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STRUCTURE AND HABITAT CORRELATIONS OF SYMPATRIC NEW ZEALAND LAND SNAIL SPECIES

Alan Solem[1] & Frank M. Climo[2]

ABSTRACT

The land snail fauna of the Manukau Peninsula near Auckland, North Island, New Zealand, is known to consist of 89 species, only four of which are European imports. At least 71 of the native species *could* co-exist essentially microsympatrically in a single remnant bush patch. Sixty species have been found in one bush patch.

On a typical adult of each species, measurements were made of shell height, diameter, whorl count, umbilical width or condition, major radial ribs on the body whorl, spire height, and body whorl descension. Ratios were calculated for height/diameter, diameter/umbilical width, and radial ribs/mm on the body whorl. Status of whorl contour, periostracal sculpture extensions, and shell color were noted. The volume of space occupied by the shell was calculated.

Taxonomic distributions of each measurement or character state were plotted, and an attempt was made to determine correlation between each major variation and shelter site preference of species.

Punctids were found to be smaller than charopids, and more elevated, with fewer whorls, generally narrower umbilicus, more often without prominent radial sculpture, more frequently with angulated or carinated periphery, and more frequently monochrome in coloration.

Shelter preference site correlations were few. Development of a peripheral keel is associated with sheltering in open ground space under deep, wet litter; periostracal fringes are most frequent in inhabitants of friable, broken-down litter; variegated color is represented better in arboreal taxa; and light or dark brown monochrome coloration in friable, broken-down litter. None of the above correlations are based on monophyletic lineages.

Number and spacing of major radial ribs were not size-associated and did not correlate with habitat preference site. Compared with Northern Hemisphere faunas, the Manukau Peninsula species are small and when shell height is plotted against shell diameter, there is one scatter, rather than the dual scatter reported for most other land snail faunas.

The distribution of shell volume among the species is not unimodal. Species with the same shelter site preference differ by at least 40% in volume. It is suggested that there is strong selective pressure for this pattern of shell volume difference, but in the absence of comprehensive ecological data on all of the species, determination of the reasons for this are at present impossible.

Key words: land snails; sympatric; structure and habitat; shell sculpture; shell shape.

INTRODUCTION

Land snail diversity on the Manukau Peninsula, southwest of Auckland, New Zealand, was surveyed by Solem, Climo & Roscoe (1981). They concluded that more than 70 of the 85 resident species of native land snails and slugs could be expected to coexist in 2 hectare patches of generalized and undisturbed lowland bush such as Jones Bush near Waiuku, from which 60 species have been collected. A diagram of typical bush facies has been published in Solem, Climo & Roscoe (1981: fig. 2) and descriptions of the special habitats are given in that report. From 45% to 75% of the total species present in a particular bush patch are found in each small (20 × 30 cm) bag of selected litter collected from an area of 0.2–0.3 m^2. All such samples were taken under a single tree or clump of saplings belonging to one plant species. Many species were collected in all basic litter types, from the drier upper fringes to the very wet streamside piles of fern fronds. Very few taxa seem to be restricted to a specialized space niche, although most species have characteristic preferences as to moisture and space conditions (*i.e.,* where they are most

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easily collected alive) (Solem, Climo & Roscoe, 1981: appendix 1). The 70 species are essentially microsympatric.

This level of sympatric land snail diversity so greatly exceeds that found in other areas of the world, which normally is five to twelve species (Solem, in preparation), that a preliminary review of correlations among habitat preferences, taxonomic groups, and aspects of shell size, shape, proportions, volume, sculpture, and color seemed desirable. Limitations of knowledge concerning the biology of individual species are a severe handicap. We do not know the life history of any of the species discussed. We have no data on seasonal abundance fluctuations, and no clear indication as to differentiation into shelter, foraging, or mating niches for any of the species. Our own sampling program included a mixture of shelter and foraging niches, since rains ended a short drought of about two weeks at the start of our field work and continued at intervals through the program.

Despite these limitations, we have developed several testable hypotheses to explain the nature of this high diversity (Solem, Climo & Roscoe, 1981). First, we have demonstrated that this fauna is 80.5% composed of species whose ranges extend considerably north and south of the study area, 19.5% of species whose ranges are at or near a north, south or west limit. Second, the fauna is not composed of a few species blooms. Only four of the 85 resident Manukau Peninsula land snail species are interpretable as being products of a cladistic bifurcation. The other 81 species have separate "nearest relatives" and often not even demonstrable "grandparental" ties. Thus, this faunal diversity is not packed through recent union of two or more allopatric faunas, nor is it the result of species swarms or blooms resulting from a few colonizations of virgin territory. Third, we suggest that this is a mature community of land snails, accumulated gradually over time, resulting primarily from highly favorable and stable conditions of moisture and space retention in the litter. This snail community ranges from somewhere north of Auckland south through the Central North Island cave region. Fourth, the number of sympatric land snail species declines sharply in other parts of New Zealand. We have presented hypotheses based on patterns of moisture interruption, changes in litter space quality, recentness of area colonization, degree of exposure to desiccating winds, changes in rock substrate, topographic variation, and alterations in litter acidity to account for these reductions in diversity.

In this paper we attempt to relate physical characters of the snail shells with observed habitat preferences. We regard shell size as a rough predictor of snail body size, since the snail can withdraw its body completely into the shell for all but a few of the New Zealand native taxa. We have chosen to exclude from this analysis the very large slug-like carnivore *Schizoglossa worthyae* Powell, 1949, which has an ear-shaped shell remnant into which the animal cannot possibly withdraw, and the medium to large, native, shell-less slugs of the family Athoracophoridae. We also are omitting three species that we did not collect personally on the Peninsula (*Paryphanta busbyi*) [Gray, 1840], a human introduction; *Egestula egesta* [Gray, 1850], a northern species recorded once from the Manukau Peninsula tip; and "*Phrixgnathus*" n. sp. 55, recorded in beach wash), and two taxa whose absence from our collections surprised us ("*Phrixgnathus*" n. sp. 61 and *Otoconcha dimidiata* [Pfeiffer, 1853]). We thus are basing this analysis on 82 species of shelled land snails, four introduced from Europe (Table 1), instead of the 89 species reviewed by Solem, Climo & Roscoe (1981: appendix 1).

The number of described New Zealand land snail species, 315, is much less than the 670 now represented in the collections of the National Museum of New Zealand (Climo, unpublished). Thus, many species are undescribed and must be indicated by either a number or "n. sp. aff." Each such undescribed taxon is referenced to a National Museum of New Zealand catalogued lot (Solem, Climo & Roscoe, 1981: appendix 1). Similarly, generic units in the Charopidae and Punctidae are in a state of flux. Current units are "form genera" without phyletic coherence. Citation of a species as "*Charopa*" is to give both an idea of general shell morphotype, and also to indicate that the species probably is not congeneric with the generotype.

A monograph of the New Zealand Punctidae is partly completed (Climo, in preparation) and data as to subfamily and generic units have been tabulated for use in testing structural associations. This is in too preliminary a form for publication and is subject to revision in final manuscript. Nevertheless, it does enable us to state that certain features of the punctid variation patterns are not monophyletic. Knowledge of the New Zea-

land Charopidae is much less advanced, and we are not prepared to make phylogenetic predictions in relation to this complex.

To our knowledge, this is the first such attempt to analyze sympatric land snail species associations in this manner. We recognize the defects inherent because life history data and precise ecological knowledge are lacking. We hope that this preliminary report will stimulate others to test our predictions through instigation of such studies and to apply the techniques we use here toward study of land snail faunas in other areas of the world.

MATERIAL STUDIED

All measured and observed specimens for this study are deposited in the National Museum of New Zealand, Wellington. Most of the specimens used are those reviewed by Solem, Climo & Roscoe (1981). There are a few exceptions. Certain species collected on the Manukau in low numbers were represented only by dead, worn adults or subadult examples. Other species were represented only by specimens with the shell surface so covered by debris that observation and measurement of critical features was not possible. Jones Bush, near Waiuku, had the most diverse fauna of all the patches that we sampled, and is the primary source of material used in this study. Where adequate specimens from Jones Bush itself were available, they were used. The many studies of Cumber (1960, 1961, 1962, 1964, 1967a–d) amply demonstrated that there is considerable local geographic variation in both size and sculpture counts within New Zealand land snail species. Therefore, when we lacked adequate Jones Bush or Manukau Peninsula material, we had to select material for measuring that fairly represented the Manukau morphotype.

Recent studies on the Endodontidae (Solem, 1976), Pacific Basin Charopidae (Solem, 1983), and Western and central Australian Camaenidae (Solem, 1979, 1981a, b) have demonstrated that under desert, savannah, and tropical rain forest conditions, size distributions within large samples of shells are unimodal. The mean will shift from population to population as local variation determines, and allochronic variation is a real phenomenon, but the principle of unimodal distribution within a population is well established. Most species of New Zealand bush snails are collected in low numbers, so that sample size generally was small. For a few species (Table 11) we have measured series and provide variation data. For most species we have simply selected a typical adult example to represent the Manukau morphotype of that species.

The authors consulted together on the selection of "an average adult" to represent the species. Most New Zealand land snail species show nondeterminate growth. There is, however, an easily recognizable short zone of gerontic shell growth and increased body whorl descension that represents "adult shell increment." For the Charopidae, Punctidae, Achatinellidae, and Rhytididae, this is the common growth pattern. In the Liareidae, Hydrocenidae, Valloniidae, Cionellidae and Helicidae, termination of shell growth is marked by the formation of a reflected lip. For the introduced *Oxychilus,* a definite narrowing of the shell aperture is an equivalent indicator of shell maturity. Thus, selection of an adult shell that is average in size and form is easily accomplished.

All representative adult specimens were selected and measured before any analysis was attempted. We are confident that our representative specimens lie within 1.5 standard deviations on each side of the population mean. Since we are concerned with size differences greater than 40% for shell volume and generally even larger differences for particular measurements, we consider that our data base is adequate for the intended study. We lacked sufficient series of adults to make comprehensive measurements for each species. Appendix 1 reviews data on variation in some species in comparison with the average adult selected.

BASIC MEASUREMENTS

For the pulmonates, which are hermaphroditic, basic measurements were made on an adult shell; for the dioecious prosobranchs, measurements were taken on both a male, which is consistently smaller, and a female shell. Shells over 5 mm in maximum dimension were measured with a vernier caliper, shells under 5 mm with an ocular micrometer. Accuracy of measurement is as summarized by Solem (1976: 15). Fig. 1 indicates how the basic measurements were taken:

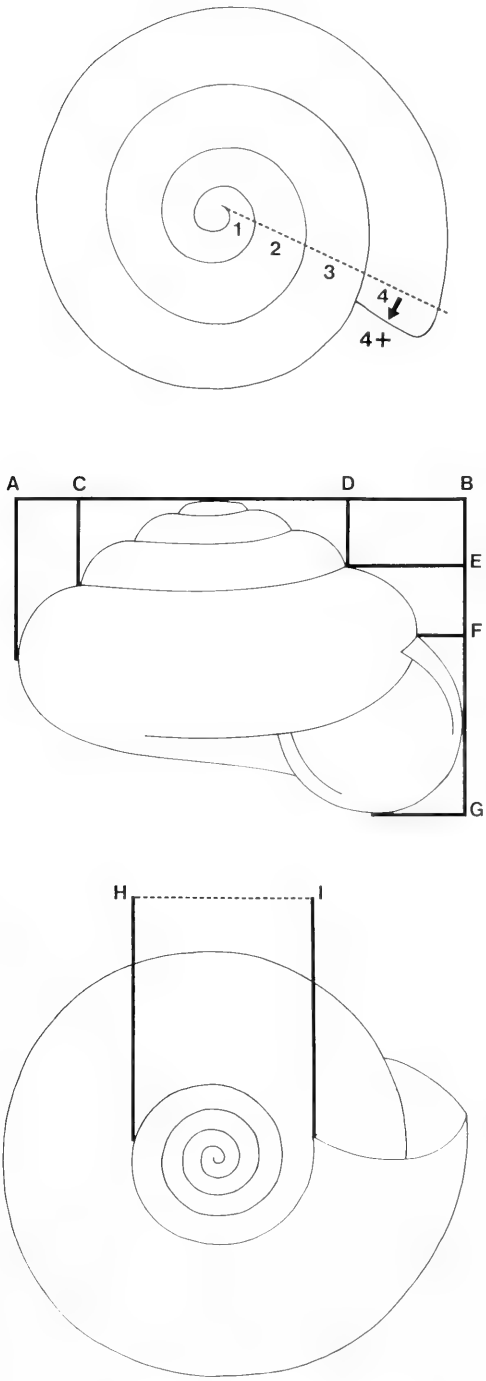

FIG. 1. Basic measurements of shells.

Whorls were recorded to the nearest ⅛th;
Shell diameter is A to B distance;
Spire diameter is C to D distance;
Shell height is B to G distance;
Spire elevation is B to E distance;
Body whorl height is E to G distance;
Body whorl descension is E to F distance;
Umbilical width is H to I distance.

Two standard ratios were calculated:

H/D ratio, a measure of proportionate shape obtained by dividing Shell height by Shell diameter; and

D/U ratio, an indication of proportionate Umbilical width obtained by dividing Shell diameter by Umbilical width.

Where the radial sculpture was clear enough and large enough to be counted at 60× magnification, the number of major ribs on the body whorl was counted, and then an index of rib spacing, *Ribs/mm on Body whorl,* calculated by the formula:

$$\frac{\text{radial ribs on body whorl}}{\pi \times \text{shell diameter}} = \text{Ribs/mm}$$

to provide data on rib frequency and spacing. The minor error inherent in this index has been discussed by Solem (1976: 42–43).

The above measurements are standard in systematic malacology and are useful in systematic discrimination of taxa. They are of less utility in trying to indicate the relative size of species.

Nobody has devised an acceptable volume measure of a crawling snail. The foot varies in length from roughly the diameter of the shell aperture in such taxa as *Cytora, Laoma,* and "*Phrixgnathus*" to the very long, slender foot with prominent mucous pore and "horn" seen in such genera as *Allodiscus* or *Otoconcha.* The body size of snails varies dramatically with water content. Temporary water volume loss of 30% with concomitant body shrinkage is not unusual. This fact produces an unacceptable margin of error in measuring animals. Material in preservative varies so much in degree of contraction that no comparable measurements can be made. Methyl alcohol produces three to four times the shrinkage occurring when ethanol is used. We have not attempted to make live or preserved specimen body measurements.

We suggest that a more accurate predictor of normal body size is to prepare estimates of shell volume. The reasons for this are simple. When the snail is extended and crawling, the

pallial cavity inside the shell body whorl contains sufficient space for the head and foot to be withdrawn completely. If the animal is partly desiccated, it, plus a reserve store of water, can retract for a significant part of a whorl. If the snail is fully hydrated, it may have to eject water in order to complete withdrawal. Thus, the volume of space inside the shell may be taken as an average approximation of actual body volume. It is not a perfect estimate. Species with greatly increased whorl count, such as *Laoma leimonias* (Fig. 3d) with 7½ whorls, may have the soft parts absent from the first two or three shell whorls, and in many species there is a fraction to a whole whorl of empty space above the apex of the soft parts. As the snail has added later whorls, the apical soft parts have been withdrawn from the original apical whorl(s). There is thus a small amount of empty space in the upper spire of the shell. Short of working out individual growth curves from analysis of cross-sectional views prepared of each species and correcting for this empty space, any internal volume estimate will be subject to major error. If we were dealing with actual biomass, then calculation of such internal shell space volumes would have been appropriate. Our concern is with occupied habitat space—how much space in the litter will be needed by the typical adult snail shell. We thus have prepared estimates of total shell volume for this study, rather than biomass or internal shell volume.

While these total shell volume calculations probably are roughly proportional to snail biomass, differences in shell thickness, external contours and growth pattern would cause variation. Total shell volume is intended to be an indication of the physical space needed by the snail in its habitat and should not be interpreted otherwise.

The method of preparing shell volume estimates is given in Appendix 1, together with evidence as to individual species size, shape and volume variability.

TAXON-LINKED VARIATION IN BASIC PARAMETERS

Most readers will be unfamiliar with the appearance of these species. Figs. 2–4 contain side view line drawings of some charopids and all punctids mentioned in this report. Table 1 gives a reference to illustrations of all native New Zealand species, referring to Figs. 2–4, the standard monographs of Suter (1915) and Powell (1976, 1979), or a few technical reports when no other illustrations have been published. Inclusion also of top and basal views would have been useful, but this was not practical as no stock of such figures was available to us. Few of the figures are of Manukau Peninsula specimens. Some may reflect local geographic or clinal differences from the discussed Manukau morphotype. Nevertheless, the figures do give the general aspect of each species, although they are not intended to represent the precise details of spire elevation, rib spacing, body whorl contour, or color patterns.

Tables 2–6 outline taxonomically linked patterns of shell variation in the Manukau Peninsula fauna. Two families predominate, the Charopidae with 40 species and the Punctidae with 27 species. The 15 "other" taxa include four European imports (species 79–82 in Table 1), one Hydrocenidae (*Omphalorissa purchasi*), six Liareidae (species 2–7 in Table 1), one Achatinellidae (*Lamellidea novoseelandica*), and three Rhytididae (two *Delos* and one *Rhytida*). Of these, the introduced *Cionella, Omphalorissa, Lamellidea,* both *Liarea, Cytora pallida* and *C. torquilla* are elongated to turritelliform in shape with H/D ratios of 1.358–2.075; the introduced *Helix aspersa,* and native taxa *Cytora cytora* and *C. hedleyi* have nearly equal heights and diameters; *Rhytida greenwoodi* has the height two-thirds of the diameter; the introduced *Vallonia* and *Oxychilus,* plus both *Delos* have more nearly planiform shells, with the height about half the shell diameter. None of these species show especially unusual shell dimensions, allowing for increased whorl count in lanceolate-shaped taxa (*Liarea* and elongated *Cytora*).

Most of these species are strictly terrestrial, except for *Lamellidea* which is arboreal, and *Omphalorissa* which is both arboreal and terrestrial. There is no correlation between habitat preference and shell shape in this grouping. Total size, as indicated by the Adjusted Shell Volume (ASV), has the arboreal *Lamellidea* (1.30 mm^3), ambivalent *Omphalorissa* (0.95 mm^3), and terrestrial *Cytora torquilla* (1.01 mm^3) at the lower size range. The carnivorous *Rhytida greenwoodi* (3,523 mm^3) and herbivorous *Helix aspersa* (6,148 mm^3) have ten to eighteen times the ASV of the next largest species (the charopid *Allodiscus dimorphus*, 340 mm^3). *Vallonia* and two *Cytora* (*cytora* and *hedleyi*) are in the

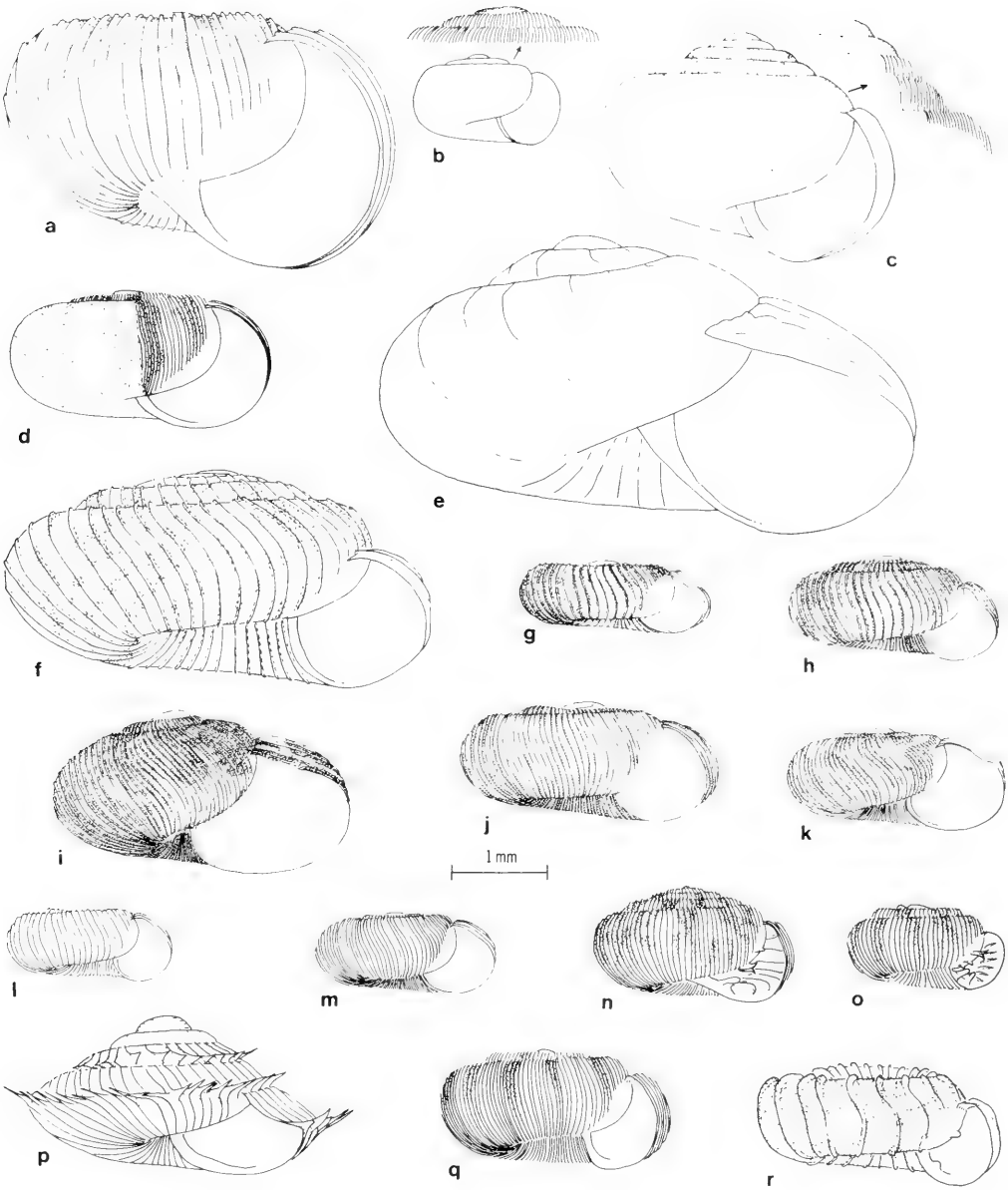

FIG. 2. Shells of some Manukau Peninsula charopids: a, *Allodiscus dimorphus*; b, "*Allodiscus*" *urquharti*; c, *Allodiscus* n. sp. aff. *granum*; d, *Allodiscus planulatus*; e, *Flammulina perdita*; f, *Charopa coma*; g, "*Charopa*" n. sp. aff. *pseudanguicula*; h, "*Charopa*" *pseudanguicula*; i, "*Charopa*" *costulata*; j, "*Charopa*" *ochra*; k, "*Charopa*" *pilsbryi*; l, "*Charopa*" *fuscosa*; m, "*Charopa*" *chrysaugeia*; n, *Huonodon pseudoleiodon*; o, *Huonodon hectori*; p, *Therasiella* n. sp. aff. *neozelanica*; q, "*Mocella*" n. sp. aff. *maculata*; r, *Fectola mira*. Scale line equals 1 mm. Drawings by F. M. Climo from unpublished manuscripts.

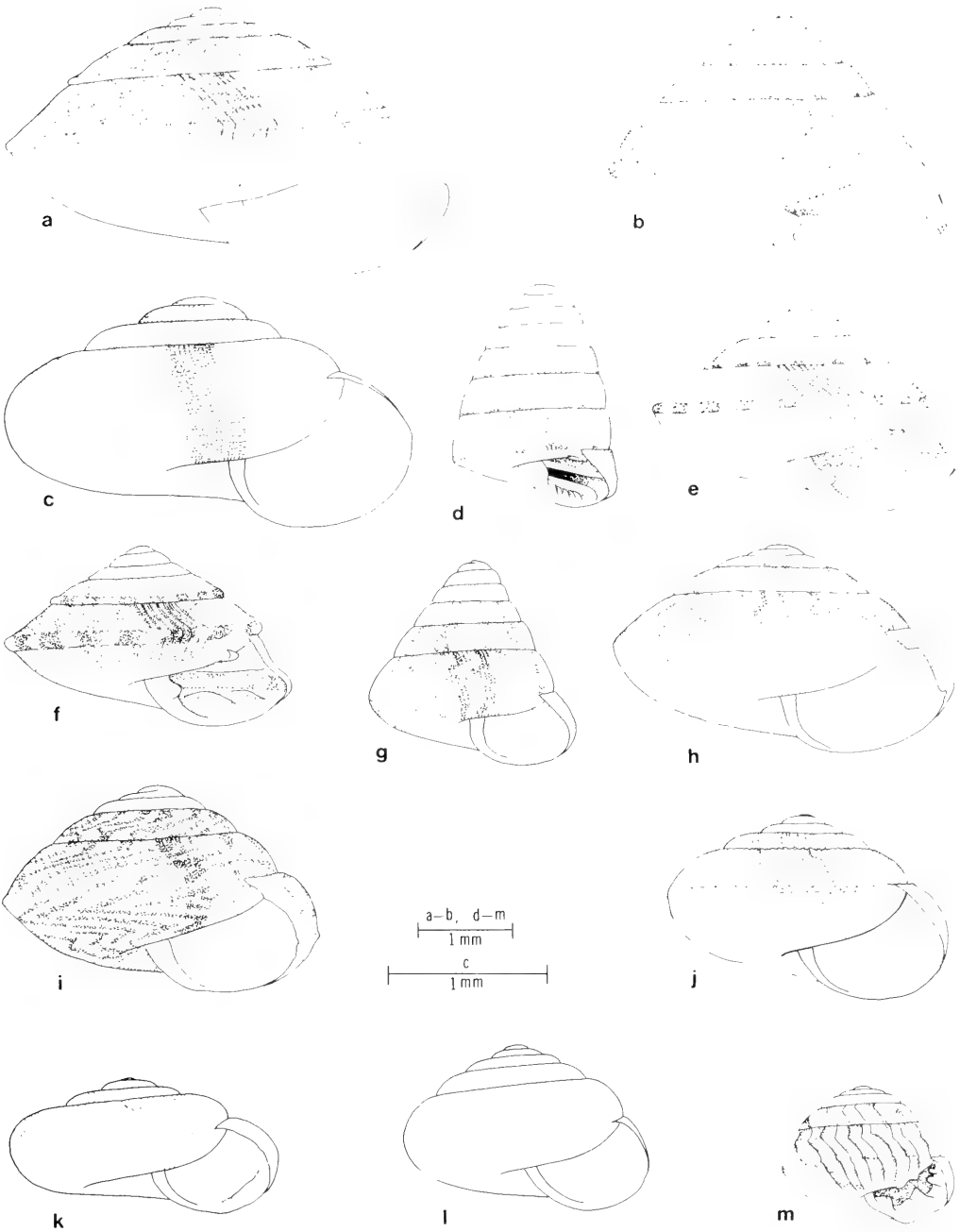

FIG. 3. Shells of Manukau Peninsula punctids: a, "*Laoma*" *mariae*; b, "*Phrixgnathus*" *poecilosticta*; c, "*Phrixgnathus*" *moellendorffi*; d, *Laoma leimonias*; e, *Laoma* n. sp. aff. *marina*; f, *Laoma marina*; g, "*Phrixgnathus*" *erigone*; h, "*Phrixgnathus*" *levis*; i, "*Phrixgnathus*" *conella*; j, "*Phrixgnathus*" *ariel*; k, "*Phrixgnathus*" n. sp. 59; l, "*Phrixgnathus*" *glabriusculus*; m, "*Phrixgnathus*" *pirongiaensis*. Scale lines as marked. Drawings by F. M. Climo from monographic review of New Zealand punctids that is in preparation.

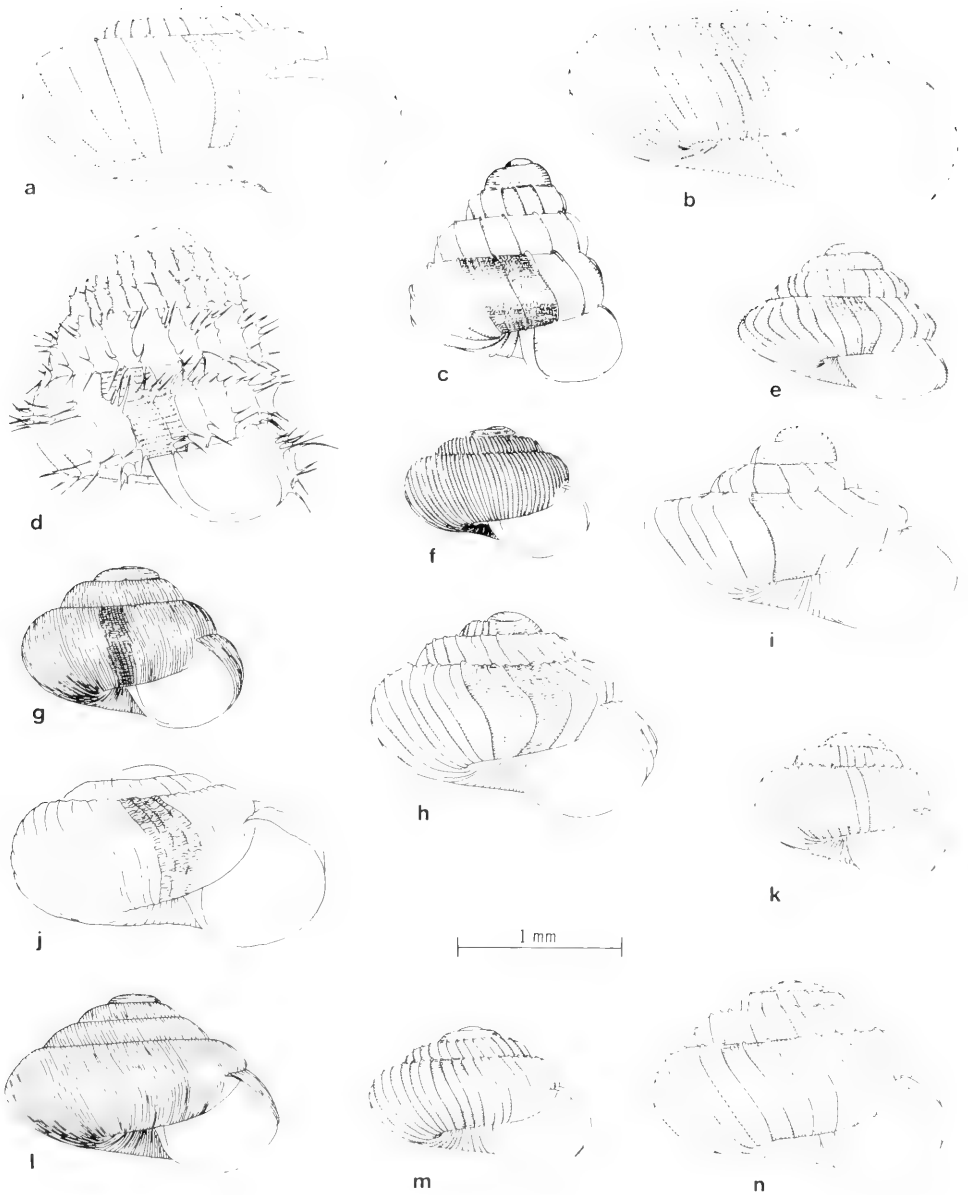

FIG. 4. Shells of Manukau Peninsula punctids: a, *Paralaoma caputspinulae*; b, "*Paralaoma*" n. sp. 40; c, "*Paralaoma*" n. sp. aff. 33; d, "*Paralaoma*" *francesci*; e, "*Paralaoma*" *serratocostata*; f, "*Paralaoma*" n. sp. 1; g, "*Paralaoma*" n. sp. 33; h, "*Phrixgnathus*" *elaiodes*; i, *Obanella rimutaka*; j, *Pasmaditta jungermanniae*; k, "*Paralaoma*" n. sp. 8; l, "*Paralaoma*" n. sp. 38; m, "*Paralaoma*" n. sp. 29; n, "*Paralaoma*" *lateumbilicata*. Scale line equals 1 mm. Drawings by F. M. Climo from monographic review of New Zealand punctids that is in preparation.

TABLE 1. List of taxa discussed.

Family Hydrocenidae
1. *Omphalorissa purchasi* (Pfeiffer, 1862)—(Powell, 1979: 85, fig. 13-1)

Family Liareidae
2. *Liarea hochstetteri carinella* (Pfeiffer, 1861)—(Powell, 1976: pl. 35, fig. 7)
3. *L. egea egea* (Gray, 1850)—(Powell, 1976; pl. 35, fig. 4)
4. *Cytora cytora* (Gray, 1850)—(Powell, 1979: 85, fig. 12-3)
5. *C. hedleyi* (Suter, 1894)—(Powell, 1979: 85, fig. 12-5)
6. *C. pallida* (Hutton, 1883)—(Powell, 1979: 85, fig. 12-2)
7. *C. torquilla* (Suter, 1894)—(Powell, 1979: 85, fig. 12-4)

Family Achatinellidae
8. *Lamellidea novoseelandica* (Pfeiffer, 1853)—(Powell, 1979: 302, fig., 74-1)

Family Rhytididae
9. *Delos coresia* (Gray, 1850)—(Powell, 1979: 348, figs. 81-2-4)
10. *D. jeffreysiana* (Pfeiffer, 1853)—(Powell, 1979: 348, figs. 81-1)
11. *Rhytida greenwoodi* (Gray, 1850)—(Powell, 1979: pl. 64, figs. 1-2)

Family Charopidae
12. *Cavellia buccinella* (Reeve, 1852)—(Powell, 1979: pl. 57, fig. 8)
13. *C. roseveari* (Suter, 1896)—(Suter, 1915: pl. 10, figs. 5a, b)
14. *Mocella eta* (Pfeiffer, 1853)—(Climo, 1981: fig. 1A-C)
15. "*M.*" n. sp. aff. *maculata* (Suter, 1891)—Fig. 2q
16. "*M.*" n. sp. aff. *manawatawhia* (Powell, 1935)—(aff. Powell, 1979: pl. 57, fig. 10)
17. "*Charopa*" *pseudanguicula* Iredale, 1913—Fig. 2h
18. "*C.*" *chrysaugeia* (Webster, 1904)—Fig. 2m
19. "*C.*" n. sp. aff. *pseudanguicula* Iredale, 1913—Fig. 2g
20. "*C.*" *fuscosa* (Suter, 1894)—Fig. 2l
21. "*C.*" *pilsbryi* (Suter, 1894)—Fig. 2k
22. *C. coma* (Gray, 1843)—Fig. 2f
23. "*C.*" *costulata* (Hutton, 1883)—Fig. 2i
24. "*C.*" *ochra* (Webster, 1904)—Fig. 2j
25. *Fectola mira* (Webster, 1908)—Fig. 2r
26. *F. unidentata* Climo, 1978—(Climo, 1978: fig. 4)
27. *F. infecta* (Reeve, 1852)—(Climo, 1978: fig. 3)
28. *Huonodon pseudoleiodon* (Suter, 1890)—Fig. 2n
29. *H. hectori* (Suter, 1890)—Fig. 2o
30. *Allodiscus dimorphus* (Pfeiffer, 1853)—Fig. 2a
31. *A. tessellatus* Powell, 1941—(Powell, 1979: pl. 58, figs. 1-2)
32. "*A.*" *urquharti* Suter, 1894—Fig. 2b
33. *A.* n. sp. aff. *granum* (Pfeiffer, 1857)—Fig. 2c
34. *A. planulatus* (Hutton, 1883)—Fig. 2d
35. *Geminoropa cookiana* (Dell, 1952)—(Climo, 1981: fig. 2A-C)
36. *Serpho kivi* (Gray, 1843)—(Powell, 1979: pl. 65, fig. 9)
37. *Flammulina perdita* (Hutton, 1883)—Fig. 2e
38. *F. chiron* (Gray, 1850)—(Suter, 1915: pl. 26, figs. 13a-b)
39. "*F.*" *feredayi* (Suter, 1915: pl. 9, figs. 10a-b)
40. "*Thalassohelix*" *ziczac* (Gould, 1848)—(Powell, 1979: pl. 65, fig. 10)
41. *Therasia decidua* (Pfeiffer, 1857)—(Suter, 1915: pl. 50, fig. 8)
42. *Suteria ide* (Gray, 1850)—(Powell, 1979: pl. 65, figs. 1-2)
43. *Phenacohelix giveni* Cumber, 1961—(Powell, 1979: pl. 65, fig. 11)
44. *P. pilula* (Reeve, 1852)—(Suter, 1915: pl. 26, figs. 7a-b)
45. "*P.*" n. sp.—(Suter, 1915: aff. pl. 26, figs. 5a-b—without color pattern)
46. *P. ponsonbyi* (Suter, 1897)—(Suter, 1915: pl. 26, figs. 8a-b)
47. *Therasiella neozelanica* Cumber, 1967—(Cumber, 1967d: fig. 1G-I)
48. *T. serrata* Cumber 1967—(Cumber, 1967d: fig. 1A-C)
49. *T.* n. sp. aff. *neozelanica* Cumber, 1967d—fig. 2p
50. *T. celinda* (Gray, 1850)—(Cumber, 1967d: fig. 3)
51. *T. tamora* (Hutton, 1883)—(Cumber, 1967d: fig. 2)

TABLE 1 (Continued)

Family Punctidae
52. *Obanella rimutaka* Dell, 1952—Fig. 4i
53. "*Laoma*" *mariae* (Gray, 1843)—Fig. 3a
54. *L.* n. sp. aff. *marina* (Hutton, 1883)—Fig. 3e
55. *L. marina* (Hutton, 1883)—Fig. 3f
56. *L. leimonias* (Gray, 1850)—Fig. 3d
57. "*Phrixgnathus*" *erigone* (Gray, 1850)—Fig. 3g
58. "*P.*" *ariel* (Hutton, 1883)—Fig. 3j
59. "*P.*" *elaiodes* Webster, 1904—Fig. 4h
60. "*P.*" *moellendorffi* Suter, 1896—Fig. 3c
61. "*P.*" *conella* (Pfeiffer, 1862)—Fig. 3i
62. "*P.*" *poecilosticta* (Pfeiffer, 1852)—Fig. 3b
63. "*P.*" *glabriusculus* (Pfeiffer, 1853)—Fig. 3l
64. "*P.*" *pirongiaensis* Suter, 1894—Fig. 3m
65. "*P.*" *levis* (Suter, 1913)—Fig. 3h
66. "*P.*" n. sp. 59 (= *glabriusculus* of authors)—Fig. 3k
67. *Pasmaditta jungermanniae* (Petterd, 1879)—Fig. 4j
68. "*Paralaoma*" n. sp. 38—Fig. 4l
69. "*P.*" n. sp. 29—Fig. 4m
70. "*P.*" *lateumbilicata* (Suter, 1890)—Fig. 4n
71. "*P.*" n. sp. 1—Fig. 4f
72. "*P.*" n. sp. 8—Fig. 4k
73. "*P.*" *serratocostata* (Webster, 1906)—Fig. 4e
74. "*P.*" n. sp. 40—Fig. 4b
75. *P. caputspinulae* (Reeve, 1852)—Fig. 4a
76. "*P.*" *francesci* (Webster, 1904)—Fig. 4d
77. "*P.*" n. sp. 33—Fig. 4g
78. "*P.*" n. sp. aff. 33—Fig. 4c

INTRODUCED TAXA
79. *Vallonia* spp. (Family Valloniidae)
80. *Oxychilus cellarius* (Müller, 1774) (Family Zonitidae)
81. *Cionella lubrica* (Müller, 1774) (Family Cionellidae)
82. *Helix aspersa* (Müller, 1774) (Family Helicidae)

3.2–5.6 mm^3 range, and the remaining seven species are in the 12.1–72.8 mm^3 classes.

The punctids and charopids show more easily recognizable differences. Tables 2 and 3 show that the charopids are significantly larger in diameter (Table 2), but tend toward a lower H/D ratio (Table 3) because the shell height generally is in a reduced range in proportion to the shell diameter. The three most elevated charopid shells are those of *Serpho kivi* (H/D ratio 0.771), *Allodiscus* n. sp. aff. *granum* (Fig. 2c, H/D ratio 0.788), and "*Phenacohelix*" n. sp. (H/D ratio 0.852). The first species is normally found foraging on large leaves or on tree trunks, the latter two are terrestrial. Those punctids with H/D ratio above 0.850, include the characteristic inhabitant of new leaves on top of the litter (*Laoma leimonias*, Fig. 3d, H/D ratio 1.438), ground surface under moist litter ("*Phrixgnathus*" *poecilosticta,* Fig. 3b, H/D ratio 0.892), in drier friable litter ("*Paralaoma*" *francesci*, Fig. 4d, H/D ratio 1.035), and the characteristic tree branch and sapling species ("*Phrixgnathus*" *erigone,* Fig. 3g, H/D ratio 1.026). The way in which these increased H/D ratios is achieved differs considerably. The four punctids have increased whorl counts and strong spire protrusion; *Serpho kivi* shows moderate whorl increment and strong spire protrusion; "*Phenacohelix*" n. sp. and *Allodiscus* n. sp. aff. *granum* show a combination of spire protrusion and lateral compression of the body whorl (Fig. 2c). The latter two species lack any trace of a keel or peripheral angulation; the others have sharply angled or keeled peripheries.

Whorl count distribution (Table 4) confirms the difference, in that mean whorl count for the punctids is one interval less than for the charopids. Those few punctids with enlarged whorl counts, *Laoma leimonias* (Fig. 3d), "*Phrixgnathus*" *erigone* (Fig. 3g), and "*P.*" *poecilosticta* (Fig. 3b), also have the in-

TABLE 2. Height and Diameter distributions.

Interval	Height				Diameter			
	Other	Charopid	Punctid	Total	Other	Charopid	Punctid	Total
0.50– 1.00	—	5	6	11	—	—	1	1
1.01– 1.50	1*	10	10	21	3	—	7	10
1.51– 2.00	3	9	4	16	—	5	6	11
2.01– 2.50	2	5	4	11	3*	6	2	11
2.51– 3.00	2*	2	2	6	1*	10	6	17
3.01– 3.50	1	3	1	5	2	6	2	10
3.51– 4.00	—	2	—	2	1	2	1	4
4.01– 4.50	1	—	—	1	1	1	1	3
4.51– 5.00	1	—	—	1	—	—	1	1
5.01– 5.50	1*	1	—	2	—	2	—	2
5.51– 6.00	—	—	—	—	—	2	—	2
6.01– 6.50	—	—	—	—	—	2	—	2
6.51– 7.00	1	1	—	2	1*	1	—	2
7.01– 8.00	1	1	—	2	—	2	—	2
8.01– 9.00	—	—	—	—	—	1	—	1
9.01–10.0	—	—	—	—	—	1	—	1
10.1 –11.0	—	—	—	—	—	1	—	1
14.2	1	—	—	1	—	—	—	—
21–24	1*	—	—	1	2*	—	—	2

*Indicates an introduced species.

TABLE 3. Height/Diameter Ratio distributions.

Interval	Other	Charopid	Punctid	Total
0.400–0.450	1*	4	—	5
0.451–0.500	1	7	1	9
0.501–0.550	1	3	1	5
0.551–0.600	1*	7	2	10
0.601–0.650	—	11	5	16
0.651–0.700	1	3	4	8
0.701–0.750	—	2	7	9
0.751–0.800	—	2	3	5
0.801–0.850	—	—	—	—
0.851–0.900	—	1	1	2
0.901–0.950	—	—	—	—
0.951–1.000	1*	—	—	1
1.001–1.050	1	—	2	3
1.051–1.100	1	—	—	1
1.101–1.500	2	—	1	3
1.501–1.800	1	—	—	1
1.801–2.100	4*	—	—	4

*Indicates an introduced species.

TABLE 4. Whorl Count distributions.

Whorl counts grouped	Other	Charopid	Punctid	Total
2⅞	—	1	—	1
3¼	2*	3	1	6
3⅝	1	3	3	7
4	3*	3	4	10
4⅜	2*	8	6	16
4¾	1	5	4	10
5⅛	1	11	2	14
5½	1*	6	4	11
5⅞	1	—	—	1
6¼	1	—	2	3
6⅝	1	—	—	1
7	—	—	—	—
7⅜	1	—	1	2

*Indicates an introduced species.

creased H/D ratio discussed above. Reduction in whorl count is more frequent in the Charopidae. The "*Flammulina*"-type taxa (Fig. 2e) are early stages in a slug lineage, and hence show initial stages in shell whorl reduction. The reason why several of the "*Charopa*" group show only 3⅜ or 3¾ whorls, while the median for the Charopidae is 4¾ whorls, is unknown to us. Since the three *Flammulina* show different habitat preferences (tree trunks, undersides of logs, near ground surface in deep wet litter) and the "*Charopa*" with greatly reduced whorl counts are equally varied (*chrysaugeia,* Fig. 2m, probably arboreal; *fuscosa,* Fig. 2l, under compressed broad leaf litter; *pilsbryi,* Fig. 2k, under loosened bark of fallen logs; *costulata,* Fig. 2i, in well-decomposed, powdery litter near logs), this

type of change cannot be linked simply with habitat preference.

Umbilical proportion distributions are charted (Table 5) only for the Charopidae and Punctidae. The other taxa have closed umbilici except for the three Rhytididae, *Vallonia* and *Oxychilus*. Those with open umbilici are in the 2.70–5.04 range. The dominant families show clear differences. Charopids tend toward widely open or narrow to closed umbilici, while the punctids show basically narrow to closed, with a few moderately open. The more openly umbilicated punctids are not taxonomically clustered, and show different habitat preferences.

Adjusted Shell Volume (ASV) distribution (Table 6) again shows the Charopidae as being, in general, larger than the Punctidae, but the dispersion for each family is clearly not unimodal. The probable reasons for this are discussed below. The only really small charopid is "*Allodiscus*" *urquharti* (Fig. 2b, 1.30 mm^3), an inhabitant of broken-down litter. The node of large punctids includes one tree trunk species ("*Phrixgnathus*" *ariel,* Fig. 3j, 12.3 mm^3) and five ground surface under wet litter taxa that vary from 9.1–31.1 mm^3. Only one of these, "*Phrixgnathus*" *poecilosticta,* has a markedly increased whorl count (6¼) to explain partly the large size.

How snails get big is not a simple matter. Increase in whorl count is an obvious means, but where this occurs, the animal often withdraws from the earlier whorls. These can be sealed off by a calcareous plug in such taxa as Urocoptidae, *Rumina,* some Clausiliidae, and New World Pomatiasidae, with the early whorls breaking off subsequently. Where the shell shape is lanceolate, increased whorl number is a very practical option, producing a very high H/D ratio and permitting total whorl counts in excess of 20 to be reached. For essentially planulate taxa or those with near globular shape, now useless early whorl decollation is not a viable alternative. Massive withdrawal from early whorls has been demonstrated for the planulate helicarionid *Coxia m. macgregori* (Cox, 1870) and the endodontid *Libera f. fratercula* (Pease, 1867) (see Solem, 1976: 95, fig. 55). We predict this will be found also in such planulate taxa as the Brazilian *Polygyratia polygyrata* (Born, 1778) and the Mexican urocoptid *Hendersoniella palmeri* (Dall, 1905) (see Zilch, 1959–1960: 529, fig. 1857; 604, fig. 2119). The only Manukau Peninsula species that has increased size in this manner is the comparatively small *Laoma leimonias* (Fig. 3d), height 2.57 mm with 7½ whorls. Live specimens show that the first two to three whorls are without soft parts, although whether this space contains an air bubble or a water reservoir is unknown at present. Species with moderate whorl increments, such as "*Phrixgnathus*" *erigone* (Fig. 3g), "*P.*" *poecilosticta* (Fig. 3b), "*Paralaoma*" *francesci* (Fig. 4d), *Cavellia roseveari,* "*Charopa*" *pseudanguicula* (Fig. 2h), *C. coma* (Fig. 2f), *Allodiscus dimorphus* (Fig. 2a), *Serpho kivi,* and "*Phenacohelix*" n. sp., also can show gross increments with thickenings of the ribbing, enlagement of both the nuclear and postnuclear whorls. More frequently, size increase is a result of the latter process. How-

TABLE 5. Distribution of umbilical proportions.

Interval	Charopids	Punctids	Total
2.00–2.50	2	—	2
2.51–3.00	6	—	6
3.01–3.50	1	—	1
3.51–4.00	6	—	6
4.01–4.50	1	1	2
4.51–5.00	2	3	5
5.01–6.00	4	1	5
6.01–7.00	—	1	1
7.01–8.00	—	2	2
8.01–10.0	2	3	5
10.1–15.0	3	4	7
15.1–20.0	5	3	8
Lateral crack	4	6	10
Closed	4	3	7

TABLE 6. Adjusted Shell Volume (ASV) distribution.

Interval in mm^3	Other	Charopid	Punctid	Total
0.25–0.50	—	—	2	2
0.51–1.00	1	—	5	6
1.01–1.50	2	1	2	5
1.51–2.00	—	—	2	2
2.01–3.00	—	5	5	10
3.01–5.00	2*	5	2	9
5.01–7.00	1	4	2	7
7.01–9.00	—	6	1	7
9.01–15.0	2*	6	3	11
15.1–40.0	2	3	3	8
40.1–75.0	3*	4	—	7
75.0–150.0	—	3	—	3
150.1–350.0	—	3	—	3
3,000–6,000	2*	—	—	2

*Indicates an introduced species.

ever, none of the Manukau Peninsula taxa, except *Laoma leimonias,* show a clear and notable pattern of whorl increment change.

While there are some trend differences between the Charopidae and Punctidae in respect to individual size and shape parameters, the above brief review of departures from the norm shows no simple and direct correlations between such variations and space or moisture preferences by the species involved.

Some variations in shell contours are partly correlated with habitat specialization. Development of a keel is restricted to the punctids, and *Liarea hochstetteri carinella.* In that species, *Laoma leimonias* (Fig. 3d), "*Paralaoma*" *serratocostata* (Fig. 4e), and "*P.*" *francesci* (Fig. 4d), the keel is low on the whorl profile, a result of spire elevation and high H/D ratio. In "*Laoma*" *mariae* (Fig. 3a), *L.* n. sp. aff. *marina* (Fig. 3e), "*Phrixgnathus*" *poecilosticta* (Fig. 3b), and "*P.*" *levis* (Fig. 3h), the keel is medial on an obtusely angled periphery. Of the *Laoma* and "*Phrixgnathus*" taxa listed above, only "*P.*" *ariel* is arboreal, with the others mostly ground space associated. The remaining "*Phrixgnathus*" lack keels, with the arboreal "*P.*" *erigone* (Fig. 3g, sharply angled periphery) and "*P.*" *elaiodes* (Fig. 4h, weakly angled periphery), suspended litter in bracts ("*P.*" n. sp. 59, Fig. 3k, weakly angled), ground surface in drier litter ("*P.*" *moellendorffi,* Fig. 3c, weakly angled) and spaces in wet litter above ground level ("*P.*" *glabriusculus,* Fig. 3l, rounded periphery), suggesting that possibly possession of a keeled periphery is an advantage in occupying the ground surface under moist to wet litter, although the association is far from complete. Other taxa with sharply angled peripheries include the arboreal *Serpho kivi,* and the basically ground level *Therasiella, Cytora pallida, Therasia decidua, Obanella rimutaka* (Fig. 4i), and "*Paralaoma*" n. sp. 38 (Fig. 4l), which show different habitat preferences, but are terrestrial rather than arboreal. A few species show a weak mid-whorl angulation of the periphery—*Rhytida greenwoodi,* "*Mocella*" n. sp. aff. *manawatawhia, Paralaoma caputspinulae* (Fig. 4a), "*P.*" n. sp. 33 (Fig. 4g), and "*P.*" n. sp. aff. 33 (Fig. 4c), but the remainder show evenly rounded or slightly laterally flattened peripheries as in the larger *Allodiscus.*

The 15 species of punctids with angulated to carinated peripheries are not closely related. Climo (in preparation) refers them to 12 genera in three subfamilies. Five species in five genera in three subfamilies occupy ground surfaces under wet litter and have protruded thread-like keels. They are "*Laoma*" *mariae* (Fig. 3a), "*Phrixgnathus*" *poecilosticta* (Fig. 3b), "*Paralaoma*" *serratocostata* (Fig. 4e), *Laoma* n. sp. aff. *marina* (Fig. 3e), and "*Phrixgnathus*" *conella* (Fig. 3i). The other punctids with thread-like keels are *Laoma marina* (Fig. 3f), which lives "on leaves and twigs in wet areas"; "*Phrixgnathus*" *pirongiaensis* (Fig. 3m), found in "wet deep litter"; *Laoma leimonias* (Fig. 3d), found "in wet, undecomposed, broad-leaf litter"; "*Phrixgnathus*" *ariel* (Fig. 3j), found on "trunks and branches of larger trees"; and "*Phrixgnathus*" *levis* (Fig. 3h), found "under moist broadleaf litter" (Solem, Climo & Roscoe, 1981: appendix 1). The presence of a keel is associated with the ground surface habitat, but it is not restricted to this niche.

Where there is relatively close relationship among Manukau Peninsula punctids, true *Laoma* for example, shelter site preferences are diverse. *Laoma leimonias* (Fig. 3d) lives at the top of the litter among newly fallen leaves; "*Phrixgnathus*" *erigone* (Fig. 3g) prefers encrusting materials on saplings and small tree trunks; *L. marina* (Fig. 3f) is on leaves and twigs in wet litter above ground level; and *L.* n. sp. aff. *marina* (Fig. 3e) is on the ground surface under deep litter.

Thus, the similarity of keeled structure in ground space taxa is not caused by monophyly, and the only set of congeneric punctids differs radically in its shelter site preferences and shell forms.

POST-APICAL SCULPTURE

The post-apical sculpture found on the Manukau Peninsula species ranges from smooth, glossy surfaces from which even incremental growth irregularities are absent, to quite prominent periostracal extensions in a series of mostly unrelated taxa. This extreme is discussed first.

Five species have periostracal "hairs" or "setae" extending upward from the side or tops of radial ribs. These are easily abraded and will be partly eroded or missing in many individuals. They are microscopic in size and regularly spaced on the main ribs of "*Paralaoma*" *serratocostata* (Fig. 4e). In "*P.*" *francesci* (Fig. 4d) they are much longer and grouped into two spaced clusters of three or four each at intervals along the radial ribs above the periphery, but are single and at

TABLE 7. Radial periostracal sculpture.

	Species	Largest dimension	ASV	Periostracal structure	Habitat preference
3	*Liarea egea*	6.95 mm	28.6	ridges	wet piles
4	*Cytora cytora*	2.63	5.7	0.2 mm hairs	friable, well-drained
5	*C. hedleyi*	2.15	3.2	ridges	rimu litter
6	*C. pallida*	4.8	18.5	ridges	unknown
7	*C. torquilla*	2.13	1.2	ridges	friable, well-drained
30	*Allodiscus dimorphus*	9.2	340.2	ridges	very rotten logs
40	"*Thalassohelix*" *ziczac*	7.25	134.4	0.5 mm hairs	on ground under logs
42	*Suteria ide*	7.1	118.5	0.7 mm hairs	on ground under logs
47	*Therasiella neozelanica*	2.37	3.7	0.15 mm triangular peripheral	friable, well-drained
48	*T. serrata*	2.93	6.4	0.3 mm triangular peripheral	friable, well-drained
49	*T.* n. sp. aff. *neozelanica*	2.93	7.1	0.3 mm triangular peripheral	friable, well-drained
50	*T. celinde*	3.0	8.0	very short peripheral	underside of logs
51	*T. tamora*	3.43	11.7	0.2 mm fan-shaped peripheral	friable, well-drained
73	"*Paralaoma*" *serratocostata*	1.25	0.6	microscopic hairs	fine grain decomposition
76	"*P.*" *francesci*	1.45	0.9	clumped hairs	deep, friable podocarp

regular intervals on the shell base. In *Cytora cytora* the setae are 0.2 mm long, spaced singly along low radial ridges at intervals slightly less than their height, and all are slanted backward from the aperture at about a 45° angle. "*Thalassohelix*" *ziczac* has 0.5 mm long setae and *Suteria ide* has up to 0.7 mm long setae that arise at regular intervals from the top of narrow, low ridges and point directly outward. The form of the setae compares quite well with those found in the Hawaiian *Cookeconcha decussatulus* (Pease, 1866) (see Solem, 1976: 36, figs. 26b–c).

Simple periostracal radial ridge extensions, that are very subject to wear, are found in juveniles of *Liarea egea,* all ages of *Cytora hedleyi, C. torquilla, C. pallida, Allodiscus dimorphus* (Fig. 2a), *Therasiella celinde* and *T. tamora.* In the *Therasiella,* these ridges are extended slightly or into long curved projections from the periphery (see Cumber, 1967d: 64, figs. 2A–C; 66, figs. 3A–C). Other *Therasiella* show an intensification of this phenomenon. *T. neozelanica* has 0.15 mm long triangular projections from the periphery (Cumber, 1967d: 62, figs. 1G–I), they reach 0.3 mm long in *T. serrata* (*ibid.,* 62, figs. 1A–C), and form a nearly continuous fringe of about 0.3 mm long triangular projections in *T.* n. sp. aff. *neozelanica* (Fig. 2p).

