitat now as it is the biological traits of disjunct residual populations and the cost to achieve recovery. Low density, population isolation, and reproductive failure are the key biological factors in preventing natural recovery of these rare species, whereas required financial commitments impede the implementation of recovery plans.

Zebra Mussels

A brief overview of conservation of mussel resources in the U.S. would not be complete without mention of the exotic zebra mussel, which now threatens many mussel species in large rivers and reservoirs of the eastern U.S. From an establishment in Lake St. Clair in 1986 (Hebert et al., 1989), the zebra mussel quickly swept through the Great Lakes in five years and entered the Mississippi River system in 1991 through the Illinois River (Whitney et al., 1995). The trail of devastation to native unionoids in the Great Lakes is well documented (Nalepa & Schloesser, 1993), and now a similar scenario is being displayed in rivers with numerous commercial vessels, infested with zebra mussels and serving as vectors of dispersal. Although quantification of the mortality being suffered by native mussels in rivers is incomplete (Hunter et al., 1997), severe mortality at sites in the Ohio River is now confirmed, and various levels of mortality in the Mississippi and Illinois rivers have been reported (Tucker, 1994; Ricciardi et al., 1998). As of 1998, the zebra mussel is confirmed in at least 19 states and more than 100 lakes throughout this region of infestation (Amy Benson, USGS, Gainesville, Florida, pers. comm.). The zebra mussel continues to spread to new waterways through principally

anthropogenic means, and the worst fears of malacologists are being fulfilled. The continued existence and recovery of rare unionids in mainstem rivers is undoubtedly in jeopardy, and implementation of a national strategy to address this exotic species and other serious threats is long overdue.

COMMERCIAL HARVEST OF MUSSELS

Shell Buttons

Although the exploitation of freshwater mussels for making pearl buttons dates back to at least 1800 (Coker, 1919), a thriving industry did not develop until John Boepple set up his button business in Iowa in 1891. Species of mussels used to manufacture buttons had to meet the following requirements: white nacre, iridescent, solid crystalline structure, smooth surfaces, uniform thickness, and adequate size and shape to yield several buttons blanks (Coker, 1919). Using these criteria, commercial harvesters exploited such species as washboard (*Megaloniais nervosa*), yellow sandshell (*Lampsilis teres*), mucket (*Actinoniais ligamentina*), black sandshell (*Ligumia recta*), and ebonyshell (*Fusconaia ebena*) because of their localized abundance and excellent shell quality. The ebonyshell was the preferred species because it possessed all of the traits needed to produce top quality buttons.

As the industry expanded in production and geographic range, from the mainstem Mississippi River into major tributaries such as the Ohio River, overexploitation of mussel beds and preferred species mandated that other species be harvested to meet the demand for shells. By the late 1890s, a "shell rush" to supply the button industry swept through the Mississippi River Valley (Table 3). Although harvest records are incomplete, the extent of exploitation was intense (Claassen, 1994). As mussel beds became depleted and resource abundance declined, the market value of shells increased, stimulating further intensive and extensive harvest in old and new river reaches. The insatiable demand for buttons in the burgeoning manufactured clothing industry created an influx of new harvesters and re-exploitation of new and old mussel beds, as less desirable species became acceptable.

The frenzied harvest made use of various techniques to collect the mussels from shallow and deep water areas of lakes and rivers. Hand collecting, clam tongs, pitchforks, rakes,

TABLE 3. Tons of mussel shells harvested for button production in selected years from the Mississippi River Valley, 1898–1944.

Year	Tons of Shell
1898	3,641
1899	23,824
1908	38,133
1912	55,671
1929	27,176
1933	30,146
1937	26,993
1941	17,381
1944	20,300

and dredges were commonly used, but brailing was the predominant technique used in rivers. The standard brail bar was about 4 m long, with crowfoot hooks attached by chains to the bar. The crowfoot hooks were constructed of galvanized wires in various hook-design configurations, with four prongs and a bulbous tip on each prong to prevent mussels from slipping off the hook. The brail bar and attached hooks, suspended on a rope, was bounced along the river bottom as the boat drifted downstream. When the hook moves across a gaped mussel, the mussel closes on the hook and is pulled from the substratum. After several minutes of dragging the hooks, the brail is brought to the surface to remove attached mussels. This basic brail design and method of brailing is still in use in the Mississippi River basin, although surface-supplied air diving is much more common today.

Harvested mussels were brought ashore to shell camps where they were steamed open in large vats. It was at this stage that natural pearls were discovered and sought in the cooking vats and mussel meats. Quality pearls fetched a handsome reward from local jewelers. The shells were sorted, meats were discarded, and specimens were taken to the factories where the button blanks were cut, drilled, polished, and processed for shipment to garment factories in the eastern U.S. In its heyday, the button industry consisted of more than 100 factories and tens of thousands of workers (Claassen, 1994). However by the early 1940s the availability of plastic buttons, zippers, automated button sewing machines, strong detergents and other advancements spelled the demise of this once lucrative but resource-damaging venture. Except for small businesses working specialty buttons from shells into the 1960s, the once thriving button industry perished by the late 1940s.

Cultured Pearls

During the boom and bust of the shell button industry, researchers in Japan were attempting to implant bead nuclei into oysters to produce cultured spherical pearls. As early as the 1920s, tons of pigtoe mussels were being shipped to Japan annually to provide the raw material for bead nuclei (Claassen, 1994). By the 1950s, the U.S. became the exclusive source of raw shell for Japan's booming cultured pearl industry. Thus, the mussel populations in the Mississippi River system had only a brief reprieve from exploitation. During this second "shell rush", the lower Tennessee River became an important source of shell. Reservoirs constructed by TVA in the 1940s became repopulated and colonized by several species that were highly sought because of their thick shell, white unstained nacre, and large size. Such species as the washboard, threeridge (*Amblema plicata*), maple-leaf (*Quadrula quadrula*), and Ohio pigtoe (*Pleurobema cordatum*) were abundant and of excellent quality. Shell harvesting in reservoirs as well as in free-flowing rivers became widespread in the Mississippi, Ohio, Tennessee, Cumberland, and other rivers in the central United States.

The resurgence of musseling initially focused on rivers and mussel beds underexploited or unexploited during the intense harvest earlier this century for the pearl button industry. A flotilla of brail boats explored and exploited mussel beds in the Tennessee, Mississippi, Cumberland, Ohio, Wabash, Missouri, and other rivers with commercial stocks of mussel species. In addition to the traditional method of brailing for mussels, diving came into practice in the 1960s. Underwater harvest by surface-supplied air diving began amid a flurry of controversy, but allowed a new suite of harvesters to ply their trade particularly in reservoirs. The competition and controversies between these two user groups (brailers vs. divers) is still unresolved, but the efficiency and selective harvest by divers cannot be denied.