The taxonomic spread of hairy shells is broad, and the appearance of "hairs" clearly is of independent origin. The functional and habitat associations of the periostracal extensions are not clear. Table 7 gives the largest dimension, ASV, type and size of periostracal extension, and habitat preference of each species. In the log habitat, the two species with longest hairs, "*Thalassohelix*" *ziczac* and *Suteria ide,* occur on the ground under logs or in deep litter by logs. Their setae could be interpreted as an aid in minimizing litter accumulation on shell surfaces. The very large *Allodiscus dimorphus* (Fig. 2a) is log associated, but we cannot say if it prefers the underside of the log, as does *Therasiella celinde,* or the ground spaces under the log. It has very low periostracal extensions. The first three species are quite large, the latter of relatively small size. Intermediate-sized species that are log associated, such as *Allodiscus tessellatus* in litter or under logs, *Charopa coma* (Fig. 2f) in decay spaces on the log itself, and *A. planulatus* (Fig. 2d) found on the ground under logs, show no such periostracal

features. The latter three species range in the 11.8 to 63.4 mm^3 ASV level, thus being intermediate in size. *T. celinde,* the only log dweller in its genus, has the least prominent periostracal extensions.

According to Cumber (1967d: 61), species of *Therasiella* ". . . are rendered inconspicuous by the habit of gathering trash on all surfaces except those immediately adjacent to the aperture. It would appear that the membraneous plaits [= peripheral periostracal projections] have adhesive properties when moist, and this soon gathers a miscellaneous covering of bits and pieces. . . ." We cannot comment on *Therasiella tamora,* which was a rare species on the Manukau (Solem, Climo & Roscoe, 1981), but the other three species quite commonly are collected sympatrically and Cumber (1967d) lists a number of same day-same locality records for *tamora, serrata* and *neozelanica.* The size of the peripheral protrusions is proportionate in the four species, allowing that *T. neozelanica* is much smaller and that the shorter blades of *T. tamora* extend well above and below the actual periphery, thus offering greater surface area than the longer triangular projections of *T. serrata* and *T.* n. sp. aff. *neozelanica* (Fig. 2p). All four *Therasiella* live in friable, broken-down litter, in which they are joined by the similar-sized *Cytora cytora* with 0.2 mm slanted hairs, the very small ridged *C. torquilla,* and the microscopically haired and very small "*Paralaoma*" *serratocostata* (Fig. 4e) and "*P.*" *francesci* (Fig. 4d).

Our knowledge of their ecology does not permit defining exact niches for any of these species, but it must be emphasized that their preference sites are shared with species of similar size that do not have such special features (Solem, Climo & Roscoe, 1981: fig. 2).

The occurrence of such periostracal developments effectively spans the volume range of snail species found on the Manukau. For only two very large species, "*Thalassohelix*" *ziczac* and *Suteria ide* that live on the ground surface under logs, can we suggest an obvious function of the periostracal protrusions. For the other species, we record the existence of various types of projections in the same preferred environment, but have no knowledge of their function.

Normal "endodontoid" sculpture consists of major radial ribs between which lie a complex microsculpture. In both the Pacific Island Endodontidae (Solem, 1976) and Charopidae (Solem, 1983), there is a close correlation between shell size and spacing of the major ribs. Larger species have larger ribs that are spaced more widely; small species have smaller and more crowded ribs. For the Endodontidae, the spacing pattern for 133 species was summarized (Solem, 1976: 44, table XV) and then graphed for a monophyletic lineage (*ibid.*: 48, fig. 35). There are few departures from this normal pattern.

For the 40 species of charopids recorded from the Manukau Peninsula, the five *Therasiella* have only periostracal extensions; *Serpho kivi* and *Therasia decidua* have only weak growth irregularities; *Flammulina perdita* (Fig. 2e) has a glossy, smooth shell surface; both *Flammulina chiron* and "*F.*" *feredayi* have greatly reduced surface sculpture although reticulated remnants can be detected; and both "*Charopa*" *pilsbryi* (Fig. 2k) and "*C.*" *costulata* (Fig. 2i) have the sculpture detectable as composed of major and minor elements, but too fine to count at 60× magnification. *Serpho kivi* (ASV is 487.9 mm^3) and *Flammulina perdita* (ASV is 72.5 mm^3) are arboreal taxa; *F. chiron* (ASV is 116.3 mm^3) is found on the underside of logs; "*F.*" *feredayi* (ASV is 10.5 mm^3) is in deep litter near the ground; *Therasia decidua* (ASV is 237.6 mm^3) is a ground dweller around monocots in more open areas; "*C.*" *costulata* (ASV is 13.4 mm^3) lives in well-decomposed powdery litter near logs; and "*C.*" *pilsbryi* (ASV is 4.9 mm^3) is under the loosened bark of fallen logs. Sculpture reduction in arboreal and very large species is typical in the Charopidae (Solem, 1983), but reduction occurring in the two "*Charopa*" and "*F.*" *feredayi* is unexpected and we can assign no reason for this.

The 28 species of Charopidae in which the sculpture could be counted are graphed in Fig. 5. ASV has been used as a better indicator of size than any single dimension. With the exception of four taxa in the *Allodiscus* group, species 30–33, which do show a good linear correlation of rib spacing and size, the amazing fact is that there is no pattern to the scatter. The four *Phenacohelix* (species 43–46) maintain essentially the same rib spacing despite a sixfold size differential (ASV 10.2–60.0 mm^3), and the three *Fectola* (species 25–27) show only minor change. Similar rib spacing occurs at widely different volumes, and quite divergent rib spacing occurs at the same volume.

Considering those taxa at the 2–3 mm^3

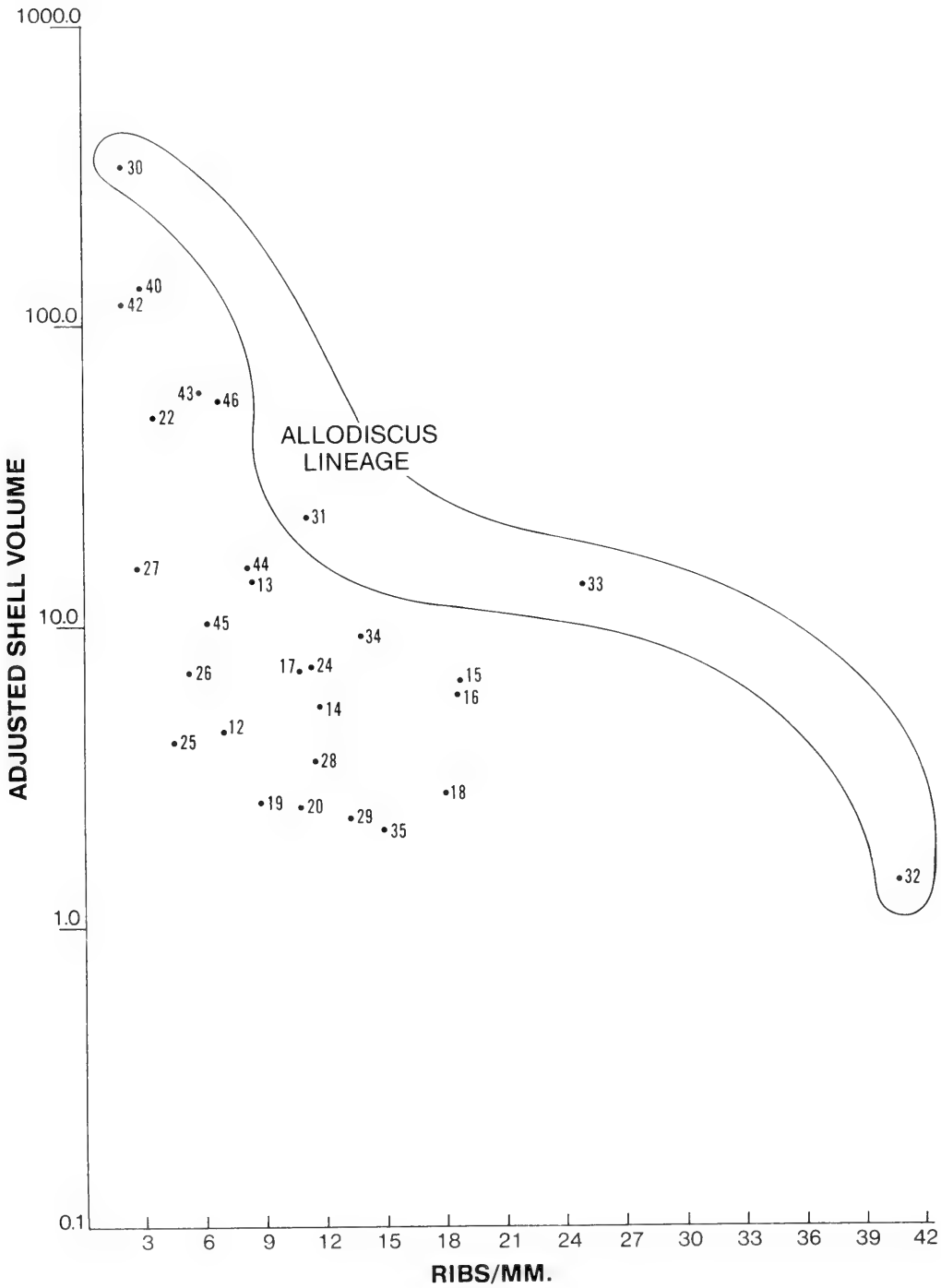

FIG. 5. Relationship between rib spacing and adusted shell volume in Manukau Peninsula Charopidae. Numbers are those used in Table 1.

ASV level, "*Charopa*" n. sp. aff. *pseudanguicula* (19, Fig. 2g) and "*C.*" *chrysaugeia* (20, Fig. 2m) are both tree trunk taxa and show the sculptural extremes; "*C.*" *fuscosa* (20, Fig. 2l) prefers compressed broad leaf litter; *Huonodon hectori* (29, Fig. 2o) is in suspended litter of tree axils and bracts; and *Geminoropa cookiana* (35) prefers the loam and grit next to the ground under logs. The slightly larger *Huonodon pseudoleiodon* (28, Fig. 2n) prefers older, but still not decayed fallen leaves, near the top of the litter.

On the Pacific Islands, the Charopidae and Endodontidae live in situations with very few sympatric species (five to ten would be normal) and their sculpture spacing is closely size linked. The Manukau Peninsula charopids show little linkage between shell sculpture spacing and size. We can detect no habitat preference correlation with sculpture spacing.

Size, prominence and shape of the radial sculpture require only a few comments. In the large species with periostracal hairs, *Suteria ide* and "*Thalassohelix*" *ziczac,* the main ribs are very low and widely spaced, and they also are small in *Allodiscus dimorphus* (Fig. 2a). *Charopa coma* (Fig. 2f) is best described as "gross" in that the ribs are very thick and prominent. Its shell is significantly enlarged in diameter over probable relatives, but is not significantly altered in whorl count. *Fectola mira* (Fig. 2r, 25) and, to a lesser extent, *Fectola infecta* (see Climo, 1978: 193, fig. 3) have enlarged and strongly sinuated sculpture combined with a tendency toward lateral whorl flattening. An interesting parallelism to this was seen in "*Charopa*" n. sp. aff. *pseudanguicula* (Fig. 2g, 19), which has sculpture shape and proportion, plus lateral whorl flattening, that closely mimics the appearance of *F. mira.* On several occasions Frank Climo and David J. Roscoe have collected samples of *F. mira* in the field only to find out when sorting under a microscope that a small proportion of the specimens were the "*Charopa*". Our Manukau samples showed that this association is less than perfect, since 9 out of 10 live "C." n. sp. aff. *pseudanguicula* were picked off tree trunks, while *F. mira* was found in slimy interfaces of nikau boles.

The situation in the Punctidae is more complex. Of the 27 species of Punctidae recorded from the Manukau Peninsula, only nine show countable radial sculpture. Two of these show periostracal extensions (see above). Only "*Phrixgnathus*" *poecilosticta* (Fig. 3b) shows raised, rounded calcareous radial ribs equivalent to those of the Charopidae. In appearance we rank it as a punctid equivalent of *Charopa coma* in appearing "gross," both in size and features. It is a ground surface dweller in good litter. Table 8 presents the data on the species of ribbed punctids, together with habitat preference information.

As in the Charopidae, we can suggest no clear indication of a correlation among sculpture spacing, size, and habitat preference. It is quite possible that a correlation does exist, but our knowledge of local ecology is in-

TABLE 8. Punctid radial sculpture.

Species	ASV in mm^3	Sculpture type	Habitat preference
62 "*Phrixgnathus*" *poecilosticta*	17.0	calcareous, 10/mm	ground surface, wet litter
64 "*P.*" *pirongiaensis*	1.8	sinuated, 9/mm, periostracal	wet deep litter
69 "*Paralaoma*" n. sp. 29	0.5	periostracal, 33/mm, also spiral corrugations	slimy litter
70 "*P.*" *lateumbilicata*	1.0	thin periostracal, 15/mm deciduous	friable dry litter
71 "*P.*" n. sp. 1	0.4	periostracal on low bases, 40/mm, spiral corrugations	deep fine grain litter
72 "*P.*" n. sp. 8	0.5	same as 71, higher, 12/mm	deep fine grain litter
74 "*P.*" n. sp. 40	4.3	periostracal, 13/mm, strong spiral corrugations	medium moist litter

TABLE 9. Punctids with reduced sculpture.

Species	ASV in mm^3	Habitat preference
53 "*Laoma*" *mariae*	31.1	slimy surfaces
54 *L.* n. sp. aff. *marina*	10.0	drier than species 53, but similar
55 *L. marina*	6.5	leaves and twigs in wet areas
57 "*Phrixgnathus*" *erigone*	2.5	tree trunks
58 "*P.*" *ariel*	12.3	more open tree trunks
59 "*P.*" *elaiodes*	2.8	trees with scaly bark
60 "*P.*" *moellendorffi*	6.8	ground surface under drier litter
61 "*P.*" *conella*	9.1	ground surface under moist litter
67 *Pasmaditta jungermanniae*	2.4	uncertain
68 "*Paralaoma*" n. sp. 38	1.5	slimy interfaces

adequate to recognize and define any such association.

Four punctids, *Obanella rimutaka* (Fig. 4i), *Paralaoma caputspinulae* (Fig. 4a), "*P.*" n. sp. 33 (Fig. 4c), "*P.*" n. sp. aff. 33 (Fig. 4g), have detectable traces of sculpture, but it is reduced to the point that primary and secondary riblets cannot be clearly distinguished. The degree of reduction is equivalent to that found in *Flammulina chiron.* Four punctids, *Laoma leimonias* (Fig. 3d, on older leaves near top of litter), "*Phrixgnathus*" *levis* (Fig. 3h) and "*P.*" *glabriusculus* (Fig. 3l) (both moist litter generalists), and "*P.*" n. sp. 59 (Fig. 3k, suspended litter in bracts), have smooth, shiny shells. The remaining ten punctids have irregular growth striae or faint remnants of radial sculpture. They include a variety of habitats and sizes (Table 9), and we are not able to make firm functional associations.

Of the non-punctids remaining, *Liarea hochstetteri carinella* has very weak sculptural features, the arboreal *Lamellidea novoseelandica* and the deep litter to arboreal *Omphalorissa purchasi* have smooth, shiny shells. Both species of *Delos* have shiny shells relieved by the incised lines typical of many smaller rhytidids (Solem, 1959: pl. 13, fig. 5). *Rhytida greenwoodi* and the shell remnant of *Schizoglossa worthyae* have malleated surfaces, rather than discrete radial or spiral sculpture.

In conclusion, we are unable to associate patterns of primary shell sculpture with either shell size or habitat preferences of the species. Study of the microsculpture requires SEM analysis and is beyond the scope of this project.

SHELL COLOR AND HABITAT

A detailed breakdown of color variation is presented in Table 10. There is a slight taxonomic difference, with the punctids being 51.9% monochrome and 48.1% variegated, while the charopids are 40.0% monochrome and 60.0% variegated. Variegated color is achieved in a variety of ways. Most commonly there is "tiger-striping" of alternating red and yellow irregular radial flammulations, often at least partially zigzagged (Figs. 2f, i, k, n, o, r, 3b–i). Variation of both actual and relative width of the color bands within samples of a species normally is very large and we could not discriminate classes of color band width to which species could be assigned. Nor could we categorize color brightness on a species basis.

A few species are unusual in their color variegation. *Suteria ide* has narrow red radials on yellow; *Serpho kivi* irregular red blotches on yellow; *Cavellia buccinella, Allodiscus tessellatus* and *A.* n. sp. aff. *granum* (Fig. 2c) white dots on red ground color; and *Liarea hochstetteri carinella, Cytora pallida,* and "*Paralaoma*" *lateumbilicata* become secondarily "flammulated" as wear and radially-oriented peel spots on the brown periostracum become yellowish in tone. The last three taxa would be effectively monochrome as juveniles, variegated as adults.

Particular color patterns or tones are not micro-habitat restricted. The unusual white dots on red ground color, for example, is found on the shells of dwellers in dry litter (*Cavellia buccinella*), under logs in wet litter (*Allodiscus tessellatus*), and in the upper sections of mamaku piles (*A.* n. sp. aff. *granum*).

There are slight proportionate differences in color, noticeable in respect to both arboreal and well-decomposed litter habitats. Of the 36 taxa with regular or irregular variegated color, six (16.7%) are arboreal and two (5.6%) inhabit arboreal litter (*Huonodon hectori* in tree forks or axils and "*Phrixgnathus*" n. sp. 59 in slime or wet debris of flax, nikau, or *Frey-*

TABLE 10. Taxonomic distribution of shell color.

Color	Family group				Total species
	Other	Rhytidids	Charopids	Punctids	
White	1	—	1	—	2
Yellow-brown	3	—	6	11	20
Light brown	1	—	7	2	10
Dark brown	3	—	2	1	6
White dots on red	—	—	3	—	3
Red and yellow flammulations	2	1	19	12	34
Brown, wear spots yellow	2	—	—	1	3
Irregular red on yellow	—	—	1	—	1
Greenish yellow	—	2	—	—	2
Narrow red radials on yellow	—	—	1	—	1
Totals	12	3	40	27	82

cinetia banksii), whereas of the 30 with yellow brown or light brown color, only three (10%) are arboreal ("*Phrixgnathus*" *elaiodes, Flammulina perdita, Lamellidea novoseelandica*). The latter two are found on exposed surfaces, while the first shelters under scaling bark. Two monochrome species (6.7%), "*Charopa*" *chrysaugeia* and *Omphalorissa purchasi*, are sometimes found on arboreal surfaces. Thus, only 16.7% of the monochrome taxa, but 22.3% of the variegated taxa are arboreal.

Where the leaves, fronds and twigs have been fragmented into deep friable litter, a larger difference in color distribution exists. Of the 36 flammulated taxa, only "*Charopa*" *costulata* is in wet powdery litter near logs. Four of the ten light brown colored species, two of the six brown taxa, but only one ("*Paralaoma*" n. sp. 8) of the 20 yellow brown taxa prefer this habitat.

All major color variations are found in the wet litter habitats, but we could detect no significant proportionate differences.

The predominance of the monochrome taxa in decomposed litter, a basically monochrome habitat, is not surprising, but the scatter of variegated taxa through exposed and cryptic ground surface habitats was unexpected. To what extent this will correlate with foraging into more exposed sites and sheltering in hidden, dark sites can only be determined after extensive studies of movements of species. Certainly the efficacy of variegated color in aiding concealment in many groups of animals is well documented, but we have no knowledge concerning predators on the New Zealand bush snails, and the significance of this color pattern is unknown. Compared with other land snail faunas known to the authors, the high proportion of variegated color patterns is matched only by the New Caledonian fauna, which is predominantly composed of charopids and rhytidids, compared with the charopid-punctid dominance in New Zealand.

SHELL VOLUME AND HABITAT

We are not aware that shell volume has been used previously in trying to establish niche preferences in land snails. When members of a fauna range in shell shape from planiform to globular to lanceolate, neither maximum shell diameter nor shell height will describe adequately actual shell size. We present two main analytic portraits of volume relationships. Fig. 6 graphs Adjusted Shell Volume (ASV) against shape, using H/D ratio as the shape indicator. Fig. 7 attempts to correlate shape and habitat preferences for the species actually collected in Jones Bush, the site with the largest number of species.

The few truly planulated endemic snails, three *Fectola* (25–27, Fig. 2r) and "*Charopa*" n. sp. aff. *pseudanguicula* (19, Fig. 2g, which may have a mimetic relationship with *F. mira*, Fig. 2r, see Solem, Climo & Roscoe, 1981: 465), have a H/D ratio of less than 0.450, which also is the situation in the introduced *Vallonia* (79). Nine species are between 0.450 and 0.500; 15 between 0.500 and

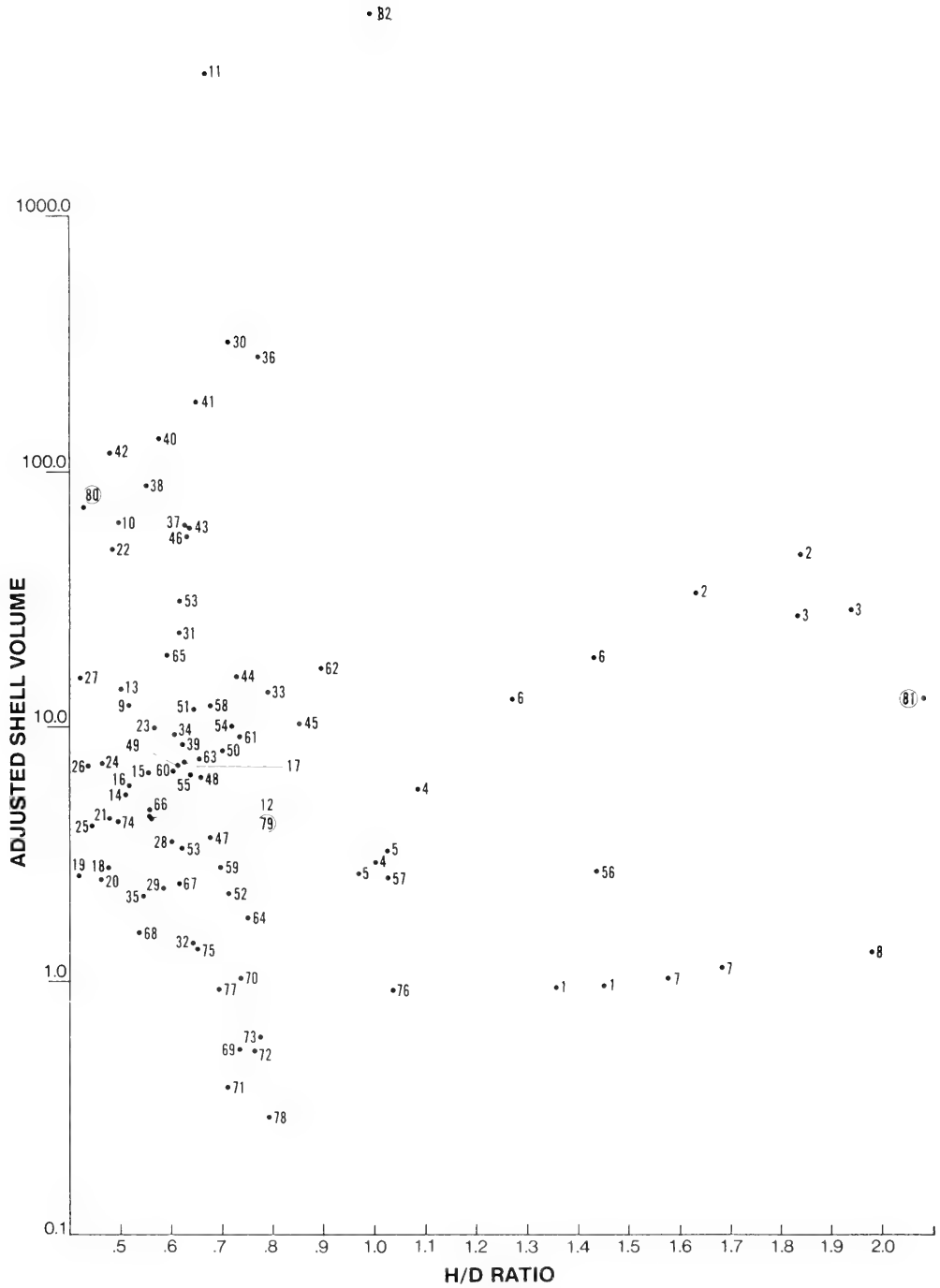

FIG. 6. Relationship between shell shape and adjusted shell volume. Numbers are those used in Table 1. Circled numbers are introduced species.

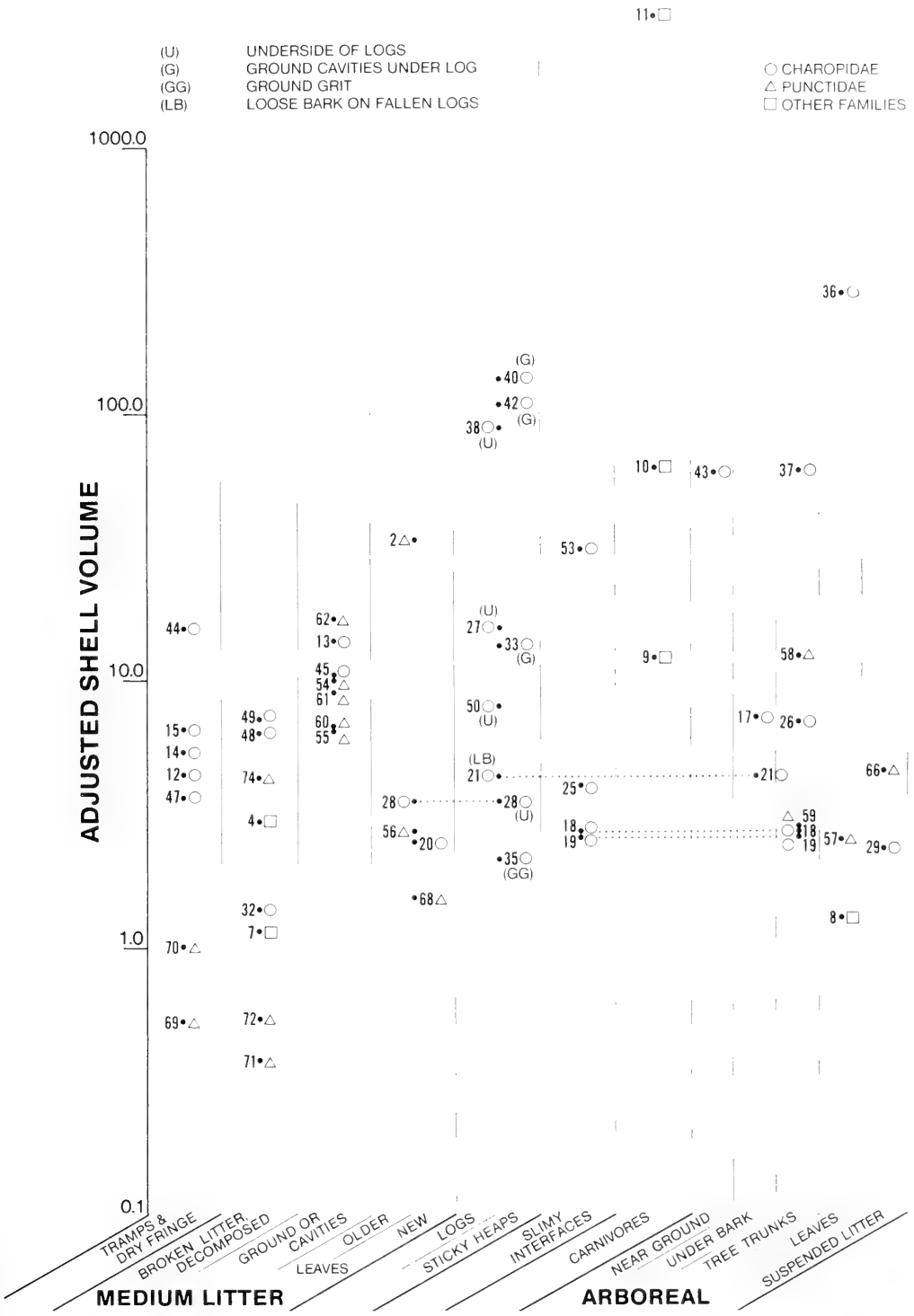

FIG. 7. Adjusted shell volume distribution within shelter site niches for species found in Jones Bush. Numbers are those used in Table 1.

0.600; 24 between 0.600 and 0.700; 14 between 0.700 and 0.800; two, "*Phenacohelix*" n. sp. (45) and "*Phrixgnathus*" *poecilosticta* (62, Fig. 3b) between 0.800 and 0.900; and 13 species above 0.900. The latter group includes the introduced *Cionella lubrica* (81) and *Helix aspersa* (82), two *Liarea* (2, 3), four *Cytora* (4–7), *Lamellidea novoseelandica* (8), *Omphalorissa purchasi* (1), and three punctids (*Laoma leimonias* [56], "*Phrixgnathus*" *erigone* [57], and "*Paralaoma*" *francesci* [76]). For the prosobranchs, both male (smaller) and female (larger) specimens have been plotted. There is a tendency for the very small, under 1 mm^3, and very large, over 120 mm^3, species to be globular, but no obvious pattern for those of intermediate size.

There are no simple habitat-shape correlations in this fauna. Of the eight truly elongated species, H/D ratio over 1.200, two (*Omphalorissa purchasi* sometimes, *Lamellidea novoseelandica* always) are arboreal, the other six (*Cionella lubrica, Laoma leimonias,* two *Liarea,* two *Cytora*) are strictly terrestrial. Of the five nearly globular species, *Helix aspersa,* "*Paralaoma*" *francesci,* and two *Cytora* are terrestrial, and only "*Phrixgnathus*" *erigone* is arboreal.

The overall taxonomic distribution of ASV is shown in Table 6, with the native and introduced faunal elements separated. The intervals are not intended to be equal, but rather to suggest where natural breaks seem to be occurring and thus to emphasize natural rather than arbitrary clusters.

The taxonomic distribution is significant, with the ASV of the Punctidae clearly less on the average than for the Charopidae. The only punctid species to exceed 30 mm^3 is "*Laoma*" *mariae* (Fig. 3a), an inhabitant of the wettest niche, slimy interfaces near the ground surface in very wet piles of litter. Both "*Phrixgnathus*" *poecilosticta* (Fig. 3b) and "*P.*" *levis* (Fig. 3h) are in the 17–19 mm^3 size range; *Laoma* n. sp. aff. *marina* (Fig. 3e), "*Phrixgnathus*" *ariel* (Fig. 3j), and "*P.*" *conella* (Fig. 3i) in the 9–12.3 mm^3 range; and the other 21 species are less than 7.5 mm^3. The only very small charopid is "*Allodiscus*" *urquharti* (Fig. 2b), 1.4 mm^3, while 10 species (25%) exceed 31 mm^3. We do not comment on the probable reasons for this situation, since no published phylogenetic interpretation of the charopid-punctid lineage exists, and this is not the place to try to fill that gap.

When volume relationships (ASV) are organized on a habitat basis for the 56 shelled species of native snails we actually collected in Jones Bush (Fig. 7), there is clear suggestion that species are rarely occupying the same space at the same size. Wherever there is less than a 40% volume difference, except for the tramp and dry fringe species, it is possible to show clear differentiation of preferred habitat. Of those taxa with a preference for decomposed, friable litter, *Therasiella serrata* (48) prefers wetter heaps of litter than *T.* n. sp. aff. *neozelanica* (49), while *Cytora torquilla* (7) prefers well-drained slopes and "*Allodiscus*" *urquharti* (32) is in very damp litter. The ground or cavity taxa appear clumped, but significant preferences do exist. Kneeling while collecting *Laoma* n. sp. aff. *marina* (54) results in soaked pants, but handpicking "*Phrixgnathus*" *poecilosticta* (62) and "*P.*" *conella* (61) produces only wetted pants. The latter two species are very different in volume (17.0 and 9.1 mm^3, respectively). *L. marina* (55) is on twigs and leaves above ground level in wet areas, while "*Phrixgnathus*" *moellendorffi* (60) prefers the ground surface on drier slopes. *Cavellia roseveari* (13) is on soil surface in medium moisture conditions, but seems often to be absent when these larger punctids are present. "*Phenacohelix*" n. sp. (45) is on medium to wet slopes with nikau and flax, often in cavities. Thus, all seven species, although clumped in size and general *type* of habitat preferred, are recognized by skilled collectors as being most frequently found in slightly to moderately different moisture regimes, or if occurring in the same conditions, show clear size differences.

Species inhabiting the upper part of the litter, freshly fallen to compacted but still undecomposed leaves, sort out vertically ("*Charopa*" *fuscosa,* 20, on older leaves and *Laoma leimonias,* 56, on newly fallen leaves), or occur in a variety of wet litter types (*Huonodon pseudoleiodon,* 28), including under stones and under sides of logs, as well as the older leaf litter.

Log or sticky pile taxa sort out nicely by shelter, except for the two largest species. Both "*Thalassohelix*" *ziczac* (40) and *Suteria ide* (42) are in cavities next to the ground under large logs, share long periostracal hairs, and reduced primary sculpture. The former is a quite rapidly moving species, while the latter is very slow moving (*teste* Winston Ponder), so that there may be highly significant foraging differences with only resting space clearly shared.

The dry fringe and tramp species *Cavellia buccinella* (12), *Mocella eta* (14), "*M.*" n. sp. aff. *maculata* (Fig. 2q, 15), and *Therasiella neozelanica* (47) form a group, differing from nearest neighbor only by about 20% in volume. There are habitat differences under conditions of limited abundance (Solem, Climo & Roscoe, 1981: 477–478), but they are not separable where common.

The only other cluster, of three tree trunk taxa, may be an artifact caused by lack of knowledge. "*Charopa*" *chrysaugeia* (Fig. 2m, 18), is a rare species that in Jones Bush was taken live on a tree trunk (one specimen) and on nikau boles (three specimens). "*C.*" n. sp. aff. *pseudanguicula* (Fig. 2g, 19) in Jones Bush had nine specimens from tree trunks and one from a nikau bole. "*Phrixgnathus*" *elaiodes* (Fig. 4h, 59) is generally found on tree trunks with scaling bark.

Allowing for such imperfections of knowledge as have just been cited, it seems that ASV is a good indicator of niche specialization, with taxa of similar volume showing different specializations, and generally only taxa with more than 40% volume difference occupying the same litter space by preference. There is no obvious differentiation of size among shelter habitats. Considerable study of exact preferences and spot abundance of these species will have to be made before this hypothesis can be confirmed or rejected, but it does suggest numerous opportunities for field study in the North Island of New Zealand.

A number of other Manukau Peninsula species that might also be found in Jones Bush were identified by Solem, Climo & Roscoe (1981). For 12 of these it was possible to specify habitat preferences. None of these conflict in volume with those currently recorded from Jones Bush, although in the interest of simplicity and understandability of Fig. 7, species 5, 23, 24, 30, 31, 34, 39, 41, 46, 51, 64 and 76 have not been plotted. For species 67 and 77, habitat preference cannot be specified at present, and "*Phrixgnathus*" n. sp. 55, although recorded from drift at Wattle Bay, Manukau Peninsula by Norman Douglas, has not been included in our discussions here.

We thus suggest that a 71 native species land snail fauna for Jones Bush would be harmonious in that a "same site" volume differential of at least 40% would exist among species. Why this volume differential exists remains to be determined. An important indication that selective forces are involved comes from the spaced distribution of ASV (Table 6), which contrasts significantly with other parameters. The spaced nature of the dispersions for the Charopidae and Punctidae strongly suggest selection for differences. We do not think that competition for food is a meaningful possibility. The massive quantities of litter available, small size of snails, presence of few carnivorous snails (two *Delos* and one *Rhytida*), lack of dominant species in terms of individual numbers, and wide movements through microhabitats, all suggest general, rather than specialized, feeding behavior.

We suggest that investigation of ASV relationships among sympatric taxa in other areas of the world may yield highly significant results in regard to coexistence of land snail species.

SHELL SHAPE AND HABITAT

The shape distribution of land snails in a number of faunas has been reviewed by Cain (1977, 1978a–b, 1981a–b). He demonstrated a typical bimodal pattern of scatter when maximum height is plotted against maximum diameter. The high-spired, generally multiwhorled shells have the height significantly greater than the diameter and occupy an upper scatter, while the discoidal to low-spired shells, generally with comparatively few whorls, occupy a low scatter. Except for the fauna of the Philippines and New Guinea (Cain, 1978a), which show in part an intermediate scatter indicating a near globose shape, Cain's observations are of clear-cut shape dichotomy. His suggestion that the "tall" taxa are arboreal, or at least forage on vertical surfaces, has received some support in regard to the British fauna by the studies of Cameron (1978) and Cain & Cowie (1978).

An equivalent diagram for the Manukau Peninsula taxa is presented in Fig. 8. Direct comparisons with Cain's diagrams are complicated by the necessity to use a double logarithmic plot for the Manukau taxa. Cain used arithmetic scales as he, in general, was dealing with relatively large taxa. The Helicidae, for example, are in the 5–50 mm size range (Cain, 1981b; 151, 156, fig. 10). Even when faunistic diagrams include smaller Orthurethra and aulacopod taxa, for example Cain (1981b: 158–164, figs. 11–17), most of the species are larger than 10 mm in diameter.

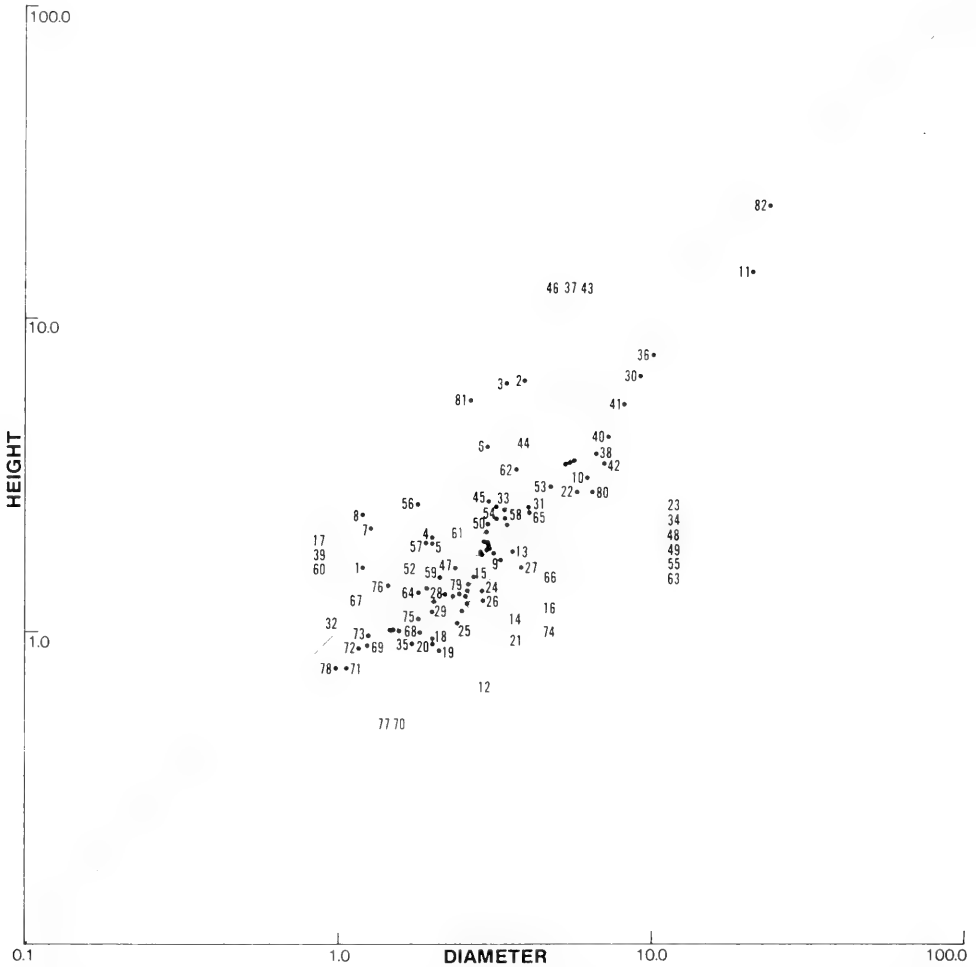

FIG. 8. Relationship between shell height and shell diameter in Manukau Peninsula land snails. Numbers are those used in Table 1.

The Manukau taxa, in contrast, are quite small. Only two native species, *Rhytida greenwoodi* (11) and *Serpho kivi* (36) plus the European *Helix aspersa* (82) exceed 10 mm in diameter, and two native species, *Allodiscus dimorphus* (Fig. 2a, 30) and *Therasia decidua* (41) are 8–10 mm in diameter. Of the 82 species, 68 (82.9%) are less than 5 mm in diameter and 74 (90.2%) are less than 5 mm in height. The Manukau fauna is thus composed of much smaller snail species than in the faunas analyzed by Cain. Not only is there an order of magnitude size differential, but the Manukau taxa do not break up into separate scatters. Most species lie just below or moderately below the mid-line, significantly above the "low scatter" position of Cain's diagrams. The few taxa above the mid-line are phyletically diverse, comprising the hydrocenid *Omphalorissa purchasi* (1); both *Liarea* (2, 3) and two of the four *Cytora* (6, 7) in the Liareidae; the only achatinellid species, *Lamellidea novoseelandica* (8); a punctid, *Loama leimonias* (56); and the introduced *Cionella lubrica* (81). Of these eight "tall taxa," only *Lamellidea* is primarily arboreal. *Omphalorissa* is occasionally arboreal and the remaining six species are litter dwellers. Since only about

⅓th of the Manukau taxa is arboreal, this habitat distribution of "tall taxa" is normal.

The greater central tendency of the Manukau scatter and the absence of a clear dichotomy is highly distinctive. We have very little understanding of the physical forces exerting selective pressures in the litter. It is quite probable that small sized species are more affected by adhesive tendencies of moist granules or decayed fragments of plants, can move through litter regardless of shell shape with relative ease, and are more sensitive to micro-differences to moisture than the generally larger taxa of the Northern Hemisphere faunas charted by Cain.

The reasons for the difference in the Manukau fauna remain to be investigated, but the existence of this altered pattern is obvious.

DISCUSSION

Surprisingly few correlations between particular structures and habitat preference were identified in this analysis. Presence of a sharp keel was associated with taxa occupying the ground surface under thick, moist to wet litter, although peripheral angulation of the shell was found in taxa occupying a variety of habitats. There was no phyletic basis to the keel-habitat association. Periostracal fringes or hairs occurred mostly in species sheltering in friable, broken-down litter, but many species in this habitat did not have periostracal extensions. There was a slight overrepresentation of shells with variegated color pattern among arboreal taxa, and a stronger association of light brown or dark brown monochrome taxa with friable, broken-down litter.

We must stress that a majority of the species has been found in several microhabitats (Solem, Climo & Roscoe, 1981: 463, table 5) and our own collecting demonstrated that taxa sheltering in slime-filled interfaces of nikau boles or mamaku fronds at least sometimes forage on tree trunks. Other taxa may be more sedentary, i.e., *Therasiella, Liarea, Cytora,* but until information is accumulated on species movements, more precise correlation of structure and habitat will not be possible. Many structural features may be advantageous during foraging, but neutral to mildly disadvantageous in shelter or mating areas. Thus, we consider our results to be preliminary and of value primarily in pointing out where additional work is needed. We have chosen not to discuss in detail the possible ecological significance of the color and shape correlations as deduced from equivalent observations on insects and vertebrates, since predator, diel cycle movement, and feeding data are not available for the snails.

More significance can be placed on the departures from the expected patterns that we demonstrate. In both the Endodontidae (Solem, 1976) and Charopidae (Solem, 1983) from the Pacific Islands, radial rib spacing and prominence correlates closely with shell size and habitat. Smaller species have more and more crowded ribs, larger species have fewer and more widely-spaced ribs. Large species and semiarboreal taxa have a tendency toward reduction or loss of shell sculpture. Discovering that rib prominence and spacing in the New Zealand Charopidae and Punctidae (Tables 7–9, Fig. 5) is not size or habitat correlated was surprising.

Similarly, the absence of a bifurcated scatter to the plot of shell height and shell diameter (Fig. 8) opens up many intriguing possibilities for investigation both in New Zealand and for parallel studies elsewhere. Is the smaller size of the New Zealand species a major factor? Do the New Zealand species have a greater vertical foraging zone than members of northern faunas? Where do the South African and Australian faunas plot out in relation to the two observed modes? What are the mechanics of movement through litter in terms of shell size and shape? To what extent is the bimodality of plots for species from other areas a function of shell geometry and whorl number factors?

Finally, perhaps the most significant finding is the discovery of spaced distribution of shell volume (Table 6) in the Manukau fauna and the existence of clear volume differential among taxa in the same shelter preference site (Fig. 7). While it is tempting to invoke Hutchinsonian ideas of species size relationships (Hutchinson & MacArthur, 1959) to explain the volume differential, the lack of data on feeding, movement, life history, and species interactions makes such an attempt premature. In addition, the recent review of Simberloff & Boecklen (1981) questioning the reality of a size ratio among sympatric taxa suggests caution in making sweeping conclusions from our preliminary data. We choose to point out the phenomenon and suggest that investigation of volume relationships in other faunas should yield important information and ideas.

After completion of this manuscript, the re-

port of Waldén (1981) on high diversity communities of land snails in talus and boulder slopes in Sweden was received. His conclusions regarding accumulation of species in habitats parallels many of the conclusions we presented in Solem, Climo, & Roscoe (1981), but his study does not address questions we attempt to answer in this report.

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APPENDIX 1. Method of shell volume calculation and intrapopulational variability

The protruding spire of the shell is considered to be a cone, and its volume is computed using the Spire height (Fig. 1, B–E) and Spire diameter (C–D). If the spire is flat-sided as in *Liarea* and many of the larger punctids (Figs. 3a, e, f, g), this can be very accurate. When the whorls are rounded, as in many charopids (Figs. 2c, e, n), using the suture-to-suture distance results in the actual whorl profiles dipping in and out of the projected cone profile. These dips would tend to cancel each other, and we consider the variation introduced by this factor to be negligible. In only a few taxa, such as *Laoma leimonias* (Fig. 3d) with its "U"-shape, and *Obanella rimutaka* (Fig. 4i), which has concave sides to shell spire, is this calculation noticeably inaccurate. In most taxa, the spire forms a small proportion of the total shell volume, so even these errors are considered to be relatively minor.

In a separate calculation, the body whorl is treated as a cylinder, using the measurements Body whorl height (Fig. 1, E–G) and Shell diameter (A–B), to calculate its volume. This includes two major sources of error: 1) the body whorl is one volution of a logarithmic spiral, and thus does not conform to a circle; and 2) there usually is clear descension of the body whorl from the previous suture (Fig. 1, E–F), so that the aperture is deflected significantly downward from the initial plane of body whorl coiling (see Figs. 2f, q, r, 3k, l, 4b). The curvature of the whorls produces corner volume that is not an actual part of the shell. There also is a slight increase in whorl cross-sectional areas from the beginning of the body whorl to the aperture. We consider that the changes in whorl cross-section and body whorl contour are minor and would be roughly equivalent for each species. Exceptions are the few species in which the whorls are laterally flattened (Figs. 2r, 3d). Here there is less "corner volume" included in the volume calculation. Protrusion of the periphery into a keel (Figs. 2p, 3e, f, 4e, i) normally results in narrowing of the body whorl height, thus partly compensating for the increased "shell corner volume." For our purposes, these changes are considered to be minor. The error introduced by the coiling being a logarithmic spiral, rather than a circle, would be roughly the same in all species.

The significant variable among species is the degree of Spire descension (Fig. 1, E–F). This ranges from none in the planulate *Geminoropa cookiana* (see Climo, 1981: fig. A–C), up to 44% of the Body whorl height in *Serpho kivi, Cytora hedleyi,* and *C. torquilla* (see Powell, 1979: figs. 12-3, 12-5, pl. 65, fig. 9). We have adjusted the body whorl volume by deducting the percentage descension of the spire from the raw calculated volume. The adjusted body whorl volume and the spire volume were then added to produce the Adjusted Shell Volume (ASV). We utilize this figure as a complete volume estimate of the physical space occupied by the adult shell in the habitat. The included minor additional space resulting from peripheral contours and the difference between the spiral versus

circular coiling can be viewed as necessary room for the foot to protrude partially and the snail to start crawling.

The umbilical opening at the base of the shell is a small to significant volume of space. It is not enclosed by the shell in most New Zealand species. In rare cases, it is secondarily narrowed and used as an egg brood chamber (such Pacific Island Endodontidae as *Libera, Gambiodonta, Endodonta, Pseudolibera,* and *Taipidon semimarsupialis,* see Solem, 1976, and the New Zealand "*Fectola*" *marsupialis* [Powell, 1941]), or it may be used as an egg deposition site by spiders or insects. The effect of umbilical size on Adjusted Shell Volume is indicated in Fig. 9. Of the 78 native New Zealand species reported from the Manukau, 31 had a relatively open, "U"-shaped umbilicus, and nine showed in central section a more nearly straight-sided "V"-shaped umbilicus. Seven species had the umbilicus completely closed by reflection of the columellar lip, three species had it present as a lateral crack, and 28 species had the umbilicus less than 0.3 mm wide with a D/U ratio of 9.7 to 100. The volume of their umbilici would be negligible (see Fig. 9).

Calculation of the umbilical volume was done in two ways. For the taxa with "V"-shaped umbilici, Body whorl height less Body whorl descension plus Spire elevation provides an indication of umbilical depth. The space approximates that of a cone, except that the very tip would be truncated by the apical whorls. We have not compensated for this, since the tip of the cone contains trivial volume. There would be greater error by bringing the tip of the cone to the base of the apical whorls. We also recognize that the curved inner walls provide spaces that we are not measuring. Calculating the umbilicus as the volume of a cylinder provides comparative figures. Since the "V"-shaped umbilici are mostly narrow, their impact on total shell volume (Fig. 9) is quite small, and their shape produces a clear offset from the curve for the "U"-shaped umbilici.

Calculating the volume of the "U"-shaped umbilicus as that of a cylinder presents additional possibility for error. The upper end is clearly dome-shaped, not nicely truncated, and there will be some umbilical narrowing toward the apex. To compensate for this, we have reduced the "Body whorl height less Body whorl descension plus Spire elevation" measure by 20% to allow for these space losses. Utilizing these assumptions and expressing Umbilical volume as a percentage of ASV, we plot this percentage against proportionate umbilical width (Fig. 9). When the umbilical opening is less than a fifth of the shell diameter, the volume of the umbilicus is less than a twentieth of the shell itself. Only when the umbilicus is a third or more of the shell diameter, does the umbilical area represent a significant portion of the shell volume. We consider that ignoring this factor in our analysis is justified.

Data on variability within populations is presented for several species (Table 11) and compared with measurements of the representative adult used in the main analysis. The measured population samples are from the National Museum of New Zealand's mollusk

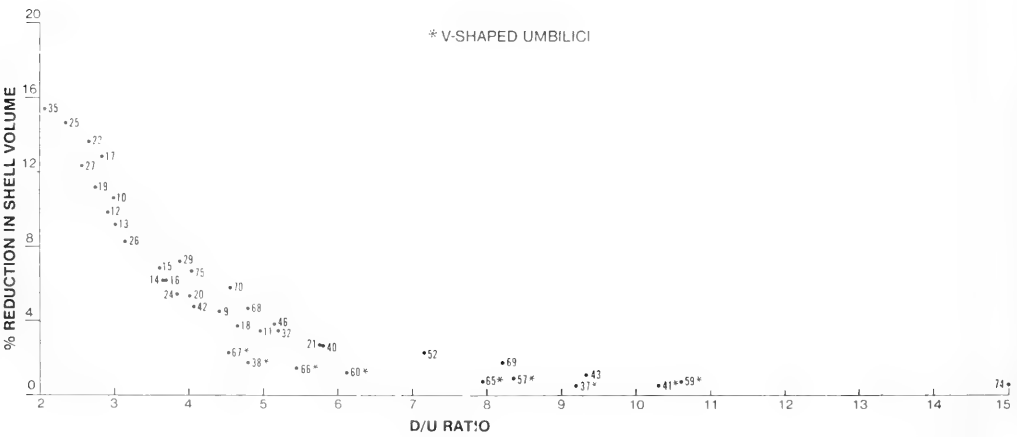

FIG. 9. Effect of umbilical size on adjusted shell volume. Numbers are those used in Table 1.

TABLE 11. Variability within selected populations.

Species and catalog number	Number of measured adults	Mean, range and standard deviation of: Shell height in mm	Shell diameter in mm	H/D ratio	Whorls	Adjusted Shell volume in mm^3
Lamellidea novoseelandica M 51794	8	2.64 ± 0.170 (2.33–2.93)	1.33 ± 0.055 (1.25–1.38)	1.987 ± 0.107 1.868–2.225)	5– (4⅝– to 5⅜)	1.67 ± 0.340 (1.20–2.14)
SELECTED ADULT		2.37	1.20	1.975	4¾	1.30
Mocella eta M 69320	10	1.22 ± 0.091 (1.05–1.35)	2.43 ± 0.094 (2.30–2.60)	0.502 ± 0.025 (0.457–0.541)	4 + (3⅞– to 4¼)	4.38 ± 0.643 (3.58–5.50)
SELECTED ADULT		1.30	2.55	0.510	4¼	5.44
"*Thalassohelix*" *ziczac* M 51728	6	4.22 ± 0.303 (4.05–4.90)	7.65 ± 0.386 7.26–8.30)	0.578 ± 0.012 (0.559–0.591)	5¼ (5⅛– to 5½–)	152.2 ± 27.51 (125–200)
SELECTED ADULT		4.20	7.25	0.579	5⅛	134.5
Phenacohelix ponsonbyi M 57366	9	3.36 ± 0.308 (3.01–3.79)	5.37 ± 0.311 (4.84–5.75)	0.625 ± 0.031 (0.568–0.667)	4⅞ + (4½ to 5¼–)	53.5 ± 7.63 (41.5–64.7)
SELECTED ADULT		3.40	5.40	0.630	5¼–	56.7
Laoma n. sp. aff. *marina* M 51790	11	2.16 ± 0.100 (2.01–2.40)	3.05 ± 0.111 (2.93–3.29)	0.707 ± 0.025 (0.660–0.744)	5¼ (5– to 5½)	8.68 ± 1.25 (7.04–11.3)
SELECTED ADULT		2.30	3.20	0.719	5½ +	10.00
"*Phrixgnathus*" *pirongiaensis* M 63499 (extralimital)	12	1.56 ± 0.059 (1.45–1.66)	1.98 ± 0.040 (1.91–2.04)	0.788 ± 0.026 (0.739–0.826)	5¼– (5 to 5⅜)	2.45 ± 0.150 (2.22–2.71)
SELECTED ADULT FROM MANUKAU PENINSULA		1.37	1.83	0.749	5¼	1.78

collection and consist of dead adult shells. They are from Jones Bush, Manukau Peninsula or near Waitomo Caves, except for "*Phrixgnathus*" *pirongiaensis*. Only two specimens of the latter species were taken from kauri litter on the Manukau (Solem, Climo & Roscoe, 1981: 470, 484). They were small and barely adult. Thus a very small adult was selected from an extralimital set with many specimens. Subsequent measuring of representative adults from that sample (Table 11) resulted in a much larger size. This example is included here to emphasize both the geographic variability within New Zealand land snail species, and the slight intrapopulational variability shown by this inhabitant of deep wet litter.

The choice of sets included in Table 11 was limited by: 1) availability of sufficient adult specimens; 2) an attempt to include a sample of the family taxa; and 3) to use materials representative of different habitat preferences. *Lamellidea novoseelandica* is an arboreal achatinellid; *Mocella eta* is a charopid found in drier fringe area litter; "*Thalassohelix*" *ziczac* lives in deep wet litter that is well shaded and is one of the larger taxa; *Phenacohelix ponsonbyi* is a charopid from well-drained slopes in moderately wet, undisturbed areas; *Laoma* n. sp. aff. *marina* typically lives on wet ground surfaces under broad leaf litter and is a large, keeled punctid; and "*Phrixgnathus*" *pirongiaensis* is a keeled, small punctid from very wet litter.

Most measurements of the selected representative adults are well within one standard deviation of the population means. Since these specimens were selected prior to the population sample measuring and analysis, we are confident that the individuals used of *all* species fairly represent the size and shape of the Manukau morphotypes. The selected *Lamellidea* is a little smaller, and the *Mocella* a little larger than the means, but these differences are much less than the basic 40% differences between species used in the analysis. We consider that the data basis is adequate for this study.

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SYSTEMATIC REVISION OF THE HYDROBIIDAE (GASTROPODA: RISSOACEA) OF THE CUATRO CIÉNEGAS BASIN, COAHUILA, MÉXICO

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ABSTRACT

This study gives detailed morphological descriptions, including aspects of shell and soft-part anatomy, for 12 species of nine genera of hydrobiid snails (Gastropoda: Rissoacea) from the isolated desert spring system of Cuatro Ciénegas, Coahuila, México. Snails were collected from 103 localities in the basin and summaries of the distribution and ecology for each species are given. One new genus and three new species are described.

The six nominal species of *Mexipyrgus* are reduced to one variable species, *Mexipyrgus churinceanus,* as there are no suites of morphological features that can consistently define separate taxa when a large number of populations is studied. A multivariate morphological analysis of *Mexipyrgus churinceanus,* involving 20 morphological characters from 33 populations in the basin, shows that the trends of variation only partly follow the distribution of populations among the drainage systems of the basin.

Contrary to previous thought, there are no subfamilies of hydrobiids endemic to the Cuatro Ciénegas basin; all taxa studied belong to either the Nymphophilinae or Littoridininae, widely distributed subfamilies. Five genera and at least nine species are endemic to the basin. Phenetic and phyletic analyses show that of the five endemic genera, three are more closely related to nonendemic genera found in the basin than to each other, suggesting a polyphyletic origin for the endemic snails. The endemic snails may also be of a more recent and local origin than once thought. Snail taxa from the Pliocene Pebas Formation of Peru, the shells of which are superficially similar to those of the Cuatro Ciénegas endemic taxa, are not Hydrobiidae and thus the conchological similarity is due to convergence.

Four of the Cuatro Ciénegas hydrobiid genera are ovoviviparous. Anatomical studies show that the evolution of this reproductive mode in the Hydrobiidae has involved modifications of the female reproductive system to separate incoming sperm from outgoing embryos, increase the amount of space available for holding embryos, and allow for control of the release of young.