Harvesters sell their catch typically alive, to buyers representing companies of the Shell Exporters Association (Table 4). This non-profit corporation was chartered in 1994 "to advance the interest in research, environmental protection, and the commercial markets which concern the freshwater mussel". Nearly all of the mussel harvest is passed through one of these companies for process-

TABLE 4. Members of the Shell Exporters Association (SEA) that export mussel shells in United States.

James Peach American Shell Company, Inc. 4805 Old Hickory Blvd. Hermitage, Tennessee 37076 Phone 1-800-251-9372 1-800-248-3064 (205) 359-4345	Nelson Cohen P.O. Box 235 Terre Haute, Indiana 47808-0235 Phone 1-800-222-6436 FAX (812) 234-6198
Butch Ballenger Mississippi Valley Shell Company, Inc. 2579 Stewart Road Muscatine, Iowa 52761 Phone 1-800-882-2526 (319) 264-5883 FAX (319) 264-2053	Tennessee Shell Company, Inc. Highway 70E P.O. Box 647 Camden, Tennessee 38320 Phone 1-800-835-0964 (901) 584-7747 FAX (901) 584-8043
Robert Leasure Leasure Shell Company, Inc. 2612 Jackson, No. 4 Bradford, Arkansas 72020 Phone (501) 523-5028	Peggy Linley Hudson Shell Company, Inc. P.O. Box 965 Decatur, Alabama 35602 Phone (205) 353-6944 FAX (205) 353-2793
Lonnie Garner U.S. Shell Company, Inc. Route 1, Box 323 Hollywood, Alabama 35752 Phone (205) 437-9784	

ing. As in earlier times, the live animals are steamed open in large cookers, passed through rotators to remove the meats, sorted by species and size, and then bagged for shipment via containers to their overseas destination.

The methods of commercial harvest of freshwater mussels have not changed appreciably in the last 30 years. Underwater divers are the principal harvesters, although brailing is still conducted in large rivers and where diving is prohibited (Kentucky). Because there are no safety regulations required of musselers, most divers work alone using principally hookah rigs of imaginative but unsafe design. Exploitation of the mussel resource remains a competitive venture, and to avoid the "tragedy of the commons," management is by restrictive regulations. Most states have no restriction on the number of harvesters for either in-state or out-of-state residents, with higher license fees for non-resident musselers. However, when shell prices are high, the license fees often are recouped during the first day of harvest.

Foreign demand for shell regulates the level of effort and number of people who participate in today's shell industry. An evaluation

of the numbers of licenses sold in key states, such as Tennessee and Kentucky, shows a direct correlation between demand (price) and number of licenses sold. When prices are high, part-time divers venture out in summer to supplement their income. When prices are low, only the full-time, hardy musselers continue to ply their trade, even in winter.

The decade of the 1990s has seen a tremendous upheaval in the shell business. Shell exports have exhibited a steady decline since 1993 (Table 5). In the early 1990s, high demand for shell by Japan and other Asian markets created employment and income opportunities for musselers of all ages. John boats dotted the surface of numerous reservoirs and rivers with sought-after species, such as washboards and threeridges. The industry, with an estimated value of \$50 million, stimulated local economies in rural towns along these waterways. However, the shell business fell on hard times beginning in 1996 when a major die-off of Akoya pearl oysters occurred in Japan. This mortality, combined with declining value of the yen and the booming Chinese pearl culture industry, has drastically reduced the demand for shell. For the last two years (1997-1998), the shell harvest

TABLE 5. Export of freshwater mussel shells and shell products from the United States to Asian countries, from October 1992–December 1997.

Year	Exported Shell ($\times 1000$ kg)*
1992	1,535
1993	6,263
1994	5,352
1995	3,565
1996	2,574
1997	1,329

*Data compiled from LEMIS (U.S. Fish and Wildlife Service)

has been minimal, and most musselers have sought employment elsewhere.

FRESHWATER MUSSEL MANAGEMENT

Management of the freshwater mussel resources in the United States has historically been the responsibility of the states, whereas shell exports are regulated and monitored by the law enforcement branch of the U.S. Fish and Wildlife Service. Shell export records are cataloged in the Law Enforcement Management Information System (LEMIS) of the U.S. Fish and Wildlife Service, and data are retained for the most recent 5–6 year period (Table 5). Nearly all shell exports were destined for Japan, to be processed into beads for the pearl oyster industry.

Like other fish and wildlife resources, the state fish and wildlife agencies should manage freshwater mussels to benefit the residents of their respective states, while assuring conservation of the resource for future generations. Although mussels occur in most rivers and lakes, from western tributaries of the Mississippi River to the East Coast of the United States, not all states allow commercial harvest (Fig. 3). Because of the paucity of viable mussel populations, law enforcement costs, and other factors, some states with commercial species, such as Indiana, West Virginia, Ohio, and Mississippi, do not permit commercial harvest. Complete protection of all species prevents possible violations of the Endangered Species Act through the incidental take of federally listed or state-protected species. Only 14 states allowed commercial harvest in 1997 and only in designated waters (Parrott, 1998). Texas was technically open to shell harvest, but a reduction in minimal daily catch to 25 lb of live mussels or 12 lb of shell essentially precluded commercial musseling.

Shell harvests are tightly regulated by the state agencies through permit and harvest reports required of both musselers and shell buyers according to the regulations of state-issued licenses. Each state specifies the species that can be harvested, minimum size limits, open and closed areas, season and time of day, method of capture, and other limitations to prevent overharvest and ensure continued reproduction of harvested species. Unfortunately, harvest regulations are not uniform and vary considerably from state to state (Table 6). Therefore, musselers who travel among states to work must be cognizant of each state's regulations, as violations of state law by ignorance or intent are treated the same.

The mussel resources in the upper Mississippi River are effectively managed by an Upper Mississippi River Conservation Committee (UMRCC), consisting of representatives from Minnesota, Wisconsin, Iowa, Illinois, and Missouri. These five states are working towards unifying management regulations for harvested species in the upper Mississippi River. Although some differences in growth rates occur among mussel subpopulations within various navigation pools of the river, standardized harvest regulations would be biologically sound, facilitate compilation of harvest data for stock assessment, and simplify law enforcement within and among states. An extension of this UMRCC effort to the lower river as well would promote holistic resource management based on biological factors rather than on political boundaries that often interfere with management of widespread species.