Key words: Hydrobiidae; Cuatro Ciénegas; systematics; morphology; endemism; evolution; ovoviviparity.

INTRODUCTION

The small (30 by 40 km) desert valley of Cuatro Ciénegas, Coahuila, México (Fig. 1) harbors a remarkable endemic biota (Contreras, 1978; Minckley, 1969). Most of the endemic taxa are associated with the extensive spring fed aquatic environments of this closed-drainage basin and include one genus and four species of crustaceans (Cole & Minckley, 1966, 1970, 1972; Holsinger & Minckley, 1971), eight–ten species of fishes (Minckley, 1977), and two species of turtles (Schmidt & Owens, 1944; Webb & Legler, 1960). In addition, three subfamilies, five genera, and 12 species of hydrobioid snails (those rissoacean snails that resemble *Hydrobia* in shell, operculum, penis, or radula) have been considered endemic to the valley (Taylor, 1966).

Apart from their high endemism, the hydrobioid snails of Cuatro Ciénegas are of interest for the following reasons: 1) a number of taxa have large, sculptured or color-banded shells whereas most hydrobioids have small, smooth shells without color bands; 2) several taxa may have been involved in coevolution with snail-eating cichlid fishes in the valley (Vermeij & Covich, 1978); 3) the snails are deployed within a nearly unique variety of spring fed aquatic environments within the desert; 4) differentiated populations of snails are found among the various springs of the valley and offer the opportunity to study evolution in a natural laboratory (Taylor, 1966; Taylor & Minckley, 1966).

The original description of the Cuatro Ciénegas hydrobioids (Taylor, 1966) stimulated this study and stands as an exemplary contribution for that time period. Credit should

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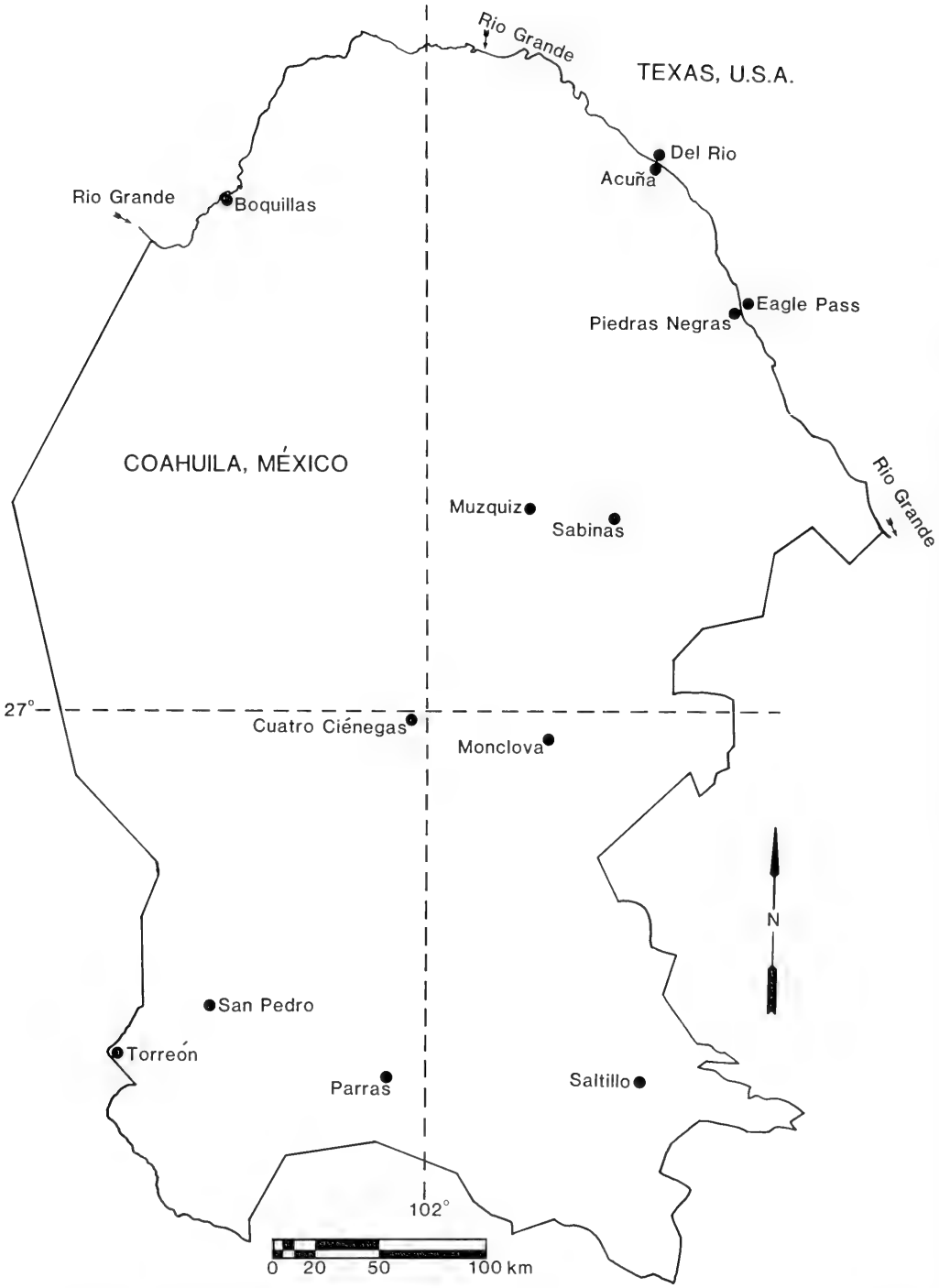

FIG. 1. Map of Coahuila, México, showing the location of the Cuatro Ciénegas Basin. The arrows indicate the direction of flow of the Rio Grande.

be given to that author for recognizing the uniqueness of both the hydrobioid fauna and the environmental setting.

The classification scheme within which the Cuatro Ciénegas hydrobioid snails were described was based on a character set restricted to shell, operculum, penis, and a few other aspects of external morphology (Taylor, 1966). It is now known, on the basis of overall soft part anatomy, that such characters have frequently converged in hydrobioid taxa that are not closely related (Davis, 1979). In a series of papers (Davis, 1968, 1979, 1980; Davis *et al.,* 1976, 1982; Davis & Pons da Silva, 1984) it was shown that the Hydrobiidae (pre-1980) are polyphyletic and that study of all aspects of soft part morphology, particularly the entire female reproductive system, is necessary to recognize convergences and clarify the systematic relationships of hydrobioid snails. As the original descriptions of the Cuatro Ciénegas hydrobioids did not include aspects of internal anatomy, these taxa's classification is suspect. The species descriptions were usually based on collections from single localities, and only 12 localities were sampled (Taylor, 1966).

This paper: 1) presents detailed morphological descriptions of the Cuatro Ciénegas hydrobioid taxa and assesses their systematic affinities, the results of which are frequently at odds with previous classification (Taylor, 1966). One new genus and three new species are described. Morphological data from populations are analyzed to resolve species problems; 2) summarizes the distribution and ecology of each species (103 localities sampled in the valley); 3) discusses the results of the above as they relate to the origin, evolution, and endemism of the hydrobioid snails of Cuatro Ciénegas; 4) discusses the evolution of ovoviviparity[1] in hydrobioid snails.

Environmental Setting

Cuatro Ciénegas lies in the mideastern section of the Chihuahuan Desert (Miller, 1977; Fig. 1). The valley receives less than 200 mm of precipitation annually (Minckley, 1969). The mean annual temperature is 23°C (Morafka, 1977), with midday summer temperatures exceeding 40°C. The valley floor is 740 m above sea level, bounded on all sides by tall (to 3000 m) peaks of the Sierra Madre Orientale. The valley floor is relatively flat.

The basin consists of two lobes, separated by the northern end of the Sierra de San Marcos (Fig. 2). There are far more springs in the eastern than in the western lobe. Springs are particularly concentrated around the Sierra de San Marcos. The springs vary: there are small seeps; small springs with spring pool areas of less than 10 m^2 that run for only tens of meters; and much larger springs with spring pool areas in excess of 900 m^2 and depths to 7 m, whose outflows are large streams. While most of the springs are limnocrenes, with spring pools at the heads, there are also rheocrenes, where water rushes out of the ground as flowing streams. Other aquatic environments include playa lakes, receiving flow from large streams; spring fed pools that have no outflows; and extensive spring fed marshes. This great diversity of desert spring fed aquatic environments can only be matched in North America by that seen in the Death Valley–Ash Meadows area (Deacon & Minckley, 1974; Soltz & Naiman, 1978).

Spring levels vary seasonally, as the water table rises in the winter and drops in the summer. The spring water is generally quite hard and high in sulphates (Minckley & Cole, 1968). Most of the springs are thermal (to 34°C), but cooler (14–25°C) springs are also found. The larger springs have fairly constant water temperatures throughout the year (Minckley, 1969), while spring runs and shallow pools can be subject to considerable variation in water temperature. For example, North Spring has a spring pool area of about 230 m^2 and a maximum depth of 1.5 m. Its waters flow into a second pool and then run as a wide shallow stream for 77 m before disappearing into a hole. The spring is thermal; 10 separate headspring temperature readings during 1981 gave a mean of 32.9°C (29.5–34.5°C). Maxi-mini thermometer readings for four days (beginning 6/18/81) had a variation of 31–34.5°C for the head and 20.6–37.8°C at the hole where the water goes underground. Thus, during this time period, the headspring water temperature varied only 3.5°C while downstream it varied 17.2°C.

The larger springs and their outflows have considerable microhabitat diversity, usually including several types of aquatic vegetation (sedges, *Nymphaea, Chara, Utricularia*); a soft sediment consisting of snail copropel and/or an algal-detritus mixture; a sand con-

[1]By ovoviviparity I mean brooding young without direct tissue connection, following Van der Schalie (1936).

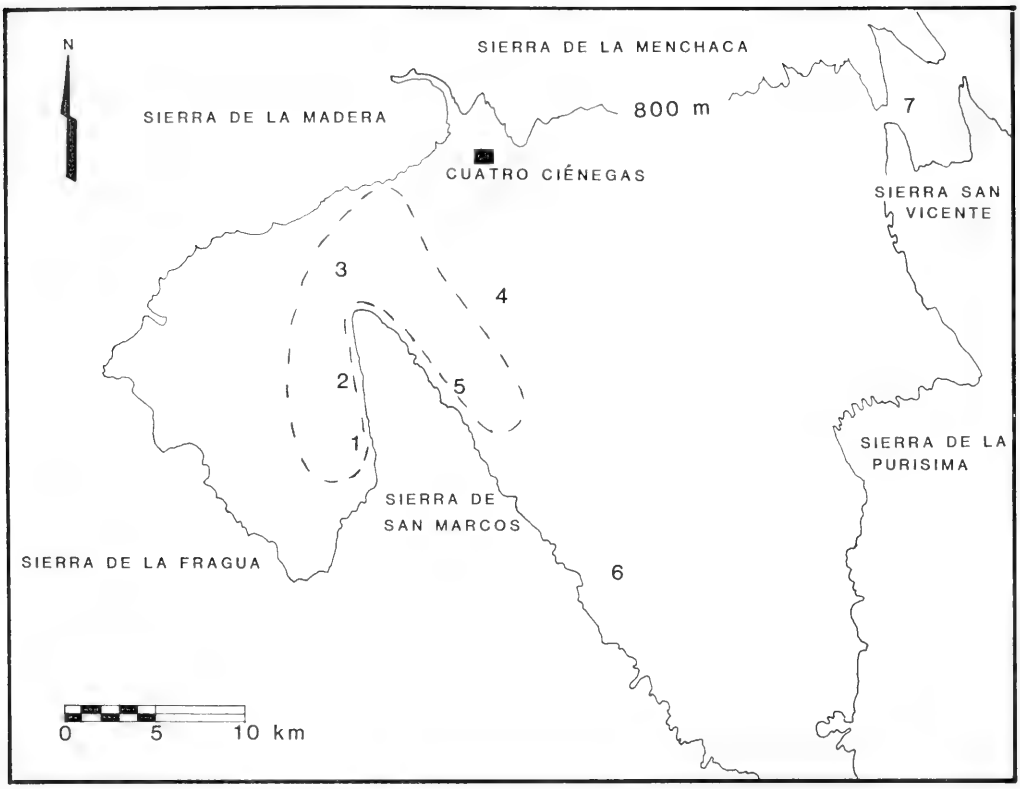

FIG. 2. Map of the Cuatro Ciénegas Basin, showing the portion of the basin that was intensively sampled (area enclosed by dashed lines). The numbers indicate the origins of the seven major drainages of the basin (from Minckley, 1969): 1, the Churince system; 2, the Becerra system; 3, the Rio Mesquites system; 4, Rio Puente Chiquito; 5, Tio Candido; 6, Santa Tecla Laguna; 7, Rio Salado de Nadadores.

sisting of travertine pieces and shell debris; large travertine blocks; and the banks, which can be gently sloping or greatly undercut. The smaller springs and their outflows have fewer microhabitats, typically including a dark organic mud, fine travertine sand, and occasional *Chara* mats.

Five to seven major drainage systems occur in the valley (Fig. 2), with possible natural connections existing between them via underground rivers, or surficial waters during rainy periods (LaBounty, 1974; Minckley, 1969). A number of irrigation canals drain water from the large springs, lowering their levels and destroying peripheral aquatic habitats (Minckley, 1969). Irrigation canals from different drainage systems are often connected, offering opportunities for gene flow between previously isolated populations. The basin currently has no surficial connection to outside drainage, but water has probably drained from the basin to the nearby Rio Salado de Nadadores (Fig. 2, Locality 7) in the past (Miller & Minckley, 1963; Minckley, 1969). More information on the aquatic environments of the basin can be found in Arnold (1972), Brown (1974), Deacon & Minckley (1974), and Minckley (1969).

MATERIALS AND METHODS

Localities

The waters of only a portion of the valley drainage (dashed line in Fig. 2), encompassing parts of four of the basin drainages, were intensively sampled. One hundred collection localities from this area are shown in Fig. 3 and described in Appendix 1. Three other localities (101–103 in Appendix 1) from other areas were also sampled. The various locali-

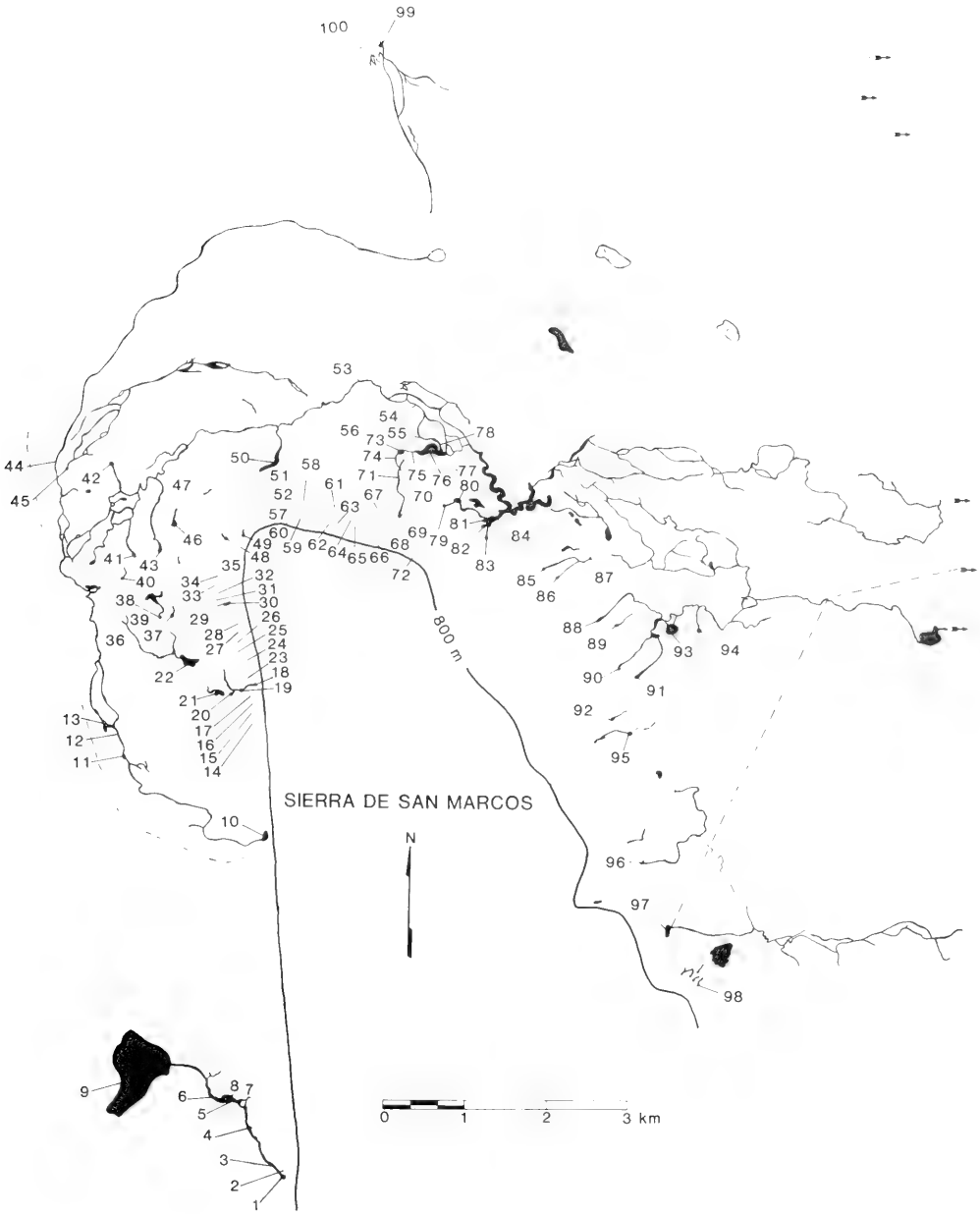

FIG. 3. Map of the portion of the basin drainage that was intensively sampled. The numbers (1–100) refer to collection localities. The dashed lines refer to irrigation canals. The arrows indicate waters that continue to flow toward the east.

ties (and ANSP catalog numbers for the lots) for each species are given in Appendix 2. Snails were collected and studied in the valley during September, 1978; April–August, 1979; April, 1980–June, 1981; and December, 1981.

Collection Methods

Fine hand sieves were used to collect snails from sediments ranging from flocculent copropel to coarse travertine sand. In some cases, the sediment itself was collected and

examined under a dissecting microscope to determine whether very small snails, which would pass through the sieves, were present. Snails were picked off large pieces of travertine using tweezers. Samples of aquatic vegetation were collected and carefully washed in a bucket to remove clinging snails. The material was then sorted under a dissecting microscope. A number of snail taxa are suspected of living in groundwater outlets or subterranean waters of the valley (Taylor, 1966). To collect such snails, ordinary domestic mops were placed into small springheads, removed after a 24 hour period, and then washed in a bucket. The snails that colonized the mops were collected and sorted under a dissecting microscope (method suggested orally by Dr. W. L. Minckley; similar method described in Holsinger & Minckley, 1971). For each small springhead, one to ten mop samples, spread out over a period of months, were taken. This method is crude as it cannot distinguish between snails living at the groundwater outlet and those being washed out from underground. A superior method, sampling only snails from subterranean waters, would be to place a fine mesh net over the groundwater outlet, essentially filtering snails from the water stream; such a method has been successfully used to sample the fauna of artesian wells (Holsinger & Longley, 1980).

Anatomy

Dissection techniques are those of Davis (1979) and Davis & Carney (1973). Several other techniques were also employed. The penis was cut from the male, examined on both sides at 50× magnification, and then wet-mounted on a slide with cover slip for study using a compound microscope fitted with an ocular micrometer. The length of the pallial oviduct is the length from the anterior end to the posteriormost point, excluding any length bent back upon itself at the posterior end. The length of the duct from the seminal receptacle is the length from the seminal receptacle body to the junction with the oviduct or sperm duct. The nervous system was studied for species of all genera except *Mexistiobia* n. gen. and *Coahuilix* and showed no variance except in size and concentration of ganglia. Therefore, the measurements of the nervous structures are not presented. For ovoviviparous species, shelled embryos were gathered by cracking the shell of an adult female, removing the brood pouch, and placing it in a drop of CLOROX for several minutes (Davis, 1969b). The embryonic shells were then counted and, in some cases, measured. In addition, the brood pouches of several snails were teased apart so the small, nonshelled embryos could be counted. Shell measurements for the various taxa are from mature adults (those having a complete aperture). The apical whorls of shells were measured using the method of Davis (1967, pl. 3, fig. 6). Radulae and shells were studied and photographed using the SEM facility at the Academy of Natural Sciences of Philadelphia. Statistical techniques, for the most part, are restricted to t-tests and correlation coefficients.

The generic descriptions are necessarily brief, as anatomical data is generally available for only one species per genus (due to monotypy or lack of studies of congeners). The descriptions will have to be altered as more data become available. Only character states unique to the various taxa, or of use in assessing their systematic status, are stressed. Other characters and their character states that are standard for the Rissoacea: Hydrobiidae (Davis, 1966, 1979; Hershler & Davis, 1980) are not mentioned and include, for example, the characteristic loop of the intestine above the style sac, the position of the salivary glands on top of the nerve ring, and the ovoid shape of the fecal pellets. Fifty-one characters and their character states that were used to distinguish genera and generic groups in the Hydrobiidae are listed in Appendix 3 with notations as to where they are figured. Common radular formulas for species studied are given in Table 1. Several characters may be unfamiliar to the reader and require explanation. The bolster and ventral channel of the pallial oviduct are defined and discussed in Davis *et al.* (1982), and Davis & Pons da Silva (1984). The caecal chamber (defined in Davis *et al.*, 1982), while apparently present in all Hydrobiidae, is reduced in some taxa so as not to project posterior to the stomach. Tentacle ciliation is discussed in Davis *et al.* (1982). The digestive gland of hydrobioid snails usually has finger-like tubercles projecting from the main body, but in small sized snails the tubercles may be mere swellings. In resolving species problems, emphasis was placed on whether purported species were sympatric or not, and whether consistent morphological differences between purported species could be found

TABLE 1. Generalized cusp formulas for the four tooth types of the radula of all species studied.

Species	Central	Lateral	Inner marginal	Outer marginal
Nymphophilus minckleyi	4(5)–1–4(5) / 3–3	2(3)–1–2(3)	12–17	15–20
Mexistiobia manantiali	4(5)–1–4(5) / 1–1	4(5)–1–3	19–24	22–26
Coahuilix hubbsi	3(4)–1–3(4) / 1–1	5–1–3(4)	16–21	16-19
Paludiscala caramba	4(5)–1–4(5) / 1–1	4(5)–1–3	18–24	16–25
Cochliopina milleri	4(5)–1–4(5) / 1–1	3(4)–1–3	18–25	19–28
Mexithauma quadripaludium	4(5)–1–4(5) / 2(3)–2(3)	3(4)–1–3(4)	10–14	12–16
Durangonella coahuilae	4(5)–1–4(5) / 1–1	4(5)–1–4(5)	19–27	20–27
Mexipyrgus churinceanus	4(5)–1–4(5) / 2(3)–2(3)	4(5)–1–4(5)	21–36	24–38

when numerous populations were studied. The descriptions of taxa not named in this paper are modified from those of Taylor (1966) and Thompson (1979).

For a multivariate analysis of *Mexipyrgus churinceanus* populations the computer program used was the June, 1974 version of the SUNY at Stony Brook numerical taxonomy program, NT-SYS (Rohlf *et al.*, 1972). Characters were standardized in the usual manner (Sneath & Sokal, 1973). In the Q-mode analysis, a taxonomic distance matrix was generated, using the unweighted pair-group method with arithmetic averaging (UPGMA). The minimum spanning tree (MST) and "subsets" components of NT-SYS were used. For the R-mode analysis, character correlations were subjected to Principal Components Analysis (PCA), with the first three components used to yield a matrix of OTU projections in principal component space. These OTU locations in the three-dimensional PCA space were used as the initial configuration for a nonmetric multidimensional scaling (MDS) placement of the Q-mode taxonomic distances between OTUs. The Prim Network was used. As the cluster analysis and phenograms generated are subject to distortion, only the ordination and MDS are presented here. Components were extracted until eigenvalues were less than 1.0. Subset solutions and the minimum spanning tree are superimposed on the ordination diagrams.

SYSTEMATIC FRAMEWORK

The basic features of rissoacean snails are reviewed in Fretter & Graham (1962). Davis (1979) has listed the features that distinguish hydrobioid snails from other rissoaceans. The definitions below are modified from those of Davis (1979, 1980) and Davis *et al.* (1982).

Family Hydrobiidae

These include hydrobioids in which sperm enter the anterior end of the pallial oviduct and pass along an internal, ciliated ventral channel to the bursa copulatrix; or, in which sperm enter a separate spermathecal duct, presumably formed by separation of the ventral channel from the pallial oviduct (not to be confused with the convergent structure of the Pomatiopsidae), and pass through it to the bursa copulatrix. The spermathecal duct is never associated with either the kidney or the pericardium (contrast the Triculinae). The mode of reproduction is oviparity or ovoviviparity. The penis may (Thompson, 1968, fig. 39) or may not (Thompson, 1968, fig. 37I) have lobes. The penis may also be without specialized glands (Hershler & Davis, 1980, fig. 4D), or may have glandular ridges consisting of an elevated area in which rows of small glands discharge through a central slit (Thompson, 1968, fig. 42); apocrine glands (Andrews, 1977, p. 82, fig. A); glandular papillae (Hubendick, 1955, fig. 88); or mammiform

glands (Fig. 44). The latter two gland types are only borne on penial lobes, whereas glandular ridges and apocrine glands can be found on the penis as well (Thompson, 1968, figs. 44, 38, respectively). There is neither a pedal crease nor a suprapedal fold (contrast the Pomatiopsinae); the snails move by ciliary gliding. The mantle collar may or may not have a pallial tubercle, filament, or numerous papillations. The tentacles may or may not have hypertrophied ciliary tufts. The eyes are located in slight swellings at the base of the tentacles. The central tooth of the radula usually has pronounced lateral angles, giving the tooth a trapezoidal shape, and one or more pairs of basal cusps that usually originate from the lateral angles (contrast the Pomatiopsidae). The stomach has a caecal chamber that usually protrudes posterior to the stomach chambers. An anterior digestive lobe may be present. The shell may or may not have wrinkled, pitted apical microsculpture.

Subfamily Nymphophilinae

These include Hydrobiidae in which the pallial oviduct has an internal ciliated ventral channel, and the penis is bilobed and bears one or more elevated glandular ridges. The tentacles do not have hypertrophied ciliary tufts. The apical whorl has wrinkled, pitted microsculpture. The only mode of reproduction thus far reported for this subfamily is oviparity (Thompson, 1968, 1977, 1979).

Subfamily Littoridininae

These include Hydrobiidae in which there is a spermathecal duct separate (at least posteriorly) from the pallial oviduct. The spermathecal duct may be short or long. The pallial oviduct often has three or four tissue types. The penis may be without specialized glands, or with large apocrine glands, glandular papillae, or mammiform glands. The tentacles often have hypertrophied ciliary tufts. The apical whorl may or may not have wrinkled, pitted microsculpture. The mode of reproduction may be oviparity or ovoviviparity.

The subfamilial placement of the Cuatro Ciénegas hydrobioids, based on anatomical study, is contrasted with that of Taylor (1966) in Table 2.

DESCRIPTION OF TAXA

Nymphophilinae

Nymphophilus Taylor, 1966

Type-species: *Nymphophilus minckleyi* Taylor, 1966.

Distribution: endemic to the Cuatro Ciénegas Basin.

Species included: *N. minckleyi, N. acarinatus* n. sp.

Description

Diagnostic features of *Nymphophilus* include the large (length, 3.5–8.3 mm) trochoid

TABLE 2. Subfamilial placement of the Cuatro Ciénegas hydrobiid genera (based on the results of this study) contrasted with that of Taylor (1966).

Taylor (1966)	This study
Family Hydrobiidae	Family Hydrobiidae
Subfamily Cochliopinae	Subfamily Littoridininae
*Coahuilix**	*Coahuilix**
Cochliopina	*Paludiscala**
Subfamily Littoridininae	*Mexithauma**
*Mexipyrgus**	*Cochliopina*
Durangonella	*Durangonella*
Subfamily Nymphophilinae**	*Mexipyrgus**
*Nymphophilus**	Subfamily Nymphophilinae
Subfamily Mexithaumatinae**	*Nymphophilus**
*Mexithauma**	*Mexistiobia*[1]
Subfamily Paludiscalinae**	Subfamily Unknown
*Paludiscala**	*Orygoceras?*[2]

*Genus endemic to the Cuatro Ciénegas basin.
**Subfamily considered endemic to the Cuatro Ciénegas Basin by Taylor (1966).
[1]New genus.
[2]Systematic status uncertain as anatomy is not yet studied.

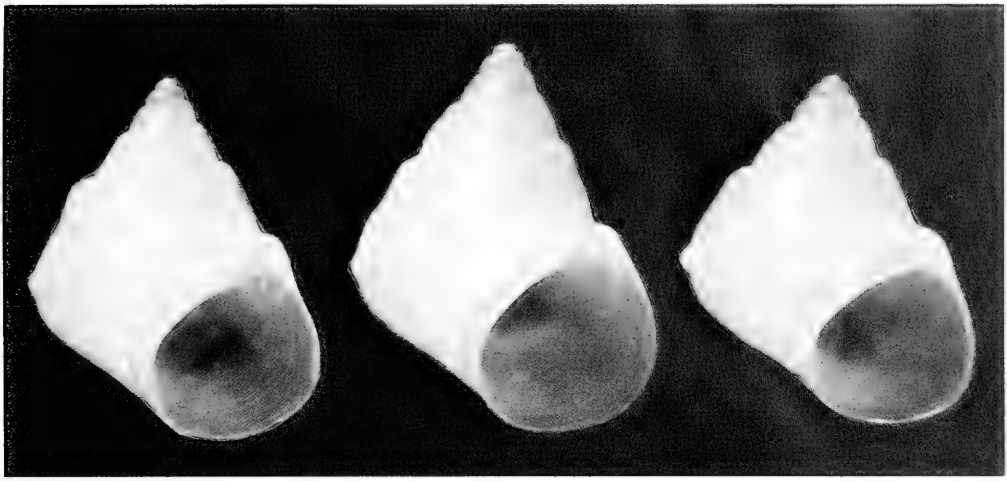

FIG. 4. Shells of *Nymphophilus minckleyi* from Locality 76. The shell on the left is 8.0 mm long; the others are printed at the same enlargement.

shell (Figs. 4, 9), multispiral operculum (Fig. 5B), elongate osphradium (30% of the ctenidium length), and bush-like male gonad (not shown).

The bursa (Bu) is positioned posterior to the pallial oviduct (Figs. 7A, B); the duct of the bursa to the common opening of the albumen gland and ventral channel is elongate; the seminal receptacle (Sr) and oviduct coils (Coi) are located anterior to the bursa (Fig. 7B); the bolster of the ventral channel is well-developed (Bvc, Fig. 7D); the pallial oviduct opens laterally as a common genital aperture (Cga, Fig. 7E); the penis (Fig. 8A) with massive, folded penial lobe (Plo) bears one to four glandular ridges (Glr) on its ventral surface.

Discussion

Among nymphophilines, *Nymphophilus* is most similar to *Marstonia,* as both taxa have a penis with few glandular ridges (for *Marsto-*

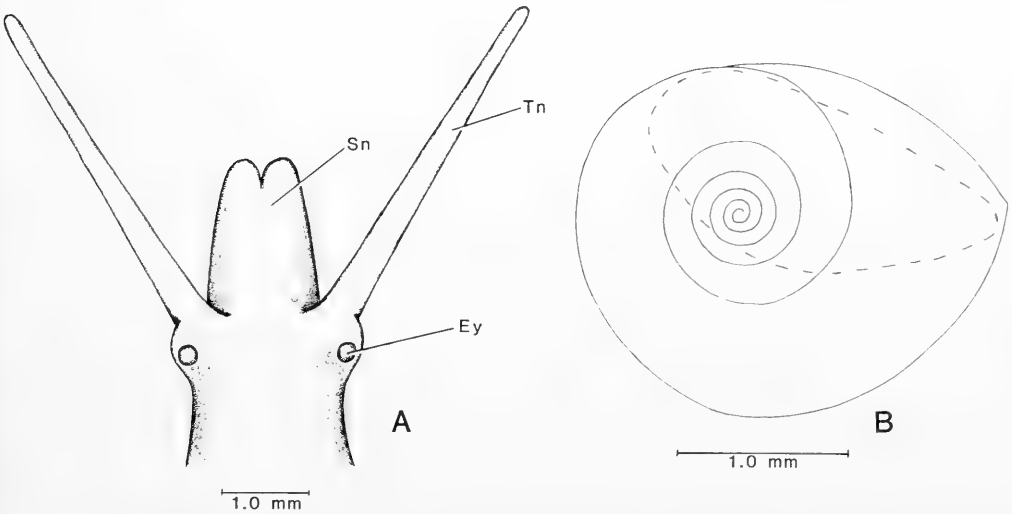

FIG. 5. Head and operculum of *N. minckleyi.* A. Head seen dorsally. B. Operculum, with dashed line indicating attachment area to operculigerous lobe. Ey—eye; Sn—snout; Tn—tentacle.

nia, see Thompson, 1977, figs. 5, 7, 11) and a large bursa positioned posterior to the pallial oviduct (for *Marstonia,* see Thompson, 1977, fig. 10).

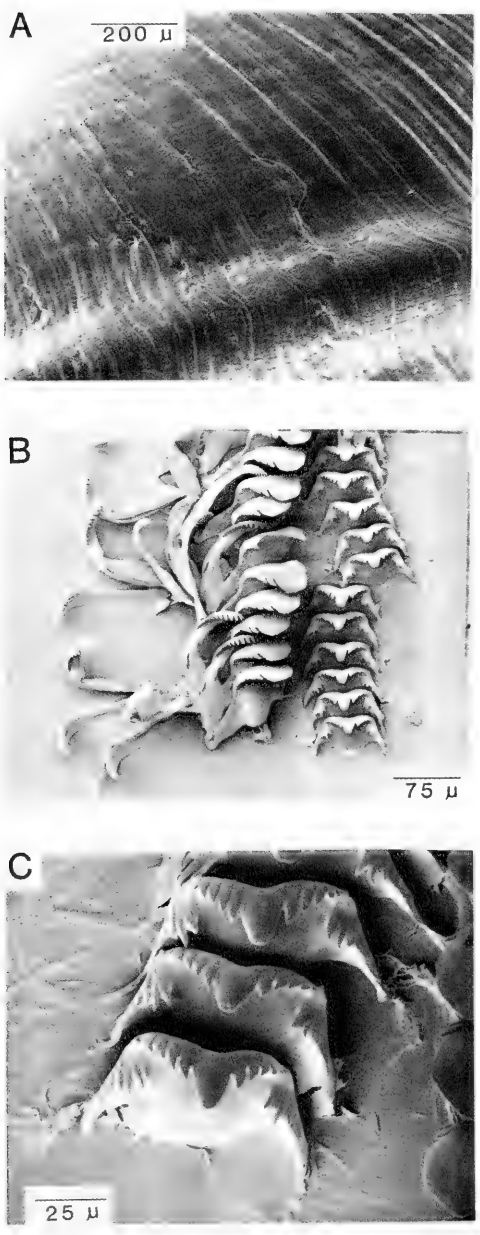

FIG. 6. SEM photos of shell and radula of *N. minckleyi.* A. Portion of body whorl showing the peripheral keel and wavy collabral microsculpture. B. Portion of radular ribbon. C. Several central teeth.

Nymphophilus minckleyi Taylor, 1966

Holotype: UMMZ (University of Michigan Museum of Zoology) 220188.

Type-locality: Locality 53.

Habitat: *Nymphophilus minckleyi* is found in large springs and their outflows. *Nymphophilus minckleyi* was rarely taken from the smaller springs; mops yielded specimens from the heads of only three of 38 such springs. In the large springs, *N. minckleyi* was collected from aquatic vegetation (*Nymphaea, Chara, Utricularia*), travertine, and, to a lesser extent, from gentle, sloping banks. On a microhabitat scale, this species is occasionally sympatric with *Mexithauma quadripaludium* and *Cochliopina milleri.* It has been suggested (Arnold, 1972) that *N. minckleyi,* as well as *Mexithauma* and *Mexipyrgus,* is nocturnal, moving about at night when the predaceous cichlid fish are inactive.

The egg capsules of this species were found on water lily (*Nymphaea*) leaves from many localities throughout the year. It was not uncommon to find over 100 capsules on a single 15 cm leaf. The capsules were rarely found on the shells of living snails. The egg capsule is hemispherical and is coated with detrital material along the sides, but the top of the capsule is clear of detritus and the yellow-colored embryo is visible inside. Usually, hydrobioid egg capsules are completely coated with either sand or detritus. For 13 egg capsules from Locality 76, the egg capsule diameter is 0.52 ± 0.40 mm. The height of the capsule is about 0.25 mm. The embryonic shells inside have 1.00–1.25 whorls.

Description

Nymphophilus minckleyi is distinctive in having a large shell (Fig. 4), with 5.5–6.0 flattened to slightly rounded whorls. There is a strong spiral keel, at or just above the suture, that fades on the body whorl and is barely noticeable at the aperture.

Shell

The spire is moderately high, the base rounded, and the umbilicus narrow. The sutures are shallow. The aperture is longer than it is wide, somewhat angled adapically, rounded abapically, and with a complete thickened peristome in adults. In adult shells, the aperture is adnate to or slightly separated from the penultimate whorl. The plane of the

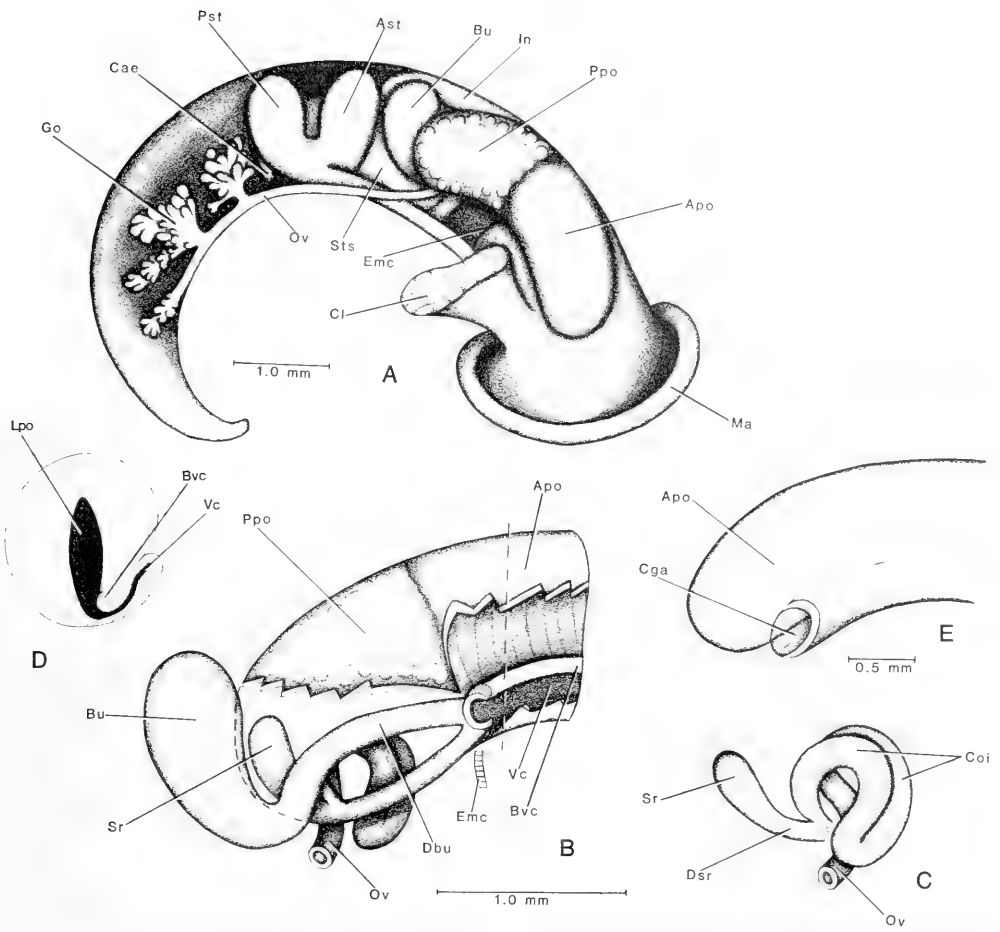

FIG. 7. Female reproductive anatomy of *N. minckleyi*. A. Snail uncoiled, exposing the ventral aspect without head and kidney tissue. Note the position of the bursa (Bu) posterior to the pallial oviduct (Apo + Ppo). B. Oriented as in A, but with a portion of the pallial oviduct cut away to reveal the bursa copulatrix complex, bolster (Bvc), and ventral channel (Vc). The left-hand dashed line indicates the posterior extent of the pallial oviduct. C. Oriented as in B, but with the bursa, its duct (Dbu) and the anterior portion of the duct of the seminal receptacle removed to reveal the oviduct coils (Coi). D. Cross-section of the pallial oviduct (looking anteriorly), cut where the right-hand dashed line indicates in B. Note the thickened bolster (Bvc) and well-developed ventral channel (Vc). E. Dorsal aspect of the capsule gland (Apo) showing the lateral opening of the common genital aperture (Cga). Apo—capsule gland; Ast—anterior stomach chamber; Bu—bursa; Bvc—bolster of ventral channel; Cae—caecum of stomach; Cga—common genital aperture; Cl—columellar muscle; Coi—coil of oviduct; Dbu—duct of the bursa; Dsr—duct of seminal receptacle; Emc—posterior end of mantle cavity; Go—gonad; In—intestine; Lpo—lumen of pallial oviduct; Ma—mantle edge; Ov—oviduct; Ppo—albumen gland; Pst—posterior stomach chamber; Sr—seminal receptacle; Sts—style sac; Vc—ventral channel.

aperture is only slightly tilted away from the coiling axis. The shell is colorless and translucent. The pitted apical microsculpture is shown in Thompson (1979, figs. 4–7). Postembryonic whorls have coarse, wavy growth lines (Fig. 6A), giving the shell a satiny sheen. Shell measurements for three populations, all from large springs or streams, are given in Table 3. Shell lengths of males are significantly larger ($p < .01$) than those of females for all three populations. Shells were removed from egg capsules from Locality 76 and their apical whorls measured. For 16 shells, the width of the tip of the apical whorl

TABLE 3. Shell measurements (mm) of males and females from three populations of *Nymphophilus minckleyi*. Snails with the dominant maximum whorl number were used. N = 9, Mean ± standard deviation. "p" refers to the significance level for the difference between shell lengths of males and females (t-test) for that population.

	Whorls	Length	Width	Length of body whorl	Length of aperture	Width of aperture	p
Locality 76							
♂	6.0	7.88 ± 0.50	5.90 ± 0.42	5.99 ± 0.49	4.38 ± 0.42	3.53 ± 0.24	<.005
♀	5.5	6.89 ± 0.28	5.51 ± 0.22	5.37 ± 0.31	3.92 ± 0.11	3.21 ± 0.20	
Locality 97							
♂	6.0	7.90 ± 0.30	5.68 ± 0.27	5.86 ± 0.27	4.05 ± 0.58	3.56 ± 0.15	<.01
♀	6.0	7.49 ± 0.32	5.58 ± 0.20	5.52 ± 0.26	2.77 ± 0.24	3.22 ± 0.18	
Locality 53							
♂	5.5	8.30 ± 0.36	6.52 ± 0.37	6.57 ± 0.39	4.81 ± 0.27	3.93 ± 0.19	<.005
♀	5.5	7.79 ± 0.18	6.33 ± 0.27	6.08 ± 0.14	4.45 ± 0.23	3.71 ± 0.20	

averaged 0.135 ± 0.017 mm; the width of the first whorl was 0.339 ± 0.040 mm. The width of the first whorl for shells from the type-locality was 0.30 mm (Thompson, 1979).

Nonreproductive Features

Details of the anatomy are from the population from Locality 76 unless otherwise indicated. Measurements of organs and structures are given in Table 4. The snout (Fig. 5A) is 1.77 mm long and relatively squat while the tentacles are thick and elongate (relative to the snout). The snout and tentacles have embedded in them yellow granules. The eyes are partially surrounded by clear, closely packed granules that extend back along the neck. A light dusting of melanin on the rostrum and tentacles was occasionally seen. The foot is large (relative to that of other species), thickened and dusted with melanin on its dorsal surface and sides. Body pigmentation consists of a dusting of reddish melanin on both dorsal and ventral surfaces. The male gonad occasionally has very dark pigmentation on its ventral surface. The operculum (Fig. 5B) has 5.5–6.0 whorls, and the nucleus is positioned at 39% of the long axis

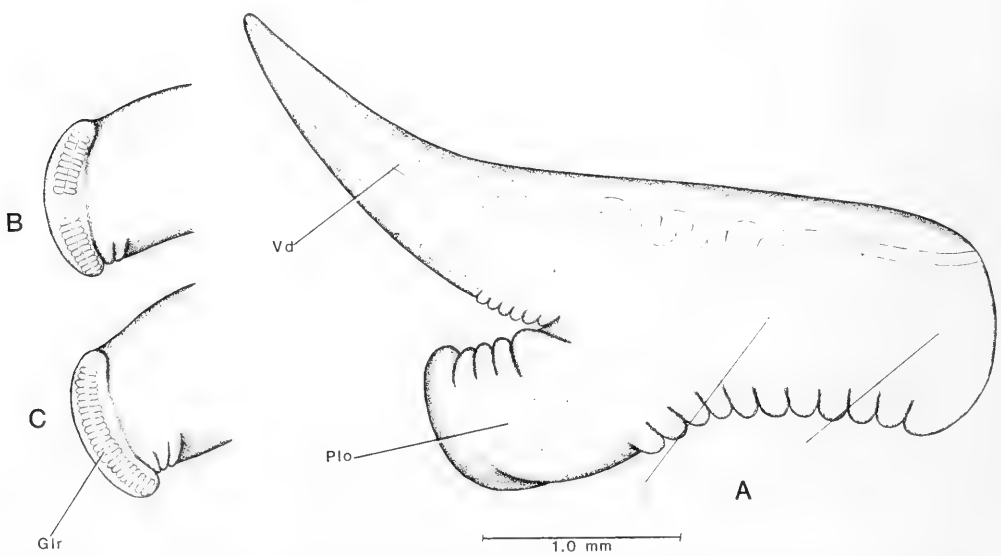

FIG. 8. The penis of *N. minckleyi*. A. Dorsal aspect of the penis. Note the large penial lobe (Plo) with numerous folds in it. B, C. Ventral aspect of the penial lobe showing the glandular ridge(s) (Glr). Vd—Vas deferens.

TABLE 4. Dimensions (mm) or counts of non-neural organs and structures of *Nymphophilus minckleyi*. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	9.30 ± 0.33	12.40 ± 0.31
Gill filament number		46.8 ± 2.17	
Osphradium	L	0.89 ± 0.13	
Gonad	L	2.49 ± 0.18	4.76 ± 0.59
	W	1.09 ± 0.09	1.19 ± 0.01
Prostate	L		1.33 ± 0.12
	W		0.65 ± 0.08
Penis	L		4.10 ± 0.46
	W		1.14 ± 0.15
Pallial oviduct	L	2.96 ± 0.35	
	W	1.16 ± 0.13	
Bursa copulatrix	L	1.01 ± 0.08	
	W	0.44 ± 0.08	
Seminal receptacle (body) (N = 6)	L	0.51 ± 0.06	
	W	0.21 ± 0.03	
Seminal receptacle (duct)	L	0.14 ± 0.10	
	W	0.12 ± 0.02	

of the operculum. The operculigerous lobe has a dusting of melanin along its perimeter. The caecal chamber (Cae) extends posterior to the stomach chambers (Fig. 7A).

Radula

The radula is shown in Figs. 6B & C. There are three pairs of basal cusps on the central tooth, arising from the lateral angles (Fig. 6C). The central cusp of the central tooth is broad and large relative to the cusps on either side. The lateral tooth also has a massive central cusp (Fig. 6B). The marginals have relatively few cusps. Radular statistics and the various cusp arrangements for the four tooth types are given in Tables 5 and 6, respectively.

Female Reproductive Anatomy

The ventral view of the uncoiled female is shown in Fig. 7A. The lobe-like gonad (Go) is short (27%) relative to body length. There are three to five gonad branches, each consisting of small lobes.

The oviduct (Ov) passes beneath the pallial oviduct just at the end of the style sac. A short gonopericardial duct is present (Thompson, 1979, fig. 15). The pallial oviduct is 32% of the body length. The two sections of this organ, the anterior capsule gland (Apo) and the posterior albumen gland (Ppo), are easily distinguishable even in unstained specimens. The posteriormost 20% of the pallial oviduct overlies the style sac (Sts, Fig. 7A). The anterior pallial oviduct ends 1.14 mm from the mantle edge. The relationships between the bursa copulatrix complex and pallial oviduct are shown in Figs. 7B, C. The bursa (Bu) is sac-like and large; 34% the length of the pallial oviduct. The duct of the bursa (Dbu) is

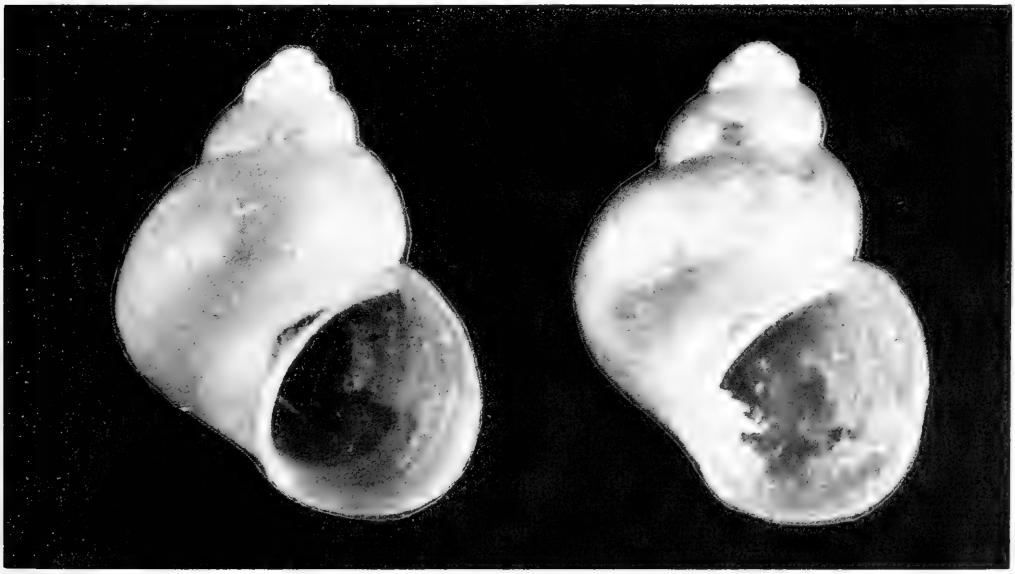

FIG. 9. Shells of *Nymphophilus acarinatus* from Locality 113. The shell on the left is the holotype (ANSP 355255) and is 4.25 mm long. On the right, printed to the same enlargement, is a paratype (ANSP 355256).

110% of the bursa length (Fig. 7B). The duct of the bursa, seminal receptacle (Sr), and oviduct coils (Coi) lie appressed to the dorsal surface of the albumen gland. The seminal receptacle and oviduct coils are largely anterior to the bursa and dorsal to the duct of the bursa. The pear-shaped seminal receptacle is relatively large; 50% of the length of the bursa. The oviduct coils twice, with the first coil dorsal to the second one, before receiving the short duct of the seminal receptacle (Dsr, Fig. 7C) and joining the duct of the bursa at the opening to the pallial oviduct (Fig. 7B).

The bursa copulatrix complex has a common opening with the albumen gland and ventral channel (Vc) at the posterior end of the mantle cavity (Fig. 7B). The ventral channel is considerably folded toward the ventral side of the pallial oviduct (Fig. 7D). The bolster of the ventral channel (Bvc) is rounded and thickened (Figs. 7B, D). Sperm masses were found in the ventral channel below the bolster. The walls of the ventral channel do not fuse anteriorly to form a tube separate from the capsule gland (compare Fig. 7C with Davis & Pons da Silva, 1984; fig. 6). The

TABLE 5. Radular statistics from 12 individuals of *Nymphophilus minckleyi*. $\bar{X}$ = mean, S = standard deviation. Measurements are in mm.

Radular feature	$\bar{X}$	S
Length	1.96	0.11
Width	0.274	0.019
Number of rows	60.7	3.47
Number of rows in formative stage	3.58	1.51
Width of central tooth (N = 28)	0.081	0.0054

TABLE 6. The various cusp arrangements for the four tooth types in 12 radulae of *Nymphophilus minckleyi*, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
3–1–3 / 3–3	8	2–1–2	33	9	18	15	50
4–1–3 / 3–3	17	3–1–2	17	11	25	16	50
4–1–4 / 3–2	17	3–1–3	75	12	42	17	50
4–1–4 / 3–3	8	3–1–4	8	13	42	18	58
4–1–4 / 4–4	8	4–1–1	8	14	58	19	25
5–1–4 / 3–2	25	4–1–2	8	15	58	20	25
5–1–4 / 3–3	50	4–1–3	58	16	58	21	17
5–1–5 / 3–2	8	4–1–4	17	17	17	24	8
5–1–5 / 3–3	67			18	8		
6–1–4 / 3–3	8						
6–1–5 / 3–2	8						
6–1–6 / 3–3	8						
7–1–4 / 3–2	8						
7–1–6 / 3–3	8						

capsule gland does not open at its anterior tip, but opens as a common genital aperture (Cga), lateral to and 0.3 mm posterior to the tip (Fig. 7E).

Male Reproductive Anatomy

The male gonad is relatively long, 38% of the body length, and extends to the posterior end of the stomach. The gonad has seven branches, each with many small lobes, giving the organ a bush-like appearance. The prostate is quite small, 11% of the body length, and largely posterior to the end of the mantle cavity. The anterior vas deferens exits from the posterior portion of the prostate.

The penis (Fig. 8) is relatively large and thickened, with an elongate penial filament. It is neither ciliated nor does it have an eversible terminal papilla. The single penial lobe (Plo) is positioned on the inner curvature slightly more toward the base of the verge than toward the tip. Thompson (1979, figs. 11–14) illustrates a much stouter penial filament than that shown here, possibly because he was studying preserved material. The penis has no pigment. The vas deferens (Vd) travels near the outer curvature of the penis and coils only during a portion of its length. The penis has numerous Gl_2 glands (see Davis, 1969a, for a discussion of gland types), particularly in the penial filament. The penis has no folds on its outer curvature, while the inner curvature has folds from the base to just beyond the penial lobe.

The penial lobe is quite stout and does not taper appreciably towards its distal end. Numerous folds extend inwards from its sides. The lobe curves both ventrally and towards the tip of the penis. Viewed from the ventral aspect (Figs. 8B, C), the distal edge of the lobe appears as a narrow projection folded above the proximal portion of the lobe. The surface of this distal edge, which cannot be seen in Fig. 8A, has one to three (see Table 7) glandular ridges (Glr) along its length (Figs. 8B, C). The third ridge (not shown) is often lateral to the other two. Taylor (1966, fig. 21) illustrates a fourth ridge near the base of the penial lobe (ventral surface); this was seen in only one of the 75 specimens studied from three poulations.

TABLE 7. Percent of individuals (N = 25) with 1, 2, or 3 glandular ridge(s) on the distal edge of the penial lobe in three populations of *Nymphophilus minckleyi.*

	Number of ridges		
	1	2	3
Locality 76	84	16	0
Locality 97	52	28	20
Locality 53	76	12	12

Nymphophilus acarinatus Hershler, n. sp.

Synonymy: *Nymphophilus* Hershler, n. sp. Hershler in press.

Etymology: the species name comes from the acarinate shell.

Holotype, ANSP 355255, Fig. 9A; paratypes (11); ANSP 355256, Fig. 9B.

Type-locality: Locality 98.

Habitat: *Nymphophilus acarinatus* is known only from empty shells from the type-locality and several specimens collected live from Santa Tecla Laguna (Locality 101). *Nymphophilus acarinatus* is allopatric to *N. minckleyi.*

Description

While there are insufficient anatomical data for a detailed account comparable to that of *N. minckleyi,* this species is placed in *Nymphophilus* because the organization of the bursa copulatrix complex and form of the penis are like those of *N. minckleyi.*

The shell (Fig. 9) differs from that of *N. minckleyi* in that it is somewhat smaller (length, 4.20 mm), has fewer whorls (to 4.8) that are quite rounded, and lacks a peripheral keel, even on early whorls. The growth lines are less pronounced than those of *N. minckleyi.* Measurements of the type and paratypes are given in Table 8.

Discussion

While the differences between *N. acarinatus* and *N. minckleyi* are few and restricted to shell features, there is no blurring of these differences in any of the populations studied. Specimens of *N. minckleyi* from small springs can be as small as *N. acarinatus,* but the whorls remain flattened and the peripheral keel is always present. The consistency of these differences suggests that the taxa are distinct species and not mere allopatric variants.

TABLE 8. Measurements (mm) of the shells of the holotype (ANSP 355255) and paratypes (ANSP 355256) of *Nymphophilus acarinatus*. All shells are from adults with 4.5–4.8 whorls.

	Shell length	Shell width	Length of body whorl	Length of aperture	Width of aperture
Holotype	4.25	3.33	3.37	2.30	1.91
Paratype	3.57	2.89	2.98	2.22	1.71
Paratype	4.13	3.26	3.30	2.38	1.91
Paratype	4.37	3.10	3.45	2.50	1.99

Mexistiobia Hershler, n. gen.