State of Tennessee

Because roughly half of the shells exported from the United States are harvested in Tennessee, I will showcase this state's management of the resource. The extent of commercial harvest, number of endangered species, presence of mussel sanctuaries and other factors in Tennessee cover the range of conflicts faced by most states in the management and conservation of freshwater mussels. In 1995, 3881 tons of mussels were harvested in Tennessee, with a wholesale market value of nearly \$15 million. Over 90% of these mussels were obtained from Kentucky Lake, touted as perhaps the most productive reservoir for mussels in the nation. Commercial species are abundant in the lake, and the quality of shell is excellent and commands a high price.



FIG. 3. States that allowed commercial harvest of freshwater mussels in 1997.

TABLE 6. Summary of commercial harvest regulations for freshwater mussels in the United States.

State	No. Species Harvestable	Size Limits ¹ (inches)	Season	Methods ²
Alabama	13	1 3/4-4	year-round	H,B,R,D
Arkansas	16	2 1/4-4	year-round	H,B,D
Georgia	0	>4	4/1-8/31	H,D
Illinois	11	2 1/2-4	4/11-8/31	H,B,D
Kansas	4	2 3/4-3	4/1-9/30	H,B,D
Kentucky	4	2 3/8-3 13/16	year-round	B
Iowa	7	3-4	4/1-8/31	H,B,D
Minnesota	1	2 3/4	5/16-8/31	H,D
Oklahoma	7	2 3/4-4	year-round	H,B,R,D
Missouri	3	2 3/4-4	4/1-8/31	H,B,D
Tennessee	11	2 3/8-3 3/4	year-round	H,B,D
Wisconsin	5	2 3/4-4	4/1-8/31	H,B,D
Louisiana	4	2 3/4-4	5/20-9/30	H,D
Texas	6	2 1/2-4	year-round	H,B,D

¹Each species has a specific minimum size limit

²H = Hand-collecting; B = Brail; D = Dive; R = Rake

This reservoir has received such intense shell-fishing pressure over the last 20 years that mussels are harvested soon after attaining legal size. This is particularly evident for the highly sought washboard and threeridge. Quantitative sampling of the populations of commercial species in Kentucky Lake during 1995 yielded the following percentages of legal-size mussels per species: ebonyshell

(14%); threeridge, pigtoe, mapleleaf (5%); and washboard (2%). Thus, most large animals have been harvested, and populations consist mostly of age classes below the legal size limits. Most mussels are cropped soon after reaching legal size, and sub-legal animals are undoubtedly handled and sized by divers, resulting in disturbance to these and other species collected incidental to sought-after

specimens. Studies by the Tennessee Wildlife Resources Agency indicate that most commercial species require 12–16 years to attain legal size (Hubbs & Jones, 1996). Because the larger species (washboard, threeridge) are at maximum exploitation levels, the more abundant smaller-size species are being harvested more intensely to meet the demand for shells. In recent years, the demand for large beads to produce large pearls by the South Pacific pearl companies has intensified the harvest of large, thick-shelled species; other populations of washboard and threeridge in Tennessee and elsewhere, therefore, have experienced higher exploitation rates.

To orchestrate Tennessee's mussel management program, a shell tax of 1.45 cents/lb of mussel shell is charged to buyers. Revenues of nearly \$99,000 in 1995 were used to monitor harvest, implement law enforcement, and conduct surveys and research to improve the status of commercial stocks. During spot-checks of harvesters, seven specimens of the federally endangered orangefoot pimpleback (*Plethobasus cooperianus*) were taken from musselers at the shell camp. This species, and the white wartyback (*Plethobasus cicatricosus*), resemble several tubercled species that were allowed to be harvested: purple wartyback (*Cyclonaias tuberculata*), pimpleback (*Quadrula pustulosa*), and wartyback (*Quadrula nodulata*). Thus, the inclusion of look-alikes on the commercial list resulted in the incidental take of an endangered species. To resolve this problem, TWRA removed these tubercled species from the commercial list in 1996. These three species comprised less than 1% of harvested mussel in Tennessee, so removal from the list did not create an economic hardship to the shell industry. Protection of the orangefoot pimpleback, white wartyback, and other tubercled species by this regulation change reduces the incidental take of rare species, especially by harvesters unskilled in shell identification.

Sanctuaries

Although commercial harvest of mussels is allowed in the Mississippi, Tennessee, Cumberland, and Duck rivers in Tennessee, certain areas are designated as sanctuaries (Table 7), and all other water bodies are off limits to harvesting. These refugia have extensive populations of endangered and other species, and provide relatively undisturbed conditions for reproduction, recruitment, and

TABLE 7. Mussel sanctuaries in Tennessee closed to commercial harvest.

River	River Mile Limits
Tennessee	140.0–141.5
Tennessee	201.9–206.7
Tennessee	465.9–471.0
Tennessee	520.0–529.9
Tennessee	416.5–424.7
Powell	55.0–115.6
Clinch	151.9–202.1
Duck	105.6–head
Cumberland	262.9–265.5
Cumberland	284.1–284.8
Cumberland	292.5–313.5
Hiwassee	53.5–65.9

population stability. The sanctuaries in the upper Tennessee River system, such as the Duck, Clinch, and Powell rivers, are critical to indigenous species residing in habitats that have not been altered significantly or destroyed by development projects.

Comprehensive Management

Because of the dual responsibilities of states where commercial harvest is legal, the various tools of resource managers must be effectively implemented to protect endangered species and regulate harvest of commercial species. Survey and monitoring of mussel populations and annual harvest records provide the information needed to assess population trends and to ensure that overharvest does not deplete the resource. Basic biological information for each species, such as reproductive period, host fish, growth rates, recruitment, age at first reproduction, longevity, and other factors, is essential to address questions on minimum size limits to maintain sustainable harvest on a species-by-species basis. Although changes in demand for the various species, driven by shell quality, thickness, bead size, and other factors have varied greatly over the last 20 years, management has continued on a biological basis rather than on an economic one. Neither mussel size limits nor total allowable harvest are modified to accommodate drastic fluctuations in the price of shell from year to year. Supply and demand for shell dictate harvest levels. Under this management strategy, the mussel resources in Tennessee and other states have been managed with sufficient success, such that no commercial species has been

jeopardized by inappropriate harvest regulation. The evidence of overharvest and depletion of mussel populations during the pearl button era has not sullied the present management regime of commercial species, nor has incidental take of rare species been shown to jeopardize the continued existence of those species. In my opinion, periodic episodes of illegal harvest are the only potentially damaging activities that reduce the effectiveness of current mussel resource management. The occasional poaching of shells from sanctuaries or closed areas, harvest of undersize shell or non-legal species, and the incidental take or habitat destruction of endangered species are all breaches to effective management. These management violations are the responsibility of state and federal law enforcement agencies authorized to enforce harvest regulations. Therefore, a balance of efforts to manage mussel populations and the mussel harvesters is essential to protect the resource and ensure long-term sustainability.

There is one additional management issue that I feel needs to be addressed by the appropriate states. A few states still allow the harvest of a variety of non-commercial species for the purpose of sale to biological supply companies. From conversations with colleagues in various states, it is apparent that modern-day market hunters can ravage a stream of its mussels and jeopardize those populations through overharvest. Although these species are unprotected by law, the destruction of viable populations for the financial gain of an unscrupulous few is unacceptable. The commercial shell industry can provide biological supply houses with all the specimens they need without resorting to the decimation of non-commercial species residing in small streams. In those states where such professional collectors can still operate, it is imperative that the regulatory agency introduce legislation to prevent indiscriminate collecting of live mussels. There is certainly adequate documentation in our nation's past, such as the cases of the bison and the Carolina parakeet, to demonstrate that unregulated harvest can result in the extinction or extirpation of species that once were abundant. With so many U.S. mussel species in a precarious condition, whether legally protected or in need of protection, the looting of state-owned resources must be reduced if comprehensive wildlife management for all species is to be the goal of natural resource agencies in the twenty-first century.

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DESCRIPTION OF GLOCHIDIA OF FIVE SPECIES OF FRESHWATER MUSSELS
(HYRIIDAE: UNIONOIDEA) FROM SOUTH AMERICA.

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ABSTRACT

The glochidia of South American Hyriidae were first described and illustrated by Lea (1868); 52 years later Ortmann (1921), followed by Bonetto (1954, 1955, 1960a, b, 1961a–c, 1962a, b) and others, described the larva of various species. However, many larvae are still unknown. The valves of five glochidia of two different types are here illustrated and described: two fish-parasitizing glochidia with the presence of a S-shaped tooth in each valve, and three glochidia that do not parasitize fish, with valves devoid of teeth or spinulae. The valves are measured and compared in: (a) length, (b) height, (c) dorsal hinge length, (d) displacement of the ventral point in relation to the middle of the hinge, and (e) angle of the ventral point in relation to the center of the hinge. Statistics of these measurements are also provided. The internal anatomy of the glochidium of *Diplodon martensi* (Ihering, 1891) and *D. koseritzi* (Clessin, 1888) are briefly described. Some of the larvae illustrated were photographed with a scanning electron microscope and some were drawn using a biological microscope with camera lucida.

Key words: descriptions, Glochidia, Hyriidae, Unionoidea, South America.

INTRODUCTION

The larvae of freshwater bivalves of the superfamily Unionoidea, called glochidia, usually parasitize fish. The glochidia aid dispersal of the mollusk upstream. When the fish passes by, the larva adheres to its scales, fins or gills, becoming a temporary ectoparasite. A cyst is formed by the fish, where the glochidium completes its development to the postlarval phase. This period of parasitism, which lasts 10 to 30 days, is usually conditioned to the times of high water during the spring when the phenomenon called “piracema” occurs in South America.

The glochidia of South American species have morphological affinity with Australian species and differ from the remaining Unionoidea species that live in the holarctic region (Parodiz & Bonetto, 1963). Their characteristics aid the identification and classification of this group of mollusks in which juvenile and adult individuals often lack interspecific diagnostic criteria.

Two types of glochidia are known among South American Hyriidae—those with teeth that develop parasitically on fish, where they form a cyst inside which the glochidium com-

pletes its development until the postlarval phase; and those without teeth, which fully and directly develop inside incubating pouches called marsupia of the female, until the postlarval phase.

Variations occur in the teeth of fish-parasitizing glochidia—in the tribe Castaliini and in the subgenus *Diplodon (Australis)* (Bonetto et al., 1986) of the tribe Diplodontiti, the tooth is triangular, short, wide at the base, and shaped like a beak. They have no spines or terminal cusps. In the remaining Diplodontini and Prisodontini, the tooth is fine, elongate, and S-shaped, with terminal cusps (Parodiz & Bonetto, 1963).

The glochidia of South American species were first observed by Lea (1868), who described and illustrated the larvae of two *Diplodon* species (“*Unio peculiaris*” and “*U. firmus*”). He mentioned the subtriangular contour and the ventral margin terminating in a point containing teeth. The teeth do not appear in the small figures drawn by Lea, and he did not offer a description of them. Ihering (1893: 47) commented on the wide differences in the form of South American glochidia compared to North American and European glochidia, and stated that the larvae observed

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by him did not have teeth or the adhesive filament which he called "bisso".

Ortmann (1921) described Hyriinae (= Hyriidae) larvae in more detail and observed for the first time the absence of teeth in many species. Furthermore, he pointed out that, when present, the teeth appear to be completely different from those of European species of the genus *Unio* and from the Anodontinae of Eurasia and North America; that is, they are long, fine and S-shaped, articulated at the base and without spinules. When describing *Castalia* glochidia, he mentioned that they appear to be different from those of *Diplodon* in having a shorter tooth, which is thicker at the base. He also observed that the toothless glochidia had growth bands on the borders of the valves, which probably represented the beginning of the permanent shell. He assumed that these glochidia developed in direct manner without a host fish. Bonetto (1955) confirmed the assumptions of Ortmann (1921) and stated that mature glochidia probably had an anatomical organization similar to that of glochidia in the post-larval stage after completing their parasitic development on fish and abandoning them.

Bonetto (1960a, b, 1961a–c), Bonetto & Ezcurra (1963, 1965) and Parodiz & Bonetto (1963) described 44 glochidia of different species based, in part, on his personal collection, on Ortmann's slides, and in part on fixed material belonging to various museum collections. Many glochidia have not yet been adequately described, especially with respect to dentition. Some belong to synonyms of ill-defined species, with the resulting presence of different types of larvae in the same "species".

More recently, Alvarenga & Ricci (1979) described the glochidium of *Diplodon besckeanus* (Dunker, 1849) from Rio de Janeiro. Mansur (1983) observed for the first time the glochidium of *D. koseritzi* (Clessin, 1888) and of *D. martensi* (Ihering, 1893) from the sub-basin of the Jacuí in South Brazil. Mansur & Campos-Velho (ms) described the glochidium of *Castalia martensi* (Ihering, 1891) from the same sub-basin. Martinez (1983) described the glochidium of *Castalia ambigua multisulcata* from Venezuela, a species considered by Mansur (1991) to be *C. orinocensis* Morrison, 1943.