Etymology: the name was formed by adding the prefix Mexi-, referring to distribution within México, to *Stiobia* Thompson & McCaleb, 1978, a very similar nymphophiline from the southeastern U.S.A.

Type-species: *Mexistiobia manantiali* n. sp.

Distribution: thus far known only from the Cuatro Ciénegas Basin and Durango, México (U.S. National Museum of Natural History 351817, labeled "*Valvata*").

Species included: monotypic. The specific status of the Durango population is not known.

Description

Among nymphophilines, the unique features of *Mexistiobia* include the position of the small bursa (Bu) anterior to the seminal receptacle (Sr, Fig. 14B), the very short duct of the bursa (Dbu), and the position of the male gonad overlying the posterior stomach chamber (not shown).

The shell (Fig. 10) is minute (length, 1.20 mm) and broadly conical; the bolster of the ventral channel is weakly developed (Fig. 14C); the capsule gland opens at its anterior tip as a common genital aperture (Cga, Fig. 14D); the penis has an elongate penial lobe (with one fold in it) bearing a single glandular ridge along its ventral length (Glr, Fig. 13D).

Discussion

Mexistiobia manantiali bears a remarkable conchological resemblance to *Stiobia* Thompson & McCaleb, 1978, a monotypic genus endemic to a spring in Alabama, yet it differs in 11 morphological features (Table 9). Two of these features (4, 6) may have been incorrectly interpreted by Thompson &

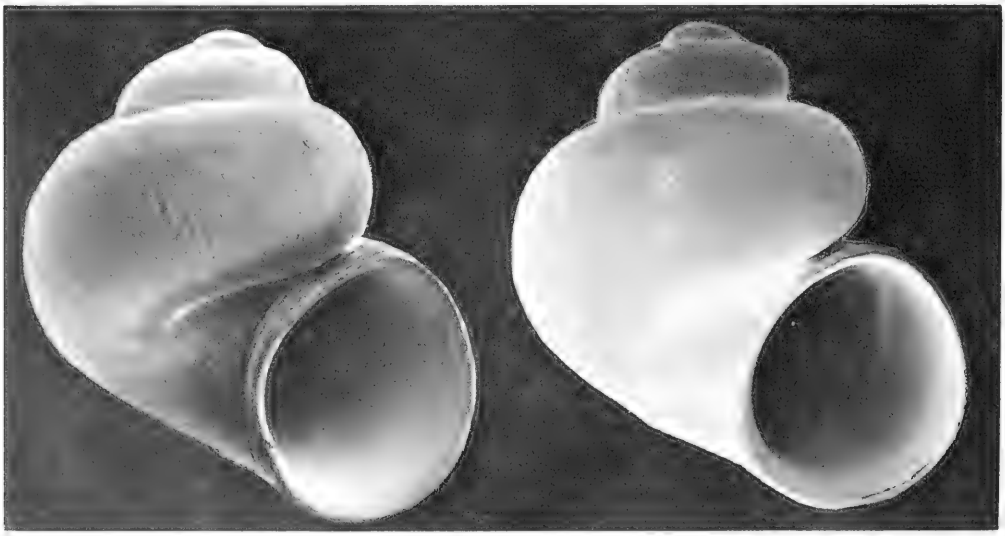

FIG. 10. SEM photos of paratype shells (ANSP A9887d) of *Mexistiobia manantiali* from Locality 51. The shell on the left is 1.15 mm long, that on the right is printed at the same enlargement.

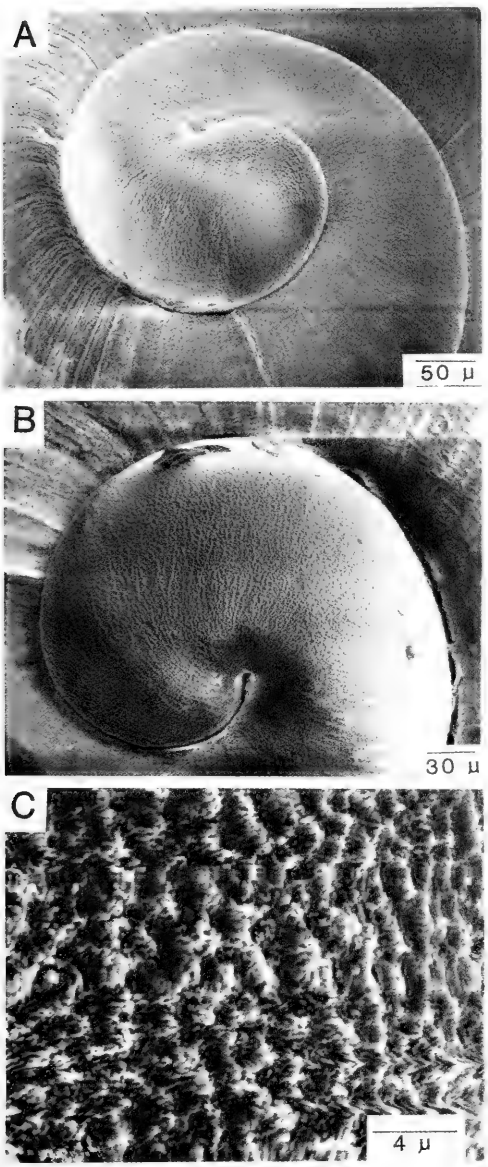

FIG. 11. SEM photos of the apical region of the shell of *Mexistiobia manantiali,* showing the wrinkled, pitted microsculpture at several magnifications.

McCaleb as these would be highly unusual traits for hydrobioid snails. Separate generic status is suggested for these two taxa because of the major differences in the position and organization of the bursa copulatrix complex, and in the form of the penis and number of glandular ridges.

While the stunted appearance of the female reproductive anatomy of *Mexistiobia* is unique among nymphophilines, a penis with few glandular ridges is also seen in *Nymphophilus* and *Marstonia* (see above).

Mexistiobia manantiali Hershler, n. sp.

Synonymy: "*Stiobia*" Hershler, n. sp. Hershler in press.

Etymology: the species name is formed from the Spanish word *manantial,* meaning spring, and refers to the spring-fed habitats of this snail.

Types: holotype, ANSP 355205; paratypes, A9887d, A9888l, 355204, Fig. 10. Because of their small size, the shells had to be photographed using the SEM, which leaves gold coating on the specimens, so the holotype was not used. The paratypes look like the holotype.

Type-locality: Locality 51, a small spring. This species was shown, but undescribed, by Taylor (1966, fig. 4).

Habitat: *Mexistiobia manantiali* is restricted to the smaller springs of the valley. It is found in association with *Durangonella coahuilae, Paludiscala caramba,* and *Coahuilix* spp. in the headsprings, and is sympatric with *D. coahuilae* in the spring runs. In terms of microhabitat, *Mexistiobia manantiali* is common in fine organic sediments and *Chara* mats, and prefers a finer sediment than does *D. coahuilae.* While found in mop samples from 20 of 38 small springheads, *Mexistiobia manantiali* probably does not live in subterranean waters as all specimens collected have eyespots and because the species is most adapted for life in open, downstream habitats (see below).

Shell

The colorless shell has rounded whorls and an open umbilicus. Adults have 3.00–3.3 whorls. The whorls sometimes have a very slight angulation at the shoulder. The latter portion of the body whorl frequently pulls away from the preceding whorl in adults. Postembryonic sculpture is restricted to strong growth lines (Figs. 10, 11A). The plane of the aperture is tilted about 10° toward the coiling axis. The apical whorl microsculpture is shown at several magnifications in Figs. 12A–C. Shell measurements from three populations are given in Table 10. The shell length for females is significantly larger than

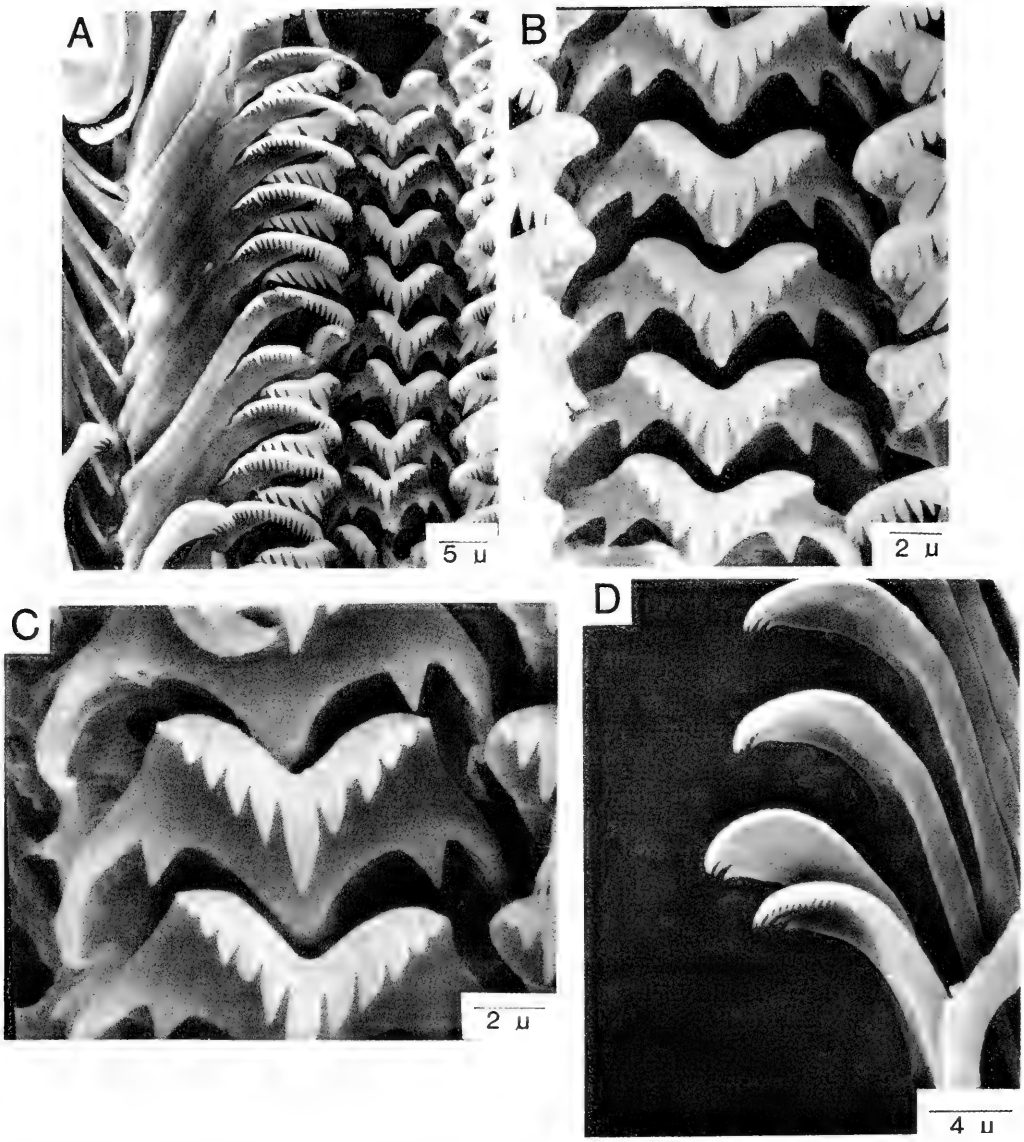

FIG. 12. SEM photos of the radula of *Mexistiobia manantiali*. A. Part of the radula ribbon. B, C. Central teeth. D. Outer marginal teeth.

that for males in two of the three populations (Table 10).

Nonreproductive Features

Observations and data on external features and anatomy are from the type population. Measurements of organs and structures are given in Table 11. The snout is elongate, while the tentacles are relatively short and thickened (Fig. 13A). A small concentration of granules partially surrounds the eyes. The snout and tentacles usually have a dark melanin dusting. Body pigmentation consists of a dark melanin dusting on the dorsal and ventral surfaces. Adults have only 10–13 gill filaments (Table 11). A prominent caecal chamber (Cae) extends posterior to the stomach

TABLE 9. List of 11 morphological differences between *Stiobia nana* and *Mexistiobia manantiali*. The morphological information on *Stiobia nana* is from Thompson & McCaleb (1978).

Stiobia nana	*Mexistiobia manantiali*
1. Shell with two spiral keels	Spiral keels absent
2. Wrinkled, pitted microsculpture on all shell whorls	Microsculpture restricted to apical whorl
3. Operculum with 2.5 whorls	Operculum with 3.5 whorls
4. Hypobranchial gland present	Hypobranchial gland absent
5. Lateral tooth of radula with massive, hoe-like central cusp	Central cusp small and dagger-like
6. Bursa and duct of bursa imbedded in the pallial oviduct	Bursa and duct dorsal to pallial oviduct
7. Duct of bursa elongate	Duct is short
8. Seminal receptacle anterior to bursa	Seminal receptacle posterior to bursa
9. Penial filament 35% of penis length	Penial filament 0.45% of penis length
10. Penial lobe massive and stout	Lobe is small and slender
11. Numerous glandular ridges over entire surface of penis	Single glandular ridge on ventral surface of penial lobe

TABLE 10. Shell measurements (mm) of males and females from three populations of *Mexistiobia manantiali*. Snails with the dominant maximum whorl number were used. N = 9, Mean ± standard deviation. "p" refers to the significance level for the difference between shell lengths of males and females (t-test) for that population.

	Whorls	Length	Width	Length of body whorl	Length of aperture	Width of aperture	p
Locality 51							
♂	3.0	1.19 ± 0.08	0.99 ± 0.04	1.08 ± 0.08	0.64 ± 0.04	0.53 ± 0.04	>.10
♀	3.25	1.21 ± 0.04	1.04 ± 0.07	1.12 ± 0.05	0.63 ± 0.03	0.62 ± 0.03	
Locality 65							
♂	3.0	0.98 ± 0.04	0.95 ± 0.04	0.84 ± 0.02	0.54 ± 0.01	0.46 ± 0.02	<.005
♀	3.25	1.10 ± 0.05	1.03 ± 0.05	0.93 ± 0.04	0.56 ± 0.03	0.47 ± 0.02	
Locality 68							
♂	3.0	1.10 ± 0.05	1.07 ± 0.04	0.94 ± 0.04	0.59 ± 0.03	0.53 ± 0.02	<.025
♀	3.25	1.15 ± 0.05	1.11 ± 0.02	0.95 ± 0.04	0.60 ± 0.03	0.52 ± 0.03	

(Fig. 14A). The operculum (Fig. 13B) has 3.5 whorls, and the nucleus is positioned at 39% of the long axis of the operculum.

Individuals with reduced body pigment were sometimes taken from the mops, while snails from downstream always have dark body pigment. The upstream pigment loss may be because the springhead is usually covered by riparian vegetation, and mimics a subterranean environment. A similar upstream-downstream pigment change is reported for the amphipod *Hyalella* in Cuatro Ciénegas (Holsinger & Minckley, 1971). The usual dark pigmentation of the snails is probably an adaptation to life in the open stream waters that are subject to great insolation.

Radula

The radula is shown in Fig. 12. The central cusps of the central and lateral teeth are blade-like. The marginals have numerous cusps. The central tooth has a single pair of basal cusps originating from the lateral angles (Figs. 12B, C). Radular statistics and the various cusp arrangements of the four tooth types are given in Tables 12 and 13, respectively.

Female Reproductive Anatomy

The organization of the female reproductive system is shown in Fig. 14. The female gonad

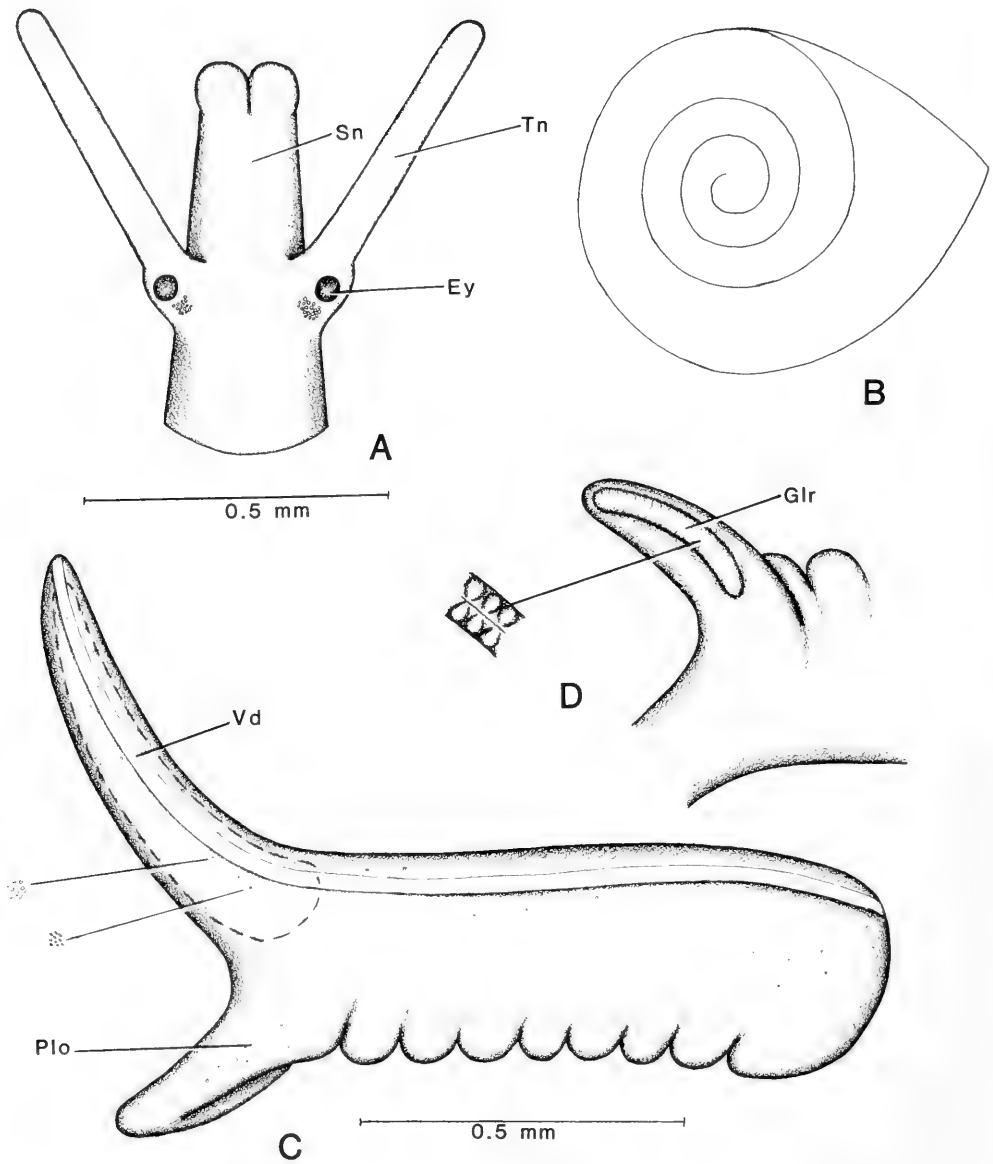

FIG. 13. Head, operculum and penis of *Mexistiobia manantiali*. A. Dorsal aspect of the head. B. Operculum. C. Dorsal aspect of the penis. Note the small, slender penial lobe (Plo). The dashed line indicates the region with melanin. D. Ventral aspect of the penial lobe showing the glandular ridge (Glr), with a close-up of the small glands. Ey—eye; Glr—glandular ridge; Plo—penial lobe; Sn—snout; Tn—tentacle; Vd—vas deferens.

(Go), a single lobed mass, occupies only 17% of the body length. The oviduct (Ov) disappears beneath the pallial oviduct (Apo + Ppo) at the end of the style sac (Sts, Fig. 14A). The pallial oviduct is divided into two equal sections: the albumen gland (Ppo) and the capsule gland (Apo). The pallial oviduct constitutes 28% of the body length. The posteriormost 30% of the pallial oviduct overlies the style sac. The anterior end of the pallial oviduct is 0.3 mm from the mantle edge.

The relationship between the bursa copulatrix complex and the pallial oviduct is shown in Fig. 14B. The bursa copulatrix complex is

TABLE 11. Dimensions (mm) of counts of nonneural organs and structures of *Mexistiobia manantiali*. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	2.57 ± 0.18	2.40 ± 0.20
Gill filament number (N = 7)		11.3 ± 0.95	
Gonad (N = 7)	L	0.45 ± 0.06	0.94 ± 0.11
	W	0.30 ± 0.02	0.29 ± 0.03
Prostate (N = 8)	L		0.39 ± 0.04
	W		0.21 ± 0.03
Penis	L		1.50 ± 0.17
	W		0.34 ± 0.04
Pallial oviduct (N = 8)	L	0.73 ± 0.09	
	W	0.31 ± 0.03	
Bursa copulatrix (N = 6)	L	0.15 ± 0.02	
	W	0.07 ± 0.02	
Seminal receptacle (body) (N = 7)	L	0.10 ± 0.02	
	W	0.06 ± 0.01	
Seminal receptacle (duct) (N = 7)	L	0.09 ± 0.01	
	W	0.04 ± 0.01	

TABLE 12. Radular statistics from 12 individuals of *Mexistiobia manantiali*. $\bar{X}$ = mean, S = standard deviation. Measurements in mm.

Radular feature	$\bar{X}$	S
Length	0.394	0.033
Width	0.069	0.005
Number of rows	56.5	6.5
Number of rows in formative stage	2.83	3.32
Width of central tooth (N = 8)	0.013	0.0004

dorsal to the pallial oviduct. The bursa (Bu) is 21% of the pallial oviduct length, and lies about 0.27 mm anterior to the end of the pallial oviduct. The seminal receptacle (Sr) and single oviduct coil are largely posterior to the bursa. In four of nine females dissected, the tip of the seminal receptacle protruded slightly posterior to the end of the pallial oviduct. The pouch-like seminal receptacle is similar in shape to and only slightly smaller than the bursa, but was easily distinguished by its pink sheen. The duct of the seminal receptacle (Dsr) is short. No gonopericardial duct was seen.

The bursa copulatrix joins the common opening of the ventral channel and albumen gland just posterior to the end of the mantle cavity (Fig. 14B). The ventral channel is only slightly folded toward the ventral side of the pallial oviduct and the bolster is small (Figs. 14B, C). The walls of the ventral channel do not fuse anteriorly (Fig. 14D).

Male Reproductive Anatomy

The male gonad is lobed and is 38% of the body length. The seminal vesicle coils on the posterior stomach chamber. The prostate overlies the mantle cavity. The anterior vas deferens exits from the posterior portion of the prostate.

The penis (Fig. 13C) has a slender, tapering penial filament, and the slender penial lobe is positioned on the inner curvature slightly closer to the tip than to the base of the penis. The penis has neither cilia, nor a ter-

TABLE 13. The various cusp arrangements of the four tooth types of *Mexistiobia manantiali*, counted from 5 radulae using SEM, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
4–1–4 / 1–1	40	4–1–3	80	19	20	22	40
5–1–5 / 1–1	60	5–1–3	80	20	40	23	40
5–1–4 / 1–1	20	5–1–4	20	21	60	24	60
6–1–5 / 1–1	20			22	60	25	80
6–1–6 / 1–1	60			23	40	26	60
				24	20		

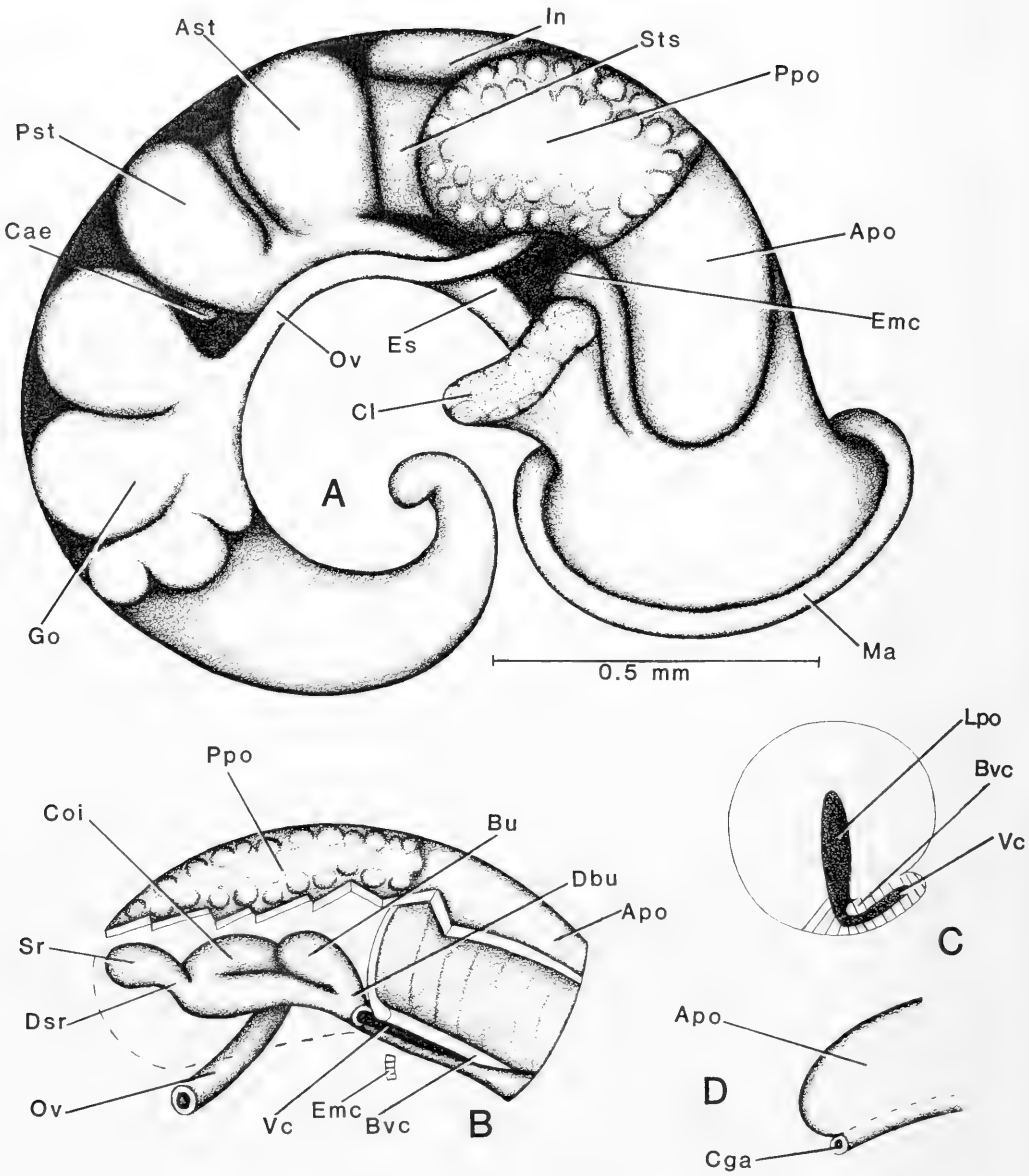

FIG. 14. Female reproductive anatomy of *Mexistiobia manantiali*. A. Snail uncoiled, exposing the ventral aspect without head and kidney tissue. B. Oriented as in A, but with a portion of the pallial oviduct removed to reveal the bursa copulatrix complex, bolster (Bvc), and ventral channel (Vc). C. Cross-section of the pallial oviduct (looking anteriorly), cut just at the common opening of the bursa copulatrix complex and albumen gland. Note the small size of the bolster (Bvc) and reduced ventral channel (Vc). D. Dorsal view of the capsule gland (Apo), showing the opening of the common genital aperture (Cga) at the anterior end of the pallial oviduct. Apo—capsule gland; Ast—anterior stomach chamber; Bu—bursa; Bvc—bolster of ventral channel; Cae—caecum of stomach; Cga—common genital aperture; Cl—columellar muscle; Coi—coil of oviduct; Dbu—duct of the bursa; Dsr—duct of the seminal receptacle; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Lpo—lumen of pallial oviduct; Ma—mantle edge; Ov—oviduct; Ppo—albumen gland; Pst—posterior stomach chamber; Sr—seminal receptacle; Sts—style sac; Vc—ventral channel.

minal eversible papilla. The penial filament is darkly pigmented (pigmented area indicated by dashed lines in Fig. 13C) and occasionally the penial lobe is also pigmented. The vas deferens does not coil in the penis. The penis has GI_1 and GI_2 glands. While the outer curvature has no folds, the inner curvature has folds from the base to the penial lobe.

The penial lobe is slender and tapers toward its distal end. Viewed from the ventral aspect (Fig. 13D), the single curved glandular ridge is seen. The ridge is elevated above the ventral surface of the penial lobe and consists of two rows of small glands that discharge through a central slit (see close-up, Fig. 13D).

Littoridininae

Coahuilix Taylor, 1966

Type-species: *Coahuilix hubbsi* Taylor, 1966

Distribution: endemic to the Cuatro Ciénegas Basin.

Species included: *Coahuilix hubbsi*, *Coahuilix landyei* n. sp.

Description

Diagnostic features (unique among littoridinines) include a minute (width, 0.85–1.40 mm) planispiral shell (Figs. 15A–G, I–K); intestine with a coil near its anterior end (Inc, Fig. 17C); basal cusps on the central tooth of the radula arising from the tooth face (Fig. 16C); coiling of the seminal vesicle on the posterior stomach chamber (Sv, Fig. 17C); and position of the prostate posterior to the mantle cavity (Fig. 17A).

The apical whorl has pitted microsculpture (Fig. 16A); the animal is blind (without eyes) and unpigmented; the digestive gland tubercles are reduced to low swellings; the caecal chamber does not protrude posterior to the stomach; gonads of both sexes are a single non-lobed mass (Figs. 17B, C), with that of the female overlying the posterior stomach chamber; the pallial oviduct is divided into three tissue types (Fig. 17B); the oviduct coils, gonopericardial duct, and seminal receptacle are absent (Fig. 17B); the spermathecal duct is elongate and opens separately from the pallial oviduct (Sd, Fig.

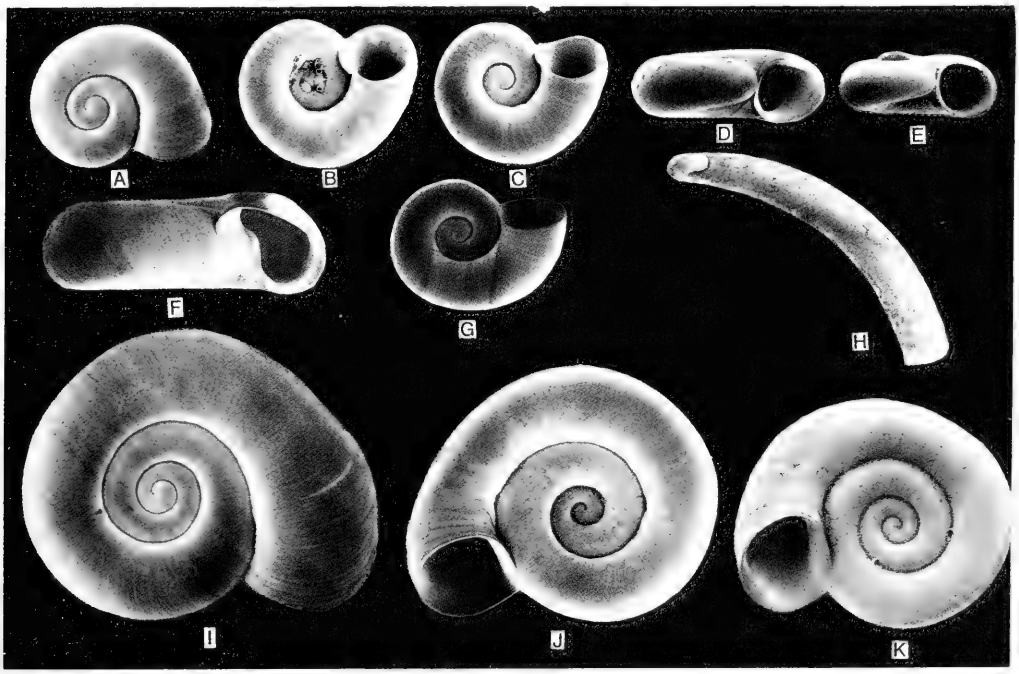

FIG. 15. SEM photos of *Coahuilix hubbsi*, *Coahuilix landyei* and *Orygoceras* (?) sp. Shells A–E are *Coahuilix hubbsi* from Locality 64; shells F, G, I, J, K are paratypes of *Coahuilix landyei* (ANSP 355211) from Locality 64; and shell H is *Orygoceras* (?) sp. from Locality 67. Shell A is 0.871 mm wide, and all others are printed to the same enlargement except H, the tube of which is 2.26 mm long.

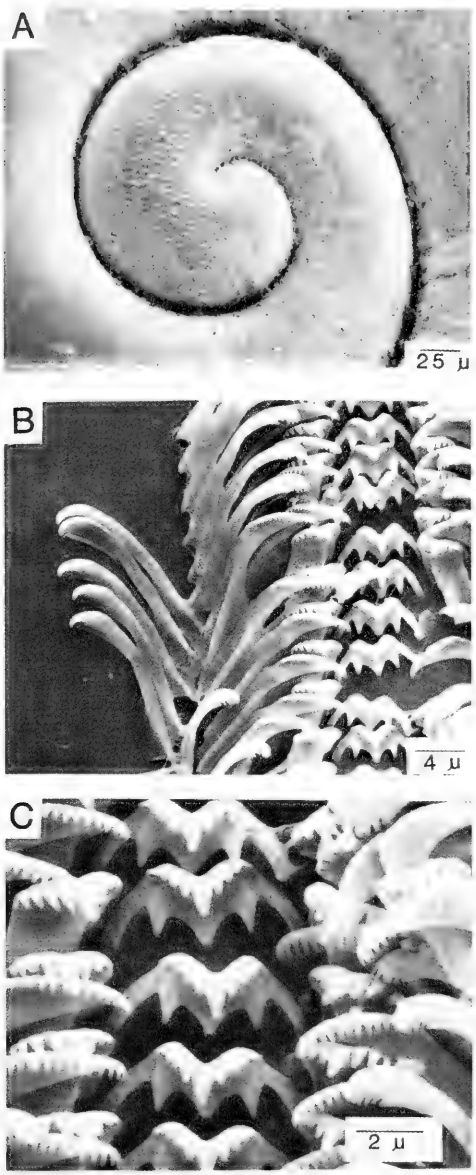

FIG. 16. SEM photos of shell and radula of *Coahuilix hubbsi*. A. Apical shell whorls, showing wrinkled, pitted microsculpture. B. Part of the radular ribbon. C. Central teeth showing the origin of the basal cusps from the face of the tooth.

17B); the females are oviparous; the penis has a bulb-like lobe bearing a large apocrine gland (Agl, Fig. 17D).

Discussion

Within the Littoridininae, *Coahuilix* and *Paludiscala* form a subgroup as they share numerous features, many related to the small size of the snails and their unique habitat (see Tables 53–55, Figs. 49, 50). The female reproductive system of these snails is characterized by loss of the oviduct coils, gonopericardial duct, and seminal receptacle. *Coahuilix* is distinguished from *Paludiscala* by the unique features listed above and by differences in the secondary sperm storage sacs (*Coahuilix*, absent; *Paludiscala*, present) and the condition of the openings of the spermathecal duct and pallial oviduct (*Coahuilix,* separate; *Paludiscala,* joined).

Coahuilix hubbsi Taylor, 1966

Holotype: UMMZ 2220180

Type-locality: Pozo de la Becerra (Locality 10): only empty shells of this species have been found in this large spring.

Habitat: Living *Coahuilix hubbsi* has been obtained only from mops placed into or just below small springheads. Downstream collecting efforts, with fine hand sieves, never yielded live specimens. Nor were they found when bottom material from the spring runs was collected and examined under the microscope. However, at Locality 64, mops were accidentally placed three meters down from the springhead, where the stream was still completely covered by riparian vegetation, and numerous living specimens were obtained.

Coahuilix hubbsi was only moderately common in the mop samples; while the species was found on mops from 15 of 38 small springheads, it never comprised more than 15% of the snails from the mops from any springhead. Only a few springs yielded more than 10 *Coahuilix hubbsi* per mop.

The fact that *Coahuilix hubbsi* is blind and unpigmented, together with its apparent restriction to groundwater outlets, suggests that the species may also live in subterranean waters in the basin. Other snail taxa in Cuatro Ciénegas with a similar habitat are *Coahuilix landyei, Paludiscala caramba,* and *Orygoceras* (?) sp.

Description

The shell (Figs. 15A–E) is less than 1.0 mm wide and has 2.3–2.5 whorls when adult. The last tenth of a whorl is slightly inflated (Fig. 15A). The aperture is inclined about 30° to the coiling axis. A small segment of the inner lip of the aperture is noticeably flared (Figs. 15B, C). Post-embryonic sculpture consists of

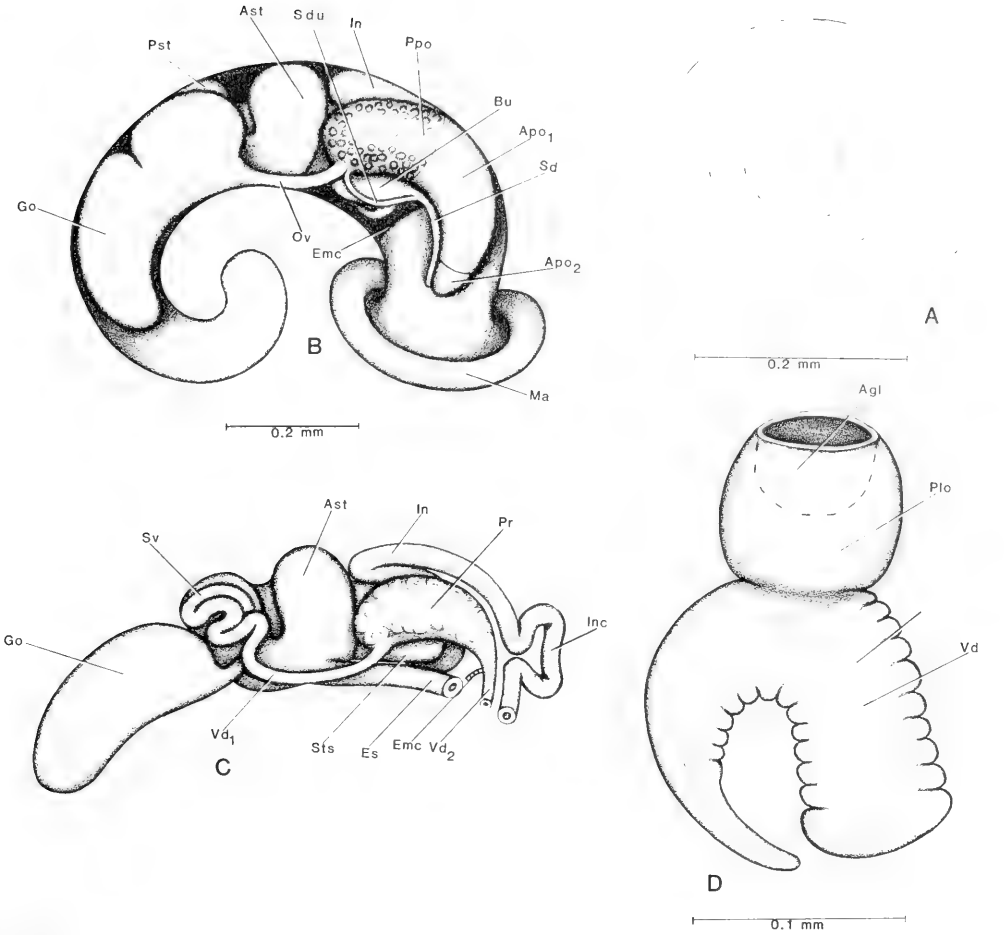

FIG. 17. Aspects of the anatomy of *Coahuilix hubbsi*. A. Operculum. B. Ventral aspect of uncoiled female without head and kidney tissue. Note the simplified gonad (Go), lack of seminal receptacle, and differentiation of the capsule gland into two tissue types (Apo_1 and Apo_2). C. Ventral aspect of uncoiled male without head and kidney tissue. Note the simplified gonad (Go), coiling of the seminal vesicle (Sv) on the stomach, small prostate (Pr) and anterior intestine coil (Inc). D. Dorsal aspect of the penis showing the large bulb-like penial lobe (Plo) with a single apocrine gland (Agl). Agl—apocrine gland; Apo_1—posterior capsule gland; Apo_2—anterior capsule gland; Ast—anterior stomach chamber; Bu—bursa; Emc—posterior end of mantle cavity; Es—esophagus; Go—gonad; In—anterior intestinal coil; Ma—mantle edge; Ov—oviduct; Plo—penial lobe; Ppo—albumen gland; Pr—prostate; Pst—posterior stomach chamber; Sd—spermathecal duct; Sdu—sperm duct; Sts—style sac; Sv—seminal vesicle; Vd—vas deferens; Vd_1—vas deferens from seminal vesicle to prostate; Vd_2—vas deferens from prostate to penis.

weak growth lines. The animal lacks gills, but has an osphradium.

Shell

Shell measurements for specimens from one population (Locality 64) are given in Table 14. The shell widths for females are greater than those for males ($p < 0.05$, Table 14). For each of three other populations (Localities 58, 38 and 67), shell widths for 20 adult specimens (sexes mixed) were measured with the following means and standard deviations: 0.822 ± 0.055 mm, 0.829 ± 0.056 mm, and 0.799 ± 0.075 mm, respectively.

The shell occasionally has a small spire (Fig. 15E). The last quarter whorl dips abapically away from the preceding whorls. The peristome is complete and slightly thickened.

TABLE 14. Shell measurements (mm) of males and females of *Coahuilix hubbsi* (from Locality 64). N = 9. Mean ± standard deviation. "p" refers to the significance level for the difference between shell widths of males and females (t-test).

	Whorls	Shell length	Shell width	Length of aperture	Width of aperture	p
♂	2.3–2.5	0.36 ± 0.02	0.85 ± 0.04	0.36 ± 0.02	0.32 ± 0.02	<.05
♀	2.5	0.37 ± 0.03	0.89 ± 0.05	0.36 ± 0.02	0.33 ± 0.02	

TABLE 15. Dimensions (mm) of non-neural organs and structures of *Coahuilix hubbsi*. N = 5. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	1.25 ± 0.08	1.24 ± 0.08
Gonad	L	0.36 ± 0.04	0.29 ± 0.03
	W	0.16 ± 0.01	0.16 ± 0.004
Prostate	L		0.19 ± 0.01
	W		0.10 ± 0.01
Penis	L		0.35 ± 0.01
	W		0.10 ± 0.01
Pallial oviduct	L	0.33 ± 0.02	
	W	0.13 ± 0.02	
Bursa copulatrix	L	0.13 ± 0.01	
	W	0.07 ± 0.02	

TABLE 16. Radular statistics from 5 individuals of *Coahuilix hubbsi*. $\bar{X}$ = mean, S = standard deviation. Measurements in mm.

Radular feature	$\bar{X}$	S
Length	0.214	0.011
Width	0.034	0.002
Number of rows	68.8	3.42
Number of rows in formative stage	5.48	0.55

No specimens were found with the extreme flaring of the aperture shown by Taylor (1966, figs. 9, 12). For nine specimens from one population (Locality 64), the width of the tip of the apical whorl averaged 0.081 ± 0.006 mm; the width of the first whorl was 0.138 ± 0.015 mm. No specimen seen had the strong growth lines shown by Taylor (1966, fig. 13).

Nonreproductive Features

Observations and data on external features and anatomy are from the population at Locality 64. Measurements of organs and structures are given in Table 15. The snout is squat and the tentacles are short and thick. The buccal mass, pink-red in color, is visible through the snout. There is a concentration of white granules and a slight pinkish color where the eyespot normally would be. The tentacles are without hypertrophied ciliary tufts. The operculum (Fig. 17A) has 3.3 whorls and the nucleus is positioned at 42% of the long axis of the operculum. A light pink color and scattered white granules are seen on the operculigerous lobe.

Radula

The radula is shown in Figs. 16B, C. The central tooth has well developed lateral angles, a small basal process, and a dagger-like central cusp. The basal cusp supports clearly arise from the face of the central tooth (Fig. 16C). Radular statistics and the various cusp arrangements for the four tooth types are given in Tables 16 and 17.

Female Reproductive Anatomy

The organization of the female reproductive system is shown in Fig. 17B. The gonad is 28% of the body length. The pallial oviduct extends to the anterior edge of the stomach and is relatively small, comprising 26% of the body length. The oviduct enters the posterior portion of the albumen gland (Fig. 17B). The capsule gland is composed of a large posterior white-colored section (Apo_1, Fig. 17B) and a smaller anterior grey-colored section (Apo_2). The sac-like bursa is positioned dorso-laterally to the pallial oviduct and has its posterior end even with that of the albumen gland. The bursa is 38% of the length of the pallial oviduct. A thin sperm duct (Sdu) issues from the anterior end of the bursa and joins the oviduct at the opening of the albumen gland (Fig. 17B). The posterior portion of the albumen gland, where the oviduct (Ov) and sperm duct (Sdu) jointly enter, has a pink sheen, indicating that sperm is inside. This region differs from the remaining albumen gland in that it is thin-walled and non-glandular: it may be a secondary sperm stor-

TABLE 17. The various cusp arrangements of the four tooth types of *Coahuilix hubbsi*, counted from five radulae using SEM, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
3–1–3 / 1–1	100	5–1–3	100	16	40	16	20
4–1–4 / 1–1	40	5–1–4	20	17	60	17	60
		6–1–4	20	18	80	18	80
				19	20	17	20
				20	20		
				21	20		

age area (as there is no seminal receptacle). The spermathecal duct (Sd) is usually tightly appressed to the pallial oviduct.

Male Reproductive Anatomy

The male gonad is 23% of the body length. The vas deferens branches off the anterior end of the gonad and the seminal vesicle consists of only a few coils (Sv, Fig. 17C). The anterior vas deferens exits from the anterior tip of the prostate.

The penis has a short penial filament. The penial lobe (Plo, Fig. 17D) is located at 46% of the length of the penis from the base (on the outer curvature), and is slightly taller than it is wide, measuring 0.098 by 0.080 mm. Folds are seen on the outer curvature of the penis from the base to the penial lobe; the inner curvature has folds for 75% of the penis length from the base. The vas deferens does not coil in the penis. Infrequent concentrations of Gl_2 glands are seen in the penis. The penis is neither ciliated, nor does it have a terminal papilla.

The single massive apocrine gland in the penial lobe occupies slightly more than one-half of the height of the lobe. The gland opening is clearly visible and almost circular in cross-section. Its detailed structure is the same as that of *Heleobops* (Thompson, 1968, figs. 38D, E).

Coahuilix landyei Hershler, n. sp.

Synonymy: *Coahuilix*, n. sp. Hershler, in press.

Etymology: named after Mr. J. Jerry Landye, a student of the freshwater molluscs of the southwestern U.S.A., and México.

Types: holotype, ANSP A9894n; paratypes (7), 355211, Figs. 15F, G, I, J, K. Again, because of their small size the shells were photographed using SEM, and therefore the holotype was not used. The paratypes used look like the holotype.

Type-locality: Locality 64.

Habitat: *Coahuilix landyei* has been collected, with one exception, only from mops placed in small springheads. In one spring (Locality 63) several specimens were taken live from *Chara* mats five meters downstream from the groundwater outlet. *Coahuilix landyei* was collected from mops from 13 of 38 small springheads. The species always comprised less than 13% of the collection from any springhead, and never totalled more than four specimens per mop.

Description

While only a few specimens of *Coahuilix landyei* were dissected, all aspects of anatomy seen were basically the same as those of *Coahuilix hubbsi*.

The shell (Figs. 15F, G, I, J–L) differs from that of *Coahuilix hubbsi* in the following respects: 1) adults have one more whorl and are larger (width, to 1.31 mm) than *Coahuilix hubbsi*; 2) the last tenth of a whorl is much more inflated than that of *Coahuilix hubbsi*; 3) the growth lines of the body whorl are much more pronounced than those of *Coahuilix hubbsi*; 4) the last third of the body whorl

TABLE 18. Shell measurements (mm) of adult *Coahuilix landyei* (>3.25 whorls, sexes mixed) from two populations. Mean ± standard deviation. The shells measured from Locality 64 are paratypes (ANSP 355211).

	Shell length	Shell width	Length of aperture	Width of aperture
Locality 64 N = 9	0.50 ± 0.04	1.28 ± 0.02	0.52 ± 0.03	0.39 ± 0.04
Locality 67 N = 7	0.49 ± 0.03	1.31 ± 0.06	0.49 ± 0.03	0.41 ± 0.03

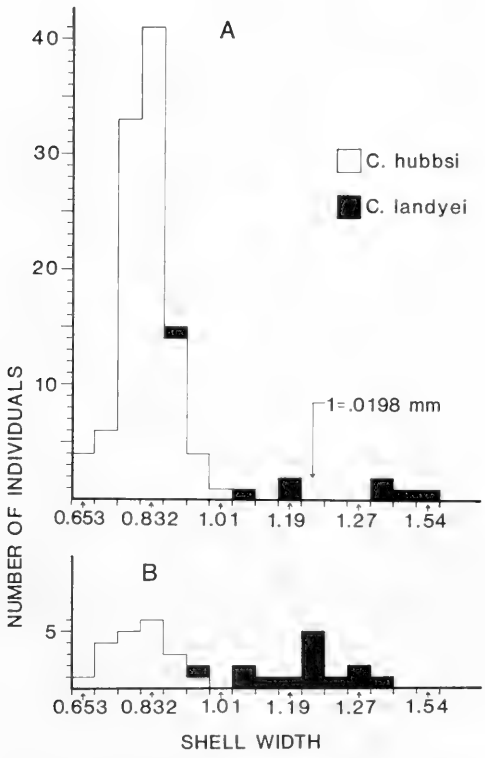

FIG. 18. Shell width frequency distributions from *Coahuilix hubbsi* and *Coahuilix landyei* collected from mops from two localities. A. Locality 67. B. Locality 64. Almost all specimens taken were adults. Note the obvious size difference between the two sympatric species (analyzed in Table 12).

overlaps the preceding whorl (Figs. 15F, J, K), while that of *Coahuilix hubbsi* merely touches the preceding whorl; 5) the aperture is much more inclined to the coiling axis than that of *Coahuilix hubbsi*; and 6) the inner lip of the aperture is much less flared than that of *Coahuilix hubbsi.* The animal has 10–12 gill filaments (*Coahuilix hubbsi* lacks gill filaments). Shell measurements for two populations of *Coahuilix landyei* are given in Table 18.

Discussion

The differences between these two species are all associated with *Coahuilix landyei* having one more shell whorl than *Coahuilix hubbsi.* Separate specific status is suggested by the two taxa being found together on mops at six localities (suggesting sympatry); in these cases the whorl count difference remained pronounced. The shell width frequency distributions (virtually all adult shells) for mop collections of the two species from two of these localities are shown in Fig. 18 and analyzed in Table 19. Note that the differences between the shell width means are highly significant for both localities. Immature *Coahuilix landyei,* with the same size and whorl number as adult *Coahuilix hubbsi,* are distinguishable from the latter as their apertures are neither thickened nor flared (Fig. 15G). Very small immature specimens (<2 whorls), were not found and probably could not be specifically identified.

Other workers have noted two small, planispiral hydrobioid species in Cuatro Ciénegas, and it is likely that the snail referred to but not described or figured as *Hauffenia* sp. (Holsinger & Minckley, 1971, p. 444) is *Coahuilix landyei.*

Paludiscala Taylor, 1966

Type-species: *Paludiscala caramba* Taylor, 1966.

Distribution: endemic to the Cuatro Ciénegas Basin.

Species included: monotypic.

Description

Unique features include the two to three prominent swellings on the coilless oviduct (Fig. 22A) and the disc-like "pouch" that

TABLE 19. Analysis of shell width frequency distributions shown in Fig. 18. "p" refers to the significance level for the difference between shell widths of the two sympatric species (t-test).

		N	Shell width (mean ± standard deviation)	p
Locality 67	*C. hubbsi*	20	0.799 ± 0.075	<.005
	C. landyei	13	1.22 ± 0.134	
Locality 66	*C. hubbsi*	103	0.818 ± 0.024	<.005
	C. landyei	8	1.37 ± 0.290	

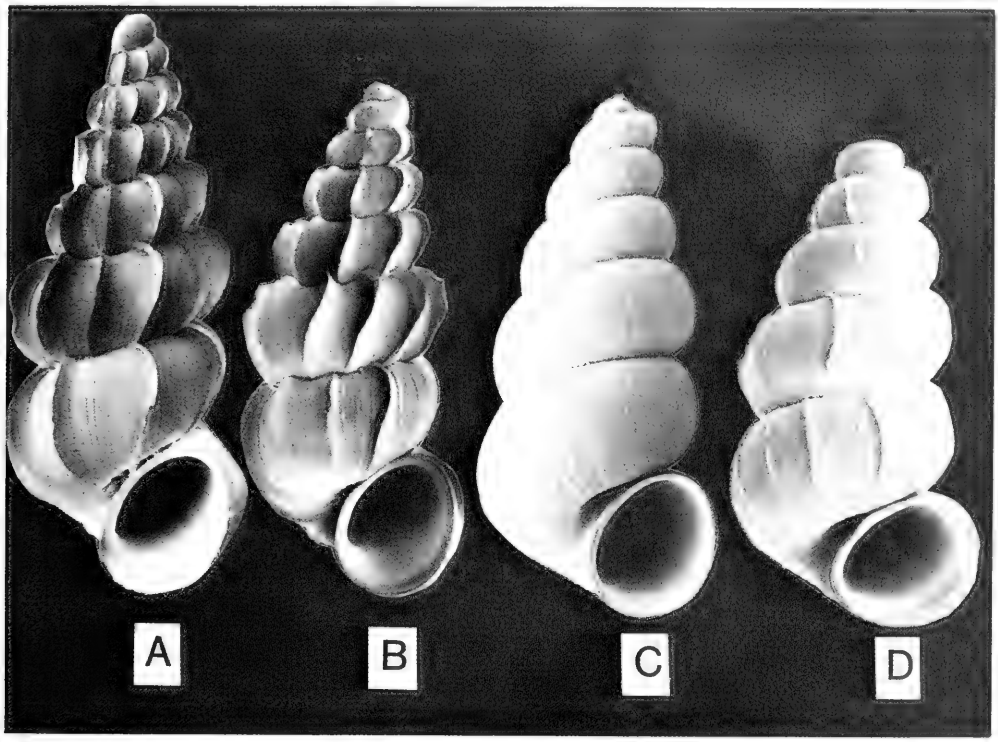

FIG. 19. SEM photos of shells of *Paludiscala caramba*. Shells A and B are from Locality 63, C and D are from Locality 38. Shell A is 2.42 mm long (B is printed to same enlargement), shell C is 1.58 mm long (D is printed to same enlargement).

bulges from the ventral surface of the albumen gland (Figs. 22A, C, D).

The shell (Fig. 19) is small (length, 1.40–2.60 mm) and turriform, with or without lamelliform costae; the apical whorl has pitted microsculpture (Fig. 20A); the animal is blind and unpigmented; the tentacles have *Hydrobia*-like hypertrophied ciliary tufts; the digestive gland tubercles are reduced to low swellings (Fig. 22B); the caecal chamber does not protrude posterior to the stomach (Fig. 22B); the pallial oviduct contains four distinct tissue sections (Fig. 22A); the seminal receptacle and gonopericardial duct are absent; the spermathecal duct (Sd) is elongate and has a common opening with that of the pallial oviduct (Fig. 22F); females are oviparous; the penis has a bulb-like lobe bearing a large apocrine gland (Agl, Fig. 21C).

Among littoridinines, *Paludiscala* is most similar to *Coahuilix* (see above; Tables 53–55, Figs. 49, 50).

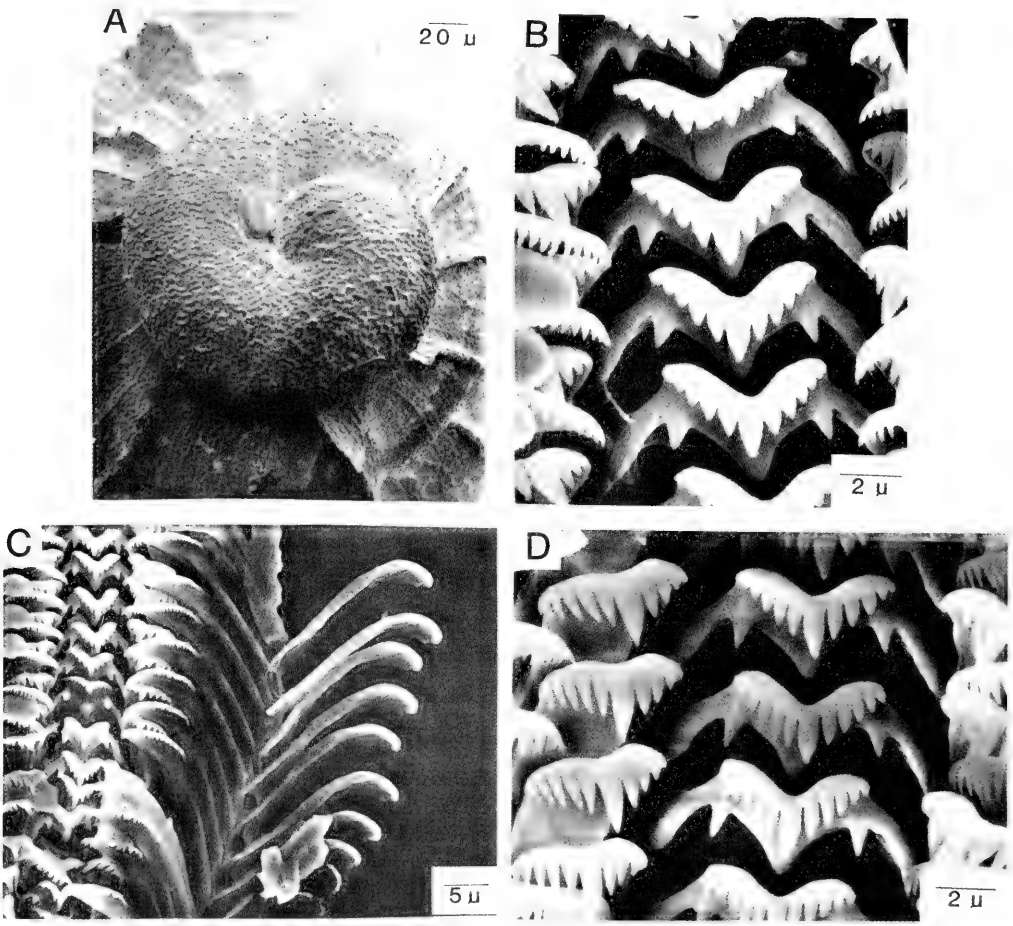

FIG. 20. SEM photos of the apical whorls and radula of *Paludiscala caramba*. A. Apical shell whorls, showing pitted microsculpture. B, D. Central teeth. C. Part of the radula ribbon.