The work intends to contribute to the knowledge of the biodiversity, morphology, and systematics of South American freshwater bivalve molluscs. Despite of their importance to the ecosystem and their close relation to fish,

they are poorly known in term of the number and distribution of species, as well as in morphology. While the lack of knowledge continues, these animals suffer from rapid anthropic growth and invasion of exotic species.

MATERIALS & METHODS

Mature glochidia collected from the marsupia of gravid females were anesthetized with menthol crystals for 48 h. The tissues were removed with commercial sodium hypochlorite (8 drops for 10 ml deionized water for 5 min). The valves were cleaned by maceration in deionized water in test tubes for 2 days, with frequent fluid changes by means of a pipette. The material was dried on filter paper, and part of its was mounted on stubs, sputtered with gold, and photographed using standard scanning electron microscopy methods. The rest was mounted on permanent optic slides. The methods for these procedures and for the measurement of the glochidial valves were those of Mansur & Campos-Velho (1990). Live samples of the glochidia of *D. martensi* and *D. koseritzi* were observed directly under the optic and stereoscopic microscope with drops of diluted methylene blue (1/1000).

Diplodon martensi (Ihering, 1893)—Bom Jardim, a small tributary from the lower part of the Cai River, between Triúnfo and Montenegro cities, Rio Grande do Sul State, southern Brazil (latitude 29°50'01"S, longitude 051°20'21"W) (MCNF mol. 6218); *Diplodon koseritzi* (Clessin, 1888)—In the lake "rio Guaíba" at the beaches Florida, Vila Elza, and Alegria, municipality of Guaíba, inside an area corresponding to 30°08'30'S and 30°09'24'S and 051°18'36"W and 051°19'18"W; at the Municipality of Porto Alegre at the bay Belem Novo, and Municipality of Viamão at the beaches of Itapuã and Porto das Pombas (MCT-PUCRS); *D. berthae*—Little lake Sheidt, near the Jacuí River and the town Cachoeira do Sul, RS (MCNF mol. 31976); *D. iheringi*—Small river Arroio do Conde, near Jacuí River and the town São Jerônimo, RS (MCNF mol. 30698); *D. charruanus*—Canal from the lake Jacaré to the Taim Ecological Station, municipality of Rio Grande, RS (MCNF mol. 8633). All studied samples and slides were preserved in the mollusc collection of the MCNF (Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul) and collection of MCT-PUCRS (Museu de Ciências e

Tecnologia da PUCRS, Porto Alegre). Some slides from Ortmann's collection were studied in the INALI (Instituto Nacional de Limnologia, Santo Tome, Santa Fé, Argentina).

RESULTS

Diplodon martensi (Ihering, 1893) (Fig. 1–3; Table 1a)

The glochidia of *D. martensi* are fish parasites. Their valves are articulated along the dorsal line, with an S-shaped tooth inserted internally close to the ventral point of each valve. The contour of the larva is subtriangular and similar to that of the parasitic glochidia described by Ortmann (1921a), Bonetto (1960a, b, 1961a–c, 1962a, b), Bonetto & Ezcurra (1965), and Alvarenga & Ricci (1979). The tooth ends with three grouped cusps, the central one being more elongate and translucent than the two lateral ones (Figs. 1–3). The outer surface of the valves is granulated, looking like an eggshell at 100 and 400 × magnification. It is perforated by minute pores visible at about 400 × magnification. Each pore is approximately 1 μm in diameter. The entire border of each valve is surrounded by a band that stands out from the general surface by being smooth (Figs. 1–3). The 40 measured glochidia of *D. martensi* varied in length from 0.28 to 0.32 mm (mode and mean 0.29); in height from 0.24 to 0.28 mm (mode and mean 0.25); and the dorsal hinge length from 0.20 to 0.23 mm (mode 0.21 to 0.22, mean 0.21). The displacement of the ventral point in relation to the middle of the dorsal hinge ranged from 0.2 to 0.5 mm (mode 0.4, mean 0.03); the angle of the ventral point in relation to the center of the dorsal hinge ranged from 12° to 19° (mode and mean 15°). The tooth length reached 0.10 to 0.11 mm (Table 1a).

The glochidia of *D. martensi* have a central adductor muscle, a posterior ciliated organ, and a long anterior filament (Figs. 1, 2). This reaches approximately four times the length of the glochidial valve and, when not distended, is rolled up two full turns inside the glochidium. Close to the base of the filament is a pair of sensory "cirros" (term used by Bonetto, 1962a), which deeply stain with methylene blue. These "cirros" are shaped like aculei with a rounded base (Fig. 1). On the inner surface of each glochidial tooth is a tuft of sensory cilia (Fig. 2). The remainder of the pallial cavity of the glochidium is lined with

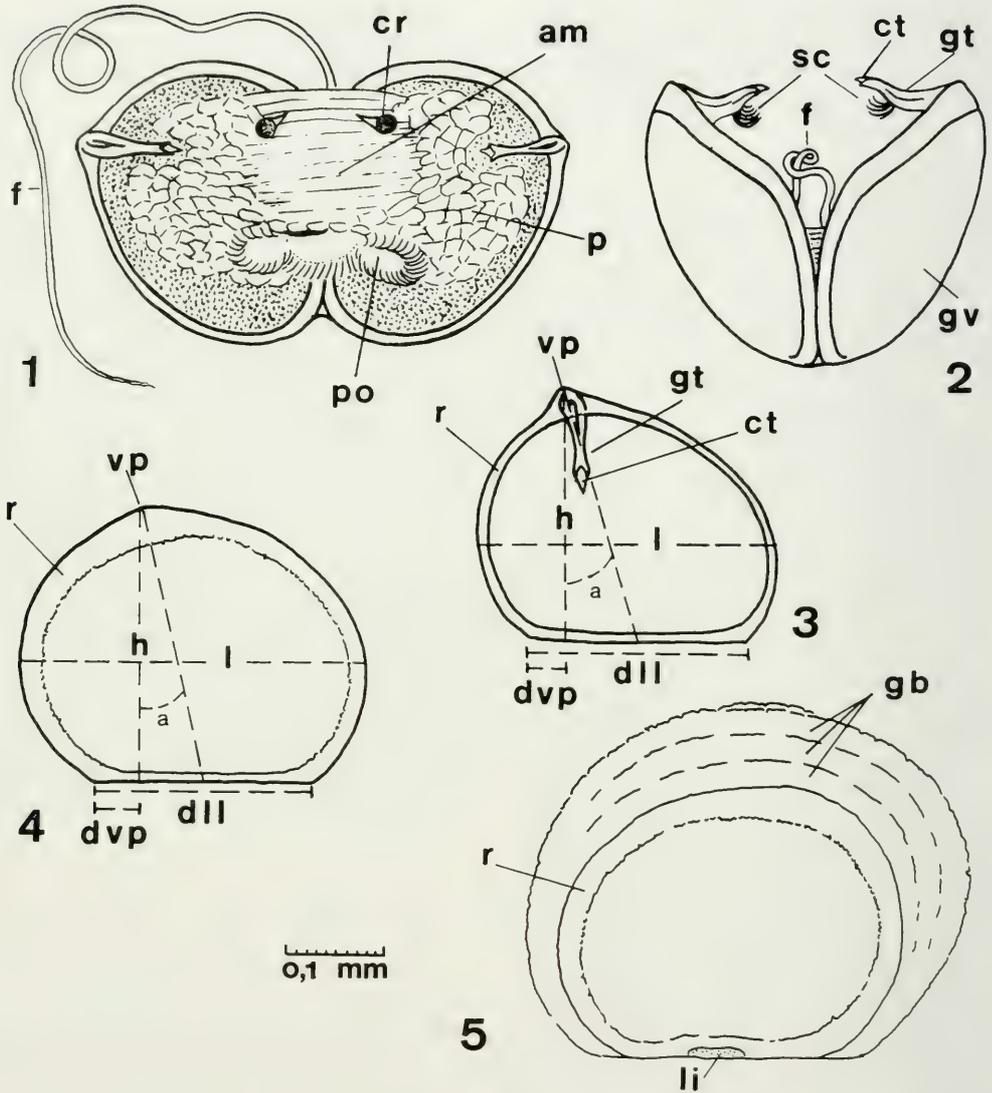
large cells-phagocytes according to Dawydoff (1928), or of the mantle according to Harms (1909)-forming a tissue of spongy appearance.

Diplodon berthae Ortmann, 1921 (Figs. 6–8; Tables 1b)

The species was considered by Parodiz (1968) as a synonym of *D. piceus* (Lea, 1860). The larva was first observed by Ortmann (1921) when describing the species. He described the larva as a fish parasite with teeth, but did not provide illustrations, because it was presumed immature. The larva is a little smaller and similar to that of *D. martensi* in shape, but its teeth are more curved inward and have a more reinforced base. One tooth has a concavity at the base (Fig. 7), and the opposite tooth has a small prominence at the same place (Fig. 8). The dimensions of the larva ranged from 0.24 mm to 0.28 mm in length (mode 0.27, mean 0.26) and 0.21 mm to 0.25 mm in height (mode and mean 0.23), with a dorsal hinge length of 0.17 mm to 0.21 mm (mode 0.19, mean 0.18). The displacement of the ventral point is 0.02 mm to 0.06 mm (mode 0.05, mean 0.04), and the angle of the ventral point in relation to the center of the dorsal hinge is 05° to 16° (mode 13°, mean 12°) (Table 1b).

Diplodon koseritzi (Clessin, 1888) (Figs. 4, 5; Tables 1c)

The glochidium presents a pair of articulated valves on the dorsal hinge and has no teeth. It has a sub-oval contour, similar to that of other non-parasitic glochidia previously described by Bonetto (1960a, b, 1961a–c, 1962a, b) and Bonetto & Ezcurra (1962, 1965). The outer surface is similar to that of *D. martensi* glochidia and is also perforated with pores. The border that surrounds each valve is wider in glochidia newly hatched from the egg and is also less smooth, occasionally showing concavities and pores. An adult *D. koseritzi* specimen collected in December had some glochidia still in the marsupium, with three growth bands in addition to the normal rim or border of the larval shell (Fig. 5). These bands do not occur on the line of valve articulation and reach the greatest height on the ventral margin, where they all reached 0.09 mm, in addition to the normal border. The existence of growth bands in addition to the nor-



FIGS. 1-3. Glochidium of *Diplodon martensi* (Ihering, 1893), of parasitic type, with "S" shaped teeth. 1. Ventral view of internal organization. 2. Frontal view, profile of teeth. 3. Glochidial valves in lateral view.

FIGS. 4, 5. Glochidium of *Diplodon koseritzi* (Clessin, 1888), non-parasitic glochidium type: 4. In lateral view, 5. With growth bands, in post-larval stage found within the marsupium.

Abbreviations: a — angle; am — adductor muscle; r — rim; l — length; li — ligament; cr — cirrus; ct — cusps of glochidial tooth; dll — dorsal hinge length; dvp — displacement of the ventral point in relation to the middle of the dorsal line length; f — filament or flagellum; gb — growth bands; gt — glochidial tooth; gv — glochidial valve; h — height; p — phagocytic cells; po — posterior ciliary organ; sc — cilia; vp — ventral point.

mal border of the glochidium, and the presence of a ligament, reveals that after they hatch from the egg, the glochidia start their post-larval development while incubated inside the marsupium (Figs. 4, 5). The glochidia measurements were as follows (without

counting the growth bands): length from 0.32 mm to 0.35 mm (mode and mean 0.34); height, 0.27 to 0.29 mm (mode 0.28, mean 0.27); dorsal line length, 0.20 to 0.22 mm (mode 0.20 to 0.21, mean 0.20); displacement of the ventral point in relation to the mid-