Paludiscala caramba Taylor, 1966

Holotype: UMMZ 220164.

Type-locality: Locality 74. Living *Paludiscala caramba* have not been found at this locality.

Habitat: *Paludiscala caramba* was by far the most common species found in the small springheads; 32 of 38 of these springs yielded this species from mops. Of the 23 springs that yielded more than 100 snails from mop collections, *Paludiscala caramba* comprised greater than 10% of the collection for spring 18, and greater than 50% of the collection for 15. Perhaps more so than *Coahuilix*, *Paludiscala caramba* can extend downstream when there is riparian vegetation covering the stream. At Locality 63, a small thermal (33–35°C) spring issues into a pool (see Brown, 1974, fig. 5), and then runs 170 m before terminating in a marsh. *Paludiscala caramba* was very abundant on plant and rock surfaces for the upper 83 m, which had virtually complete vegetative cover. Below 83 m the vegetative cover ended and no *Paludiscala caramba* were found, despite intensive collecting which yielded quantities of *Durangonella coahuilae* and *Mexistiobia manantiali*. *Paludiscala caramba* appears to have a similar pattern of distribution in other springs.

Shell

The shell of *Paludiscala caramba* has up to 7.5 rounded whorls. Shell measurements for two populations are given in Table 20. For

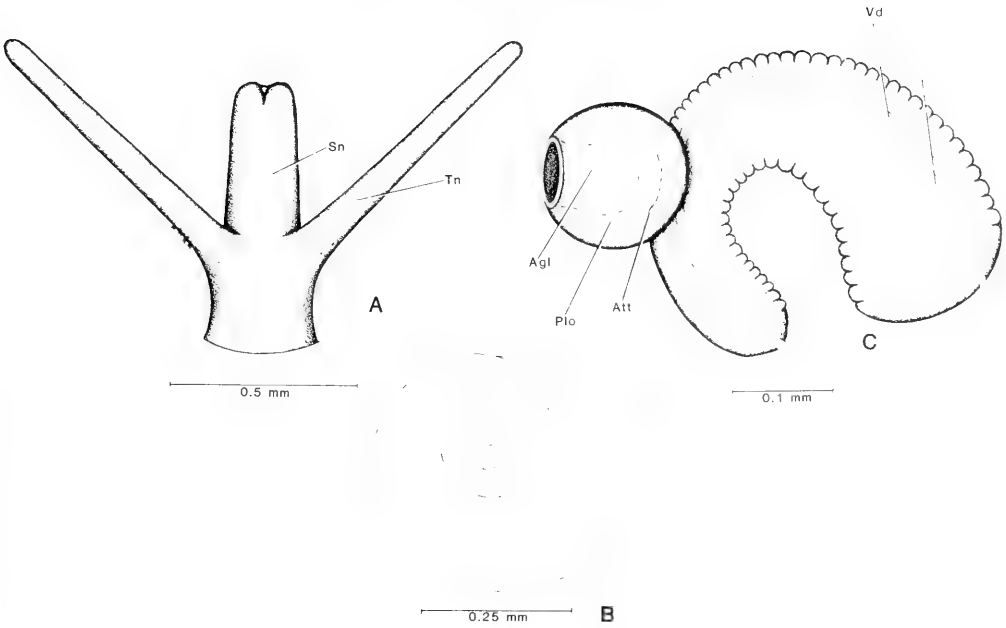

FIG. 21. Head, penis, and operculum of *Paludiscala caramba*. A. Dorsal view of the head. Note the *Hydrobia*-like hypertrophied ciliary tufts on the left tentacle and the lack of eyes. B. Operculum. C. Dorsal aspect of the penis showing the bulb-like penial lobe (Plo) with a single apocrine gland (Agl). The attachment area (Att) of the penial lobe to the penis is hidden by the lobe's curvature. Agl—apocrine gland; Att—attachment of penial lobe to penis; Plo—penial lobe; Sn—snout; Tn—tentacle; Vd—vas deferens.

both populations, there was no significant sexual dimorphism in shell length ($p > 0.1$). For most populations sampled, the shells have fairly tall, thin costae that are curved in profile (Figs. 19A, B). The costae begin after 0.8 whorls and continue to the aperture. Costae spacing is irregular: for 16 shells (sexes mixed) with 7.5 whorls from Locality 63, the penultimate whorl had 9.5 ± 1.7 costae (range of 6–12), and the body whorl had 10.5 ± 1.4 costae (range of 9–13). A few populations (Localities 38, 67) had smaller individuals (6.5 whorls, 1.4 mm shell length) with costae reduced or absent (Figs. 19C, D). The aperture is inclined only 10° to the coiling axis. The peristome is complete, slightly thickened, and adnate to or just free from the preceding whorl. The inner lip is slightly flared.

Nonreproductive Features

The anatomical description and data (Table 21) are from the population from Locality 63. The snout (Fig. 21A) is elongate, as are the tentacles. There are four or five ciliary tufts on the tentacles and the tufts are restricted to the outer edge of the left tentacle. There is a small concentration of white granules and a pink color in the areas where the eyespots normally would be. Crystalline granules are seen on the ventral body surface. The gills are reduced in number (Table 21). The operculum (Fig. 21B) has three whorls and the nucleus is positioned at 38% of the long axis of the operculum. The operculigerous lobe has a narrow band of crystalline granules and a small area of red-pink color.

Radula

The radula is shown in Figs. 20B–D. The central tooth has a single pair of basal cusps that originate from the lateral angles (Figs. 20B, D). Radular statistics and the various cusp arrangements for the four tooth types are given in Tables 22 and 23.

Female Reproductive Anatomy

The organization of the female reproductive system is shown in Figs. 22A, C, D, E, F. The

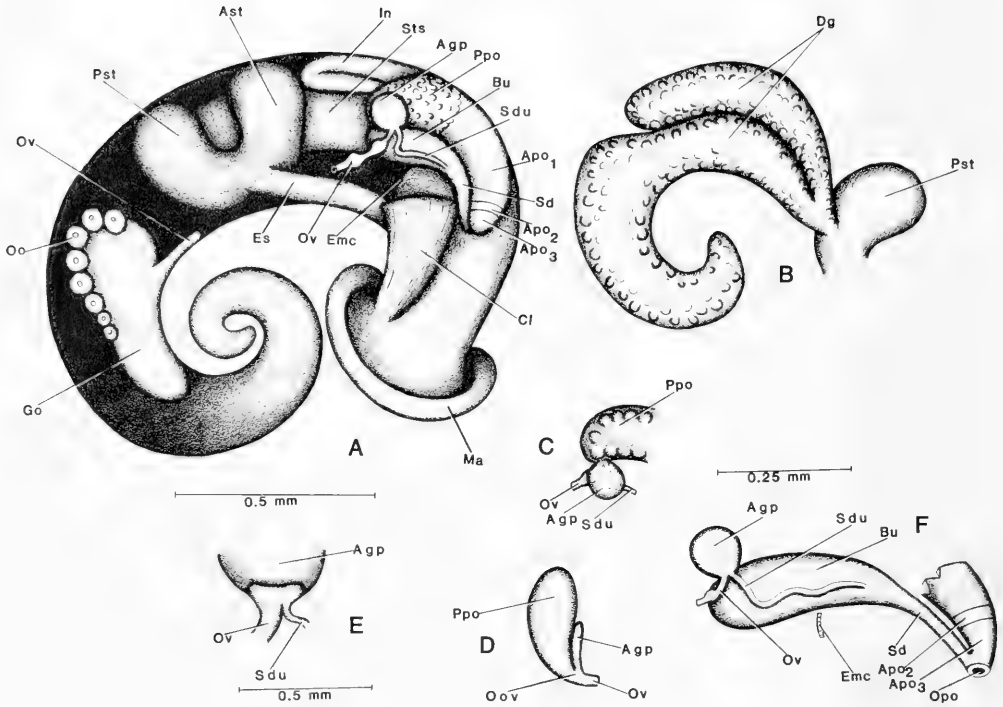

FIG. 22. Aspects of the anatomy of *Paludiscala caramba*. A. Ventral aspect of the uncoiled female without the head and kidney tissue. A section of the oviduct (Ov) has been removed. Note the swellings of the oviduct, the albumen gland pouch (Agp), and capsule gland differentiated into three distinct regions (Apo_1, Apo_2, and Apo_3). B. Ventral aspect of the digestive gland (Dg), showing the tubercles reduced to mere swellings. C. Posterior pallial oviduct oriented as in A, but with the albumen gland pouch (Agp) folded ventrally to expose its basal connection to the main portion of the albumen gland. D. Cross-sectional view of the posterior end of the pallial oviduct showing how the albumen gland pouch (Agp) and oviduct (Ov) jointly open into the albumen gland proper. E. Base of the albumen gland pouch (Agp). Note that the oviduct (Ov) and sperm duct (Sdu) join together at the opening to the albumen gland, which is differentiated from the distal portion of the albumen gland pouch. F. Oriented as in A, but with most of the pallial oviduct cut away to reveal the bursa (Bu). Note that much of the bursa is anterior to the end of the mantle cavity (Emc) and that the spermathecal duct (Sd) joins the pallial oviduct at its anterior end. Agp—albumen gland pouch; Apo_1—posterior capsule gland; Apo_2—middle capsule gland; Apo_3—anterior capsule gland; Ast—anterior stomach chamber; Bu—bursa; Cl—columellar muscle; Dg—digestive gland; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Ma—mantle edge; Oo—oocyte; Oov—opening of the oviduct; Opo—opening of the pallial oviduct; Ov—oviduct; Ppo—posterior pallial oviduct; Pst—posterior stomach chamber; Sd—spermathecal duct; Sdu—sperm duct; Sts—style sac.

gonad (Go) is a single non-lobed mass that comprises 15% of the body length. The pallial oviduct is 17% of the body length and extends slightly over the style sac. The albumen gland (Ppo) is of normal size. The capsule gland has three distinct tissue sections: a large posterior one (Apo_1, Fig. 22A), a smaller grey-colored one (Apo_2), and a somewhat larger white-colored one (Apo_3). The sac-like bursa lies dorsolateral to the pallial oviduct, and does not extend posterior to it. The bursa is 58% of the pallial oviduct length, and its anterior third lies anterior to the end of the mantle cavity (Figs. 22A, F). A thin sperm duct (Sdu) issues from the anterior end of the bursa and coils slightly on its ventral surface (Fig. 22F). The albumen gland "pouch" (Agp) appears as a disc appressed to the ventral surface of the albumen gland (Fig. 22A). When the pouch is pulled away from the pallial oviduct, its basal connection to the latter is readily seen (Fig. 22C). This is also seen in cross-section (Fig. 22D). The oviduct (Ov) and sperm duct (Sdu) jointly enter the

TABLE 20. Shell measurements (mm) of males and females from two populations of *Paludiscala caramba*. Snails with the dominant maximum whorl number(s) were used. N = 9 unless stated otherwise. Mean ± standard deviation. "p" refers to the significance level for the difference between shell lengths (t-test) for that population.

	Whorls	Length	Width	Length of body whorl	Length of aperture	Width of aperture	p
Locality 63							
♀ (n = 10)	7.0	2.26 ± 0.08	1.09 ± 0.08	1.04 ± 0.05	0.71 ± 0.04	0.53 ± 0.04	
	7.5	2.44 ± 0.09	1.13 ± 0.10	1.09 ± 0.07	0.71 ± 0.03	0.53 ± 0.03	>.1
♂	7.5	2.41 ± 0.13	1.11 ± 0.09	1.07 ± 0.05	0.71 ± 0.04	0.53 ± 0.02	
Locality 27							
♀	7.5	2.50 ± 0.10	1.18 ± 0.07	1.08 ± 0.07	0.74 ± 0.06	0.57 ± 0.03	>.1
♂	7.5	2.54 ± 0.06	1.18 ± 0.06	1.09 ± 0.06	0.74 ± 0.04	0.54 ± 0.04	

TABLE 21. Dimensions (mm) or counts of non-neural organs and structures of *Paludiscala caramba*. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	3.11 ± 0.21	3.01 ± 0.15
Gill filament number		13.6 ± 0.89	
Osphradium (N = 6)	L	0.15 ± 0.02	
Gonad	L	0.48 ± 0.07	0.47 ± 0.08
	W	0.29 ± 0.04	0.27 ± 0.03
Prostate	L		0.44 ± 0.04
	W		0.19 ± 0.03
Penis	L		0.66 ± 0.04
	W		0.19 ± 0.02
Pallial oviduct	L	0.55 ± 0.06	
(N = 7)	W	0.15 ± 0.02	
Bursa copulatrix	L	0.32 ± 0.03	
(N = 8)	W	0.14 ± 0.02	

TABLE 22. Radular statistics from 5 individuals of *Paludiscala caramba*. $\bar{X}$ = mean, S = standard deviation. Measurements in mm.

Radular features	$\bar{X}$	S
Length	0.343	0.016
Width	0.050	0.004
Number of rows	72.0	2.0
Number of rows in formative stage	5.2	1.6

TABLE 23. The various cusp arrangements of the four tooth types of *Paludiscala caramba*, counted from five radulae using SEM, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
4–1–4 / 1–1	80	4–1–3	80	18	20	16	20
5–1–4 / 1–1	40	5–1–3	20	19	20	17	20
5–1–5 / 1–1	80	4–1–4	20	20	20	18	20
6–1–4 / 1–1	20			21	40	22	40
6–1–6 / 1–1	20			22	40	23	80
				23	40	24	40
				24	20	25	20

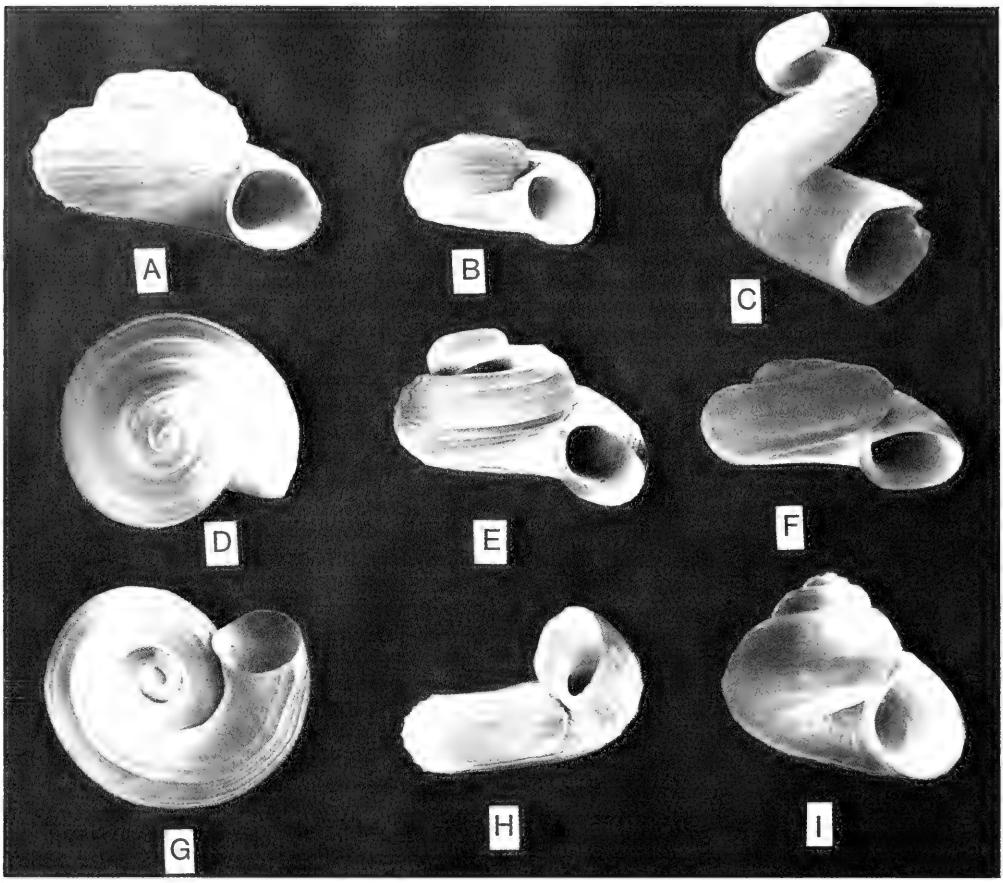

FIG. 23. SEM photos of *Cochliopina milleri* and *Cochliopina riograndensis*. Shells A–H are *Cochliopina milleri* from Locality 38, shell I is *Cochliopina riograndensis* from Locality 101. Shell A is 3.26 mm wide; the others are printed to the same enlargement.

albumen gland at the base of the pouch; this area is somewhat differentiated and appears to be a sphincter (Fig. 22E). There is no seminal receptacle of normal shape or position. The pouch, while non-glandular, is obviously of pallial oviduct origin, yet was seen to hold sperm and probably serves as a secondary seminal receptacle, as may the oviduct swellings. The spermathecal duct (Sd) may be tightly appressed to the columellar side of the pallial oviduct (Fig. 22A) or may be slightly separated from it (Fig. 22F).

The male gonad is a single non-lobed mass. The prostate is relatively large and extends considerably anterior to the end of the mantle cavity. The anterior vas deferens exists from the anterior tip of the prostate.

The penis is bluntly shaped and has neither cilia nor a terminal papilla. The penial lobe (Plo) is located at 67% of the penis length (on the outer curvature) from the base, and has a spherical shape with a diameter of 0.15 mm (Fig. 21C). The lobe overlies its attachment area to the penis, which is short and located on the outer curvature (Att, Fig. 21C). The apocrine gland (Agl) extends for slightly more than one-half of the diameter of the penial lobe. The structure of the gland is precisely that of *Coahuilix*. Folds are seen on the outer curvature of the penis from the base to the penial lobe. The inner curvature has folds for virtually its entire length. The vas deferens (Vd) does not coil in the penis. Infrequent Gl_2 glands are seen in the penis.

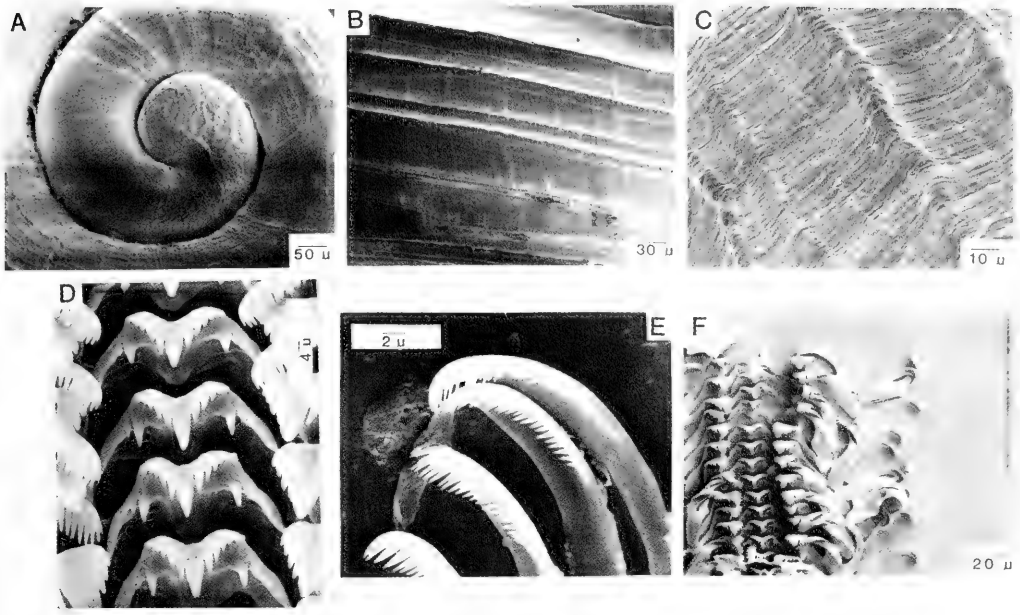

FIG. 24. SEM photos of shell and radula of *Cochliopina milleri*. A. Shell apex, showing wrinkled, pitted microsculpture. B. Portion of the body whorl showing spiral lines and collabral growth lines. C. Close-up of B. D. Central tooth of the radula. E. Outer marginal teeth. F. Part of radular ribbon.

Cochliopina Morrison, 1946

Type-species: *Cochliopina riograndensis* (Pilsbry & Ferriss, 1906).

Distribution: Rio Grande drainage, from Texas south to Panama.

Species included: 20 species listed by Taylor (1966).

Description

Cochliopina has no distinctive unique features, but is recognizable by a combination of character states (see below).

The shell (Fig. 23; Morrison, 1946, pl. 2, figs. 7–9, 11–13) is small (width, 5 mm) and planispiral to low-trochoid in form; the sculpture consists of spiral lines or cords, frequently bearing periostracal bristles; the apical whorl has pitted microsculpture (Fig. 24A); the tentacles have *Spurwinkia*-like hypertrophied ciliary tufts (Figs. 25A, B); females are ovoviviparous; the pallial oviduct has a slight posterior bend; the albumen gland is reduced in size (Ppo, Fig. 26A); the seminal receptacle opens into the oviduct (Figs. 26B, C); the oviduct and anterior end of the bursa are connected by a short sperm duct (Sdu, Figs. 26B, C); the non-muscular spermathecal duct opens just beyond the posterior end of the mantle cavity (Figs. 26A, C); the anterior end of the brood pouch is muscularized and coiled toward the columellar side (Fig. 26E); the penis is non-lobed, with an elongate penial filament, and lacks specialized glands (Fig. 25D; Morrison, 1946, pl. 3, figs. 9–15).

Discussion

Cochliopina and *Mexithauma* share numerous features (see Tables 53–55, Figs. 49, 50) relating to shell shape, sculpture, tentacle ciliation, reproductive mode, coiling of the pallial oviduct, reduction of the albumen gland size, connection between the seminal receptacle and oviduct, muscularization and coiling of the end of the brood pouch, and the form of the penis (and lack of specialized glands). Distinctive features of the female reproductive system shared by these taxa include the slight posterior bend of the pallial oviduct, the opening of the seminal receptacle into the oviduct, and the well-developed muscularization and coiling of the anterior end of the brood pouch.

The two taxa differ in the following features:

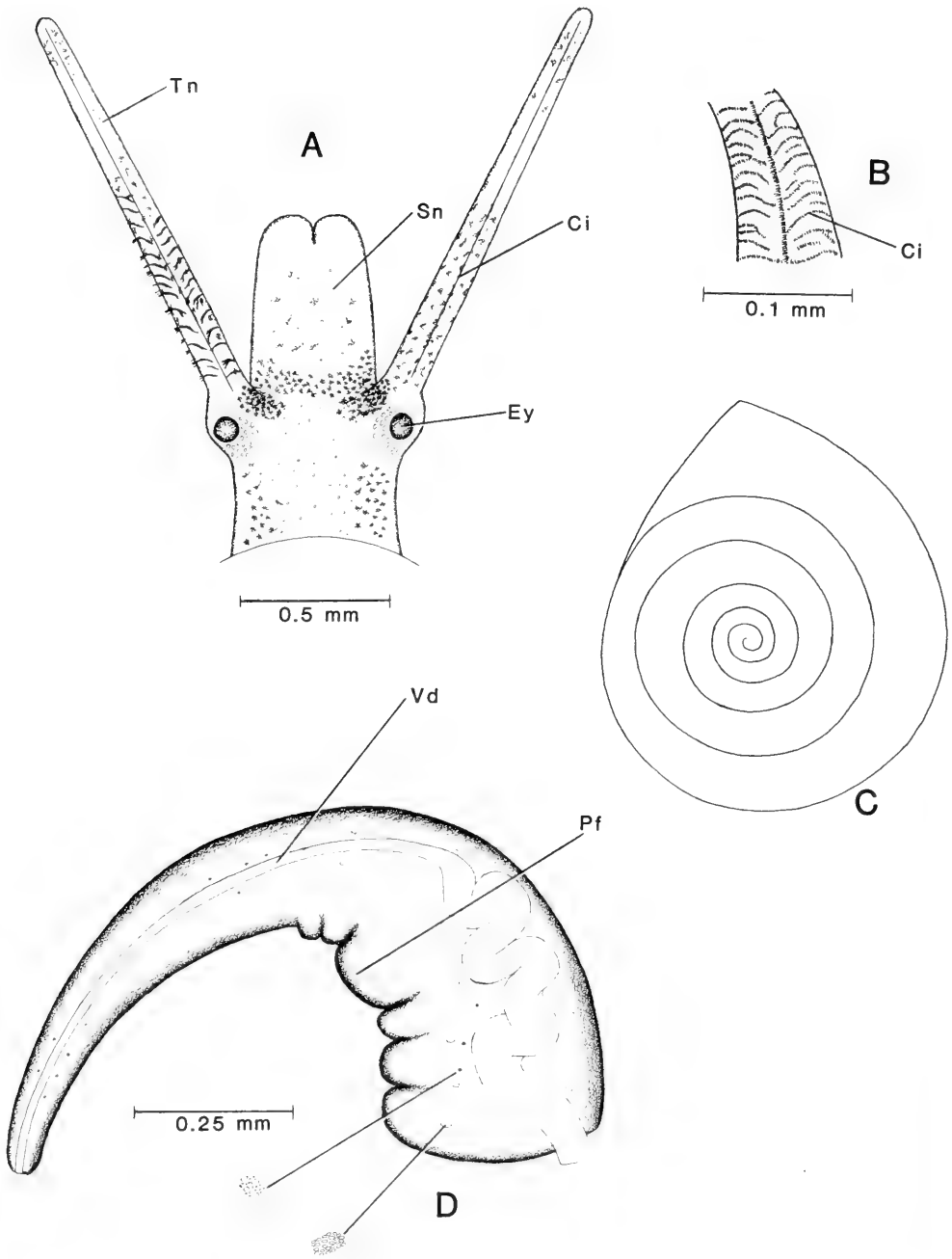

FIG. 25. Head, operculum, and penis of *Cochliopina milleri*. A. Dorsal aspect of the head. Note the *Spurwinkia*-like hypertrophied ciliary tufts (Ci) on the tentacles. B. Close-up of ciliation pattern on left tentacle. C. Operculum. D. Penis with penial folds (Pf), but no lobes. Ci—ciliary tufts on tentacle; Ey—eye; Sn—snout; Tn—tentacle; Vd—vas deferens.

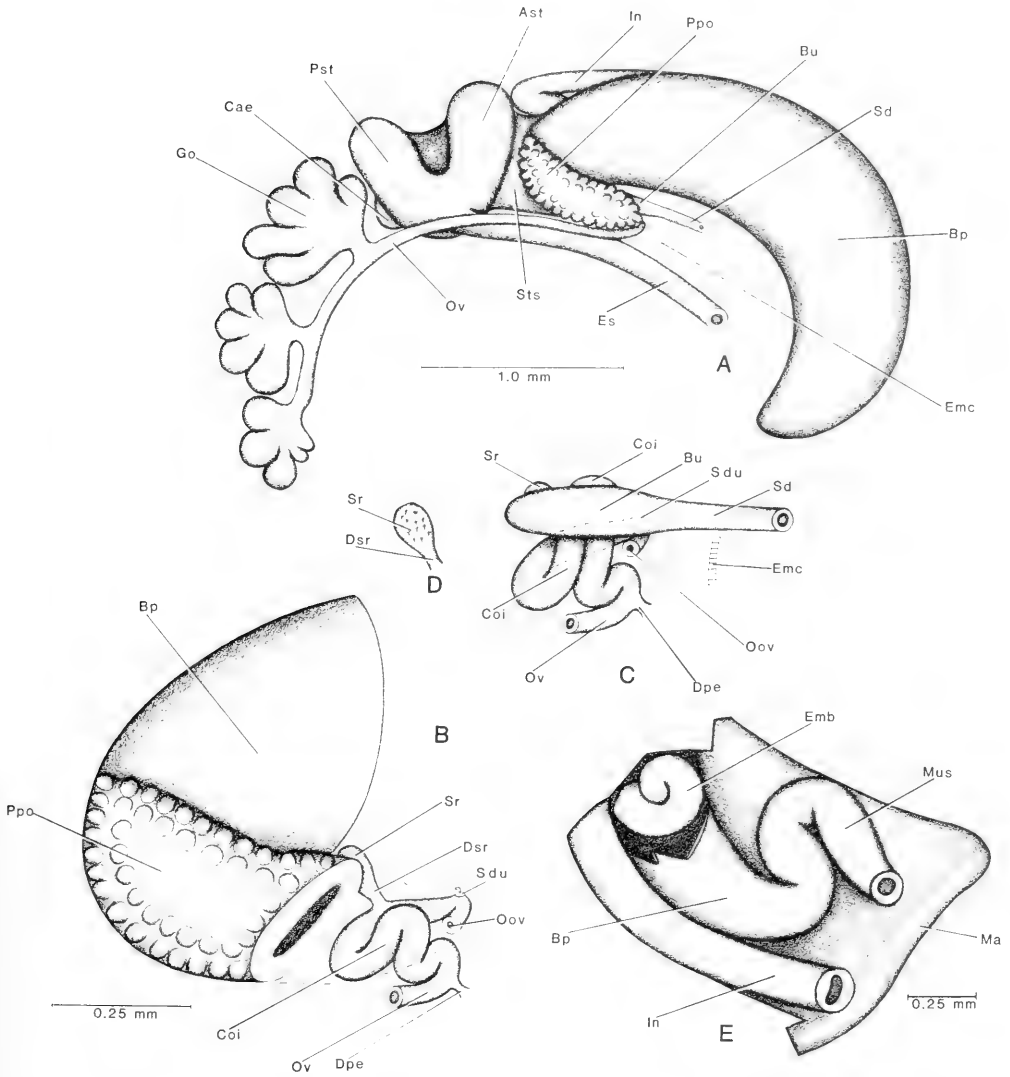

FIG. 26. Female reproductive anatomy of *Cochliopina milleri*. A. Ventral aspect of uncoiled snail without head and kidney tissue. Note the posterior bend of the pallial oviduct. B. Posterior region of the pallial oviduct, oriented as in A, but with a portion of the albumen gland (Ppo) and the bursa cut away to reveal the oviduct coils (Coi), seminal receptacle (Sr), sperm duct (Sdu), and opening of the oviduct into the albumen gland (Oov). C. Oriented as in B, but with the bursa (Bu) in place. D. The seminal receptacle (Sr) with pigment patches. E. Portion of the anterior end of the mantle cavity, showing the muscular coil (Mus) of the anterior end of the brood pouch (Bp). Ast—anterior stomach chamber; Bp—brood pouch; Bu—bursa; Cae—caecum of stomach; Coi—coil of oviduct; Dpe—gonopericardial duct; Dsr—duct of the seminal receptacle; Emb—embryonic shell; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Ma—mantle edge; Mus—muscular section of the brood pouch; Oov—opening of the oviduct; Ov—oviduct; Ppo—albumen gland; Pst—posterior stomach chamber; Sd—spermathecal duct; Sdu—sperm duct; Sr—seminal receptacle; Sts—style sac.

condition of the mantle edge (*Cochliopina,* smooth; *Mexithauma,* papillate); apical microsculpture (*Cochliopina,* pitted; *Mexithauma,* absent); relative size of the female gonad (*Cochliopina,* large; *Mexithauma,* small); length of the spermathecal duct (*Cochliopina,* short; *Mexithauma,* long); insertion of the sperm duct from the oviduct (*Cochliopina,* to bursa; *Mexithauma,* to duct of bursa); and male gonad morphology (*Cochliopina,* simple lobes; *Mexithauma,* bush-like).

Cochliopina milleri Taylor, 1966

Holotype: UMMZ 220182

Type-locality: Locality 53.

Distribution: endemic to the Cuatro Ciénegas Basin. In the eastern lobe of the basin, it has not been found south of Locality 97 (Fig. 3).

Habitat: *Cochliopina milleri* is most common in the outflows of cool (<28°C) springs, and is usually sympatric with *Nymphophilus minckleyi* on aquatic vegetation, particularly *Chara* and *Utricularia. Cochliopina milleri* was rarely found in the large spring pools, and was never found in mop or sieve collections from the small springs.

Description

The generic placement of *Cochliopina milleri* is tentative as the detailed anatomy of the type-species, *Cochliopina riograndensis,* is not known. *Cochliopina milleri* does resemble the type-species in shell form and external anatomy.

The shell (Figs. 23A–H) is thin, relatively small (length, 1.16–1.48 mm), and broadly conical to planispiral, with little whorl overlap; the narrow spiral cords are numerous, prominent, and fringed with light-colored periostracum; the aperture is nearly circular and adnate to or free from the penultimate whorl.

Shell

The shell has rounded whorls and deeply impressed sutures. The aperture is slightly angled adapically and inclined 30–45° to the coiling axis. For the population from Locality 38, coiling abnormalities were frequent and remarkable; varying from a slight loosening of the whorls (Fig. 23E), to a change in coiling direction near the end of the body whorl (Fig. 23H), to near-open coiling (Fig. 23C). Other populations show less coiling variation than that from Locality 38. There are generally 10–20 spiral cords, and numerous spiral lines on the last two whorls. Occasional specimens are almost smooth-shelled (Fig. 23F). The apical whorl microsculpture is somewhat coarser than that seen in other hydrobiid snails (Fig. 24A). Spiral sculpture begins after the first whorl. Close-ups of the spiral sculpture are shown in Figs. 24B, C. There are strong axial growth lines that become especially prominent near the aperture (Fig. 24C).

Shell measurements from the population from Locality 38 (excluding abnormally coiled specimens) are given in Table 24. Females have a greater shell width ($p < .005$) and are also relatively taller (t-test for difference be-

TABLE 24. Shell measurements (mm) of males and females from one population of *Cochliopina milleri* and two populations of *C. riograndensis.* Snails with the dominant maximum whorl number were used. N = 9 unless otherwise indicated. Mean ± standard deviation. "p" refers to the significance level for the difference between shell widths of males and females (t-test) for that population.

	Whorls	Length	Width	Length of body whorl	Length of aperture	Width of aperture	Shell length / Shell width
C. milleri (Locality 38)							
♂	3.0	1.16 ± 0.08	2.29 ± 0.15	1.11 ± 0.10	0.96 ± 0.06	0.89 ± 0.07	0.50 ± 0.03
♀	3.5	1.78 ± 0.10	3.08 ± 0.18	1.57 ± 0.11	1.19 ± 0.10	1.10 ± 0.06	0.58 ± 0.05
p = <.005							
C. riograndensis (Locality 101)							
♂	4.0	2.23 ± 0.10	2.74 ± 0.18	1.84 ± 0.09	1.48 ± 0.09	1.22 ± 0.07	0.82 ± 0.06
♀	4.5	2.78 ± 0.15	3.12 ± 0.28	2.18 ± 0.15	1.59 ± 0.14	1.36 ± 0.10	0.89 ± 0.06
C. riograndensis (Locality 102)							
♂	3.5	1.54 ± 0.04	2.14 ± 0.11	1.34 ± 0.11	1.09 ± 0.09	0.93 ± 0.08	0.72 ± 0.05
♀	4.0	2.21 ± 0.17	2.79 ± 0.15	1.86 ± 0.12	1.43 ± 0.08	1.24 ± 0.08	0.79 ± 0.05
	>4.0, <4.5	2.54 ± 0.15	3.06 ± 0.12	2.03 ± 0.08	1.54 ± 0.08	1.33 ± 0.07	0.83 ± 0.03

TABLE 25. Dimensions (mm) or counts of non-neural organs and structures of *Cochliopina milleri*. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body (N = 6)	L	6.47 ± 0.39	5.15 ± 0.31
Gill filament number		26.7 ± 2.28	
Osphradium (N = 8)	L	0.35 ± 0.05	
Gonad (N = 6)	L	1.69 ± 0.09	3.28 ± 0.15
	W	0.55 ± 0.05	0.57 ± 0.03
Prostate	L		0.56 ± 0.06
	W		0.28 ± 0.03
Penis	L		1.24 ± 0.05
	W		0.36 ± 0.04
Pallial oviduct	L	2.85 ± 0.28	
	W	0.72 ± 0.08	
Bursa copulatrix	L	0.27 ± 0.04	
	W	0.10 ± 0.01	
Seminal receptacle (body) (N = 6)	L	0.10 ± 0.01	
	W	0.06 ± 0.01	
Seminal receptacle (duct) (N = 9)	L	0.04 ± 0.01	
	W	0.04 ± 0.01	

TABLE 26. Radular statistics from 12 individuals of *Cochliopina milleri*. $\bar{X}$ = mean, S = standard deviation. Measurements in mm.

Radular feature	$\bar{X}$	S
Length	0.563	0.028
Width	0.106	0.006
Number of rows	45.6	1.96
Number of rows in formative stage	2.36	1.12
Width of central tooth (N = 21)	0.031	0.0014

tween means of shell length/width, $p < 0.005$) than males. This sexual dimorphism is seen by comparing Figs. 23A and B (adult female and male, respectively).

Nonreproductive Features

Details and data concerning anatomy are from the population from Locality 38. Measurements of organs and structures are given in Table 25. The snout is squat and the tentacles are elongate in comparison (Fig. 25A). The left tentacle has 10–12 hypertrophied ciliary tufts protruding from the outer edge and numerous ciliary tracts (Ci, Figs. 25A, B) curving inward toward the center of the tentacle from both sides. These tracts are present for 67% of the tentacle length from the base. The tentacle also has a central ciliary tract along its length. The right tentacle alone has the central ciliary tract. The snout and tentacles are dusted with melanin to varying degrees. A dark melanin patch at the base of each tentacle, across from the eyespot, is seen in most individuals. Occasional specimens had a dark melanin patch at the tentacle tips. There is a cluster of dull white granules around the eyes and smaller granules are found in the neck, snout, and tentacles.

The sides of the head-foot are sometimes darkly pigmented, and in those specimens a non-pigmented strip was seen extending from the neck to the foot. Body pigmentation for the female consists of small melanin patches on the dorsal and ventral body surfaces that interdigitate with clusters of white granules, producing a mottled appearance. The male is similarly pigmented, but has solid dark melanin on the ventral surface of the gonad. The caecal chamber is prominent.

The operculum (Fig. 25C) has 5.5 whorls and the nucleus is positioned at 42% of the long axis of the operculum. Pigmentation on the operculigerous lobe consists of two to three large melanin patches.

Radula

The radula is shown in Figs. 24D–F. The central tooth has one to three pairs of basal cusps that originate from the lateral angles. The central cusp of the central tooth is dagger-like. The marginal teeth have many cusps. Radular statistics and the various cusp arrangements for the four tooth types are given in Tables 26 and 27.

Female Reproductive Anatomy

The organization of the female reproductive system is shown in Fig. 26. The gonad (Go) has three lobate branches, and occupies 26% of the body length. The pallial oviduct occupies 44% of the body length and overlies most of the style sac. The anterior portion of the pallial oviduct is modified into a thin-walled, non-glandular brood pouch (Bp) for the storage of embryos. The pallial oviduct coils posteriorly, the distance from the posteriormost point of the pallial oviduct and the end of the coil being 0.67 mm. The small albumen gland (Ppo, Fig. 26A) constitutes this coiled portion.

The oviduct (Ov) disappears beneath the anterior portion of the albumen gland (Fig. 26A). There is a short gonopericardial duct

TABLE 27. The various cusp arrangements for the four tooth types in 12 radulae of *Cochliopina milleri*, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
4–1–3 / 2–2	8	4–1–3	83	17	8	19	40
4–1–4 / 1–1	8	4–1–4	83	18	25	20	40
4–1–4 / 2–1	8	5–1–4	8	19	50	21	40
4–1–4 / 2–2	42	5–1–5	8	20	58	22	60
4–1–4 / 3–2	17	5–1–3	8	21	92	23	80
4–1–4 / 3–3	17	3–1–3	42	22	33	24	60
5–1–4 / 2–2	17			23	58	25	60
5–1–4 / 3–2	8			24	8	26	20
5–1–5 / 2–1	17			25	25	27	20
5–1–5 / 2–2	67			26	8	28	20
6–1–5 / 2–2	25			27	8		
				28	8		

(Dpe). The bursa (Bu) is small, dorsal to, and almost entirely hidden by the albumen gland (Fig. 26A). The elongate seminal receptacle (Sr) is dorsal to and mostly hidden by the bursa. There is usually a light melanin dusting on the seminal receptacle (Fig. 26D).

The oviduct loops several times dorsolaterally to the bursa before receiving the short duct of the seminal receptacle (Dsr, Figs. 26B, C). The minuscule sperm duct (Sdu) enters the oviduct just where the latter turns to open into the end of the albumen gland (Fig. 26B). The spermathecal duct (Sd) extends 0.14 mm beyond the posterior end of the mantle cavity.

The well developed muscular loop (Mus) of the anterior brood pouch is shown in Fig. 26E. For 15 adult females, there was an average of 11.6 ± 2.97 shelled embryos in the brood pouch, as well as another eight to ten very small non-shelled embryos. For 108 shelled embryos, the range of shell width was quite narrow, 0.317–0.475 mm, and the mean was 0.400 ± 0.178 mm. Embryonic shells have up to 1.3 whorls.

Male Reproductive Anatomy

The male gonad consists of 10–12 lobed branches and fills the entire length of the digestive gland, covering the posterior stomach chamber. The gonad is 64% of the body length. The prostate is relatively small, 11% of the body length, but does overlap the mantle cavity. The anterior vas deferens exits from the anterior tip of the prostate.

The penis (Fig. 25D) has numerous folds (Pf) on the inner curvature for one half of the penis length. While one of the folds was sometimes noticeably wider than the others (as in Fig. 26D), it did not project outward as a penial lobe in any specimen. The outer curvature is without folds. Scattered throughout the penis are Gl_1 and Gl_2 gland types. The vas deferens (Vd) coils and thickens from the base of the penis until it is even with the end of the penial folds, after which it narrows and stops coiling. The penis, exclusive of the long penial filament, has a dark brown color that is not an external melanin coating, but colored tissue.

Cochliopina riograndensis
(Pilsbry & Ferriss, 1906)

Holotype: ANSP 91324.

Type-locality: debris of the Rio San Felipe near the Rio Grande, Val Verde County, Texas.

Distribution: Rio Grande drainage of Texas and northeastern México. While this species had been previously known from the Rio Salado de Nadadores, and an adjacent spring, both just east of the Cuatro Ciénegas Basin (Taylor, 1966), the author also collected it at Locality 101 in the southeastern lobe of the basin.

Habitat: This species has been found in springs and spring outlets of various sizes (Fullington, 1978; Taylor, 1966). Locality 101 is a large spring pool (the Santa Tecla Laguna) and *Cochliopina riograndensis* was abundant on unidentified vegetation along its shallow edges.

Description

This species has been amply described by Fullington (1978) and Taylor (1966). Its shell is distinguished from that of *Cochliopina milleri* by its thicker, larger, and relatively taller appearance (see Table 24: note the ratios of shell length/width). Its whorls overlap greatly and it has a more conical shape than does the shell of *Cochliopina milleri.* The aperture is angled adapically and the inner lip is partly fused to the penultimate whorl. The spiral cords are few in number and lack prominence, but the periostracal bands are darker and wider (especially in the umbilical area) than those of *Cochliopina milleri.*

Discussion

The southeastern lobe of the basin has several nonendemic taxa of undisputed Rio Grande (= Rio Bravo) origin, including the cichlid fish, *Cichlasoma cyanoguttatum* (see Minckley, 1977). The distinctive Rio Grande aspect of the fauna from this portion of the basin contrasts with the more endemic aspect of the fauna from the remainder of the basin. Hubbs & Miller (1965) suggested that the southeastern lobe had a recent surficial connection to the Rio Salado de Nadadores (Fig. 2, Number 7, a Rio Grande tributary), thus explaining this pattern. The discovery of *Cochliopina riograndensis* in the southeastern lobe of the basin supports this hypothesis.

The relationship between endemic *Cochliopina milleri* and non-endemic *Cochliopina riograndensis* is unknown as the internal

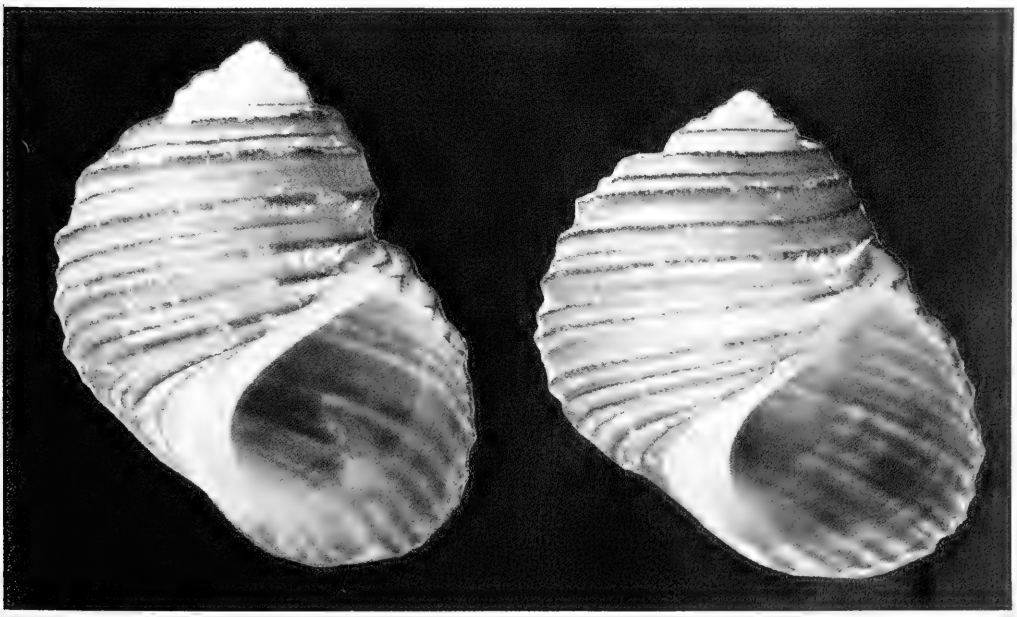

FIG. 27. Shells of *Mexithauma quadripaludium* from Locality 1. The shell on the left is 7.38 mm long, the other is printed at the same enlargement.

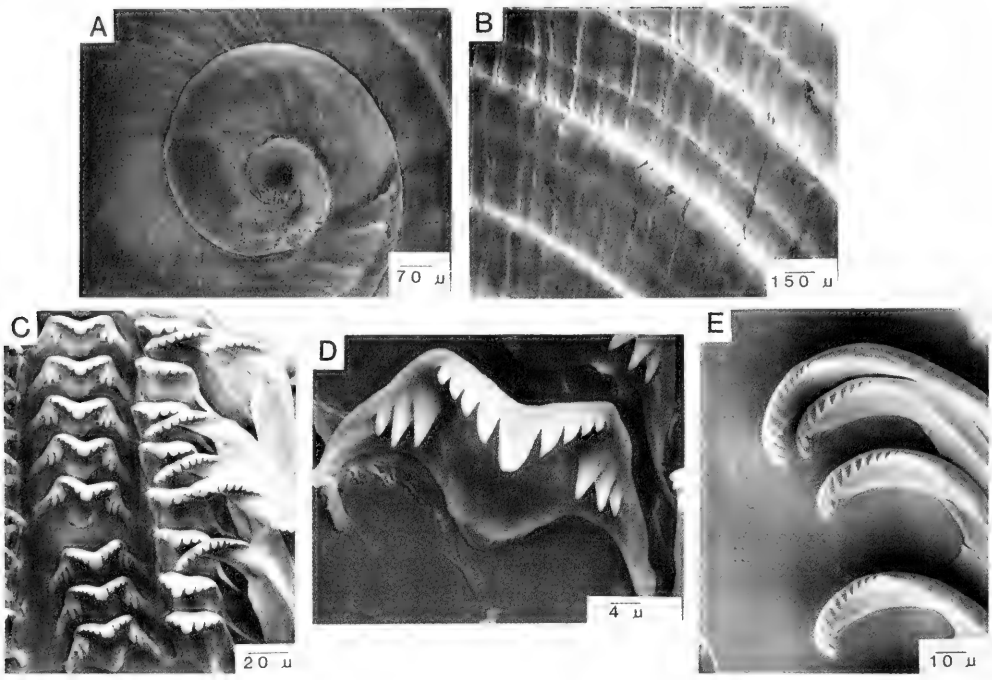

FIG. 28. SEM photos of shell and radula of *Mexithauma quadripaludium*. A. Apical whorls of shell. Note lack of microsculpture. B. Portion of penultimate whorl showing strong spiral cords and collabral microsculpture. C. Part of radular ribbon. D. Isolated central tooth. E. Outer marginal teeth.

anatomy of the latter is not known. The distinctively fragile and loosely-coiled shell of *Cochliopina milleri* may be associated with its restriction to fairly cichlid-free waters (i.e., low predation pressure).

Mexithauma Taylor, 1966

Type-species: *Mexithauma quadripaludium* Taylor, 1966.

Distribution: endemic to the Cuatro Ciénegas Basin.

Species included: monotypic.

Description

Distinctive features of *Mexithauma* include the papillate mantle edge of both sexes (Pma, Figs. 30A, 31A) and the open channel (Oc) connecting the openings of the spermathecal duct and pallial oviduct (Figs. 30C, E).

The shell (Fig. 27) is large (length, 7.0 mm), globose, without umbilicus, and with prominent spiral cords fringed with periostracum; the inner lip of the shell is thickened; the tentacles show *Spurwinkia*-like ciliation (Figs. 29A, B); females are ovoviviparous; the female gonad is very reduced in size (Go, Fig. 30A); a large pallial oviduct overlies the stomach and has a slight posterior bend (Fig. 30A); the seminal receptacle (Sr) is positioned lateral to the bursa and opens into the oviduct (Fig. 30B); the oviduct connects with the duct of the bursa via a short sperm duct (Sdu, Fig. 30B); the anterior end of the brood pouch is muscularized and coiled (Figs. 30C, E); the male gonad is bush-like (Go, Fig. 31A); the penis is non-lobed and lacks specialized glands (Fig. 31B).

Mexithauma is most similar to *Cochliopina* (see above; Tables 53–55, Figs. 49, 50).

Mexithauma quadripaludium Taylor, 1966

Holotype: UMMZ 220214.

Type-locality: Locality 97.

Habitat: *Mexithauma quadripaludium* has been found only in the larger springs and their outflows. It has been collected from all types of aquatic vegetation, sand (composed of

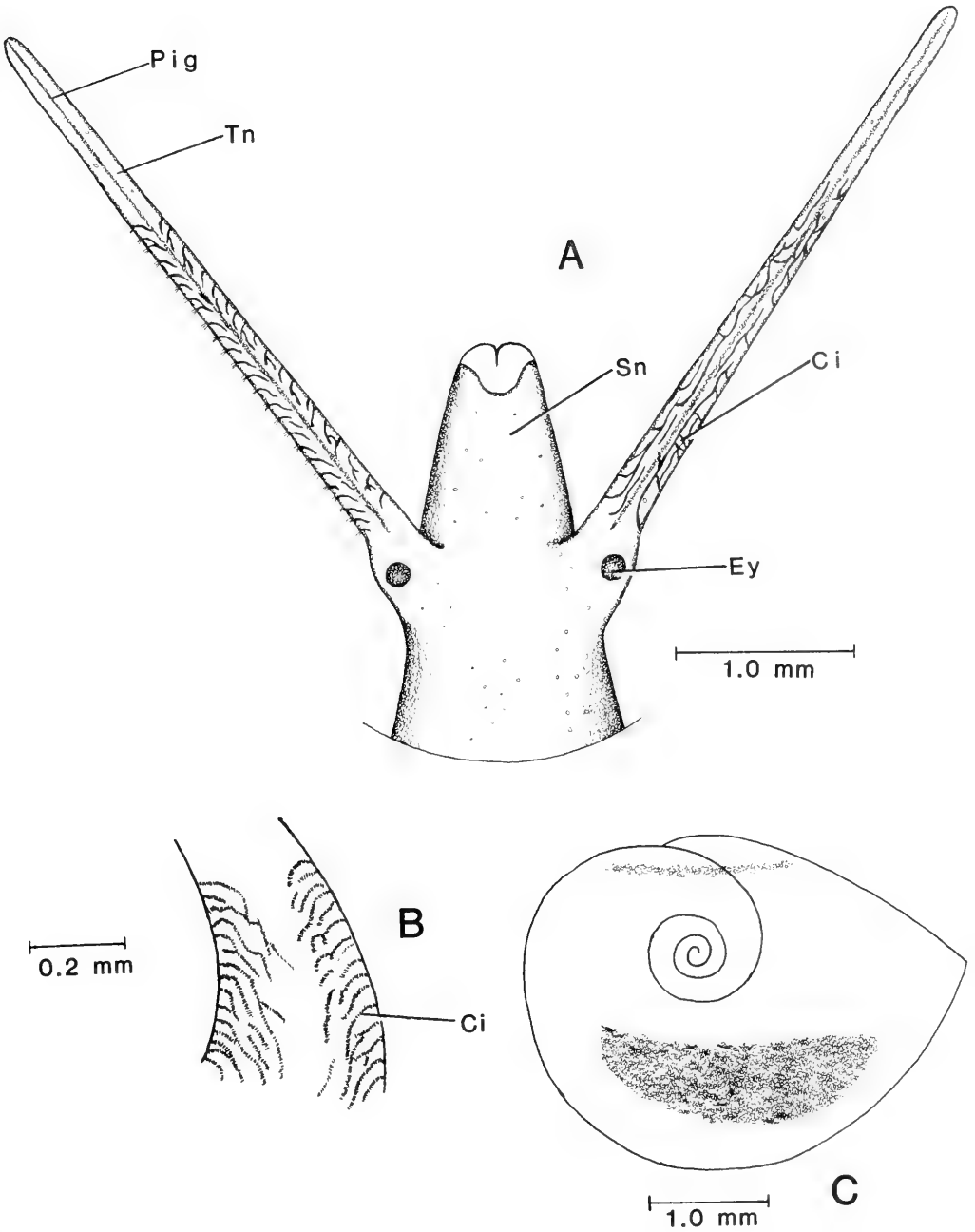

FIG. 29. Head and operculum of *Mexithauma quadripaludium.* A. Dorsal aspect of head. Note the *Spurwinkia*-like hypertrophied ciliary tufts (Ci) and the central pigment streak (Pig) on the tentacles. B. Close-up of ciliation pattern on left tentacle. C. Operculum. The pigment pattern on the operculigerous lobe is also shown. Ci—ciliary tufts on tentacles; Ey—eye; Pig—pigment; Sn—snout; Tn—tentacle.

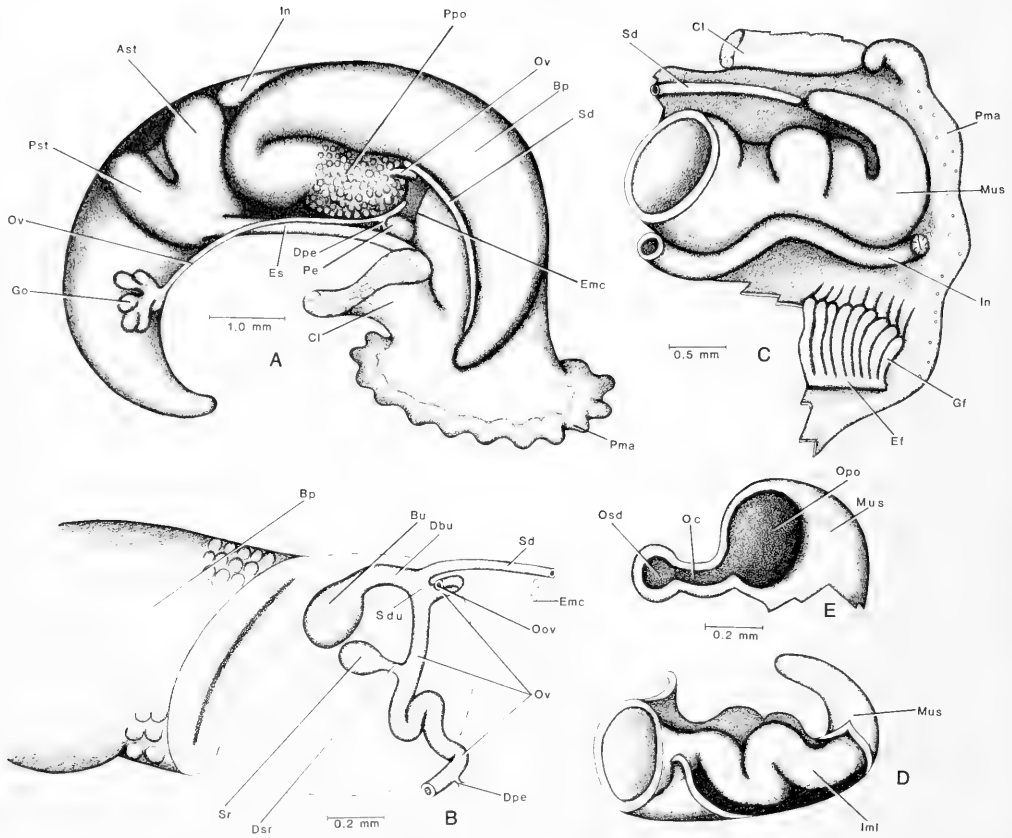

FIG. 30. Female reproductive anatomy of *Mexithauma quadripaludium*. A. Ventral aspect of uncoiled snail without the head and kidney tissue. Note the very small gonad (Go) and large pallial oviduct (Bp + Ppo) with a posterior bend. B. Oriented as in A, but with most of the albumen gland (Ppo) cut away to expose the bursa copulatrix complex. C. Anterior portion of the mantle cavity showing the muscular bend (Mus) of the anterior end of the brood pouch. D. Oriented (and scale) as in C, but with a portion of the epithelium of the anterior end of the brood pouch cut away to expose the inner muscular layer (Iml). E. Oriented as in A, showing the openings of the spermathecal duct (Osd) and pallial oviduct (Opo) connected by an open channel (Oc). Ast—anterior stomach chamber; Bp—brood pouch; Bu—bursa; Cl—columellar muscle; Dbu—duct of the bursa; Dpe—gonopericardial duct; Dsr—duct of the seminal receptacle; Ef—efferent vessel; Emc—posterior end of the mantle cavity; Es—esophagus; Gf—gill filament; Go—gonad; Iml—inner muscular layer; In—intestine; Mus—muscular section of the brood pouch; Oc—open channel; Oov—opening of the oviduct; Opo—opening of the pallial oviduct; Osd—opening of the spermathecal duct; Ov—oviduct; Pe—pericardium; Pma—papillate mantle edge; Ppo—albumen gland; Pst—posterior stomach chamber; Sd—spermathecal duct; Sdu—sperm duct; Sr—seminal receptacle.

travertine pieces and shell fragments), travertine blocks, and the gently sloping banks of spring pools. While *M. quadripaludium* and *Nymphophilus minckleyi* overlap broadly in their microhabitat usage, within any given spring they are largely allopatric on a microhabitat scale. In springs with microhabitat diversity, *N. minckleyi* is most common on *Nymphaea* and *Chara,* while *M. quadripaludium* is most common in sand or on travertine blocks. However, when one of the species is of reduced abundance or absent, usually in a spring with low microhabitat diversity, the other species may "switch" to other microhabitats, including that usually occupied by the species that is rare or absent.

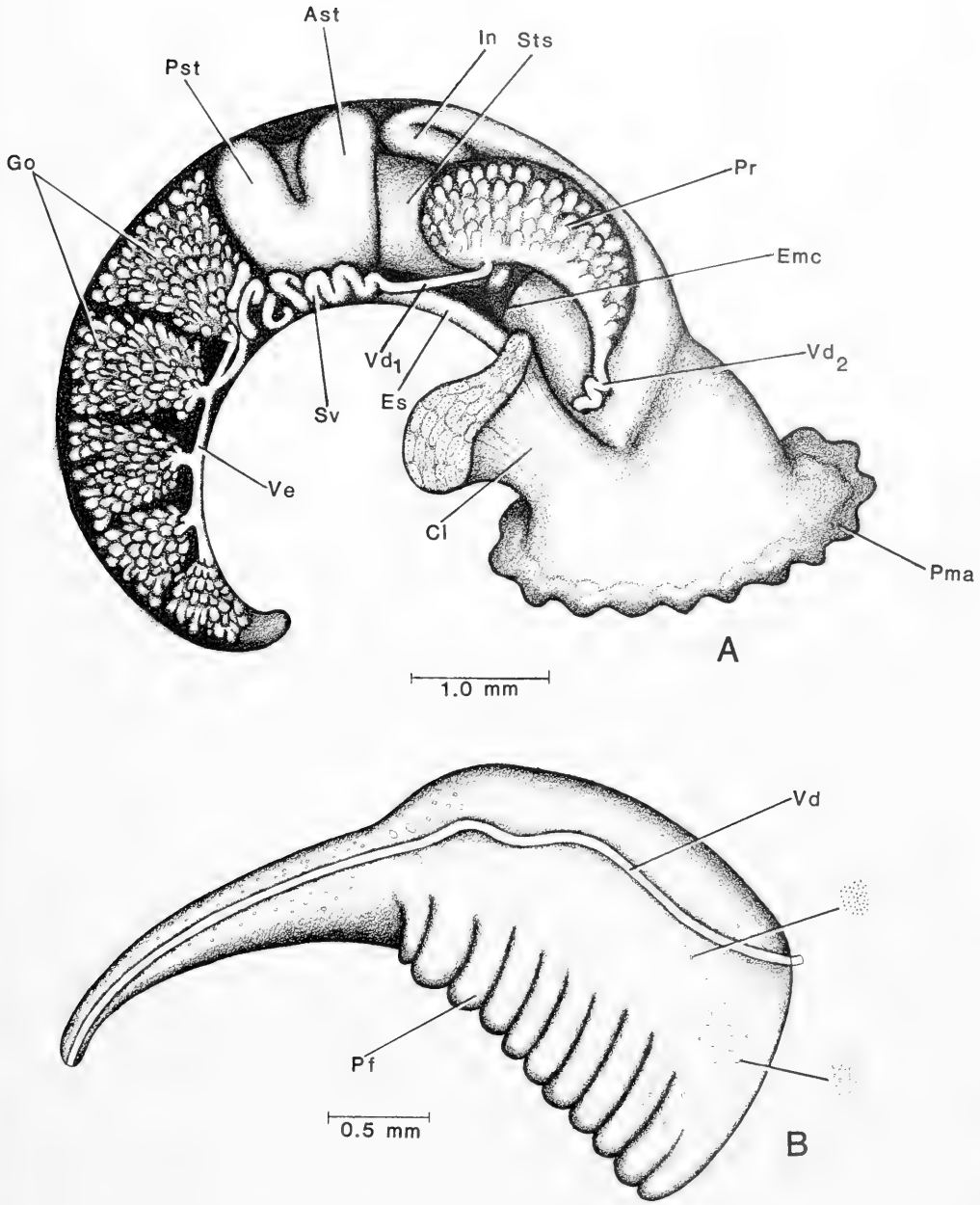

FIG. 31. Male reproductive anatomy of *Mexithauma quadripaludium.* A. Ventral aspect of an uncoiled snail without head and kidney tissue. Note the bush-like gonad (Go). B. Dorsal aspect of the non-lobed penis. Ast—anterior stomach chamber; Cl—columellar muscle; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Pf—penial fold; Pma—papillate mantle edge; Pr—prostate; Pst—posterior stomach chamber; Sts—style sac; Sv—seminal vesicle; Vd—vas deferens; Vd_1—vas deferens from seminal vesicle to prostate; Vd_2—vas deferens from prostate to penis; Ve—vas efferens.

TABLE 28. Shell measurements (mm) of males and females of *Mexithauma quadripaludium* from Locality 1. The shells measured are from the largest 10% of the population (>4.8 whorls). N = 9. Mean ± standard deviation. "p" refers to the significance level for the difference between shell lengths of males and females (t-test).

	Length	Width	Length of body whorl	Length of aperture	Width of aperture	p
♂	7.28 ± 0.18	6.08 ± 0.19	6.46 ± 0.50	4.89 ± 0.18	3.86 ± 0.31	>.10
♀	7.34 ± 0.41	6.30 ± 0.27	6.78 ± 0.31	4.94 ± 0.18	3.84 ± 0.29	

TABLE 29. Frequency distribution for number of spiral cords at the aperture of *Mexithauma quadripaludium* shells. Fifteen shells (sexes mixed) per whorl stage were used. The mean and standard deviation for the shell length of the shells used for each whorl stage are given.

		Number of spiral cords												
Whorls	Shell length	7	8	9	10	11	12	13	14	15	16	17	18	22
4.0	3.30 ± 0.32	1	3	2	4	4	1	—	—	—	—	—	—	—
4.5	5.11 ± 0.54	—	—	—	—	—	4	4	2	2	2	1	—	—
5.0+	7.44 ± 0.34	—	—	—	—	—	—	—	—	1	3	7	2	2

Shell

Shell measurements for the population from Locality 1 are given in Table 28. The exact number of whorls in large adults cannot be determined as the apex is usually somewhat eroded in such specimens. There is no significant sexual dimorphism in size of shell (Table 28). The spiral cords begin at 2.3 whorls and increase in number with shell size (Table 29). Adults have 15–22 cords on the body whorl that vary in height (Fig. 28B). Strong axial microsculpture, also fringed with periostracum, is present (Fig. 28B). The aperture is large, occupying more than one-half of the height of the body whorl, and is inclined only 10–20° to the coiling axis. The aperture is elliptical in shape, and is somewhat more angled above than below. While the inner lip is greatly thickened, the outer lip is thin. The apical whorl does not have microsculpture (Fig. 28A).

TABLE 30. Dimensions (mm) or counts of non-neural organs and structures of *Mexithauma quadripaludium*. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	11.5 ± 0.48	8.98 ± 0.32
Gill filament number		51.8 ± 1.79	
Osphradium (N = 7)	L	0.76 ± 0.08	
Gonad	L	0.88 ± 0.07	3.54 ± 0.26
	W	0.65 ± 0.06	1.29 ± 0.12
Prostate	L		1.97 ± 0.24
	W		0.95 ± 0.07
Penis	L		4.12 ± 0.22
	W		1.54 ± 0.13
Pallial oviduct	L	6.72 ± 0.35	
	W	1.91 ± 0.34	
Bursa copulatrix	L	0.26 ± 0.03	
	W	0.19 ± 0.01	
Seminal receptacle (body)	L	0.16 ± 0.01	
	W	0.13 ± 0.02	
Seminal receptacle (duct)	L	0.12 ± 0.01	
	W	0.05 ± 0.01	

Nonreproductive Features

Anatomical descriptions and data (Table 30) are from the population from Locality 1. The snout (Fig. 29A) is relatively squat, while the tentacles are thickened and elongate. Each tentacle has a central dark pigment strip, extending from just beyond the eye to the tentacle tip (Pig, Fig. 29A). On the left tentacle, there are numerous hypertrophied ciliary tufts projecting from the outer edge, as well as ciliary tracts curving inwards from both sides. The right tentacle lacks the ciliary tufts projecting from the outer side, and the ciliary tracts run along the length of the tentacle, rather than curving inward. The under-surface of each tentacle also has ciliary tracts running along its length (not figured). Small white granules are scattered in the neck, snout, and tentacles. The snout and neck have a light

dusting of melanin. The foot is large and thickened. There are distinctive pigment streaks just below the eyes and along the sides of the foot. The dorsal body surface has yellow and white pigment granules as well as melanin. The digestive gland is dark brown and has white granules scattered on its ventral surface. A prominent caecal chamber protrudes posterior to the stomach. The operculum (Fig. 29C) has 3.5 whorls and the nucleus is positioned at 41% of the long axis of the operculum. The characteristic pigment streaks on the operculigerous lobe are shown in Fig. 29C.

Radula

The radula is shown in Figs. 28C–E. The central tooth usually has three pairs of basal cusps arising from prominent lateral angles. The marginal teeth (Figs. 28C, E) have relatively few cusps. Radular statistics and the various cusp arrangements for the four tooth types are given in Tables 31 and 32.

TABLE 31. Radular statistics from nine individuals of *Mexithauma quadripaludium*. $\bar{X}$ = mean, S = standard deviation. Measurements are in mm.

Radular feature	$\bar{X}$	S
Length	1.42	0.058
Width	0.188	0.008
Number of rows	66.1	2.98
Number of rows in formative stage	4.44	1.01
Width of central tooth (N = 23)	0.047	0.0002

TABLE 32. The various cusp arrangements for the four tooth types in 11 radulae of *Mexithauma quadripaludium*, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
3–1–3 / 2–2	9	2–1–3	9	10	27	11	9
3–1–3 / 2–2	9	3–1–3	55	11	91	12	64
3–1–3 / 3–3	9	4–1–3	91	12	73	13	82
4–1–3 / 3–2	18	5–1–3	9	13	36	14	82
4–1–3 / 3–3	9	4–1–4	18	14	9	15	45
4–1–4 / 2–2	36			16	9	16	18
4–1–4 / 3–3	45					17	9
4–1–4 / 3–2	27						
5–1–3 / 3–2	9						
5–1–3 / 2–2	18						
5–1–3 / 3–3	18						
5–1–4 / 3–2	27						
5–1–4 / 3–3	18						
5–1–4 / 2–2	9						
5–1–5 / 3–2	18						

Female Reproductive Anatomy

The female gonad (Go) occupies only 8% of the body length and is a mere terminal thickening of the oviduct with a few small lobes. The pallial oviduct is 58% of the body length. The posterior bend of the pallial oviduct extends for 2.5 mm, of which 1.2 mm is albumen gland (Ppo, Fig. 30A). The remainder of the pallial oviduct is a thin-walled brood pouch (Bp). A gonopericardial duct (Dpe, Figs. 30A, B) is present. The sac-like bursa (Bu) is only 4% of the length of the pallial oviduct and is entirely dorsal to the albumen gland (Fig. 30B). The oviduct (Ov) coils once or twice before receiving the short duct from the seminal receptacle (Dsr, Fig. 30B) and then coils to enter the ventral surface of the end of the albumen gland. The spermathecal duct (Sd) is tightly appressed to the columellar side of the pallial oviduct (Fig. 30A).

In Fig. 30D, the muscularized portion of the anterior brood pouch (Mus) is slit open and the epithelium has been cut away to reveal the inner muscular layer (Iml). For 15 adult females, the number of shelled embryos in the brood sac averaged 19.4 ± 4.5 (range of 14–28), and there were an additional 22–35 small, non-shelled embryos packed in the posterior bend of the brood pouch. For 111 shelled embryos, the mean shell length was 0.48 ± 0.37 mm. The range of shell lengths was four-fold, from 0.20 to 0.87 mm. The embryonic shells have up to 2.5 whorls. The embryos have red-brown pigment splotches and yellow-white granules on the dorsal body surface.

Male Reproductive Anatomy

The male gonad (Go, Fig. 31A) has five branches and occupies 29% of the body length, almost filling the digestive gland. The prostate overlaps the mantle cavity and the anterior vas deferens exits from the anterior tip of the prostate. The seminal vesicle coils (Sv) overlap slightly onto the stomach. The penis (Fig. 31B) has an elongate penial filament. There are numerous folds on its inner curvature for slightly more than one half of its length. Where the folds end, the penis suddenly narrows on the outer curvature, giving the penis a peculiar bulging appearance at this point. The penis has numerous Gl_1 and Gl_2 glands. The vas deferens (Vd) only coils slightly in the penis. The penis has neither cilia nor a terminal eversible papilla.

Durangonella Morrison, 1945

Type-species: *Durangonella seemani* (Frauenfeld, 1863).

Distribution: known from isolated drainage systems in arid north-central México.

Species included: five species listed by Taylor (1966).

Description

Durangonella is distinguished from other littoridinine genera by a combination of character states (see below).

The shell (Figs. 32, 33; Morrison, 1945, figs. 1–4) is smooth, slender, turriform, with five to eight slowly increasing, rounded whorls; the tentacles have *Hydrobia*-like ciliation (Fig. 35A); females are ovoviviparous; the female gonad is a very small, non-lobed swelling at the end of the oviduct (Go, Fig. 36A); the pallial oviduct is large and bends posteriorly with several loops in one plane (Fig. 36A); the albumen gland is reduced to a mere glandular smear on one of the loops (Ppo, Fig. 36A); the seminal receptacle (Sr) connects with the oviduct via a short sperm duct (Sdu, Fig. 36D); the spermathecal duct is elongate with an opening separate from that

FIG. 32. SEM photos of shells of *Durangonella coahuilae* from Locality 6. The shell on the left is 3.60 mm long; the other one is printed at the same enlargement.

of the pallial oviduct (Figs. 36A, F, G); the anterior end of the brood pouch is weakly coiled and muscularized (Figs. 36F, G); the penis has a blunt, ciliated tip, a terminal eversible papilla, and one (Fig. 35D) or two (Morrison, 1945, fig. 5) simple lobes that lack specialized glands.

Discussion

Durangonella is most similar to *Mexipyrgus* (see Tables 53–55, Figs. 49, 50), and these taxa share the following distinctive features: pallial oviduct with posterior coil in several loops; albumen gland reduced to a mere

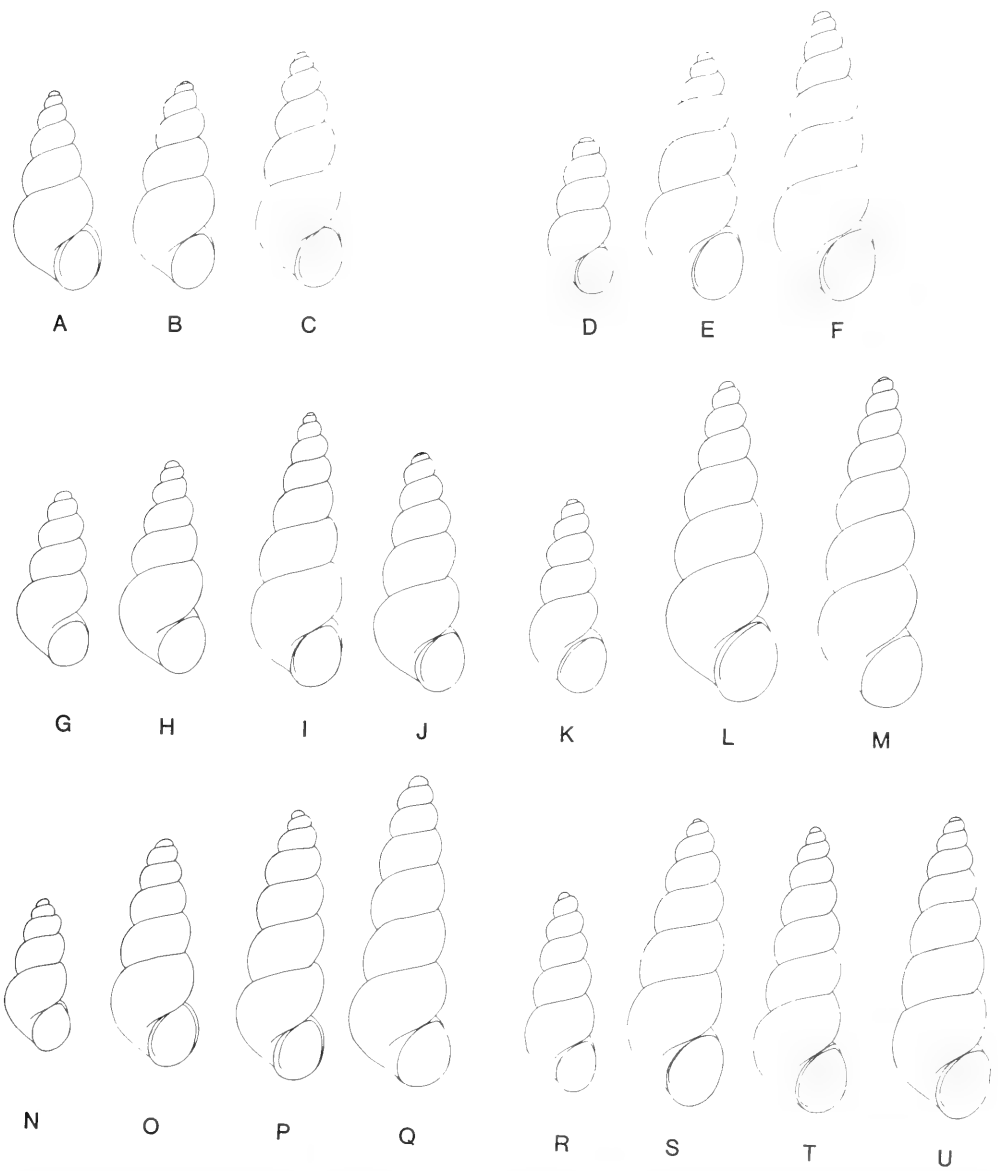

FIG. 33. Camera lucida drawings of shells from six populations of *Durangonella coahuilae*. The shells are from the following localities: A–C, Locality 6; D–F, Locality 14; G–J, Locality 9; K–M, Locality 38; N–Q, Locality 13; R–U, Locality 65. Shell A is 2.42 mm long, and the others are at the same scale.

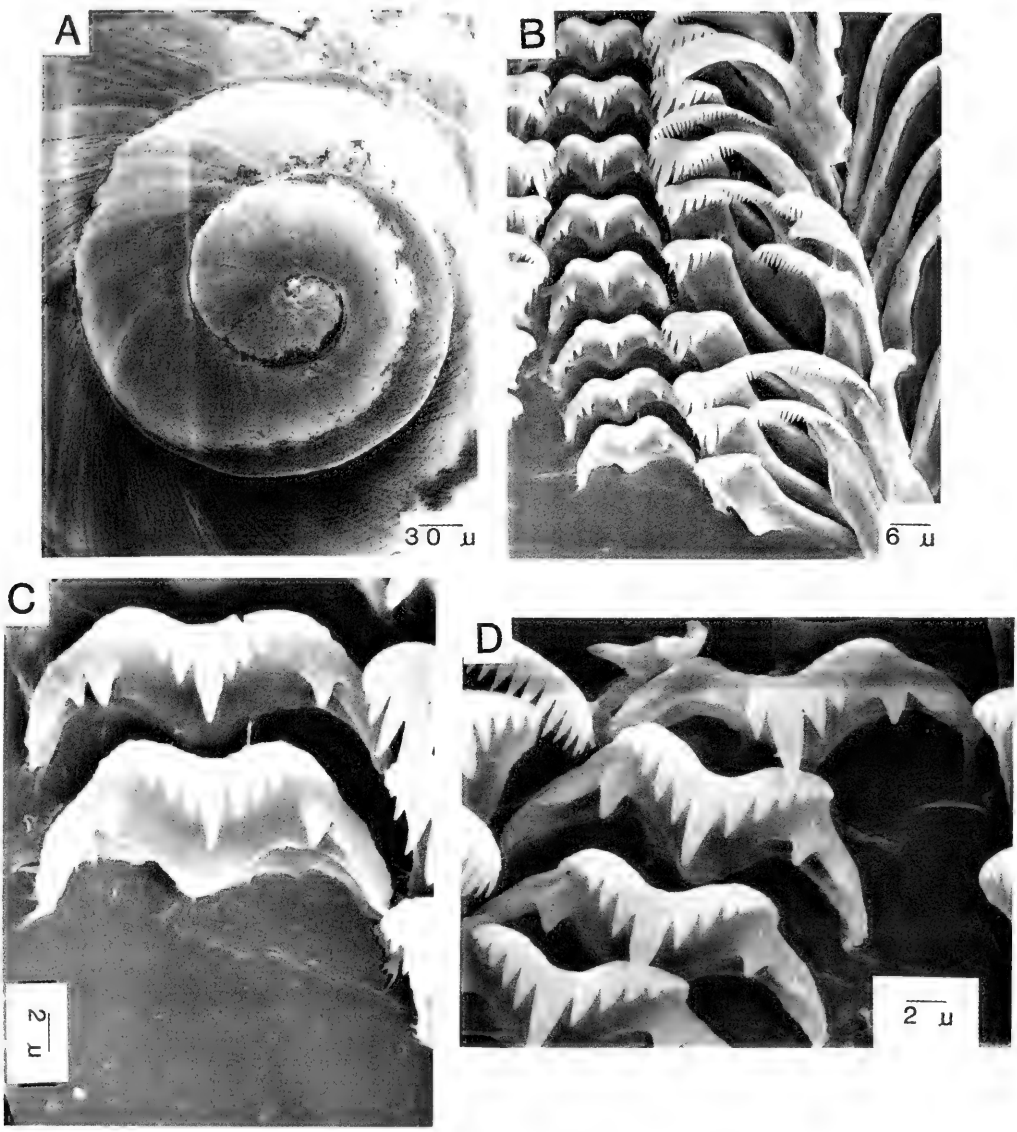

FIG. 34. SEM photos of shell and radula of *Durangonella coahuilae*. A. Apical whorls of the shell. Note the lack of microsculpture. B. Part of the radular ribbon. C, D. Isolated central teeth.

glandular smear; seminal receptacle connected to the oviduct via a short sperm duct; anterior end of the brood pouch weakly muscularized and coiled; penis with a blunt, ciliated tip and a terminal eversible papilla.

Durangonella differs from *Mexipyrgus* in the following features: penis glands (*Durangonella,* absent; *Mexipyrgus,* mammiform); ciliary tufts on tentacles (*Durangonella, Hydrobia*-like; *Mexipyrgus,* absent); female gonad (*Durangonella,* small and non-lobed; *Mexipyrgus,* large and lobed); posterior coils of pallial oviduct (*Durangonella,* in one plane; *Mexipyrgus,* in several planes); duct of seminal receptacle (*Durangonella,* not coiled; *Mexipyrgus,* coiled); spermathecal duct (*Durangonella,* short; *Mexipyrgus,* long).

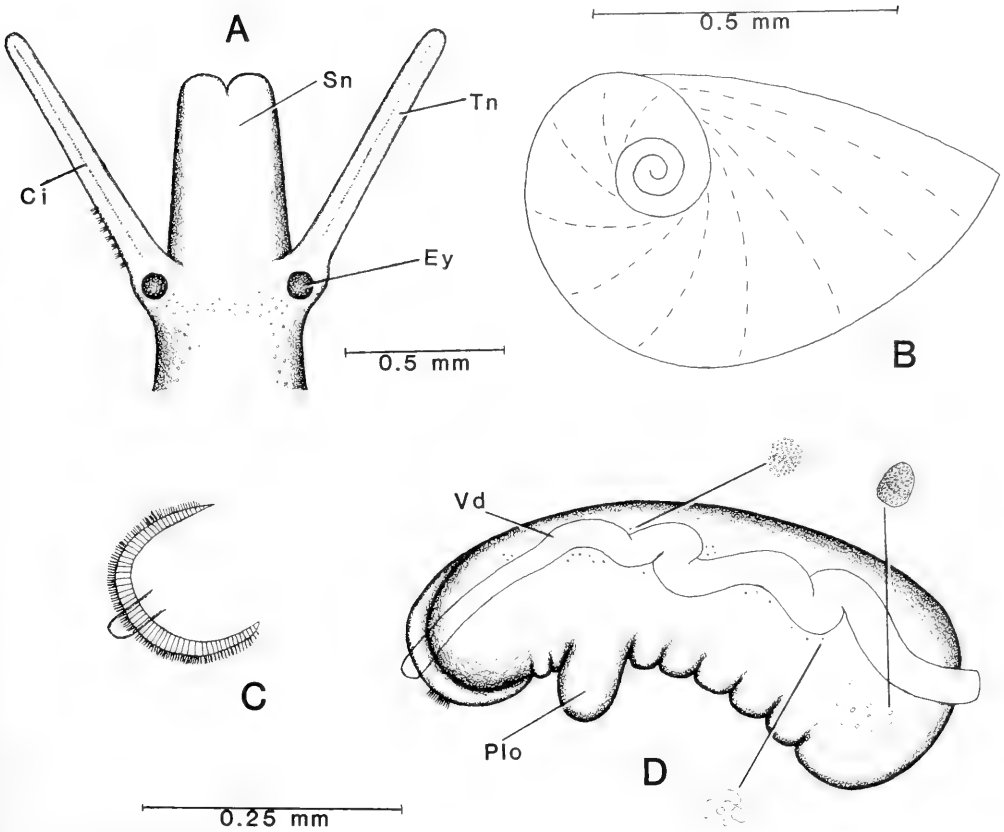

FIG. 35. Head, operculum and penis of *Durangonella coahuilae*. A. Dorsal aspect of head showing the *Hydrobia*-like ciliation of the left tentacle and central ciliary bands (Ci) on both tentacles. B. Operculum. C. Tip of the penis showing the ciliated columnar epithelium. D. Dorsal aspect of the penis showing the blunt tip and small penial lobe (Plo). Note the reduced ciliation (compared to C) of this specimen. Ey—eye; Sn—snout; Tn—tentacle; Vd—vas deferens.

Durangonella coahuilae Taylor, 1966

Holotype: UMMZ 220159.

Type-locality: Locality 9.

Distribution: endemic to the Cuatro Ciénegas Basin.

Habitat: *Durangonella coahuilae* is found in the basin in a wide variety of aquatic environments that include a playa lake, pools formed where the water table is at ground level, small spring-fed pits without outflows, marshes, and springs and streams of all sizes. *Durangonella coahuilae* was found on mops from 23 of 38 small springheads, but, as with *Mexistiobia manantiali,* it probably does not inhabit subterranean waters as it has eyespots and body pigment, and is very common downstream. *Durangonella coahuilae* is most common in soft organic sediments, but was also found in *Chara* mats and on marl pieces. More so than the other hydrobiids of the basin, *D. coahuilae* is found in waters whose temperatures fluctuate greatly on a diurnal and seasonal basis. For example, *D. coahuilae* was collected from pools with seasonally fluctuating levels (Localities 7, 8) that had water temperature ranges of 9.5–35.6°C during 1980. Snails disappeared from the pools only when they went temporarily dry during an arid period.

Description

The shell (Fig. 33) is not readily distinguishable from those of the other *Durangonella* spp. (Morrison, 1945, figs. 1–4): it varies in

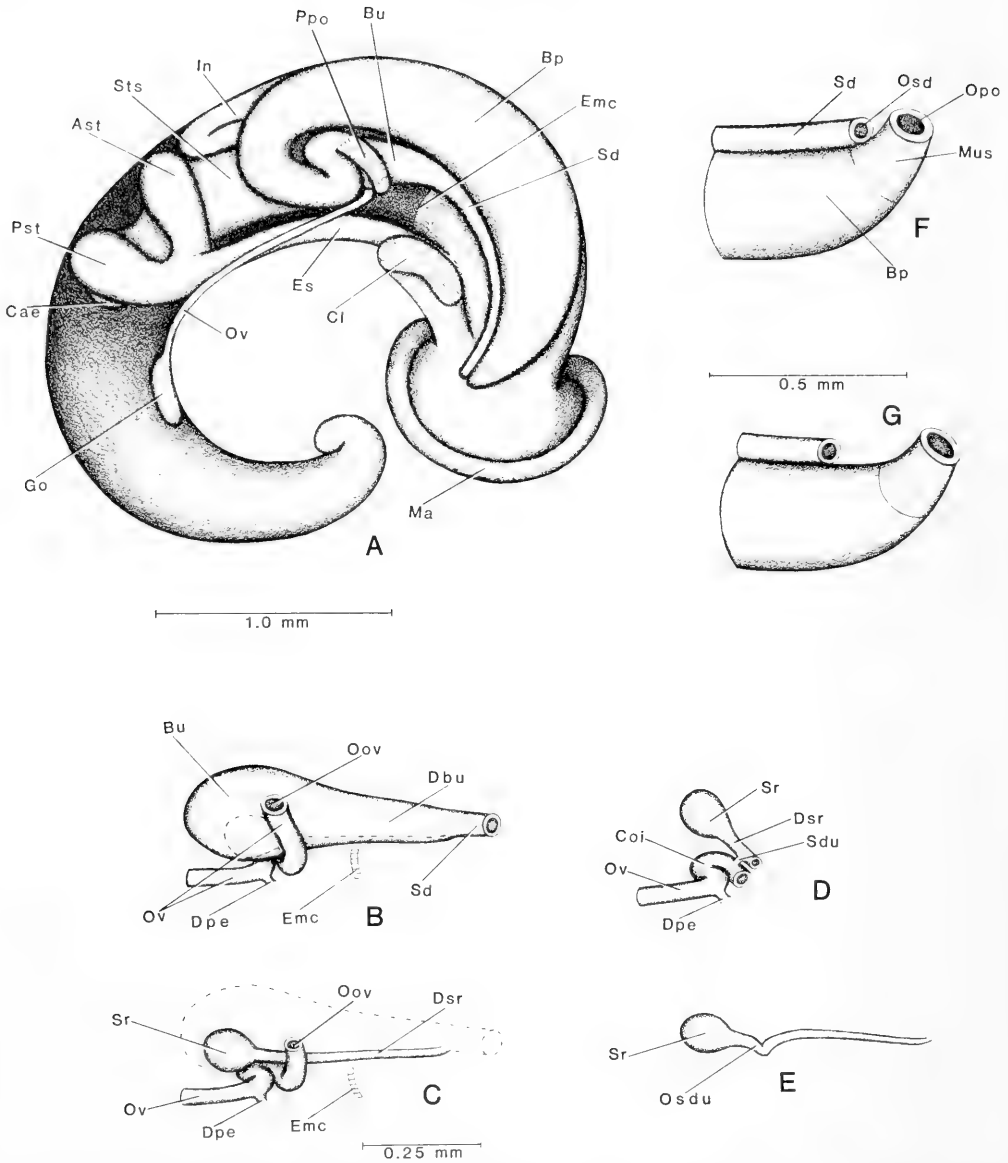

FIG. 36. Female reproductive anatomy of *Durangonella coahuilae*. A. Ventral aspect of uncoiled female without head and kidney tissue. Note the small gonad (Go), posterior coiling of the pallial oviduct (Bp + Ppo), and very small albumen gland (Ppo). B. Oriented as in A, but with the pallial oviduct cut away to expose the bursa (Bu). The spermathecal duct (Sd), which is elongate, has been cut. C. Oriented as in B, but with the bursa removed to expose the seminal receptacle (Sr), its duct (Dsr) and oviduct (Ov). D. Oriented as in C, but with the seminal receptale (Sr) rotated slightly to expose the short sperm duct (Sdu). E. Frequently-seen kink in the duct of the seminal receptacle. F, G. Variation in the position of the end of the spermathecal duct relative to the opening of the pallial oviduct (Opo). Ast—anterior stomach chamber; Bp—brood pouch; Bu—bursa; Cae—caecum of stomach; Cl—columellar muscle; Coi—coil of oviduct; Dbu—duct of the bursa; Dpe—gonopericardial duct; Dsr—duct of the seminal receptacle; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Ma—mantle edge; Mus—muscular section of the brood pouch; Oov—opening of the oviduct; Opo—opening of the pallial oviduct; Osd—opening of the spermathecal duct; Osdu—opening of the sperm duct; Ov—oviduct; Ppo—albumen gland; Pst—posterior stomach chamber; Sd—spermathecal duct; Sdu—sperm duct; Sr—seminal receptacle; Sts—style sac.

size, number and roundness of whorls, and depth of sutures to such an extent that individuals can be found that correspond to the types of all of the nominal species.

Durangonella coahuilae does differ from *D. seemani* (the type of the genus) in terms of number of penial lobes (*D. coahuilae*, 1; *D. seemani*, 2), and number of basal cusps on the central tooth of the radula (*D. coahuilae*, 1; *D. seemani*, 2).

Shell

The shell varies in length from 2.30–5.10 mm and has 5.5–8.5 whorls. The aperture is crescent-shaped. The peristome is entire in adults and the end of the body whorl frequently pulls away from the penultimate whorl. The umbilicus varies from a slight chink to an open slit. Growth lines are prominent (Fig. 34A). Populations vary not only in size of shell and number of whorls, but also in relative shell width, relative size of the body whorl, whorl roundness, suture depth and angle, and degree of sexual dimorphism (Fig. 33). The shell has a smooth apical whorl (Fig. 34A). Shell measurements from nine populations are given in Table 33. For all populations, females are clearly larger than males and have more whorls.

Nonreproductive Features

The measurements of organs and structures for snails from four populations are

TABLE 33. Shell measurements (mm) of males and females from nine populations of *Durangonella coahuilae*. Snails with the dominant maximum whorl number(s) were used. Mean ± standard deviation. The localities are the following types of aquatic environments: 6, fluctuating pool semi-connected to stream; 9, playa lake; 13, 38, 8, large streams; 14, 51, 74, 65, 43, small streams.

	Whorls	N	Length	Width	Length of body whorl	Length of aperture	Width of aperture
Locality 6							
♂	5.5	7	2.58 ± 0.17	1.16 ± 0.07	1.43 ± 0.10	0.82 ± 0.06	0.56 ± 0.03
♀	6.5	9	3.54 ± 0.17	1.45 ± 0.04	1.77 ± 0.07	0.99 ± 0.05	0.68 ± 0.04
Locality 9							
♂	6.0	9	2.75 ± 0.17	1.21 ± 0.07	1.50 ± 0.08	0.85 ± 0.04	0.61 ± 0.05
	6.5	7	3.11 ± 0.13	1.31 ± 0.05	1.62 ± 0.09	0.91 ± 0.06	0.63 ± 0.06
♀	6.5	9	3.40 ± 0.28	1.44 ± 0.11	1.78 ± 0.14	1.01 ± 0.09	0.71 ± 0.07
Locality 13							
♂	5.5	10	2.35 ± 0.14	0.99 ± 0.06	1.26 ± 0.08	0.74 ± 0.05	0.49 ± 0.03
♀	7.0	10	3.75 ± 0.16	1.38 ± 0.07	1.65 ± 0.08	0.97 ± 0.05	0.66 ± 0.05
	7.5	9	4.15 ± 0.20	1.43 ± 0.10	1.72 ± 0.10	1.00 ± 0.06	0.69 ± 0.03
Locality 14							
♂	5.5	9	2.51 ± 0.11	1.07 ± 0.08	1.37 ± 0.06	0.80 ± 0.04	0.58 ± 0.04
	6.0	7	2.73 ± 0.11	1.09 ± 0.05	1.42 ± 0.08	0.84 ± 0.06	0.56 ± 0.04
♀	7.0	9	3.85 ± 0.17	1.51 ± 0.06	1.85 ± 0.08	1.08 ± 0.05	0.74 ± 0.03
Locality 38							
♂	6.0	10	2.85 ± 0.11	1.18 ± 0.05	1.52 ± 0.08	0.91 ± 0.04	0.61 ± 0.02
♀	8.0	8	4.83 ± 0.18	1.61 ± 0.09	2.00 ± 0.10	1.14 ± 0.07	0.79 ± 0.04
	8.5	10	5.08 ± 0.30	1.58 ± 0.07	1.99 ± 0.10	1.11 ± 0.07	0.79 ± 0.04
Locality 43							
♂	5.5	10	2.75 ± 0.18	1.20 ± 0.08	1.52 ± 0.09	0.90 ± 0.05	0.61 ± 0.04
♀	6.5	9	4.07 ± 0.19	1.64 ± 0.07	2.05 ± 0.10	1.19 ± 0.07	0.83 ± 0.04
Locality 51							
♂	7.0	9	3.55 ± 0.11	1.26 ± 0.06	1.58 ± 0.08	0.89 ± 0.04	0.63 ± 0.02
♀	7.5	8	4.36 ± 0.16	1.53 ± 0.05	1.89 ± 0.09	1.08 ± 0.04	0.74 ± 0.04
Locality 74							
♂	6.5	7	3.06 ± 0.18	1.14 ± 0.07	1.48 ± 0.07	0.82 ± 0.05	0.58 ± 0.04
♀	7.0	9	3.96 ± 0.27	1.47 ± 0.09	1.86 ± 0.08	1.04 ± 0.06	0.72 ± 0.04
Locality 65							
♂	6.5	12	3.28 ± 0.19	1.25 ± 0.07	1.56 ± 0.10	0.92 ± 0.07	0.63 ± 0.04
♀	7.0	10	3.90 ± 0.25	1.44 ± 0.09	1.79 ± 0.11	1.06 ± 0.07	0.71 ± 0.05

TABLE 34. Dimensions (mm) or counts of non-neural organs and structures of *Durangonella coahuilae* from Locality 6. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	5.04 ± 0.39	3.35 ± 0.12
Gill filament number		26.7 ± 2.80	
Osphradium	L	0.21 ± 0.03	
Gonad	L	0.33 ± 0.08	1.13 ± 0.09
	W	0.08 ± 0.02	0.38 ± 0.004
Prostate (N = 6)	L		0.68 ± 0.09
	W		0.34 ± 0.04
Penis	L		0.55 ± 0.05
	W		0.21 ± 0.03
Pallial oviduct	L	2.20 ± 0.23	
	W	0.51 ± 0.06	
Bursa copulatrix	L	0.33 ± 0.04	
(N = 7)	W	0.22 ± 0.01	
Seminal receptacle	L	0.11 ± 0.01	
(body) (N = 6)	W	0.09 ± 0.01	
Seminal receptacle	L	0.04 ± 0.01	
(duct) (N = 8)	W	0.04 ± 0.01	

TABLE 35. Dimensions (mm) or counts of non-neural organs and structures of *Durangonella coahuilae* from Locality 9. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	5.09 ± 0.43	4.46 ± 0.30
Gill filament number		26.4 ± 1.82	
Osphradium	L	0.20 ± 0.03	
Gonad	L	0.26 ± 0.04	1.30 ± 0.11
	W	0.09 ± 0.01	0.43 ± 0.03
Prostate (N = 6)	L		0.72 ± 0.06
	W		0.34 ± 0.04
Penis (N = 6)	L		0.56 ± 0.04
	W		0.21 ± 0.03
Pallial oviduct	L	2.10 ± 0.21	
	W	0.53 ± 0.05	
Bursa copulatrix	L	0.29 ± 0.02	
	W	0.18 ± 0.02	
Seminal receptacle	L	0.13 ± 0.02	
(body) (N = 8)	W	0.10 ± 0.02	
Seminal receptacle	L	0.05 ± 0.01	
(duct) (N = 8)	W	0.04 ± 0.01	

TABLE 36. Dimensions (mm) or counts of non-neural organs and structures of *Durangonella coahuilae* from Locality 13. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	5.50 ± 0.27	4.08 ± 0.26
Gill filament number		31.5 ± 1.76	
Osphradium	L	0.21 ± 0.04	
Gonad	L	0.36 ± 0.05	1.74 ± 0.18
	W	0.10 ± 0.02	0.44 ± 0.03
Prostate	L		0.74 ± 0.10
	W		0.32 ± 0.03
Penis	L		0.42 ± 0.04
	W		0.16 ± 0.02
Pallial oviduct	L	2.39 ± 0.21	
	W	0.47 ± 0.05	
Bursa copulatrix	L	0.30 ± 0.03	
(N = 9)	W	0.17 ± 0.03	
Seminal receptacle	L	0.13 ± 0.01	
(body) (N = 10)	W	0.10 ± 0.02	
Seminal receptacle	L	0.03 ± 0.01	
(duct) (N = 6)	W	0.04 ± 0.01	

TABLE 37. Dimensions (mm) or counts of non-neural organs and structures of *Durangonella coahuilae* from Locality 14. N = 5 unless stated otherwise. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	6.44 ± 0.63	4.76 ± 0.34
Gill filament number		34.7 ± 1.75	
Osphradium	L	0.21 ± 0.04	
Gonad	L	0.41 ± 0.02	1.70 ± 0.23
	W	0.09 ± 0.02	0.47 ± 0.04
Prostate (N = 7)	L		1.00 ± 0.09
	W		0.40 ± 0.04
Penis	L		0.46 ± 0.04
	W		0.21 ± 0.04
Pallial oviduct	L	2.19 ± 0.25	
	W	0.59 ± 0.06	
Bursa copulatrix	L	0.34 ± 0.04	
(N = 6)	W	0.19 ± 0.02	
Seminal receptacle	L	0.12 ± 0.02	
(body) (N = 7)	W	0.11 ± 0.02	
Seminal receptacle	L	0.04 ± 0.002	
(duct)	W	0.03 ± 0.004	

given in Tables 34–37. The anatomical drawings and radula photographs are from specimens from Locality 6. The snout (Fig. 35A) is elongate and the tentacles are relatively short. In addition to the seven to nine hypertrophied ciliary tufts on the outer edge of the left tentacle, each tentacle has a central ciliary tract (Ci, Fig. 35A). Small granules are seen around the eyespots and in the neck. The snout may or may not be dusted with melanin. The sides of the head-foot usually have a light melanin dusting, and an unpigmented strip, extending from the eye to the base of the foot, can be seen. Body pigment can be red or black. The male gonad always has dark melanin on its ventral surface. Pop-

ulations may have snails devoid of other body pigment (Locality 6), or with a dark melanin coating (Localities 9, 14), or a spotted pattern (Localities 13, 39) on the ventral body surface. The caecal chamber protrudes posterior to the stomach (Cae, Fig. 36A). The paucispiral operculum (Fig. 35B) has 3.3 whorls and the nucleus is positioned at 26% of the operculum length. The operculigerous lobe has several melanin streaks.

Radula

Radular statistics for specimens from the type-locality (Locality 9) and a second locality (Locality 6) are given in Table 38. The cusp arrangements for the four tooth types (for specimens from Locality 6) are given in Table 39. The central tooth of the radula has one (and occasionally a second) pair of basal cusps that arise from prominent lateral angles (Figs. 34C, D).

Female Reproductive Anatomy

The female gonad (Go, Fig. 36A) occupies only 6% of the body length. Oocytes were frequently seen in the gonad throughout the year. The pallial oviduct occupies 34-44% of the body length, depending on the population. The length of the posterior bend of the pallial oviduct is 0.6 mm, and the bend extends to

TABLE 38. Radular statistics from individuals of *Durangonella coahuilae* from two populations. $\bar{X}$ = mean, S = standard deviation. Measurements in mm.

	Locality 9 (N = 13)		Locality 6 (N = 5)	
Radular feature	$\bar{X}$	S	$\bar{X}$	S
Length	0.444	0.035	0.380	0.023
Width	0.087	0.007	0.074	0.006
Number of rows	48.6	3.25	51.4	2.07
Number of rows in formative stage	3.0	1.3	4.0	1.2
Width of central tooth (N = 14)			0.020	0.001

TABLE 39. The various cusp arrangements for the four tooth types of *Durangonella coahuilae*, counted from 5 radulae using SEM, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
4–1–4 / 1–1	20	3–1–3	20	19	40	20	20
4–1–4 / 2–1	20	4–1–3	40	20	40	21	40
5–1–4 / 2–1	60	4–1–4	40	22	40	22	60
5–1–5 / 1–1	80	5–1–5	40	23	40	23	40
5–1–5 / 2–1	100			24	40	24	40
6–1–5 / 1–1	20			25	40	25	40
6–1–5 / 2–1	20			26	20	26	20
6–1–4 / 2–2	20			27	20	27	20

within 0.13 mm of the end of the mantle cavity. The albumen gland (Ppo) is no more than a glandular smear on the posterior-most 0.28 mm of the pallial oviduct. The remainder of the pallial oviduct serves as a brood pouch (Bp).

A gonopericardial duct (Dpe) is present (Figs. 36B–D). The sac-like bursa (Bu) is only 12–16% of the length of the pallial oviduct. The seminal receptacle (Sr), dorsal to the bursa, is circular in outline and 44% the length of the bursa. The oviduct (Ov) has a single coil dorso-lateral to the seminal receptacle (Figs. 36B, C) and then receives the short sperm duct (Sdu) from the duct of the seminal receptacle (Dsr). The length of the duct of the seminal receptacle to the opening of the seminal receptacle is short, 0.03–0.05 mm, and then the duct travels (dorsal to and hidden by the bursa) for 0.41 mm until it joins the duct of the bursa (Dbu, Fig. 36C). This juncture occurs 0.20 mm anterior to the end of the mantle cavity. The duct of the seminal receptacle often has a kink in it just after the opening of the sperm duct (Fig. 36E). The opening of the spermathecal duct is 0.08–0.20 mm posterior to that of the pallial oviduct (Figs. 36F, G).

Data for number of shelled embryos brooded for females from six populations are given in Table 40. To these numbers can be added one to two non-shelled embryos that were dissolved in the Clorox. For 39 embryonic shells (Locality 6), shell length averaged 0.384 ± 0.158 mm, with an eightfold range in lengths from 0.079–0.693 mm. The largest embryonic shells have 2.5–2.8 whorls.

Male Reproductive Anatomy

The lobed male gonad has four to five branches and constitutes 29–43% of the body length. The prostate is 0.16–0.21% the body length, and overlaps the mantle cavity. The anterior vas deferens exits from the anterior tip of the prostate.

The single penial lobe (Plo, Fig. 35D) is located at 61% the penis length from the base. Folds are present on the inner curvature from the base to just beyond the penial lobe. The blunt tip of the penis has tall columnar cells extending back 0.10 mm to where the penial folds end. Ciliation of these cells is variable; for the population from Locality 6, one of the five penes studied had no cilia, and the other four had a small ciliated patch (Fig. 35D). Other populations, particularly those with large-sized males, usually had the entire columnar-celled area ciliated (Fig. 35C). The vas deferens (Vd) coils only slightly in the penis. The penis has both Gl_1 and Gl_2 gland types common. Some populations have males with a small pigmented patch near the penis tip.

Discussion

Among the *Durangonella* species, only *D. coahuilae* has received complete anatomical study. The penis and radula of *D. seemani* have been figured (see above), while the other four species are known only from the shell. Anatomical study of these allopatric species is necessary to resolve their systematic status.

Durangonella coahuilae had been previously known (Taylor, 1966) only from Laguna Grande (Locality 9), the playa lake that is the terminus of the stream from Laguna Churince, a large spring (Locality 1). It has been suggested that other populations in the basin may represent new *Durangonella* species (Holsinger & Minckley, 1971; Taylor, 1966).

The author collected undoubted *D. coa-*

TABLE 40. Data for number of shelled embryos brooded by females from six populations of *Durangonella coahuilae*. The mean shell length (for shells with maximum dominant whorl number) for adult females of each population is also given.

	Mean shell length (mm)	Number of young/females			
		N	$\bar{X}$	SD	range
Locality 9	3.40	15	1.87	0.64	1–3
Locality 6	3.54	15	2.60	1.40	1–5
Locality 14	3.85	13	3.77	1.69	1–6
Locality 43	4.07	14	8.14	1.41	6–10
Locality 13	4.15	13	5.64	1.67	2–8
Locality 38	5.08	15	5.60	1.55	2–8

huilae not only from Laguna Grande, but also in pool areas along the stream feeding it (Localities 7, 8), groundwater-fed pools near the stream (Localities 4, 5, 6) and from a mop placed in a small seep near Laguna Churince (Locality 2).

The shells of *D. coahuilae* from populations from the above localities of the Churince system (Figs. 33A–C, G–J), which is currently isolated from other waters of the basin, do differ from shells from other populations (Figs. 33D–F, K–U) in that the females have fewer whorls and smaller shells, and the shells are relatively wider with a relatively larger body whorl (Fig. 33; Table 33). The shell differences may be partly allometric, as the Churince shells are small, but in some cases Churince shells that are smaller (in length) than those from other populations are also absolutely wider.

Despite these differences, the Cuatro Ciénegas *Durangonella* is not being split into several species because: 1) there are no qualitative anatomical differences among the populations studied; 2) the Churince aquatic environments differ from those from which other populations were sampled; and 3) the above shell differences are not always pronounced and the author can not confidently separate out "species" when lots are mixed.

Mexipyrgus Taylor, 1966

Type-species: *Mexipyrgus carranzae* Taylor, 1966.

Distribution: endemic to the Cuatro Ciénegas Basin.

Species included: reduced to monotypy (see below).

Description

Distinctive features of *Mexipyrgus* are as follows: 1) the massive pallial oviduct that extends onto the stomach and then bends into a series of loops that coil progressively dorsal to one another (Figs. 42, 43), partially enveloping the bursa and restricting the space for the kidney (Ki) and pericardium (Pe, Fig. 42A); and 2) the greatly coiled duct of the seminal receptacle (Dsr, Figs. 42B, D, E).

The shell (Fig. 37) is large (number of whorls, 5.5–7.5; length, 3.03–8.45 mm), usually thickened, and elongate-conic in shape; low spiral welts and noded ribs may or may not be prominent on the last two whorls (Figs. 37, 38); periostracal color bands may or may not be present and (when present) vary from one to thirty distinct bands, to a single wide solid band; the marginal tooth of the radula has numerous cusps (Figs. 39B, C); females are ovoviviparous; the albumen gland (Ppo) is reduced to a glandular smear on the dorsalmost loop of the pallial oviduct (Figs. 42, 43); the bursa is enlarged and elongate (Bu, Figs. 42A, 43); the seminal receptacle is dorsal to the bursa and connects with the oviduct via a short sperm duct (Sdu, Fig. 42C); the spermathecal duct (Sd) is short, muscularized, and separated from the bursa by a slight constriction (indicated by arrow in Fig. 42B); the anterior end of brood pouch is weakly muscularized and coiled (Mus, Fig. 41B); the penis (Fig. 44A) has a blunt, ciliated tip, terminal papilla, and lobes (outer curvature, one; inner curvature, one or two) bearing mammiform glands (Mg, Fig. 44A).

Mexipyrgus is most similar to *Durangonella* (see above; Tables 53–55, Figs. 49, 50).

Mexipyrgus churinceanus Taylor, 1966

Holotype: UMMZ 220150.

Type-locality: Locality 1.

Synonymy: *M. churinceanus* Taylor, 1966
M. escobedae Taylor, 1966
M. lugoi Taylor, 1966
M. carranzae Taylor, 1966
M. mojarralis Taylor, 1966
M. multilineatus Taylor, 1966

The name *Mexipyrgus churinceanus*, rather than *M. carranzae* (type of the genus in Taylor, 1966), is applied to this species because the type population for the former has received the most morphological study.

Habitat: *Mexipyrgus churinceanus* is found almost exclusively in the larger springs and streams of the basin. The species was never found in sieve collections from smaller streams, and only a single specimen was taken (Locality 65) from mops from 38 small springheads. *Mexipyrgus churinceanus* is restricted to soft sediments which appear (at 50×) to be composed of snail copropel or decaying plant matter. To a lesser extent, specimens were taken from a mixture of soft sediment and coarse travertine sand. Densities of snails were determined using a box core sampler (22.5 cm square bottom) and ranged up to 49,000/m^2 (Locality 30). The only species found sympatric with *M. churinceanus* in its microhabitat was *Durangonella coahuilae*.

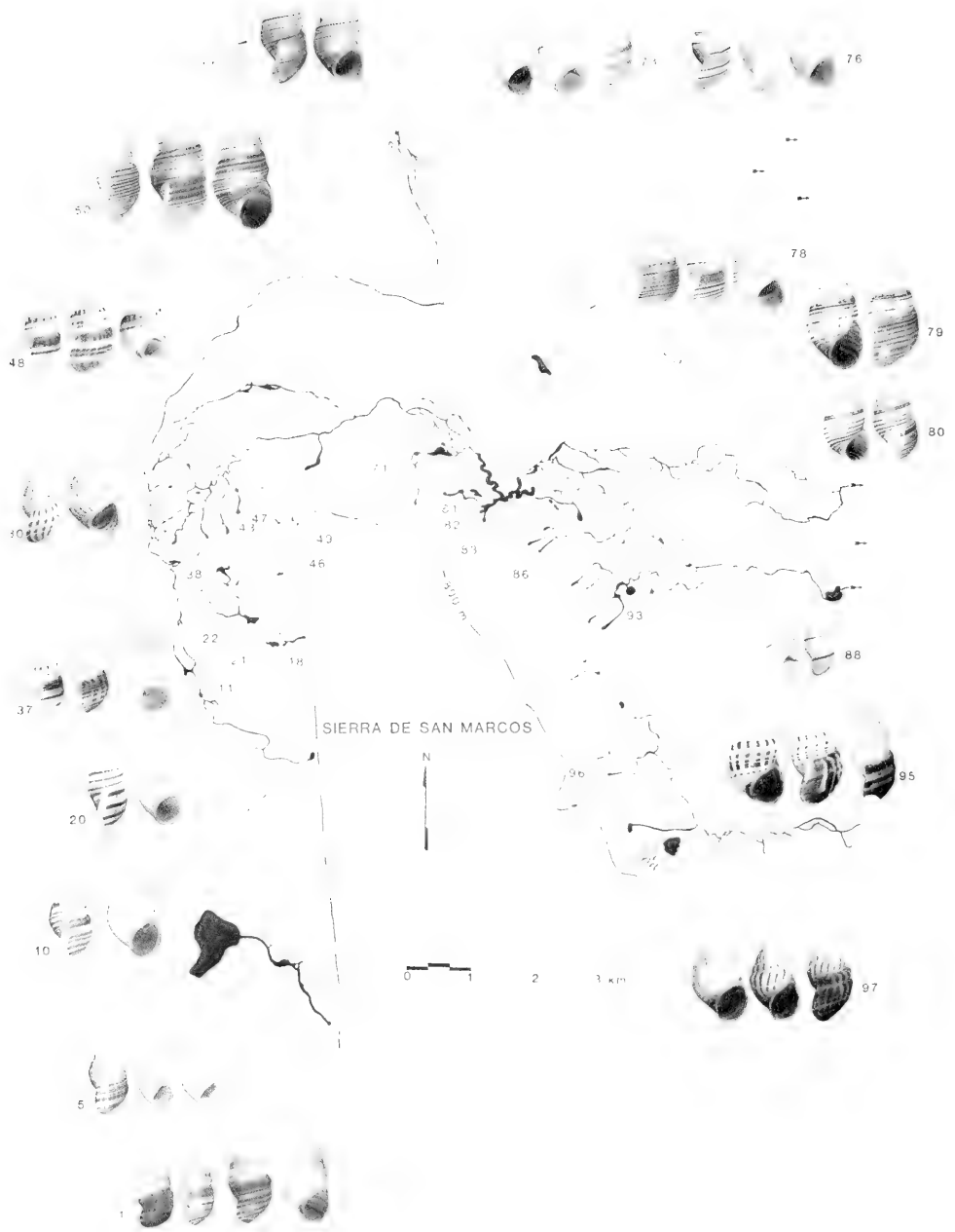

FIG. 37. Localities from which samples of *Mexipyrgus churinceanus* were taken for the multivariate analysis, with photos of shells from selected populations. The photos of shells from Localities 1, 5, 37, 50, 99, 76, 78, 79, 80, and 97 are printed at the same enlagement. The shell on the left for Locality 1 is 5.36 mm long. The photos of shells from Localities 10, 20, 30, 48, 73, 88, and 95 are printed at the same enlargement. The photo on the left for Locality 10 is 4.29 mm long.