TABLE 1. Statistics of glochidia measurements

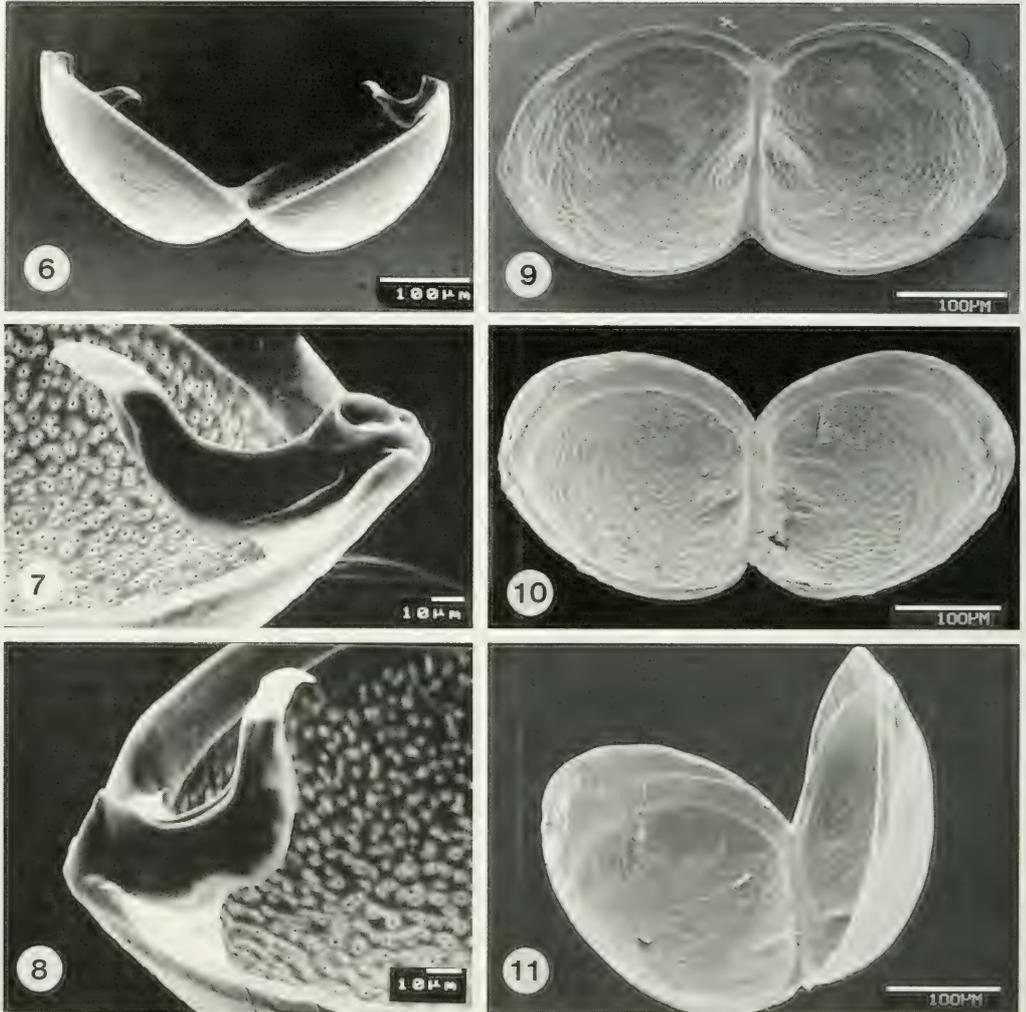
a. <i>Diplodon martensi</i> (Ihering, 1893)					
n = 40	Range	Mode	Mean	s	cv%
Length	0.28–0.32	0.29	0.297	0.0114	3.8396
Height	0.25–0.28	0.25	0.258	0.0095	3.2166
DLL	0.20–0.23	0.21–0.22	0.214	0.0093	4.3435
DVP	0.02–0.04	0.04	0.035	0.0071	20.3026
A°	12°–19°	15°	15	1.39	8.99
b. <i>Diplodon berthae</i> Ortmann, 1921					
n = 26	Range	Mode	Mean	s	cv%
Length	0.24–0.28	0.27	0.26	0.0092	3.47
Height	0.21–0.25	0.23	0.23	0.0089	3.89
DLL	0.17–0.21	0.19	0.18	0.0112	5.97
DVP	0.02–0.06	0.05	0.04	0.0114	24.83
A°	05°–16°	13°	12	2.4446	20.11
c. <i>Diplodon koseritzi</i> (Clessin, 1888)					
n = 40	Range	Mode	Mean	s	cv%
Length	0.32–0.35	0.34	0.34	0.0081	2.4041
Height	0.27–0.29	0.28	0.276	0.0065	2.3697
DLL	0.20–0.22	0.20–0.21	0.205	0.0059	2.8885
DVP	0.01–0.05	0.04	0.033	0.0111	33.6337
A°	10°–18°	12°	13	2.01	14.42
d. <i>Diplodon iheringi</i> Simpson, 1900					
n = 35	Range	Mode	Mean	s	cv%
Length	0.26–0.30	0.28	0.28	0.0096	3.41
Height	0.23–0.25	0.24	0.24	0.0058	2.41
DLL	0.17–0.21	0.20	0.19	0.0120	6.37
DVP	0.02–0.06	0.05	0.04	0.0098	21.87
A°	5°–15°	10°	10	1.99	18.93
e. <i>Diplodon charruanus</i> (Orbigny, 1835)					
n = 26	Range	Mode	Mean	s	cv%
Length	0.27–0.29	0.28	0.28	0.0061	2.20
Height	0.22–0.25	0.25	0.24	0.0091	3.77
DLL	0.18–0.20	0.19	0.19	0.0074	3.93
DVP	0.04–0.06	0.05	0.05	0.0082	16.34
A°	9°–15°	12°	12	1.74	14.11

DLL, Dorsal hinge length; DVP, Displacement of the ventral point in relation to the middle of the hinge length; A°, Angle of the ventral point in relation to the center of the hinge.

dle of the dorsal line ranged from 0.01 mm to 0.05 mm (mode 0.04, mean 0.03); angle of the ventral point in relation to the center of the dorsal line ranged from 10° to 18° (mode 12°, mean 13°) (Table 1c). The ventral point was distinguished with difficulty by being highly rounded even in newly hatched glochidia, this being the probable reason for the wider variation in the angle.

The recent released glochidium of *D. koseritzi* has the same morphological characteristics of the young bivalve picture for *D. variabilis* by

Parodiz & Bonetto (1963). It can move using its foot. No hooks or spines were observed on the valves. The foot is well developed and is able to stretch the length of the shell. Behind the foot, two short branchial filaments are visible on each side. The mantle borders are evident and thick. Many broods were observed within marsupia under a stereomicroscope. The egg has a transparent membrane, permitting the observation of the embryo, which is a white mass in its first phase. Later, small, white valves appear. When the embryos are more



FIGS. 6–8. Glochidium of *Diplodon berthae* Ortmann, 1921, of parasitic type, with “S” shaped teeth. 6. Frontal view of gaping glochidial valves. 7, 8. Details of glochidial teeth. FIGS. 9–11. Glochidium of non-parasitic type, with gaping valves. 9. Glochidium of *D. iheringi* Simpson, 1914; 10, 11. Glochidia of *Diplodon charruanus* (Orbigny, 1835), found in the marsupium, in post-larval stage. 10. With poorly developed growth bands. 11. With well-developed growth bands.

developed and ripe to be released, the valves become pale yellow and the border is visible without hooks. The valves close and open when stimulated mechanically and the foot moves between them, even inside the egg.