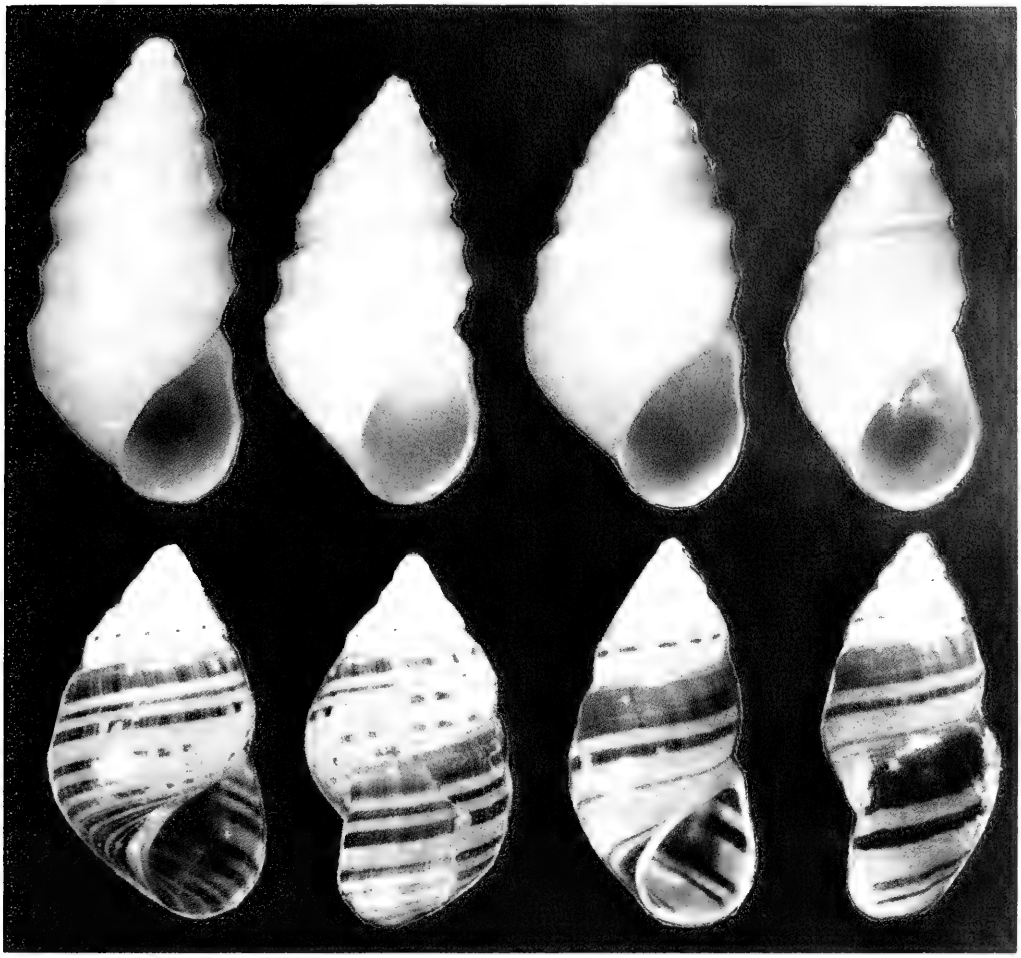

FIG. 38. Photos of shells of *Mexipyrgus churinceanus*. The top row has shells (periostracum removed) from Locality 1, showing sculptural variation. The shell figured on the left is 7.20 mm long. The others in this row are printed at the same enlargement. The bottom row has shells from Locality 90 (eastern lobe of the basin, Fig. 3) showing the thickened sutural periostracal band. The shell on the left is 3.76 mm long and the others in this row are printed to the same enlargement.

Shell

Shell measurements and other data for adults from 33 populations are given in Table 49. The shells are usually white-colored and opaque, but in a few populations they are colorless and transparent. The whorls are flattened and the sutures are not very impressed. The apical whorl is smooth (Fig. 39A). Sculpture begins at or just before the beginning of the third whorl (Fig. 39A). Spiral cords dominate the third whorl while axial ribs predominate on the fourth whorl. Sculpture on the last two whorls is variable both within (Fig. 38, top row) and among (Fig. 37) populations. After the fourth whorl, noded ribs may be prominent, reduced, or absent; and the spiral sculpture usually consists of two low welts. Cancellate sculpture was rarely seen. In populations with adult shells having prominent axial sculpture, 12–20 ribs were seen on the penultimate whorl. In some cases, a small number of narrow spiral cords is seen below the suture on the body whorl. Sculpture is generally reduced on the body whorl relative to that of the penultimate whorl. Axial growth

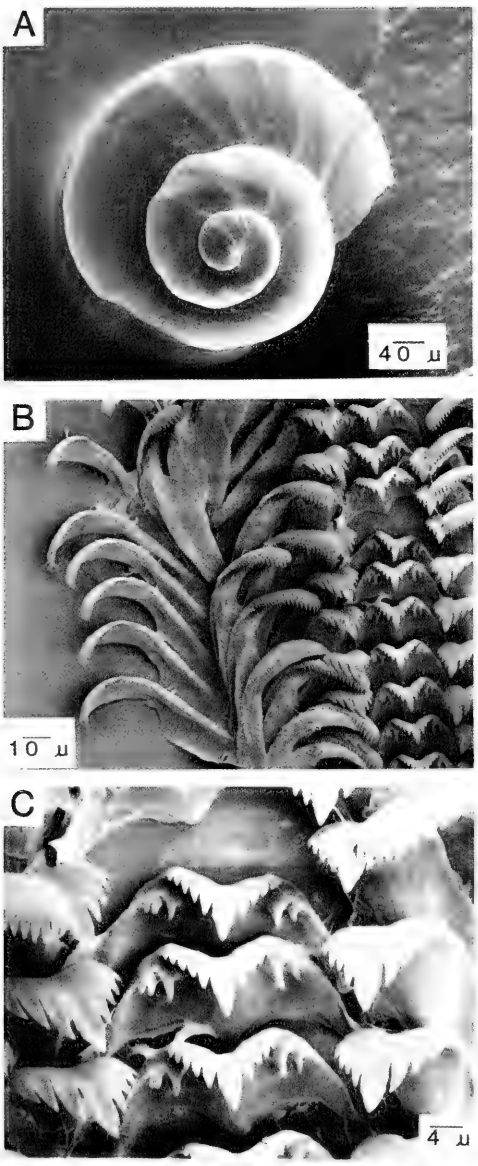

FIG. 39. SEM photos of shell and radula of *Mexipyrgus churinceanus*. A. Apical view of embryonic shell. Note smooth apex. B. Part of radular ribbon. C. Close-up showing central teeth.

lines are usually prominent. Adult females generally have larger and relatively wider shells than males, as well as more prominent sculpture. The aperture is elongate and somewhat pyriform above. The outer lip usually has a pronounced sinuation. The umbilicus is a narrow slit.

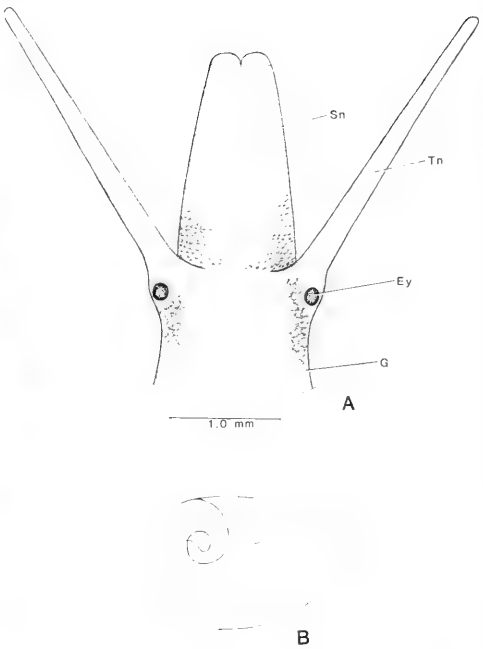

FIG. 40. Head and operculum of *Mexipyrgus churinceanus*. A. Dorsal aspect of the head. Note the concentration of glandular units (G) and darker color streaks. B. Operculum with the dashed lines showing the attachment area to the operculigerous lobe. Ey—eye; G—glandular unit; Sn—snout; Tn—tentacle.

TABLE 41. Dimensions (mm) of non-neural organs and structures of *Mexipyrgus churinceanus* from Locality 1. N = 5. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	8.33 ± 0.36	6.82 ± 0.34
Osphradium	L	0.30 ± 0.02	
Gonad	L	1.66 ± 0.23	2.68 ± 0.11
	W	0.59 ± 0.03	0.75 ± 0.08
Prostate	L		1.50 ± 0.12
	W		0.90 ± 0.08
Penis	L		2.61 ± 0.17
	W		1.15 ± 0.05
Pallial oviduct	L	4.35 ± 0.34	
	W	1.08 ± 0.10	
Bursa copulatrix	L	1.25 ± 0.16	
	W	0.36 ± 0.05	
Seminal receptacle (body)	L	0.30 ± 0.04	
	W	0.09 ± 0.01	
Seminal receptacle (duct)	L	0.13 ± 0.01	
	W	0.06 ± 0.002	

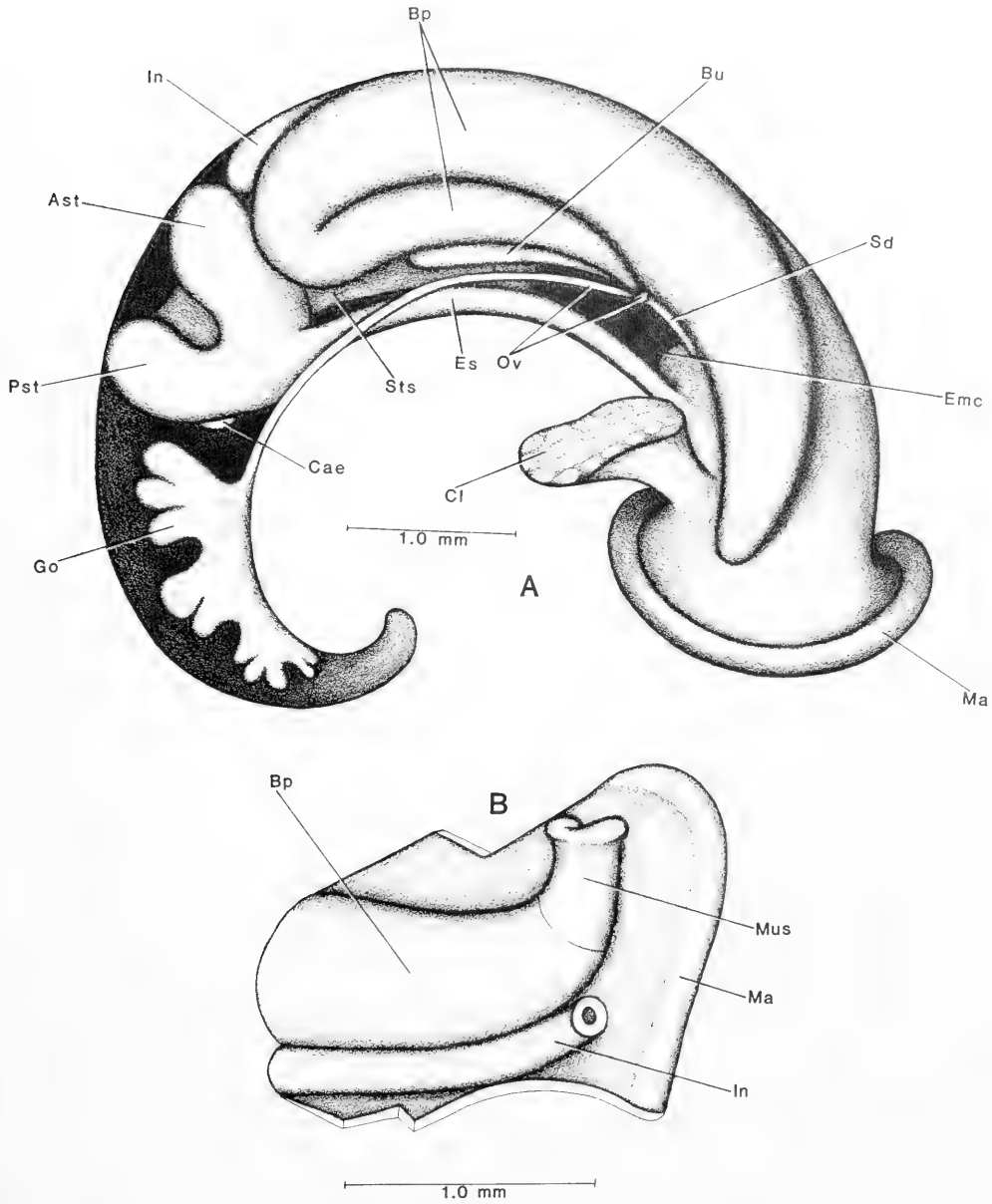

FIG. 41. General organization of the female reproductive anatomy of *Mexipyrgus churinceanus*. A. Ventral aspect of uncoiled snail without the head and kidney tissue. Note the posterior bend of the brood pouch (Bp). B. Portion of the anterior end of the mantle cavity showing the slight muscular twist (Mus) of the anterior end of the brood pouch. Ast—anterior stomach chamber; Bp—brood pouch; Bu—bursa; Cae—caecum of stomach; Cl—columellar muscle; Emc—posterior end of the mantle cavity; Es—esophagus; Go—gonad; In—intestine; Ma—mantle edge; Mus—muscular section of brood pouch; Ov—oviduct; Pst—posterior stomach chamber; Sd—spermathecal duct; Sts—style sac.

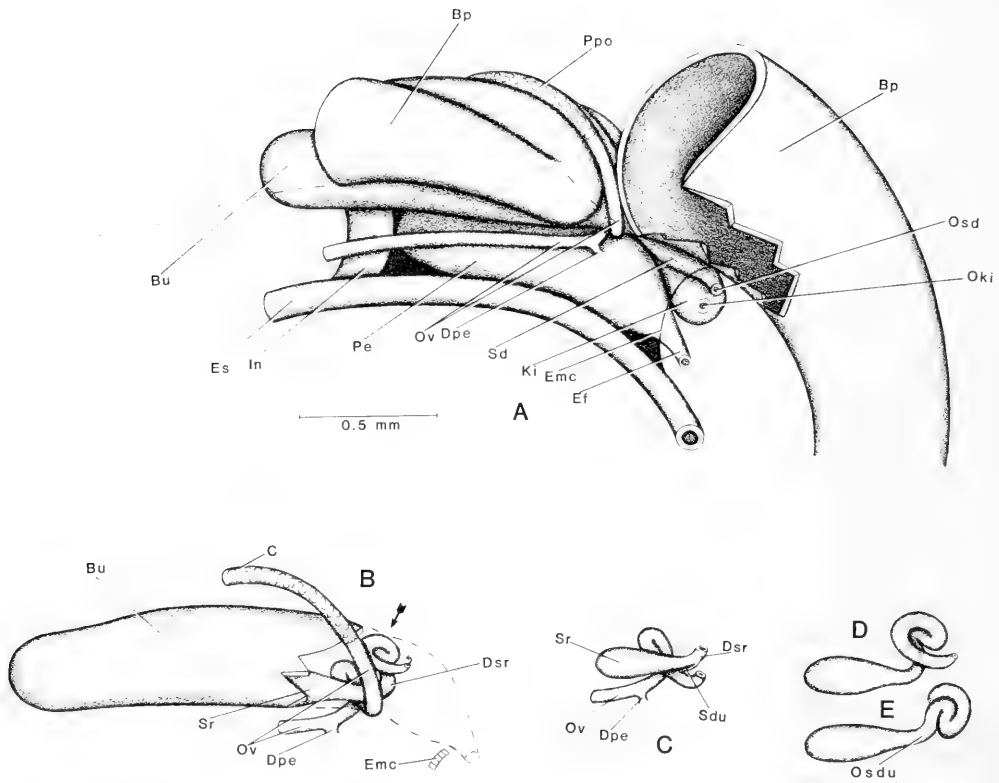

FIG. 42. Female reproductive anatomy of *Mexipyrgus churinceanus*. A. Oriented as in Fig. 41A, but with much of the brood pouch (Bp) cut away to expose the bursa (Bu) and continuation of the pallial oviduct coils dorsal to the part that has been cut away. Note the reduction of the size of the albumen gland (Ppo) and the infringement of the bursa (Bu) and pallial oviduct onto the space occupied by the pericardium (Pe) and kidney (Ki). B. Oriented as in A, but with portion of the brood pouch (Bp) and bursa (Bu) cut away to expose the oviduct (Ov), seminal receptacle (Sr), its duct (Dsr), and the opening of the duct of the seminal receptacle into the dorsal side of the bursa (indicated by small arrow). The large arrow indicates the constriction between the bursa (Bu) and spermathecal duct (Sd). Point C is included for comparison with Fig. 43. C. Oriented as in B, but with part of the oviduct cut to expose the slender sperm duct (Sdu). D, E. Oriented as in C to show variation in the coiling pattern of the duct of the seminal receptacle. Bp—brood pouch; Bu—bursa; Dpe—gonopericardial duct; Dsr—duct of the seminal receptacle; Ef—efferent branchial vessel; Emc—posterior end of the mantle cavity; Es—esophagus; In—intestine; Ki—kidney; Oki—opening of the kidney; Osd—opening of the spermathecal duct; Osdu—opening of the sperm duct; Ov—oviduct; Pe—pericardium; Ppo—albumen gland; Sd—spermathecal duct; Sdu—sperm duct; Sr—seminal receptacle.

Nonreproductive Features

Measurements of organs and structures from three populations are given in Tables 41–43. The description of external features and anatomy is largely based on study of the type population (from Locality 1). The snout (Fig. 40A) is elongate and the tentacles are thin and short by comparison. The tentacles are without hypertrophied ciliary tufts. Large, milk-white granules (G, Fig. 40A) are concentrated in the rostrum and neck. A light dusting of melanin may or may not be seen on the rostrum and neck. The paucispiral oper-

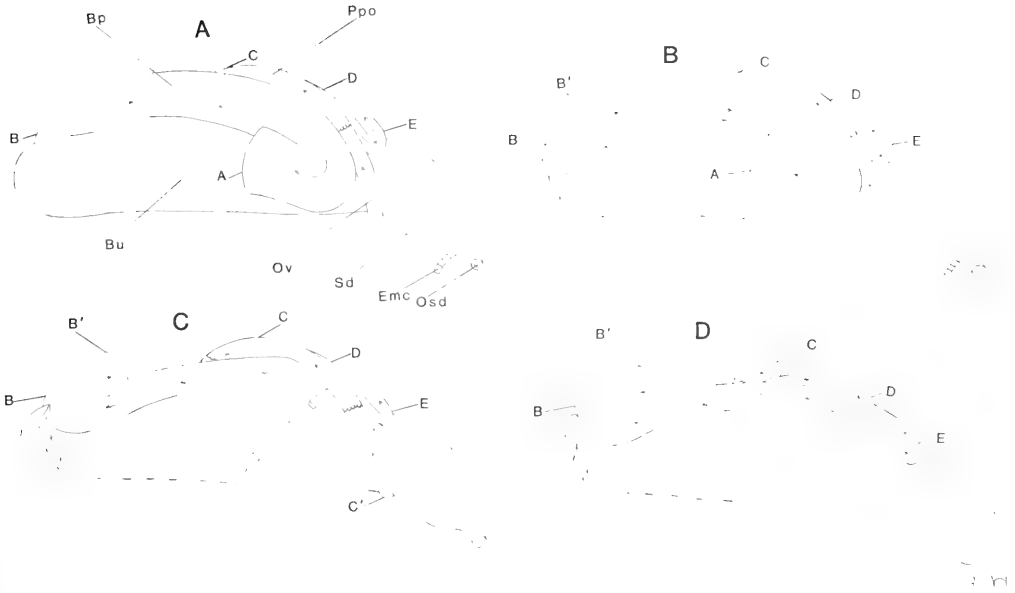

FIG. 43. The nature of the coils of the posterior pallial oviduct as revealed by progressive removal of portions of the coils. Orientation as in Fig. 42A. A. Pallial oviduct cut at point A. B. Part of the bursa (Bu) is cut away to expose the coils on its dorsal side. C. Section A–B′ of the pallial oviduct has been removed. D. Section C–C′ has been removed. The small arrows indicate the direction of movement of eggs and embryos through the pallial oviduct coils. Bp—brood pouch; Bu—bursa; Emc—posterior end of the mantle cavity; Ov—oviduct; Ppo—albumen gland; Sd—spermathecal duct; Osd—opening of the spermathecal duct.

TABLE 42. Dimensions (mm) of non-neural organs and structures of *Mexipyrgus churinceanus* from Locality 30. N = 5. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	5.46 ± 0.14	5.01 ± 0.21
Osphradium	L	0.22 ± 0.03	
Gonad	L	0.80 ± 0.06	1.66 ± 0.10
	W	0.46 ± 0.05	0.54 ± 0.07
Prostate	L		1.00 ± 0.13
	W		0.53 ± 0.06
Penis	L		1.86 ± 0.17
	W		0.79 ± 0.07
Pallial oviduct	L	2.38 ± 0.14	
	W	0.70 ± 0.13	
Bursa copulatrix	L	0.80 ± 0.06	
	W	0.21 ± 0.03	
Seminal receptacle (body)	L	0.15 ± 0.02	
	W	0.07 ± 0.02	
Seminal receptacle (duct)	L	0.10 ± 0.01	
	W	0.06 ± 0.01	

TABLE 43. Dimensions (mm) of non-neural organs and structures of *Mexipyrgus churinceanus* from Locality 50. N = 5. Mean ± standard deviation. L = length, W = width.

		Females	Males
Body	L	9.58 ± 0.33	9.10 ± 0.28
Osphradium	L	0.38 ± 0.13	
Gonad	L	1.42 ± 0.17	3.26 ± 0.76
	W	0.73 ± 0.08	0.76 ± 0.09
Prostate	L		1.71 ± 0.11
	W		0.98 ± 0.06
Penis	L		3.24 ± 0.23
	W		1.52 ± 0.23
Pallial oviduct	L	5.43 ± 0.23	
	W	1.10 ± 0.08	
Bursa copulatrix	L	1.41 ± 0.10	
	W	0.38 ± 0.05	
Seminal receptacle (body)	L	0.36 ± 0.07	
	W	0.16 ± 0.02	
Seminal receptacle (duct)	L	0.21 ± 0.05	
	W	0.08 ± 0.004	

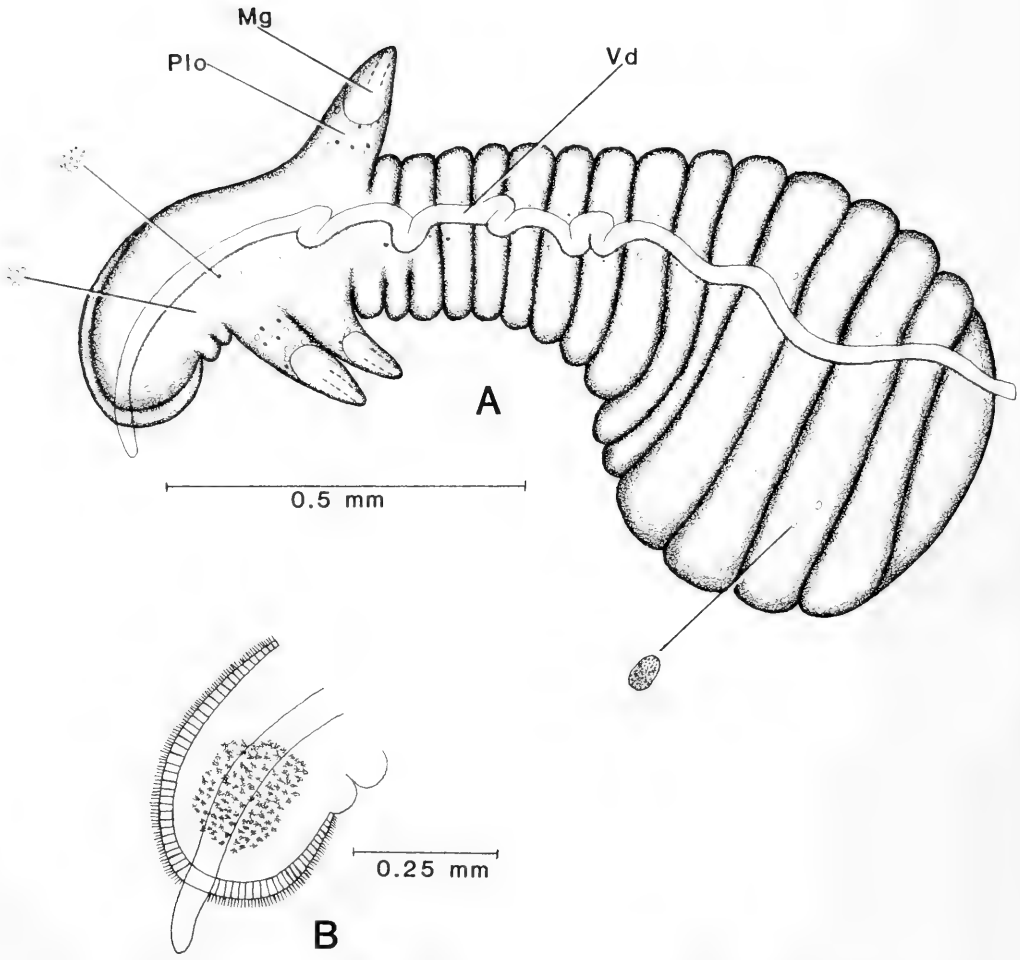

FIG. 44. The penis of *Mexipyrgus churinceanus* from Locality 1. A. Dorsal aspect of the penis. Note the folds in the penis, the blunt tip with ciliated columnar epithelium, and slender penial lobes (Plo) with mammiform glands (Mg). B. The tip of the penis showing the ciliated cells and pigment patch. Mg—mammiform gland; Plo—penial lobe; Vd—vas deferens.

TABLE 44. Radular statistics for females from 6 populations of *Mexipyrgus churinceanus*. Measurements are in mm. Mean ± standard deviation. "r" and "p" refer to the correlation coefficient (and significance level) between that particular radular feature and mean adult female shell length (from Table 33), for the 6 populations.

		Radular statistics				
	Shell length	N	Length	Width	No. rows	Width of central tooth (N)
Locality 73	4.10	10	0.57 ± 0.02	0.09 ± 0.001	49.4 ± 1.49	0.030 ± 0.0012 (32)
Locality 95	5.29	9	0.65 ± 0.03	0.11 ± 0.006	53.3 ± 2.96	0.033 ± 0.0011 (33)
Locality 76	6.72	9	0.78 ± 0.03	0.13 ± 0.005	54.1 ± 2.76	0.038 ± 0.0017 (27)
Locality 1	7.24	9	0.70 ± 0.03	0.12 ± 0.007	53.2 ± 1.92	0.034 ± 0.0013 (46)
Locality 97	7.51	9	0.71 ± 0.02	0.12 ± 0.005	54.7 ± 1.80	0.034 ± 0.0015 (29)
Locality 50	8.25	9	0.77 ± 0.02	0.14 ± 0.005	54.1 ± 1.96	0.039 ± 0.0018 (22)
			r 0.860		0.833	0.758
			p <.025		<.025	<.05

TABLE 45. The various cusp arrangements for the four tooth types in 18 radulae of *Mexipyrgus churinceanus*, with the percentage of radulae showing that arrangement at least once.

Central		Lateral		Inner marginal		Outer marginal	
anterior cusps / basal cusps	%	cusps	%	cusps	%	cusps	%
4–1–4 / 2–2	6	4–1–4	58	21	11	24	11
4–1–4 / 3–2	17	5–1–4	58	22	6	25	11
4–1–4 / 3–3	28	5–1–5	58	23	33	26	22
5–1–4 / 3–2	33	6–1–4	25	24	11	27	44
5–1–4 / 3–3	44	8–1–4	8	25	33	28	33
5–1–4 / 2–2	22	6–1–5	42	26	56	29	33
5–1–4 / 2–1	11	7–1–4	8	27	61	30	67
5–1–5 / 2–1	6	6–1–6	8	28	56	31	56
5–1–5 / 2–2	28			29	39	32	33
5–1–5 / 3–2	33			30	39	33	39
5–1–5 / 3–3	44			31	17	34	28
6–1–4 / 3–2	6			32	28	35	11
6–1–5 / 2–2	17			33	28	36	17
6–1–5 / 3–3	11			34	17	37	17
6–1–6 / 2–1	6			35	11	38	11
6–1–6 / 2–2	6			36	11		
6–1–6 / 3–2	11						
6–1–6 / 3–3	28						
7–1–5 / 2–2	6						
7–1–5 / 3–3	11						
7–1–6 / 2–1	6						
7–1–6 / 3–3	11						
7–1–7 / 3–3	6						
7–1–7 / 4–3	6						
8–1–5 / 3–3	6						
6–1–4 / 3–3	6						

TABLE 46. Common (≥40% of radulae studied) central tooth cusp formulae for 6 populations of *Mexipyrgus churinceanus*.

	Formulae
Locality 73	4–1–4/2–2, 5–1–4/2–2
Locality 95	5–1–4/2–2
Locality 76	5–1–5/2–2, 6–1–5/2–2
Locality 1	5–1–4/3–3, 5–1–5/3–3
Locality 97	5–1–5/3–2
Locality 50	5–1–4/3–3, 5–1–5/3–3

culum (Fig. 40B) has 2.0–2.5 whorls and the nucleus is positioned at 23% of the long axis of the operculum. The body pigmentation consists of thin bands of dark melanin on the dorsal surface, and yellow and white granules on the ventral body surface. The operculigerous lobe has several thin red-purple melanin streaks as well as a large central cluster of white granules. The caecal chamber extends posterior to the stomach (Cae, Fig. 41A).

Radula

The radula is shown in Figs. 39B, C. The central tooth has one to three pairs of basal cusps that arise from the lateral angles. Radular statistics for the type populations of the six nominal species are given in Table 44. Radulae were removed from large females of each population. As seen in Table 44, the length of the radula ribbon, number of rows of teeth, and width of the central tooth are all highly correlated with the average shell lengths of the females for the populations ($p < 0.05$). The cusp arrangement for the four tooth types for the population from Locality 1 are given in Table 45. Common formulae for the cusp arrangements for the central tooth for the same six populations as above are given in Table 46. Note that only the three populations with large-sized females commonly have three pairs of basal cusps.

Female Reproductive Anatomy

The female gonad (Go) consists of four to six lobed branches (Fig. 41A) and is only 15–20% of the body length. The pallial oviduct is 44–57% of the body length, and the posterior coils extend to within 0.38 mm of the end of the mantle cavity. When part of the non-reflected portion of the pallial oviduct is dissected away, it is seen that the posterior bend continues to loop dorsally (Fig. 42A). Sections of these loops are progressively cut away in Figs. 43A–D to reveal their complex nature and the way that they partially envelop the bursa (Bu).

The bursa is elongate, with a narrowed posterior section, and is 26–34% of the pallial oviduct length. The seminal receptacle (Sr) is appressed to the dorsal side of the bursa near its anterior end (Fig. 42B). The oviduct, after giving off a short gonopericardial duct (Dpe), disappears beneath the bursa and coils once before receiving the narrow sperm duct (Sdu) from the duct of the seminal receptacle (Dsr, Figs. 42B, C). The oviduct then loops back to the ventral side of the bursa to enter the posterior end of the pallial oviduct. The sperm duct is tightly appressed to the duct of the seminal receptacle. After the juncture with the sperm duct, the duct of the seminal receptacle coils variably several times before entering the dorsal side of the anterior end of the bursa (Figs. 42B–E).

The large bursa and massive coils of the pallial oviduct cover the kidney (Ki) and a small portion of the pericardium (Pe, Fig. 42A). The kidney and pericardium are relatively small and flattened compared to those of other hydrobiid snails.

Data for number of embryonic shells obtained from adult females from 10 populations are given in Table 47. Non-shelled embryos were rarely seen in dissected specimens. The correlation between shell length and number of brooded young (data from Table 47) is 0.882 and highly significant ($p < 0.005$). For 100 embryonic shells from females from Locality 1, shell length averaged 0.386 ± 0.190 mm, with a five-fold range from 0.119–0.634 mm. The embryos have red pigment on the dorsal body surface.

Male Reproductive Anatomy

The male gonad has five to six lobed branches, filling most of the digestive gland and comprising 33–39% of the body length. The prostate is 26–34% of the body length, overlaps the mantle cavity, and has the anterior vas deferens exiting from its anterior tip.

The penis is shown in Fig 44A. It has deep folds over most of its length. The single penial lobe on the outer curvature is located at 67% the penis length from the base. One or (more commonly) two lobes are on the inner curvature, again beginning at 67% of the penis length from the base (Fig. 45). In one population (from Locality 76) a few specimens had a

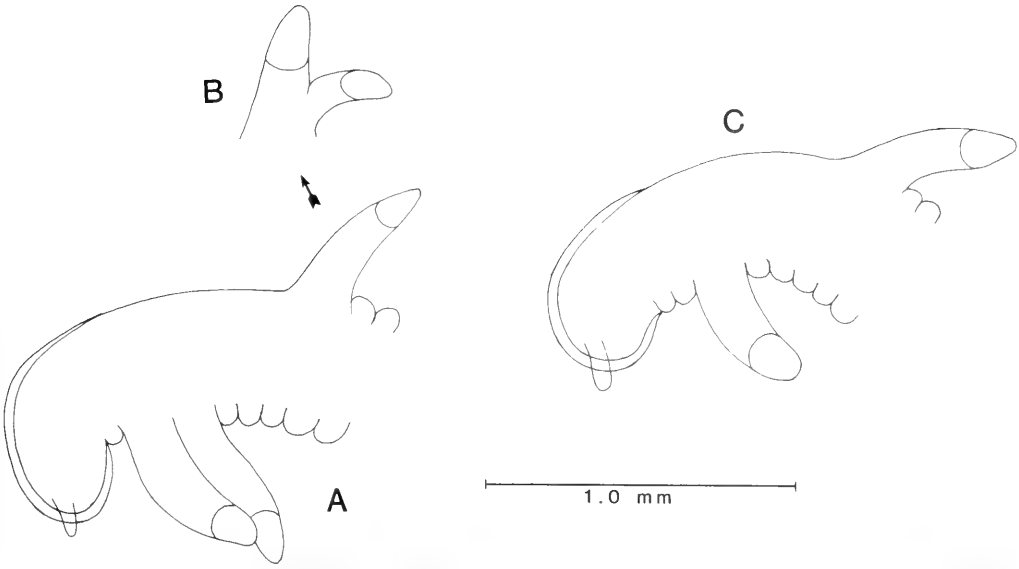

FIG. 45. Variation in the penial lobation of *Mexipyrgus churinceanus*. A. The most common penis type with two lobes on the inner curvature and one lobe on the outer curvature. B. Presence of a second, "bud-like" penial lobe (with mammiform gland) on the outer curvature, seen only in specimens from Locality 76. C. Rare penis type with one lobe on both the inner and outer curvatures, seen only in specimens from Localities 73, 76, and 99.

TABLE 47. Data for number of shelled embryos brooded by females from 10 populations of *Mexipyrgus churinceanus*. The mean shell length (for shells with the maximum dominant whorl number) for adult females for each population is also given.

	Shell length (mm)	Number of young/female			
		N	$\bar{X}$	SD	range
Locality 6	3.93	16	3.56	1.67	0–7
Locality 16	4.44	15	3.60	3.00	0–12
Locality 10	5.10	14	9.21	3.07	6–17
Locality 18	5.10	17	8.88	3.95	4–17
Localtiy 21	5.13	15	8.87	3.14	6–18
Locality 11	5.90	13	7.31	2.81	3–14
Locality 5	5.99	14	9.14	1.03	8–11
Locality 22	6.04	16	4.75	1.29	3–8
Locality 1	7.42	15	19.1	3.08	14–24
Locality 50	8.25	15	22.1	5.44	14–35

second small bud-like lobe with mammiform gland on the outer curvature (Fig. 45B).

The mammiform gland occupies about one-half the length of the penial lobe (Mg, Fig. 44A). While an apocrine gland is circular in shape and has both a very large central lumen and large terminal opening (see Thompson, 1968, fig. 38E), the mammiform gland is more conical in shape and has a very narrow central lumen (surrounded by a muscular layer) and a small, pore-like terminal opening. The penis of *Pyrgophorus coronatus* also has glands that would be considered mammiform (see Fullington, 1978, fig. 16).

The vas deferens (Vd) coils for most of the length of the penis. The tip of the penis has ciliated columnar epithelia extending back to the end of the folds on the inner curvature,

TABLE 48. Data matrix for the multi

Character	1	5	10	11	18	20	21	22	37	38	43	46	47
1. Max. no. whorls, ♂	7.0	6.0	6.0	6.5	6.0	6.0	6.0	6.5	5.5	6.0	5.5	6.0	6.0
2. Max. no. whorls, ♀	7.5	6.5	6.5	7.0	6.5	6.0	6.5	7.0	6.0	7.0	6.0	6.5	6.5
3. Shell length, ♂	5.76	4.77	4.64	5.22	4.14	4.25	4.73	5.48	4.77	5.74	4.58	4.83	4.41
4. Shell length, ♀	7.24	5.99	5.10	5.90	5.10	3.93	5.13	6.04	6.16	7.28	5.69	5.65	5.06
5. Shell width, ♀	3.35	3.04	2.72	3.05	2.72	2.24	2.76	3.16	3.48	4.10	3.46	2.99	2.91
6. Length of body whorl	4.53	3.92	3.47	3.83	3.41	2.78	3.57	4.01	4.30	4.99	4.00	3.85	3.50
7. Length of aperture	2.78	2.45	2.19	2.33	2.17	1.74	2.28	2.36	2.72	3.16	2.69	2.48	2.27
8. Width of aperture	1.79	1.53	1.42	1.60	1.49	1.24	1.51	1.74	1.94	2.19	1.80	1.61	1.60
9. No. of gill filaments	55.0	44.5	52.3	46.0	45.8	45.8	47.0	48.4	50.8	53.4	48.0	50.8	45.2
10. Body whorl with spiral cord	0	0	0	0	0	0	0	0	0	0	0	0	0
11. Shell with periostracal bands	2	0	0	0	1	0	0	0	1	1	2	0	2
12. Banded shells with thick sutural band	2	2	2	2	2	2	1	2	2	2	2	0	2
13. Penis with 1 lobe on inner curvature	0	0	0	0	0	0	0	0	0	0	0	0	0
14. Penis with 2 lobes on inner curvature	1	1	1	1	1	1	1	1	1	1	1	0	1
15. Rostrum pigmented	0	0	0	0	2	0	0	0	2	0	0	1	1
16. No. of periostracal bands	1	1	1	1	0	0	0	1	1	1	1	0	0
17. Freq. of sculpture score—1	.02	0	.14	.02	.03	.33	.03	0	0	0	0	0	.02
18. Freq. of sculpture score—2	.12	0	.33	.14	.13	.48	.46	.02	.10	0	.08	.04	0
19. Freq. of sculpture score—3	.54	.50	.49	.72	.83	.19	.51	.62	.60	.66	.92	.51	.72
20. Freq. of sculpture score—4	.32	.50	.04	.12	.01	0	0	.36	.30	.34	0	.45	.26

and 0.3 mm back on the outer curvature (Fig. 44). A pigmented patch is sometimes seen near the tip of the penis (Fig. 44B.) The penis has Gl_1 and Gl_2 glands.

Discussion

While complete anatomical data are provided for only three populations, specimens from numerous other populations (including those of the types for all nominal species) were dissected as well (see Table 48), yet no qualitative differences in soft-part anatomy were seen. The main difference between populations is in shell features, especially size, and anatomical features correlated with size, such as number of young brooded by females, radular statistics, and number of gill filaments. The purported differences (shell and anatomy) between nominal species are blurred when numerous populations are studied. For example, one of the diagnostic features of *Mexipyrgus mojarralis* (sensu Taylor, 1966), which is considered endemic to Locality 73), is a penis with a single lobe on the inner curvature (the usual number is two). Yet in the Mojarral East Laguna (Locality 76), which has a stream connection with Locality 73, individuals assignable to *M. multilineatus* (sensu Taylor, 1966) may also have this penis type (see Table 49).

As there are no morphological criteria by which separate species can be recognized, the six nominal species are reduced to one, *Mexipyrgus churinceanus.* A detailed analysis of morphological variation of *M. churinceanus,* and its relation to the species problem, is presented below.

Subfamily Unknown

Orygoceras Brusina, 1882

Type-species: *Orygoceras cornucopiae* Brusina, 1882.

Distribution: the single living species is restricted to two localities in the southwestern deserts of North and Central America (see below). Late Cenozoic fossils are known from eastern Europe (Brusina, 1882) and the northwestern United States (Dall, 1925; and others).

Species included: the living species remains undescribed. Numerous fossil species have been described.

Description

The shell is variable in size (width, 2.0–12.0 mm), but always uncoils after a whorl or so, producing a tube-like shape (Fig. 13H). Axial sculpture may (Brusina, 1882, pl. 11) or may not (Fig. 13H) be present.

Orygoceras (?) sp.

Distribution: restricted to Roaring Springs, Real County, Texas (Taylor, 1974); and a single spring in the Cuatro Ciénegas Basin (see below).

variate analysis (measurements in mm).

48	49	30	50	78	99	76	73	71	80	79	81	82	83	86	88	93	95	97	96
6.5	6.5	6.5	7.0	6.5	6.5	6.5	6.0	6.5	6.5	7.0	6.5	6.5	6.5	6.0	5.5	6.5	6.5	7.0	6.0
6.5	7.0	6.5	7.5	7.0	7.0	7.0	6.5	6.5	7.0	7.5	6.5	6.5	7.0	6.5	5.5	7.0	6.5	7.0	6.0
4.25	4.76	4.17	7.03	6.23	5.37	6.03	3.80	5.44	6.24	7.31	5.74	5.96	5.44	4.53	3.14	5.64	4.82	6.58	4.36
4.65	5.54	4.44	8.25	7.34	6.53	6.72	4.10	5.90	6.65	8.45	6.93	6.26	6.96	6.34	3.03	6.53	5.29	7.51	4.51
2.47	2.73	2.43	4.28	3.91	3.28	3.39	2.36	3.34	3.37	4.36	3.84	3.26	3.48	3.47	1.74	3.49	2.76	4.03	2.57
3.02	3.51	2.99	5.45	4.85	4.33	4.47	2.75	4.10	4.41	5.37	4.70	4.21	4.59	4.35	2.08	4.32	3.50	4.87	3.14
1.85	2.18	1.88	3.46	2.99	2.68	2.78	1.66	2.59	2.63	3.41	3.00	2.61	2.73	2.74	1.33	2.73	2.18	3.00	1.93
1.29	1.50	1.32	2.20	2.10	1.80	1.83	1.21	1.76	1.80	2.31	2.04	1.76	1.92	1.90	0.92	1.86	1.51	2.08	1.35
46.8	51.6	44.8	65.6	53.2	58.0	53.4	41.8	53.2	53.8	64.6	51.6	52.6	62.6	63.6	34.2	46.2	47.0	60.6	48.4
0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
2	2	2	2	2	2	0	2	2	0	1	1	0	0	2	0	0	2	2	1
2	2	2	0	0	0	0	1	1	1	0	0	0	1	2	0	1	2	1	2
0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	2	0	0	0	1	0	1	2	2	0	0	0	0	0	2	2
0	0	0	2	2	2	2	0	2	2	2	2	2	1	2	0	1	2	1	1
.08	.04	0	.88	.24	.96	.56	.46	.38	.34	.24	.02	.44	.50	.03	.14	.02	.02	0	0
.24	.23	0	.10	.27	.04	.38	.36	.33	.34	.38	.02	.44	.38	.21	.56	.06	.08	0.	0
.66	.73	.18	.02	.46	0	.06	.16	.29	.32	.30	.42	.12	.12	.62	.30	.60	.74	.13	.23
.02	0	.82	0	.03	0	0	.02	0	0	.08	.54	0	0	.14	0	.32	.16	.87	.77

Habitat: restricted to small springheads. Taylor (1974) found one living specimen close to the source of the spring after heavy rains. I collected three live specimens and two empty shells from mops placed at the small springhead at Locality 67. The rarity of live specimens at these localities and the fact that the snail is blind and unpigmented, suggest that its main habitat is subterranean.

Description

The shell (Fig. 13H) uncoils after 1.3 whorls and produces a tube shape that is very similar in all specimens seen. For two specimens from Cuatro Ciénegas, the shell lengths (parallel to the coiling axis) are 1.70 and 1.58 mm, and the widths are 2.26 and 2.06 mm, respectively. Adult Roaring Springs specimens are about 2.0 mm wide (Taylor, 1974). The apical whorl has pitted microsculpture, while the later whorls have strong growth lines. The operculum is paucispiral.

The animal is blind and unpigmented. The buccal mass and operculigerous lobe have a red-pink color similar to that of *Coahuilix* and *Paludiscala*. The intestine has a loop near its anterior end (Taylor, 1974, fig. 1). While not dissected, the animal is hydrobioid in its external appearance.

Discussion

There are three distinct groups of *Orygoceras* species: 1) large (width, to 8 mm), sculptured species from Late Cenozoic Balkan lake beds; 2) large (to 12 mm), smooth-shelled species from Miocene-Pliocene Idaho lake beds; and 3) the very small (to 2 mm), smooth-shelled living species found in small springheads. The relationships among these three groups remain obscure: the living species is probably not closely related to the fossil taxa as it differs greatly in shell size and habitat.

MORPHOLOGICAL DIFFERENTIATION AMONG POPULATIONS OF *MEXIPYRGUS CHURINCEANUS*

Populations of *Mexipyrgus churinceanus* show considerable variation of shell features (Fig. 37). The six nominal species of *Mexipyrgus* were described by Taylor (1966), all but one from single localities, based on characters involving shell size, shape, sculpture, number and thickness of periostracal bands, and the number of penial lobes. I collected *Mexipyrgus* from over 40 localities in the basin, and noted patterns of variation inconsistent with the species concepts of Taylor (1966). To analyze these patterns of variation and to assess the similarities and differences among populations, a data base of 20 characters (16 from shell, one from body pigment, three from soft parts), including most of those employed in the diagnoses of the nominal species, from 33 populations (OTUs) was subjected to multivariate analysis. These

TABLE 49. Characters used in assessing similarities and differences between populations of *Mexipyrgus churinceanus*. Characters 3–9 represent means (of which 4–9 are for females only). In (0, 1) pairs, 0 represents absence of a character-state, 1 represents its presence. Characters 17–20 were also scored only from shells of females as those characters exhibit sexual dimorphism.

1. Maximum number of whorls, males; 2. Maximum number of whorls, females; 3. Shell length, males; 4. Shell length, females; 5. Shell width; 6. Length of body whorl; 7. Length of aperture; 8. Width of aperture; 9. Number of gills; 10. Prominent spiral cord on body whorl (Fig. 37, shells of Locality 73) (0, 1); 11. Shell with periostracal bands (0, 0–33% of shells banded; 1, 34–67%; 2, 68–100%); 12. Banded shells with thick sutural band (Fig. 38, bottom row) (0, 0–33% of banded shells with thick sutural band; 1, 34–67%; 2, 68–100%); 13. Penis with one lobe on the inner curvature (Fig. 45c) (0, 1); 14. Penis with two lobes on the inner curvature (Fig. 45a) (0, 1); 15. Rostrum pigmented (0, 0–33% of population; 1, 34–67%; 3, 68–100%); 16. Number of periostracal bands at the shell aperture (0, <8; 1, 8–14; 2, >14); 17–20. Frequency of shells with the following axial sculpture development at the end of the penultimate whorl; 17. Absent (see Fig. 37, Localities 76, 88); 18. Low ribs of low nodes without ribs (Fig. 37, Locality 48); 19. Moderately high, noded ribs (Fig. 37, Locality 1); 20. High, noded ribs (Fig. 37, Localities 30, 94).

TABLE 50. List of *Mexipyrgus churinceanus* populations (used in the multivariate analysis) according to drainage systems.

Drainage	Localities (see Fig. 37)
I	1, 5
IIa	10, 11
IIb	18, 20, 21, 22, 37, 38, 30, 43, 46, 47, 48, 49
III	99
IVa	50, 78, 71, 73, 76, 79, 80, 81, 82, 83, 86
IVb	88, 93, 95
V	96, 97

characters are listed and explained in Table 49. The entire data set is given in Table 48. The locations of the 33 populations, together with photographs of shells from many of them, are shown in Fig. 37.

The various populations are listed (by locality number), drainage by drainage, in Table 50. Drainage 1, terminating in a shallow playa lake (Locality 9), is currently isolated from other waters of the basin. Drainage 2a consists of the large thermal limnocrene, the Pozo de la Becerra (Locality 10), and its outflow, the Rio Garabatal. Drainage 2b consists of the large number of springs in the area known as El Garabatal, to the north of the Pozo de la Becerra, and to the east of the Rio Garabatal. These springs all flow to the north or west and may have joined the Rio Garabatal in the recent past. While some of these springs flow into the waters of Drainage 4a (see below), the downstream portion of these spring outflows are fast flowing over a hard bottom and no *Mexipyrgus* was found in them during an intensive survey during 1981. Drainage 3 consists of the isolated Anteojo spring complex (Locality 99) which, prior to alterations, may have flowed south to join Drainage 4a. Drainage 4 consists of the large rheocrene, the Rio Mesquites (originating at Locality 50), and nearby springs that flow into it (together constituting Drainage 4a); and the large thermal limnocrene, Laguna Escobedae (Locality 95), and nearby springs that join the Rio Mesquites well downstream (together constituting Drainage 4b). Drainage 5 consists of the large thermal limnocrene, Laguna Tio Candido (Locality 97), and a nearby spring, that flow to the south of Drainage 4.

Five principal components account for 79.93% of the variation. The first component accounts for 40.41%; the second 17.89% (accumulated 58.30%); the third 9.69% (accumulated 68.00%); the fourth 6.66% (accumulated 74.66%); the fifth 5.27%. Character loading for each component is given in Table 51. Characters are considered highly correlated if their load is greater than 0.60. Characters with values of greater than 0.50, but less than 0.60 were assigned to the principal component for which the characters had the highest value.

Characters highly correlated within the first component are number of whorls for males (Character 1, Table 51), measurements from shells of females (Characters 3–8), gill number (9), and number of periostracal bands on the shell (16). This component is one of size: the number of periostracal bands on the shell logically correlates with adult shell length. The second component has highly correlated characters of shell sculpture (10, 17, 19, 20), incidence of a thickened periostracal band (12), and number of penial lobes (13, 14).

TABLE 51. Factor loading of characters for the five principal components that collectively account for 79.93% of the variation in the multivariate analysis.

Character	Principal components				
	1	2	3	4	5
1	0.653	−0.080	0.061	−0.004	0.401
2	−0.406	0.180	0.251	−0.011	−0.527
3	0.947	−0.007	−0.082	0.058	0.001
4	0.969	0.110	0.034	−0.093	−0.106
5	0.951	0.173	0.076	−0.072	−0.132
6	0.971	0.124	0.031	−0.077	−0.158
7	0.958	0.151	0.031	−0.085	−0.156
8	0.945	0.181	0.060	−0.081	−0.170
9	0.865	−0.058	−0.006	−0.143	0.124
10	−0.277	−0.543	0.458	−0.152	−0.006
11	0.147	0.061	0.546	−0.395	0.512
12	−0.477	0.597	0.095	−0.430	0.114
13	−0.006	−0.764	0.421	−0.128	−0.227
14	0.121	0.591	−0.593	−0.011	0.238
15	0.234	0.248	0.177	0.646	−0.022
16	0.765	−0.137	−0.071	0.004	−0.079
17	0.388	−0.826	−0.060	−0.075	0.099
18	−0.158	−0.571	−0.684	0.018	−0.000
19	−0.301	0.690	−0.032	−0.458	−0.261
20	0.016	0.520	0.544	0.511	0.159

These characters do not involve size. The third component includes highly correlated characters of incidence of periostracal banding (11), number of penial lobes (14) and shell sculpture (18). The sole characters highly correlated within the fourth and fifth components are pigmentation of the rostrum (15) and number of whorls for females (2), respectively.

Ordination diagrams following non-metric three-dimensional scaling are given in Fig. 46 (1st vs. 2nd component), Fig. 47 (1st vs. 3rd component), and Fig. 48 (2nd vs. 3rd component). The various populations are referred to by locality numbers in the ordination diagrams. The stress was low (0.0010). The matrix correlation between taxonomic distance and distances in the three dimensional scaling was 0.988.

In Fig. 46, the ordination of component 2 versus component 1, the smaller-shelled populations are in Quadrants I and IV, and the larger-shelled populations are in Quadrants II and III. Populations in Quadrants III and IV are sculptured, have thickened sutural bands and are from four of the drainages, especially Drainages 1 and 2. Populations in Quadrant II are with large, smooth shells, without a thickened sutural band, and sometimes with males having a single lobe on the inner curvature of the penis (from localities 76, 99); and are exclusively from Drainages III and IVa. In Quadrant I are populations with small-sized shells from Drainages II and IV. Of the nominal species, the populations in Quadrant II would be considered by Taylor as *Mexipyrgus lugoi* or *M. multilineatus* (type populations from localities 50 and 76, respectively). Quadrants III and IV have the type population of *M. churinceanus* (1), *M. escobedae* (95) and *M. carranzae* (97), that differ largely in shell size and sculptural development. The type population of *M. mojarralis* (73) appears as an outlier in Quadrant I as its members have very small shells, with a prominent spiral cord on the body whorl, and males with a single lobe on the inner curvature of the penis.

In Fig. 47, the ordination of component 3 versus component 1, the smaller-shelled populations are again in Quadrants I and IV, and the larger-shelled populations are in Quadrants II and III. Populations with sculptured shells and a high incidence of periostracal banding are in Quadrants III and IV, while smoother-shelled populations with a lower incidence of periostracal banding are in Quadrants I and II. Quadrant II has only populations from Drainage 4 while the other three

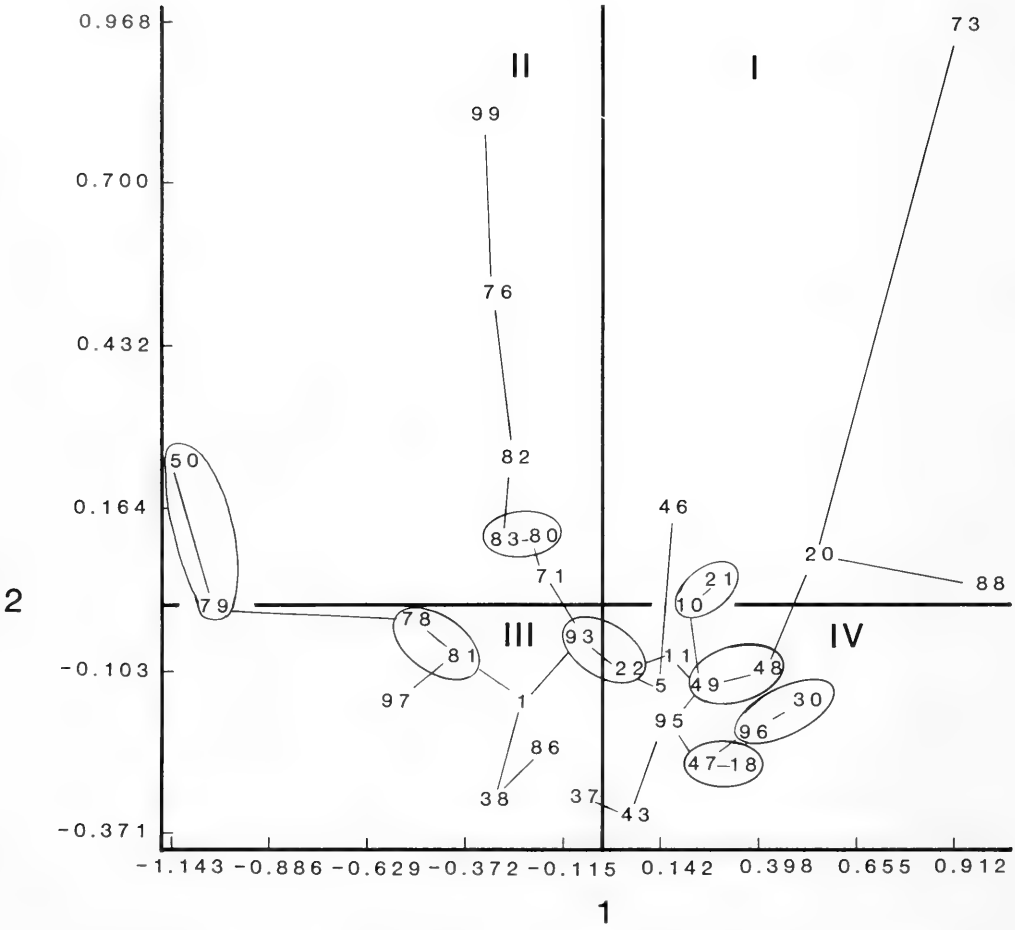

FIG. 46. Ordination diagram of 1 × 2 principal components, drawn according to non-metric multidimensional scaling. The numbers refer to the 33 populations of *Mexipyrgus churinceanus* (Fig. 37). The minimum spanning tree and subset solutions have been superimposed.

quadrants have populations from several drainages each. The population from Locality 88 appears as an outlier in Quadrant I because its members have a very small, smooth, non-banded shell.

In Fig. 48, the ordination of component 3 versus component 2, the influence of size is removed. In this case, the populations group to an even lesser degree by drainage. In Quardant I, smooth-shelled populations from Drainage 2 (10, 20, 21) group with those of Drainage 4. In quadrants II and III, populations with sculptured shells with thickened sutural bands are found: note that these include populations from Drainages II, IV and V. The populations from Localities 99 and 73 are outliers in Quadrant IV as their shells are usually banded and the males have a single lobe on the inner curvature of the penis.