Diplodon iheringi Simpson, 1900
(Fig. 9; Table 1d)

Specimens of this species were erroneously considered to be *D. charruanus* or *D.*

koseritzi by Bonetto & Dreher-Mansur (1970). The glochidia are similar to those described above but slightly smaller in height and angle, with the following measurement ranges: 0.26 to 0.30 mm in length (mode and mean 0.28 mm); 0.23 to 0.25 mm in height (mode and mean 0.24 mm); 0.17 to 0.21 mm in dorsal hinge length (mode 0.20, mean 0.19 mm); 0.02 to 0.06 in the displacement of the ventral point (mode 0.05, mean 0.04); and the angle from 5° to 15° (mode and mean 10°). The ven-

tral point is not very pointed but more distinguishable than that of *D. koseritzi*. The rim of the glochidial valves very conspicuous and thick. Well-developed growth bands were observed on some larvae inside the marsupium.

Diplodon charruanus (Orbigny, 1835)
(Fig. 10–11; Table 1e)

The larvae of *D. charruanus* from the Taim canal are relatively small, measuring (without growth bands) from 0.27 mm to 0.29 mm in length (mode and mean, 0.28 mm); height was 0.22 mm to 0.25 mm (mode 0.25 mm, mean 0.24 mm); their dorsal hinge length ranged from 0.18 to 0.20 mm (mode and mean 0.19 mm); the displacement of the ventral point in relation to the middle of the dorsal line ranged from 0.04 mm to 0.06 mm (mode and mean 0.05 mm); and the angle of the ventral point in relation to the center of the dorsal line ranged from 9° to 15° (mode and mean 12°). The ventral point is also rounded, even in newly hatched glochidia, but more distinguished than those in *D. koseritzi*. Some of the recent delivered glochidia of the same female had developed growth bands and a small ligament (Figs. 10, 11).

DISCUSSION AND CONCLUSIONS

On the basis of the dimensions reported by Ortmann (1921a), Bonetto (1960a, b, 1961a–c, 1962a, b), Bonetto & Ezcurra (1965), and Alvarenga & Ricci (1979) for parasitic glochidia and those described herein, we conclude that *D. martensi* glochidia are close to the largest glochidia known, that is, those of *D. paulista* Ihering, 1893, the measurements of which are: 0.32 mm in length, 0.26 to 0.27 mm in height, 0.22 mm in dorsal line length, 0.03 mm in ventral point displacement, 18° to 19° obliquity angle, and 0.10 mm in tooth length. The glochidia of *D. martensi* have a considerable height in relation to length, but are less high than those of *D. piceus* (Lea, 1860), with a length and height of about 0.28 and 0.29 mm (Ortmann, 1921). They are proportionally larger than the glochidia of *D. besckeanus* (Dunker, 1849) and, even though they achieve the length of *D. multistriatus* (Lea, 1831) and *D. decipens* Ortmann, 1921, the latter are a little less high, with a respective height of 0.22 and 0.24 mm. The glochidia of *D. imitator* Ortmann, 1921, with a length and height of approximately 0.27

and 0.28 mm and a tooth length of 0.09 mm, are similar to *D. martensi* in height but are less elongate than the latter.

Parodiz (1968) considered *D. berthae* to be synonymous with *D. piceus* (Lea, 1860) and denotes this species as “the black form” of the Uruguay River. Specimens from the Sheidt Lagoon identified as *D. berthae* fully fit the description of this form. We prefer to retain the species *D. berthae* as valid for the Atlantic basin of southern Brazil, because the species *D. piceus* from the Uruguay River was confused with other species by Bonetto (1964, 1965) and Haas (1930, 1969).

Comparing the dimensions given by Ortmann (1921), Bonetto (1960a, b, 1961a–c, 1962a, b), and Bonetto & Ezcurra (1962, 1965) for non-parasitic glochidia, we state that *D. koseritzi* is among the largest, comparable to those of *D. hasemani* and *D. hildae*. In turn, the larvae reported as being *D. iheringi* Simpson *sensu* Bonetto (1961b) also have dimensions close to those of the glochidia measured here (i.e., 0.31 mm in length, 0.26 mm in height, 0.20 mm in dorsal line length, 0.02 to 0.03 mm in dorsal hinge displacement and a 15° to 17° angle). During a visit to the National Institute of Limnology, Santa Fé, Argentina, it was possible to examine material identified by Bonetto as *D. iheringi* for Gualaíba, which were identical to the specimens of *D. koseritzi*. The dimensions of the glochidia were less than those of the specimens we measured, because they were immature or incomplete, with the border of the embryonic valve barely outlined. In turn, the *D. iheringi* of Bonetto (1961b) is synonymous with *D. charruanus*, according to Bonetto & Dreher-Mansur (1970).

The glochidia of *D. charruanus*, according to Ortmann (1921), measuring 0.31 mm in length and 0.26 mm in height, do not fully fit the dimensions of the specimens described here, and he did not mention the presence of growth bands. According to Bonetto (1962b), the glochidia of this species have growth bands and measure 0.30 mm in length, 0.26 mm in height, 0.18 mm in dorsal hinge length, a displacement of the ventral point of 0.03 to 0.05 mm, and an obliquity angle ranging of 9° to 14°. The glochidia samples of the species of the Taim canal are a little smaller than those mentioned by Bonetto (1962b), but in general the measurements fit with *D. charruanus* especially considering the angle.

Ortmann (1921) also described the glochidia of *D. hildae*, which measured 0.29 to

0.30 mm in length, 0.26 mm in height not counting the growth bands, and 0.34 to 0.35 mm in length by 0.28 to 0.29 mm in height counting the growth bands. On the basis of the above data, it is probable that the specimens of *D. charruanus* are closer to those of *D. hildae* than to those of *D. koseritzi*.

Internally, the hooked glochidia of Hyriidae differ little from those of the remaining Unionoidea described by Dawidoff (1928) and Harms (1909). The nonhooked glochidia about to be released from the gravid female look like young bivalves or the postlarva of the hooked glochidia after completing the parasitic phase in the fish (Parodiz & Bonetto, 1963). The young bivalve can move with its foot on substratum. Even embryos ready or about to be released but still enclosed by the egg membrane, have a foot between the hook-less valves. No information is available on the complete organogenesis of glochidia that do not parasitize fish and which develops directly in the marsupium of the gravid female just before the postlarval phase. The presence of growth bands in some recently released non-fishparasitic glochidia show that they can grow as post-larvae during a short time in the marsupia.

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