The multivariate analysis indicates that, of the characters used, there is no consistent pattern of geographic variation among the various populations in relation to drainage. Of the eight subsets formed, three are formed among populations of Drainage 2 (10–21, 48–49, 18–47), three are formed among populations of Drainage 4 (50–79, 80–83, 78–81), but two are formed among populations from widely separated drainages: 22–93 (Drainages 2b and 4b) and 30–96 (Drainages 2b and 5); and these two subsets illustrate the problem of recognizing different "species" of

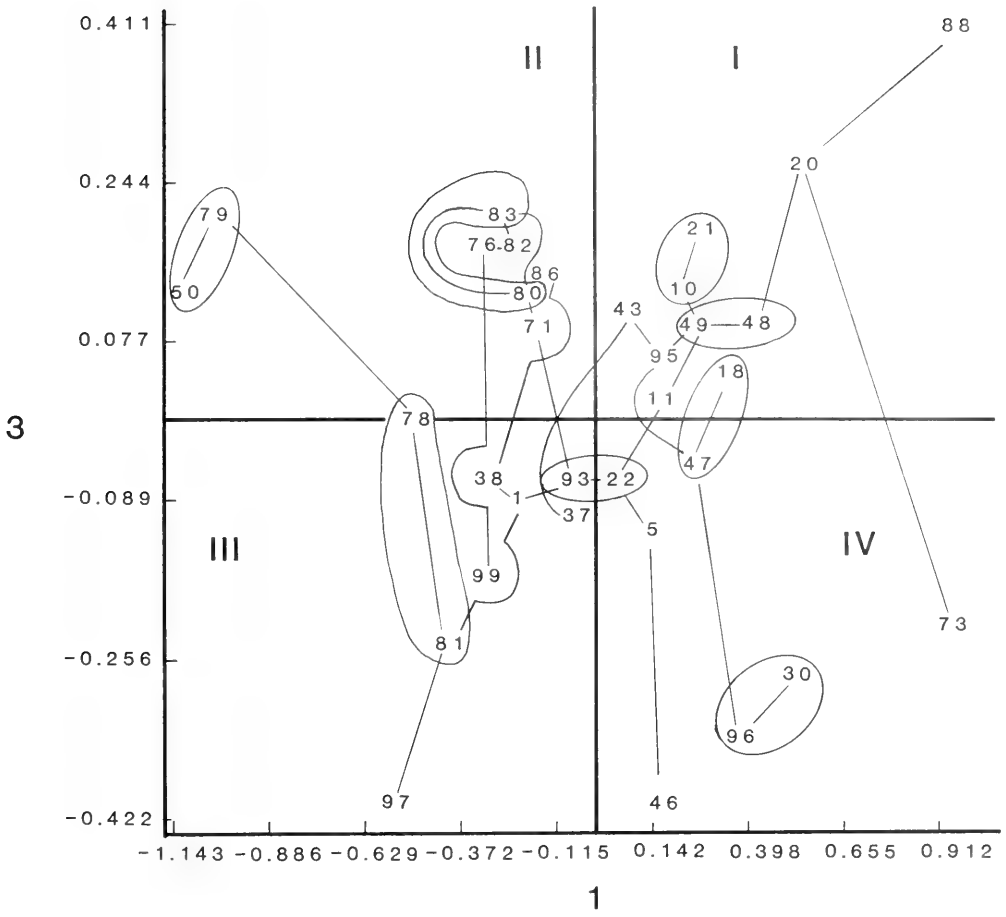

FIG. 47. Ordination diagram of 1 × 3 principal components.

Mexipyrgus. The two populations of Drainage 5 are highly sculptured and are referable to *M. carranzae* (*sensu* Taylor, 1966). Yet one sees a remarkably similar-shelled population (30) from Drainage 2b, on the other side of the tall Sierra de San Marcos, with a low probability of previous connection between the two drainages (Fig. 37). In the other case, while populations from Drainages I and II, in general, are moderately sculptured, with thickened sutural bands, and are referable to *M. churinceanus* (*sensu* Taylor, 1966), populations with similar features are seen in Drainage 4 (71, 78, 81, 86, 93, and Locality 90, Fig. 38, bottom row), supposedly the drainage harboring *M. lugoi* (*sensu* Taylor, 1966, smooth-shelled, without a thickened sutural band). While some of these Drainage 4 populations (particularly 71) are located close enough to the low, northern tip of the Sierra de San Marcos (Fig. 37) that they could have been founded by snails from western lobe waters (and hence their *M. churinceanus*-like features) given a previously different topography, the other populations (86, 93) are considerably to the south and east of the mountain tip and probably could not have been founded in such a fashion.

One must conclude, therefore, that despite some geographic differentiation of *M. churinceanus* populations, separate species cannot be distinguished, as similar morphological features involving shell size, sculpture and banding pattern have apparently been independently acquired in separated populations. The pattern seen in the ordination

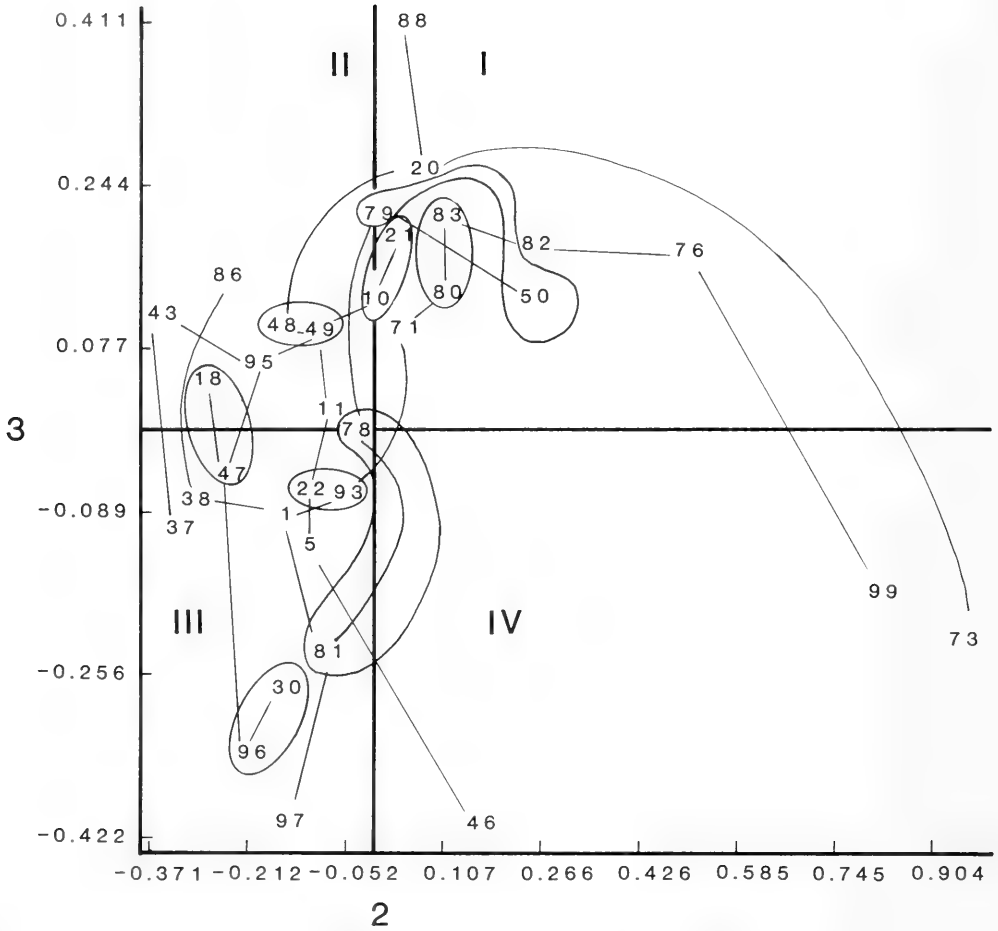

FIG. 48. Ordination diagram of 2 × 3 principal components.

diagrams, with many populations clustered together and a few outliers with unusual features, is one of a single variable species. While the six nominal species must be reduced to one, there may be three races or subspecies, corresponding to *M. churinceanus, M. lugoi* and *M. carranzae* (*sensu* Taylor, 1966), as populations assignable to each are concentrated in different portions of the basin drainage; Drainages I and II, III and IV, and V, respectively.

The shell features of *Mexipyrgus churinceanus* may be somewhat plastic phenotypically and dependent on environmental factors. While populations located close to one another may be quite similar (such as subset 48–49), in other cases populations from close-by springs (even those with an aquatic connection) are quite dissimilar on shell characters, especially when the springs differ in size, temperature, or substrate type. For example, the populations from Localities 80 and 79 are from springs (one small-sized and cold, the other large and warm) separated by only 100 m of stream, but differ greatly in shell size and frequency of a thickened sutural band (Table 49, Fig. 37). Populations from Localities 76 and 73 are also from close-by springs (one large and warm, the other small and very warm) with a short stream connection, but differ enough to have been placed in different species (Fig. 37; Taylor, 1966). However, when I collected and studied snails from a transect along the stream connection between the springs, intergradation of the two forms was apparent (Hershler, in preparation). In several cases I visited springs that had been altered by dredging. In these cases

the living specimens differed greatly in size and sculpture from sub-fossil specimens found in the dredged sediment alongside the spring, suggesting that (as the dredging was recent) the habitat change quickly resulted in a shell change.

Shell features may also depend on whether the population lives in a lentic (spring) versus lotic (stream) habitat: note that the spring-head and downstream populations in two instances (from Localities 1 and 5, 10 and 11) differ greatly in size, sculpture, and banding fequency. Banding frequency corrrelates with substrate color: populations from springs with light-colored sediments are generally unbanded while those from springs with darker sediments are usually banded. A banded shell may appear cryptic in dark sediment, making it more difficult to be seen by predaceous cichlid fish as they disturb and search the sediment when feeding.

RELATIONSHIPS AMONG THE CUATRO CIÉNEGAS HYDROBIIDS

The results of anatomical study of the Cuatro Ciénegas hydrobiids show that all of the taxa belong to the Nymphophilinae or Littoridininae (see Table 2) are widely distributed subfamilies. Thus while there are endemic genera, there are no subfamilies of hydrobiids endemic to the Cuatro Ciénegas Basin. While the relationships among the various hydrobiid taxa of Cuatro Ciénegas are discussed below, and shed some light onto the origin of the endemic taxa, the discussion is necessarily limited as the hydrobiids of the southwestern United States, Mexico, and Central America are almost entirely unknown in terms of soft part anatomy, with many taxa still undescribed.

The two nymphophiline genera of the basin, *Mexistiobia* and endemic *Nymphophilus,* differ in at least 10 morphological features (Table 52). However, many of these differences may be simple correlates of the great size difference between snails of these taxa, and the two genera may, in fact, be closely related.

A comparison of the six littoridinine genera of the basin, involving 36 characters, is given in Table 53. Of these characters, six (17%) are from the operculum or shell, seven (19%) are from nonreproductive aspects of anatomy, eight (22%) are from the male reproductive anatomy, and fifteen (42%) are from the female reproductive anatomy. A matrix of percent difference between these taxa is given in Table 54 and was constructed by simply counting differences between taxa pairs and dividing by the total number of characters shared by the two taxa. In instances where for a given character two taxa both share a character state and have a different character state (e.g., 1, 2 versus 1, 3), the difference is scored as 0.5. A phenogram, based on simple averaging of differences between taxa, is given in Fig. 49.

The phenogram (Fig. 49) indicates that there are three groups of littoridinines in the basin, each constituting a pair of genera. Two of the groups consist of very similar genera (≤29% difference), the groups themselves linking at 46% difference. The taxa of the third group, *Paludiscala* and *Coahuilix,* link at 41%

TABLE 52. List of 10 morphological differences between *Mexistiobia* and *Nymphophilus.*

Mexistiobia	*Nymphophilus*
1. Operculum with 3.5 whorls	5.5–6.0 whorls
2. Osphradium short	Osphradium elongate
3. Central tooth or radula with 1 pair of basal cusps	3 pairs
4. Male gonad overlaps stomach	Male gonad posterior to stomach
5. Male gonad a single lobed mass	Male gonad bush-like
6. Penis with single glandular ridge	1–3 glandular ridges
7. Penial lobe slender, with single fold	Penial lobe stout, with many folds
8. Bolster and ventral channel poorly developed	Bolster and ventral channel well-developed
9. Bursa small (21% of pallial oviduct length), dorsal to pallial oviduct, with a short duct	Bursa large (32% of pallial oviduct length), posterior to pallial oviduct, with a long duct
10. Opening of common genital aperture at end of pallial oviduct	Opening of common genital aperture lateral to pallial oviduct

TABLE 53. Comparison of the six littoridinine genera of Cuatro Ciénegas involving 36 characters. *Pal.* = *Paludiscala, Coah.* = *Coahuilix, Cochl.* = *Cochliopina, Mexith.* = *Mexithauma, Dur.* = *Durangonella, Mexip.* = *Mexipyrgus.*

Character	*Pal.*	*Coah.*	*Cochl.*	*Mexith.*	*Dur.*	*Mexip.*
Shell						
1. Shape: a) planispiral (0) b) trochoid-globose (1) c) ovate-conic (2) d) turriform (3)	3	0	0,1	1	3	2
2. Sculpture: a) ribs (0) b) spiral cords (1) c) absent (2)	0,2	2	1	1	2	0,1
3. Apical whorl microsculpture: a) pitted (0) b) absent (1)	0	0	0	1	1	1
4. Shell with periostracal bands (0,1)	0	0	1	1	0	1
5. Shell aperture flared (0, 1)	0	1	0	0	0	0
External Features						
6. Tentacle ciliation: a) absent (0) b) *Hydrobia*-like (1) c) *Spurwinkia*-like (2)	1	0	2	2	1	0
7. Snail blind, unpigmented (0,1)	1	1	0	0	0	0
8. Mantle edge papillate (0, 1)	0	0	0	1	0	0
9. Position of operculum nucleus along long axis: a) <0.30 (0) b) ≥ 0.30 (1)	1	1	1	1	0	0
Digestive System						
10. Digestive gland tubercles as low swellings (0, 1)	1	1	0	0	0	0
11. Intestine with anterior loop (0, 1)	0	1	0	0	0	0
12. Caecal chamber extends posterior to stomach (0, 1)	0	0	1	1	1	1
13. Origin of basal cusps of central tooth of radula: a) from face of tooth (0) b) from lateral angles (1)	1	0	1	1	1	1
Male Reproductive Anatomy						
14. Male gonad morphology: a) simple lobes (0) b) bush-like (1) c) non-lobed mass (2)	2	2	0	1	0	0
15. Seminal vesicle coils on stomach (0, 1)	0	1	0	0	0	0
16. Prostate posterior to end of mantle cavity (0, 1)	0	1	0	0	0	0
17. Penis with slender penial filament (0, 1)	0	1	1	1	0	0
18. Penial lobe(s): a) absent (0) b) bulb-like (1) c) simple (2)	1	1	0	0	2	2
19. Penis ciliated (0, 1)	0	0	0	0	1	1
20. Penis with terminal eversible papilla (0, 1)	1	0	0	0	1	1
21. Penis with specialized gland(s) (0, 1)	1	1	0	0	1	1

TABLE 53 (Continued)

Character	*Pal.*	*Coah.*	*Cochl.*	*Mexith.*	*Dur.*	*Mexip.*
Female Reproductive Anatomy						
22. Female gonad overlaps stomach (0, 1)	0	1	0	0	0	0
23. Female gonad: a) relatively large, lobed (0) b) relatively large, nonlobed (1) c) relatively small, a mere thickening of oviduct (2)	1	1	0	2	2	0
24. Reproductive mode: a) oviparity (0) b) ovoviviparity (1)	0	0	1	1	1	1
25. Length of pallial oviduct/length of body: a) <0.30 b) ≥0.30	0	0	1	1	1	1
26. Length of bursa/length of pallial oviduct: a) <0.20 (0) b) ≥0.20 <0.40 (1) c) ≥0.40 (2)	2	1	0	0	0	1
27. Albumen gland: a) normal size (0) b) reduced in size (1) c) very reduced in size (2)	0	0	1	1	2	2
28. Posterior pallial oviduct: a) with bend (0) b) with simple bend (1) c) with complex bend in more than one plane (2)	0	0	1	1	1	2
29. Normal seminal receptacle present (0, 1)	0	0	1	1	1	1
30. Secondary seminal receptacle present (0, 1)	1	0	0	0	0	0
31. Oviduct without coil (0, 1)	1	1	0	0	0	0
32. Spermathecal duct: a) long (0) b) short (1)	0	0	1	0	0	1
33. Of ovoviviparous taxa, anterior end of pallial oviduct: a) with slight muscular coil (0) b) with well-developed muscular coil (1)	—	—	1	1	0	0
34. Of taxa with a normal seminal receptacle, the seminal receptacle opens into: a) the oviduct directly (0) b) the oviduct via a sperm duct (1)	—	—	0	0	1	1
35. Of taxa with a normal seminal receptacle, the length of the seminal receptacle/length of bursa: a) <0.30 (0) b) ≥0.30 <0.50 (1) c) ≥0.50 (2)	—	—	1	2	1	0
36. Of taxa with a long spermathecal duct, the openings of the spermathecal duct and pallial oviduct are: a) separate (0) b) joined (1) c) separate, but with an open channel between them (2)	1	0	—	2	0	—

TABLE 54. A matrix of percent difference between pairs of the 6 littoridinine genera from Cuatro Ciénegas (based on data from Table 53).

	Paludiscala	*Coahuilix*	*Cochliopina*	*Mexithauma*	*Durangonella*	*Mexipyrgus*
Paludiscala	—	41	69	73	59	67
Coahuilix		—	77	82	82	84
Cochliopina			—	19	43	44
Mexithauma				—	44	53
Durangonella					—	29
Mexipyrgus						—

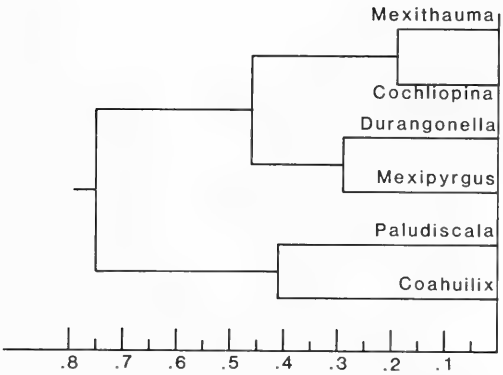

FIG. 49. Phenogram based on distance values derived from Tables 53 and 54.

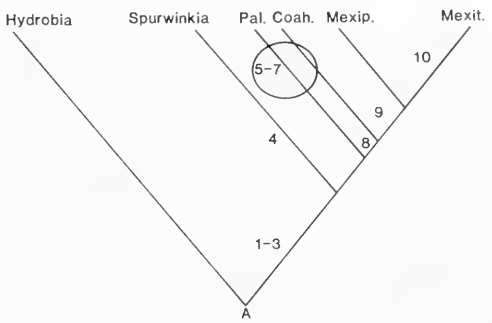

FIG. 50. Cladogram based on character-states listed in Table 55. Pal. = *Paludiscala*, Coah. = *Coahuilix*, Mexip. = *Mexipyrgus-Durangonella* group, and Mexit. = *Mexithauma-Cochliopina* group. *Paludiscala* and *Coahuilix* share character-states 5–7 (hence the circle enclosing both taxa).

difference. This group links with the other four genera at 75% difference.

An hypothesis of the phyletic relationships among *Hydrobia* (Hydrobiinae), the six Cuatro Ciénegas littoridinine genera, and *Spurwinkia*, the only other North American littoridinine known from entire soft-part anatomy (see Davis *et al.*, 1982), is shown in Fig. 50. The numbers indicate presumed derived character states, listed in Table 55, used to define clades. Several of these character states, relating to the female reproductive system, are illustrated in Fig. 51. "A" represents an hypothetical ancestral hydrobiid, with the female reproductive anatomy of *Hydrobia*.

In *Hydrobia* (Fig. 51A), sperm pass along the ciliated ventral channel of the pallial oviduct, which connects with the lumen of the pallial oviduct via a narrow slit. The ventral channel bifurcates at the posterior end of the mantle cavity; one branch leads to the bursa and the other is the anterior end of the oviduct. This groundplan of the female reproductive system is found in all Hydrobiinae, Nymphophilinae and Lithoglyphinae (Davis *et al.*, 1982).

Davis *et al.* (1982) suggest that *Spurwinkia* (Fig. 51B) evolved from an ancestor with an *Hydrobia*-like female reproductive system by having the ventral channel close off and partly separate from the pallial oviduct (character state 1). As the eggs need to reach the albumen gland, a connection to the albumen gland from the oviduct formed (character state 2); and the duct from that point to the duct of the bursa became an extension of the duct of the seminal receptacle (character state 3). The ventral channel is only partly separated from the pallial oviduct, suggesting that *Spurwinkia* is only a step removed from a snail with an *Hydrobia*-like female reproductive system. *Spurwinkia* has the additional derived feature of holding egg capsule chains in the anterior end of the capsule gland (character state 4).

While in *Paludiscala* the ventral channel is still only partly separated from the pallial oviduct, in *Coahuilix* the channel has separated entirely and constitutes a spermathecal duct (character state 8). *Coahuilix* and *Paludiscala*

TABLE 55. Presumedly derived character states serving to define clades as shown in Fig. 50.

1. Partial separation of the ventral channel from the pallial oviduct.
2. The oviduct enters the posterior portion of the albumen gland.
3. The duct of the seminal receptacle elongates, connecting to the duct of the bursa. A sperm duct connects the oviduct with the duct of the seminal receptacle.
4. Egg capsule chains are retained within the anterior end of the capsule gland.
5. Loss of eyes and pigment.
6. Loss of oviduct coils.
7. Loss of seminal receptacle.
8. Complete separation of the ventral channel from the pallial oviduct, forming a spermathecal duct.
9. Assumption of ovoviviparity: the anterior pallial oviduct is enlarged and modified into a thin-walled brood pouch, with the albumen gland reduced in size, and with the development of a muscular sphincter at the anterior end of the brood pouch.
10. Shift of ducting: the duct of the seminal receptacle shortens to open directly into the oviduct, and a short duct forms between the bursa (or duct of the bursa) and oviduct.

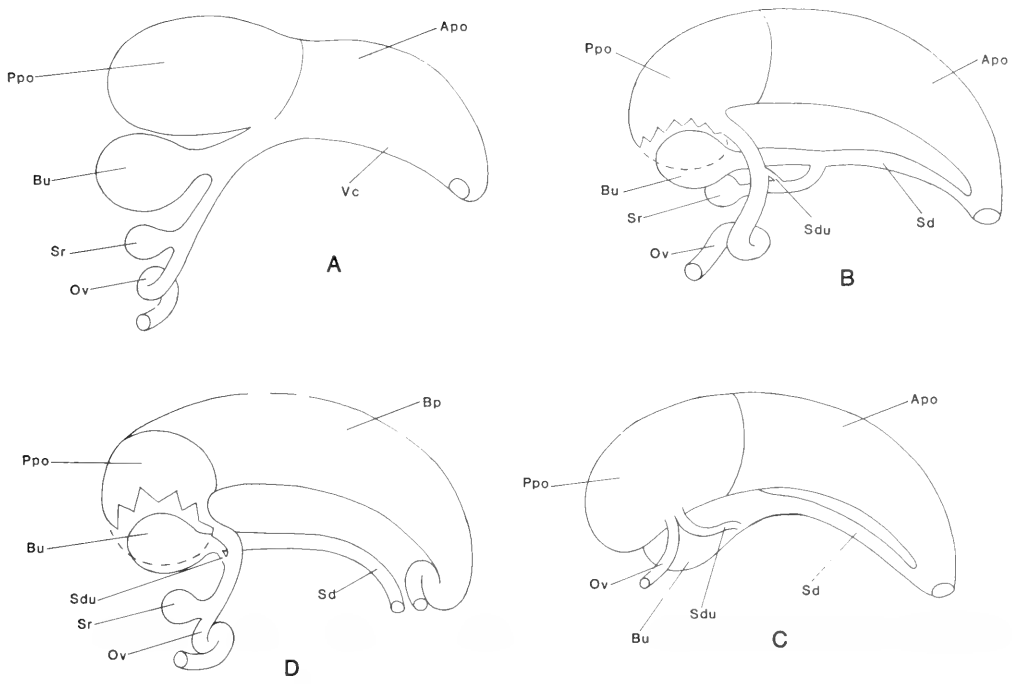

FIG. 51. Schematic drawings of the female reproductive morphologies of *Hydrobia* (A), *Spurwinkia* (B), *Coahuilix* (C), and *Mexithauma* (D). Lettering as on earlier figures.

are considered closely related and specialized; the evolution of these taxa has involved loss or reduction or morphological features, either associated with the very small size of the snails (character states 6, 7) or associated with the unusual groundwater habitat shared by these taxa (character state 5). Hydrobioid snails with a presumed groundwater habitat are known from many parts of the world and are frequently small-sized, blind, and without body pigment (Boeters, 1979; Climo, 1974; Ponder, 1966). The bursa copulatrix complex of *Paludiscala* and *Coahuilix* (Fig. 51C) could have been derived from that of *Spurwinkia* by loss of the seminal receptacle and elongation of the sperm duct so as to

connect with the oviduct at the opening into the albumen gland.

The remaining four littoridinines of Cuatro Ciénegas all have the female reproductive anatomy modified as the result of the assumption of ovoviviparity (character state 9). In the *Mexipyrgus-Durangonella* group, the organization of the bursa copulatrix complex is basically as in *Spurwinkia*: a short sperm duct connects the duct of the seminal receptacle with the oviduct.

In the *Mexithauma-Cochliopina* group ducting is different: the seminal receptacle opens directly into the oviduct and a short duct connects the bursa (*Cochliopina*) or duct of the bursa (*Mexithauma,* Fig. 51D) and the oviduct. These taxa share a puzzling mosaic of character states: while the opening of the seminal receptacle directly into the oviduct is also seen in *Hydrobia* (but not *Spurwinkia*; see above) and is arguably a primitive character state suggesting a close relationship among these taxa, the spermathecal duct is entirely separate from the pallial oviduct, a derived character state indicating that *Mexithauma* and *Cochliopina* evolved from a *Spurwinkia*-like intermediate. It seems probable that the opening of the seminal receptacle directly into the oviduct, within the littoridinine groundplan, is a derived condition and convergent with that of *Hydrobia.* Note that, among littoridinines, this condition has only been found in *Mexithauma* and *Cochliopina,* whereas in other littoridinines from various parts of the world for which the anatomy has been studied, the seminal receptacle connects with the oviduct via a sperm duct (Davis *et al.,* 1982). The latter, more widespread condition is probably the primitive one. The condition seen in *Mexithauma* and *Cochliopina* could have been derived from that of *Spurwinkia* by a shortening of the duct of the seminal receptacle, so as to open into the oviduct, and the development of a duct from the oviduct to the bursa or duct of the bursa (character state 10).

Character states from the female reproductive system have been emphasized in the phyletic analysis because of the complexity of the system (relative to other organ systems in hydrobioid snails), with several organs and ducts functionally organized to receive and hold sperm, fertilize eggs, and provide passage for the eggs or embryos through the pallial oviduct. Precise convergences should be unlikely in such a system. Yet the phyletic analysis indicates that, with the present data base, character states from the female reproductive system cannot always be confidently scored as primitive or derived; the hypothesized phylogeny is therefore tentative. Anatomical data are needed for more taxa to refine the phyletic analysis. If, for instance, littoridinine taxa are found that have the seminal receptacle opening into the oviduct as in *Mexithauma* and *Cochliopina,* but also with the ventral channel only partly separated from the pallial oviduct (as in *Spurwinkia*) then a rearrangement is suggested so that *Mexithauma* and *Cochliopina* are placed closer to "A" than *Spurwinkia.*

Convergence is not entirely unknown among features of the female reproductive system of hydrobioid snails: the Pomatiopsidae and Littoridininae both have spermathecal ducts, but of separate ontogenetic origins. The duct of the Pomatiopsidae forms as a bud from the bursa (Davis *et al.,* 1976) while that of the Littoridininae presumably forms as the ventral channel closes off and separates from the pallial oviduct (Davis *et al.,* 1982). The organization of the bursa copulatrix complex of *Mexithauma* (Fig. 30B) is, in fact, virtually identical to that of *Pomatiopsis* (Davis, 1967, pl. 8). This must be because of convergence, as *Mexithauma* lacks the following diagnostic pomatiopsine features: eyes in pronounced swellings at the bases of the tentacles, presence of a pedal crease and suprapedal fold, and basal cusps arising from the face of the central tooth of the radula (Davis, 1979). Character states involved with brooding young are unreliable, in themselves, for defining clades as the evolution of ovoviviparity involves simple, functionally correlated morphological changes (see below), and has occurred iteratively among many groups of gastropods (Fretter & Graham, 1962). Among hydrobioids, ovoviviparity has evolved at least twice: in the littoridinines and in *Potamopyrgus* which have an *Hydrobia*-like female reproductive system (Fretter & Graham, 1962, fig. 186H). Other characters whose character states could be scored as primitive or derived with even less confidence, and hence were excluded from this analysis, include penial form and gland type, length of the spermathecal duct, and tentacle ciliation pattern.

The data (summarized in the phenogram and cladogram) suggest a polyphyletic origin for the endemic hydrobiids of Cuatro Ciénegas. Of the five endemic genera, each of three (*Nymphophilus, Mexithauma, Mexipyrgus*) is more similar to a non-endemic genus

found in the basin than to the other endemic taxa, suggesting that the endemic hydrobiid fauna may be comprised of at least four separate lineages.

ORIGIN OF THE ENDEMIC SNAILS OF CUATRO CIÉNEGAS

The idea that the endemic hydrobiids of Cuatro Ciénegas are an ancient fauna, with most taxa not closely related to other hydrobiids of the region, is prevalent in the literature (Minckley, 1969, 1977; Taylor, 1966). This hypothesis is based on the supposed endemism of subfamilies of snails that have diverged from a common ancestor, necessitating an origin dating back to the Tertiary period based on the usual slow rate of freshwater snail evolution (Taylor, 1966). Does information on the geological history of the region, coupled with the (above) results of systematic study of the snails, support this hypothesis?

It is known from study of fossil plants from packrat middens that the Chihuahuan Desert is of recent origin, the change from woodlands to desert having occurred during the past 12,000 years (Van Devender, 1976, 1977; Wells, 1977). There is faunal and structural evidence that a number of internal drainages of the Chihuahuan Desert once integrated with the Rio Grande system, and have since been isolated, perhaps due to decreased discharges associated with recent aridity (Morafka, 1977; Smith, 1981). The fauna of these now-isolated drainages is characterized by relictualism and local endemism (Miller, 1977; Milstead, 1960; Morafka, 1977).

A good summary of the geological history of the Cuatro Ciénegas area and its effects on isolation of the basin drainage is given by Minckley (1969). While it is known that the Sierra Madre Orientale chain began to form in the early Tertiary, the age of the Cuatro Ciénegas Valley is unknown. It is known, from a study of pollen from cores taken from the valley, that aquatic environments have existed in the valley for at least 40,000 years (Meyer, 1972, 1973). The basin waters have had past connections with the Rio Grande drainage via the Rio Salado de Nadadores, which heads just east of the valley. The fish fauna of Cuatro Ciénegas has numerous Rio Grande elements (Minckley, 1977). Of the snails, *Cochliopina riograndensis,* a species with a Rio Grande distribution, is found in the basin; and *Nymphophilus,* one of the endemic genera, has been found as a fossil from Pleistocene-Holocene deposits alongside the Rio Monclova (a Rio Grande tributary), 70 km east of Cuatro Ciénegas (J. Landye, personal communication, 1981). Waters from the southern Rio Nazas-Aguanaval system may have also connected with the Cuatro Ciénegas drainage in the past (Conant, 1977; Minckley, 1969). Two of the non-endemic genera of the basin, *Durangonella* and *Mexistiobia*, are known from the Rio Nazas-Aguanaval drainage, but not the Rio Grande.

The above evidence, suggesting that the waters of the basin have had a recent connection to outside drainage, coupled with the discovery of a lower level of endemism than once thought, with no endemic subfamilies and three of five endemic genera closely resembling non-endemic taxa found in the basin, suggests that the endemic snails may be of a more recent and local origin than previously thought.

The Rio Grande drainage of Texas and Mexico, and other waters of southwest Texas, do harbor littoridinine and nymphophiline taxa. Genera from this area assigned to the Littoridininae, on the basis of a penis with stalked, specialized glands (not glandular ridges), include *Texadina* (penis figured in Andrews, 1977: 82–83), *Littoridinops* (Andrews, 1977: 84), and *Pyrgophorus* (Fullington, 1978, fig. 16). The distinctive penis type shared by *Mexistiobia* and *Nymphophilus,* with an elongate penial filament and small number of glandular ridges, is seen in *Fontelicella* (penis discussed in Gregg & Taylor, 1965; figured in Russell, 1971), recently found in a Rio Grande tributary not far from Cuatro Ciénegas (Lytle, 1972). Anatomical study of the above taxa is needed to help determine the origin of the endemic hydrobiids of Cuatro Ciénegas.

I predict that two of the endemic genera, *Paludiscala* and *Coahuilix,* will eventually be found living in waters outside of the basin (see below for discussion of possible *Coahuilix* from Texas). Mexico is undercollected for fresh-water snails and most workers have not employed the methods necessary to collect tiny snails from groundwater outlets. This prediction is supported by the fact that of the three blind, unpigmented crustacean genera originally described from (and considered endemic to) groundwater outlets in Cuatro Ciénegas, two, *Mexiweckelia* and *Mexistenasellus,* were later dis-

covered in cave waters in more southerly parts of Mexico (Argano, 1974; Holsinger, 1973; Magniez, 1972). The discovery of *Orygoceras* (?) sp., previously known from a single spring in southwest Texas, in Cuatro Ciénegas further attests to the potential for a widespread distribution of groundwater-dwelling taxa.

Of the other three endemic genera, one, *Nymphophilus,* may be relict as it has been found fossilized outside the basin, and the other two could have evolved in the basin from the non-endemic taxa that they closely resemble. It is now known that the rate of evolution of fresh-water snails can be quite rapid (Davis, 1979, 1981; Stanley, 1979) and thus one need not invoke an ancient origin for these endemic genera.

The amount of differentiation seen among the snail genera of the basin is slight (only 12 species are known); no genus has more than two species, and only one genus has sympatric congeners. Such minimal differentiation does not support the idea of an ancient snail fauna present in the basin for tens of millions of years, although perhaps in even such a long time span one would not expect great differentiation in so small a basin with apparently plastic drainage patterns.

Members of the endemic Cuatro Ciénegas snail fauna have been linked with those of two other faunas and these possibilities are now discussed. Numerous peculiar-shelled snail taxa have been described from the Pliocene Pebas and other formations from the Upper Amazon Valley in Peru (Boettger, 1878; Conrad, 1871, 1874a, b; Gabb, 1869; de Greve, 1938; Pilsbry, 1944). Some of these taxa have not only been placed in the Hydrobiidae, but have also been considered closely related to some of the Cuatro Ciénegas endemic taxa (Kadolsky, 1980; Parodiz, 1969). These taxa include *Tropidebora* (Pilsbry, 1944), similar to *Nymphophilus*; and *Eubora* Kadolsky, 1980 (= *Ebora* Conrad, 1871), similar to *Mexithauma.* However, examination of types of the Pebas taxa shows that they cannot be hydrobiids as they have a siphonal notch (Fig. 52), or an otherwise peculiarly-angled aperture unknown in living hydrobiids. Similarities between Pebas and Cuatro Ciénegas taxa must therefore be due to convergence.

The Edwards Aquifer in southwest Texas harbors one of the world's most diverse subterranean aquatic faunas (Longley, 1981). Included in this fauna are a number of tiny, blind, unpigmented snails (Karnei, 1978) including species with lamelliform costae on the shell that were assigned to *Paludiscala* (Fullington, 1978, fig. 17). Alcohol specimens of these species, stored at Southwest Texas State University, were studied during January, 1982. These snails differ from *Paludiscala* in at least seven features, with the female reproductive anatomy still unstudied: 1) the shell is much smaller (length 1.1 mm) and has only 3.3–3.5 whorls; 2) strong spiral lines, not seen in *Paludiscala,* run between the costae (Fullington, 1978, fig. 17); 3) the aperture is greatly flared all around; 4) the operculum has a slight internal swelling or peg, not known for any other North American hydrobioid; 5) the intestine has an anterior loop; 6) the penis has neither lobes nor specialized glands; 7) there is no ctenidium. These differences rule out there being *Paludiscala.* The shell of these snails is much more similar to that of *Lanzaia* Brusina, 1906 (figured in Bole, 1970, fig. 6), a European hydrobiine.

While *Paludiscala* may not exist in the waters of the Edwards Aquifer, it is possible that *Coahuilix* does. The type of *Horatia micra* (Pilsbry & Ferriss, 1906), described from stream drift (probably washed out of a spring) of the Guadalupe River, New Braunfels, Texas, is remarkably similar to *Coahuilix hubbsi,* with similar slight apertural flaring. *Horatia micra* is also reported from the artesian well at San Marcos, Texas, and a subterranean stream in Manitou Cave, near Fort Payne, Alabama (Hubricht, 1940). Undescribed *Horatia* are reported from Salamander Cave, Travis County, Texas (Reddell, 1965) and Roaring Springs, Real County, Texas (Taylor, 1974).

EVOLUTION OF OVOVIVIPARITY IN HYDROBIOID SNAILS

It has been suggested that the initial step in the evolution of ovoviviparity in hydrobioid snails involves a simple morphologic change: separation of the ventral channel from the pallial oviduct to form a spermathecal duct, thus keeping separate the functions of receiving sperm and storing embryos (Davis *et al.,* 1982). The need for such a prerequisite is suggested by there being numerous taxa worldwide which lack this separation, only one (*Potamopyrgus*) is known to brood young. *Spurwinkia* and *Littoridinops* are apparent intermediates in the evolution of

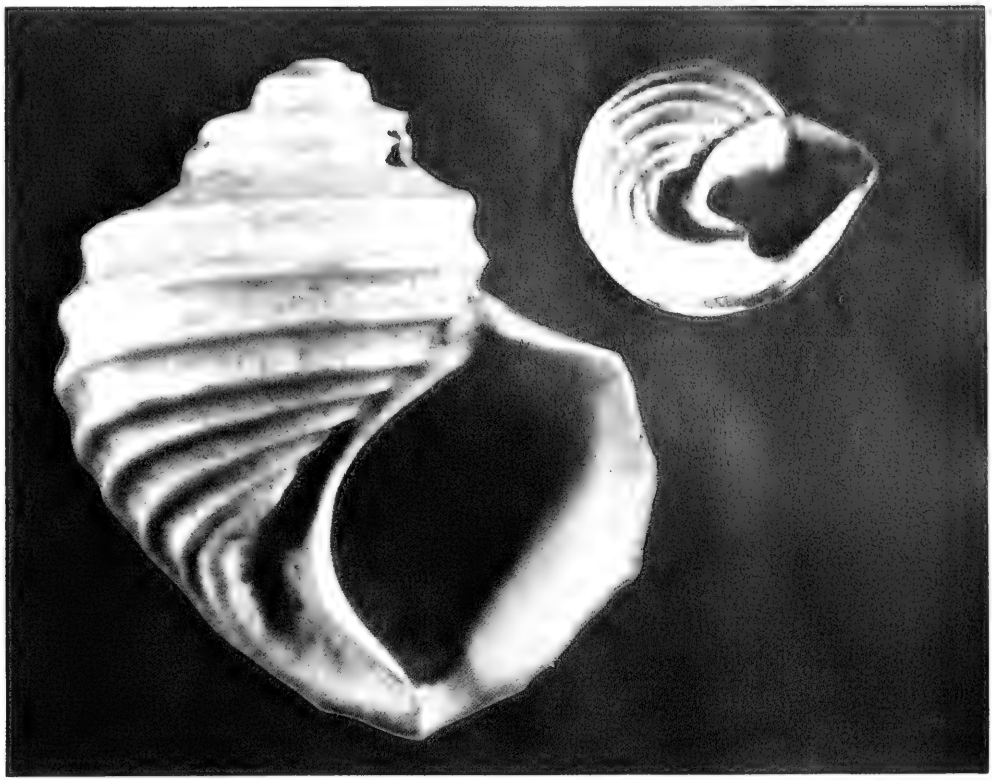

FIG. 52. Holotype (NYSM 9193) of *Eubora bella* (Conrad, 1871). The shell is 7.58 mm long. Note the siphonal notch in the basal view (lower magnification).

ovoviviparity, as they have spermathecal ducts (although that of the former is still connected anteriorly to the pallial oviduct) and, while they do not brood young, they hold egg capsules in the anterior end of the pallial oviduct before depositing them on the substrate (Davis *et al.*, 1982). The littoridinines of Cuatro Ciénegas include taxa that have further modification of the female reproductive system associated with the assumption of ovoviviparity.

Of the six littoridinine taxa of Cuatro Ciénegas, two, *Coahuilix* and *Paludiscala*, are egg layers, but do not hold egg capsules in the anterior pallial oviduct. The other four share features associated with the evolution of ovoviviparity from a *Spurwinkia*-like condition. All have an enlarged pallial oviduct (to 58% of the body length; for *Spurwinkia* it is 40%, Davis *et al.*, 1982) that often overlaps part of the stomach, and that bends posteriorly to varying degrees (*Spurwinkia* has a slight posterior bend of the pallial oviduct). *Mexipyrgus* represents the pinnacle of this trend as the length of the brood pouch is vastly increased by a series of coils that are progressively dorsal to one another. Whereas egg laying hydrobioids have a thick walled, glandular pallial oviduct with a slit-like central lumen, the ovoviviparous littoridinines all show at least some reduction in the size of the albumen gland, with the other (much larger) section of the pallial oviduct modified into a thin-walled, non-glandular brood pouch. These features collectively increase the amount of space available in the pallial oviduct for holding embryos. In addition, all of these taxa have the anterior end of the brood pouch muscularized, giving it the ability to stretch as large sized embryos are released, and perhaps the ability to control timing of embryo release. Ovoviviparous snails may not need great egg production at any one time and this may explain why female gonads of three of

the four ovoviviparous taxa are unusually small, filling only a portion of the length of the digestive gland at all times of the year.

The ovoviviparous Cuatro Ciénegas hydrobiids appear to represent two different brooding strategies, each represented by two taxa of the same clade. *Cochliopina* and *Mexithauma* brood a relatively large number of similar-sized embryos, with only a two- and four-fold range in embryo shell lengths, respectively. *Durangonella* and *Mexipyrgus* brood relatively fewer young with a greater (five- and eight-fold, respectively) range of embryo shell lengths. The latter two genera, perhaps holding embryos for long time periods (hence the great range in embryo shell lengths), may require a very long brood pouch, and they do, in fact, have a greater complexity of pallial oviduct coiling than the other two genera. It is not known why the anterior end of the brood pouches of *Cochliopina* and *Mexithauma* is much more reflected and muscularized than in *Durangonella* and *Mexipyrgus*: the former two taxa do not release relatively large young, but perhaps they release numerous young at the same time, necessitating greater stretching of the brood pouch.

Thus the evolution of ovoviviparity in these littoridinines has involved modifications of the female reproductive anatomy to increase the amount of space available for holding embryos, and to allow and control the release of large-sized embryos. Some of these brooding features parallel those described for other fresh-water gastropods. The two brooding strategies outlined above were described for species of the cerithiacean *Semisulcospira* (Davis, 1969b). The great development of posterior pallial oviduct coiling in *Mexipyrgus,* while unique among hydrobioid snails, is paralleled by that seen in several Viviparidae (Rohrbach, 1937).

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APPENDIX 1

Collection localities for this study. Localities 1–100 are shown in Fig. 3. 1. Laguna Churince (southernmost of the three Posos Bonitos), 19.5 km SSW of Cuatro Ciénegas along the highway. 2. small seep feeding the middle of the three Posos Bonitos. 3. Rio Churince, 100 m downstream from Laguna Churince. 4. Rio Churince, wide pool area, 1000 m downstream from Laguna Churince. 5. Rio Churince, very large pool area due E of Laguna Grande. 6. Small pool at NW edge of (5). 7. Small pool in marshy area, N of (5). 8. Small pool in marshy area, W of (7). 9. Laguna Grande, playa lake terminus of Rio Churince. 10. Posos de la Becerra, 4 km S of the tip of Sierra de San Marcos along the highway. 11. Stream from cool springs at Los Chiceros. 12. Small spring feeding stream at Los Chiceros. 13. Stream at Los Chiceros, below warm water inflow from canal from Posos de la Becerra. 14. Small spring, 2.24 km S of tip of Sierra de San Marcos along the highway, at SE corner of the springfield. 15. Small spring, 3 m W of (14), feeding same stream. 16. Small spring, 40 m N of (14). 17. Small spring, 146 m N of (16). 18. Large

spring, 130 m N of (17). 19. Large spring, just W of marsh terminus of (18). 20. Large spring, 10 m W of (19). 21. Large spring, 25 m NW of (20). 22. Juan Santos Laguna, NW of (21). 23. Small spring, 62 m N of (18). 24. Small springhole (no outflow), 370 m NNE of (23). 25. Small spring, 52 m NNW of (24). 26. Small spring, 88 m N of (25). 27. Small spring, 165 m NNW of (26). 28. Small spring at SE corner of vegetated area, 24 m N of (27). 29. Small spring (flowing N), about 300 m W of (28). 30. North Spring, 960 m S of tip of Sierra de San Marcos along the highway. 31. Small spring, 57 m W of (30). 32. Small spring, 27 m N of (31). 33. Small spring, 54 m N of (32). 34. Small spring, 60 m NW of (33). 35. Small spring, 328 m N of (30). 36. Small spring, 300 m NW of (22). 37. Large cold spring, 800 m S, 1 km N of tip of Sierra de San Marcos. 38. Large cold spring, about 50 m W of (37). 39. Small spring hole (no outflow), 10 m S of (38). 40. Small spring, 800 m S, 1.6 km W of tip of Sierra de San Marcos. 41. Large spring, 800 m S, 1.25 km W of tip of Sierra de San Marcos. 42. Large spring, due W of junction of streams from (41) and (43). 43. Large spring, 800 m S, 1.22 km W of tip of Sierra de San Marcos. 44. Rio Garabatal, due W of (42). 45. Small spring, due W of (42). 46. Large spring, 230 m NE of (43). 47. Small spring, just W of marsh terminus of (46). 48. Large spring, 800 m S, 400 m W of tip of Sierra de San Marcos. 49. Large spring, 700 m S of tip of Sierra de San Marcos along the highway. 50. Rio Mesquites, 200 m upstream from junction with springs from the west. 51. Small spring, 100 m S of Rio Mesquites at house of Tierra Blanca. 52. Small spring, just N of highway, 320 m NE of tip of Sierra de San Marcos. 53. Rio Mesquites at the highway, 9.3 km SSW of Cuatro Ciénegas. 54. Rio Mesquites, where small marshy stream branches off the Mojarral area. 55. Small stream branching from (54), due E of (76). 56. Small spring, 320 m S of Rio Mesquites at the highway. 57. Small spring, 370 m S of tip of Sierra de San Marcos along dirt road on the east side. 58. Small spring, due E of (59). 59. Small spring, due E of (60). 60. Small spring, 670 m S of tip of Sierra de San Marcos along dirt road. 61. Small spring, due E of (62). 62. Small spring, 150 m NE of (63). 63. Large spring, 1.12 km S of tip of the Sierra de San Marcos along dirt road. 64. Small spring, 40 m S of (63). 65. Small spring, 72 m S of (64). 66. Small spring, 60 m S of (65). 67. Small spring, 1.4 km S of tip of Sierra de San Marcos along dirt road. 68. Small spring, 1.6 km S of tip of Sierra de San Marcos along dirt road. 69. Small spring, due W of (70). 70. Large spring, 400 m E, 60 m S of (68). 71. Stream from (70), 130 m above mash. 72. Small spring, 2.7 km S of tip of Sierra de San Marcos along dirt road. 73. Mojarral West Laguna, about 200 m N of (76). 74. Stream draining marsh due W of (73). 75. Stream draining (73). 76. Mojarral East Laguna, 10.2 km S of Cuatro Ciénegas along the highway, 1.4 km S of highway. 77. Small spring, feeding SW corner of (76). 78. Pools 30 m downstream from (55). 79. Large spring, 2.4 km S of tip of Sierra de San Marcos along dirt road, 900 m E of road. 80. Large spring (receiving flow from 79), 100 m S of (79). 81. Los Remojos, northern spring, 500 m S of (80). 82. Los Remojos, middle spring. 83. Los Remojos, southern spring. 84. Los Remojos, large pool receiving inflows from (81), (82), and (83). 85. Large spring, 1.2 km S of (83). 86. Large spring, 400 m S of (85). 87. Large spring, 115 m SE of (86). 88. Large spring, about 1.2 km S of (86). 89. Large spring, about 100 m S of (88). 90. Large spring, 60 m SW of (89). 91. Large spring, 200 m S of (90). 92. Large spring, about 200 m N of (95). 93. Large spring, due E of (90). 94. Large spring, due SE of (98). 95. Laguna Escobedae, 6.72 km S of tip of Sierra de San Marcos along dirt road, 1 km E of road. 96. Large spring, 6.9 km S of tip of Sierra de San Marcos along dirt road. 97. Laguna Tio Candido, 9.3 km S of tip of Sierra de San Marcos along dirt road. 98. Large spring, 10.7 km S of tip of Sierra de San Marcos along dirt road. 99. Laguna Anteojo. 100. Smaller spring, due W of (99). 101. Santa Tecla Laguna (= La Tecla), 22.2 km S of tip of Sierra de San Marcos along dirt road, 1.6 km SE of road. 102. Spring alongside the Rio Salado de Nadadores, 3.04 km N of Sacramento, Coahuila. 103. Rio Salado de Nadadores, at Carino de la Montana, 3.84 km E of Sacramento, Coahuila.

APPENDIX 2

The material collected by the author and examined during this study is listed below by species. For each species, the lots are listed in order of locality number (1-103), with the catalog numbers and dates of collection following. All of the material is deposited in the Department of Malacology at the Academy of Natural Sciences of Philadelphia (ANSP). The initial letter A refers to specimens in alcohol. Other catalog numbers refer to dry shell lots.

1. *Nymphophilus minckleyi. 1:* A9879-A, 23 Mar. 1979; A9879-E, 17 Dec. 1981; A9878-I, 19 Dec. 1981. *3:* A9878-G, 23 Mar. 1979. *5:* A9879-B, 16 May 1980. *10:* A9878-H, 4 Aug. 1979. *13:* A9877-B, 2 June 1979; A9878-A, 17 Nov. 1980; A9878-E, 9 Feb. 1981. *18:* A9878-D, 13 June 1979. *30:* A9878-F, 9 Apr. 1979. *37:* A9878-B, 11 July 1980. *38:* A9877-C, 12 Apr. 1979; A9877-F, 18 July 1980. *40:* A9877-A, 27 May 1981. *41:* A9879-G, 17 Feb. 1981. *42:* A9879-D, 10 Apr. 1981. *43:* A9878-C, 8 July 1979. *53*: 355196, A9877-D, 26 July 1979; A9876, 23 Apr. 1979. *64:* A9879-F, 4 June 1981. *71:* A9879-C, 1 May 1981; A9879-H, 12 Dec. 1981. *76:* A9877-H, 7 May 1979; 355197, A9877-E, 7 Apr. 1981. *79:* A9877-G, 3 Apr. 1979. *97:* 355195, A9879-J, 21 May 1979.

2. *Nymphophilus acarinatus. 98:* holotype: 355255, 20 Dec. 1981; paratypes: 355256, 20 Dec. 1981. *101:* A9929-B, 13 July 1980; A9929-C, 28 May, 1981.

3. *Mexistiobia manantiali. 14:* A9888-E, 25 Nov.

1980. *16:* A9887-B, 11–14 June 1979; A9886-K, 5 June 1980; A9888-J, 27 Nov. 1980. *17:* A9887-L, 7 Dec. 1980. *25:* A9887-G, 11 Feb. 1981. *27:* A9888-I, 14 Feb. 1981. *28:* A9887-L, 17 Jan. 1981. *29:* A9888-B, 7 Apr. 1979. *31:* A9887-A, 18 May 1981. *36:* A9887-K, 20 June 1981. *38:* A9886-I, 8 Jan. 1981; A9887-H, 16 Jan. 1981, A9887-F, 5 Feb. 1981. *43:* A9886-M, 13 July 1979. *51:* holotype: 355205, 13 July 1979; paratypes: A9887-D, 20 Apr. 1979; 355204, A9888-L, 13 July 1979. *52:* A9886-D, 21 June 1981. *57:* A9886-C, 15 June 1981. *59:* A9886-B, 19 June 1981. *64:* A9886-N, 31 May 1979; A9887-E, 30 May 1981; A9886-G, 4 June 1981; A9886-L, 21 June 1981. *65:* A9886-H, 31 May 1979; 355206, A9888-F, 28 Apr. 1981. *67:* A9886-F, A9887-J, 30 Apr. 1981. *68:* A9889-K, 2 May 1981. *72:* A9887-C, 19 May 1979. *74:* A9888-C, 5 Apr. 1980. *77:* A9886-J, 16 June 1981.

4. *Coahuilix hubbsi. 16:* A9892-A, 11–14 June 1979; A9892-M, 5 Jan. 1980; A9882-H, 27 Nov. 1980. *18:* A9892-C, 19 Dec. 1981. *24:* A9892-J, 14 Dec. 1981. *31:* A9892D, 18 May 1981. *33:* A9892-B, 21 May 1981. *35:* 355210, 9 May 1981. *38:* A9893-C, 8 Jan. 1981. *58:* A9892-N, 17 Jan. 1981. *61:* A9892-I, 18 June 1981. *64:* A9893-B, 26 Apr. 1981; A9892-G, 4 June 1981; A9892-K, 21 June 1981; A9892-E, 13 Dec. 1981. *66:* A9892-L, 28 Apr. 1981. *67:* 355209, A9893-A, 30 Apr. 1981. *68:* A9892-F, 1 May 1981.

5. *Coahuilix landyei. 16:* A9894-M, 27 Nov. 1980. *17:* A9894-I, 10 June 1979. *24:* A9894-A, 13 Feb. 1981. *28:* A9894-L, 7 Apr. 1979; A9894-C, 17 Jan. 1981. *31:* A9894-F, 18 May 1981. *38:* A9894-E, 8 Jan. 1981. *59:* A9894-H, 19 Jan. 1981. *63:* A9894-K, 16 Apr. 1981. *64:* holotype: A9894-N, 29 Apr. 1981; paratypes: 355211, 27 Apr. 1981. *65:* A9894-B, 26 Apr. 1981. *61:* A9894-J, 30 Apr. 1981. *69:* A9894-G, 6 May 1981.

6. *Cochliopina milleri. 36:* A9884-E, 5 Feb. 1981. *38:* A9884-J, 3 Sept. 1978; A9884-C, 10 Apr. 1981; 355200, A9884-K, 5 Jan. 1981. *41:* A9884-D, 17 Feb. 1981. *42:* A9884-C, 10 Apr. 1981. *43:* A9884-B, 8 July 1979. *50:* A9884-I, 1 Sept. 1978. *53:* A9884-F, 23 Apr. 1979. *55:* A9884-G, 7 May 1979. *97:* A9884-H, 21 May 1979.

7. *Cochliopina riograndensis. 101* A9885-D, 10 July 1979; A9885-E, 13 July 1980; 355202, 28 May 1981. *102:* 355201, A9885-A, 25 May 1981. *103:* A9885-B, A9885-C, 24 July 1979.

8. *Mexithauma quadripaludium. 1:* A9883, 23 Mar. 1979; 355198, A9881-F, 25 Aug. 1980. *10:* A9880-E, 9 Apr. 1979; A9881-C, 4 Aug. 1979. *18:* A9880-D, 13 June 1979. *20:* A9880-G, 20 June 1979. *22:* A9880-B, 3 Apr. 1979. *30:* A9880-I, 23 Mar. 1979. *42:* A9881-E, 10 Apr. 1981. *49:* A9881-D, 23 Feb. 1981. *50:* A9880-C, 1 Sept. 1978; A9880-F, 26 Feb. 1981. *71:* A9880-A, 1 May 1981; A9881-H, 12 Dec, 1981. *76:* A9880-H, 9 May 1979; A9882, 26 July 1979; A9881-A, 10 Apr. 1981. *97:* 355199, 23 May 1981. *98:* A9881-G, 20 Dec. 1981. *99:* 355257, 10 Apr. 1981. *100:* A9881-B, 10 Apr. 1981. *101:* A9880-J, 28 Mar. 1981.

9. *Durangonella coahuilae. 2:* A9922-J, 13 Dec. 1981. *4:* A9922-I, 15 May 1980. *5:* A9922-G, 16 May 1980, A9924-B, 19 Nov. 1980. *6:* A9922-K, 17 May 1980; 355244, A9928-K, 8 Oct. 1980. *7:* A9922-H, 20 May 1980. *8:* A9922-L, 27 May 1980. *9:* A9922-D, 29 Mar. 1979; A9922-B, 16 May 1979; A9922-C, 30 May–10 June 1980; A9922-E, 23 Aug. 1980; A9922-F, 10 Oct. 1980; 355249, 22 Oct. 1980. *12:* A9924-L, 19 Dec. 1980. *13:* 355250, A9923-B, 13 Nov. 1980. *14:* A9923-E, 25 Nov. 1980; 355246, A9928-M, 27 Nov. 1980. *16:* A9926-M, 9 June 1979; A9928-J, 27 Nov. 1980; A9925-E, 6 Dec. 1980. *17:* A9927-C, A9928-B, 10 June 1979; A9927-K, 7 Dec. 1980. *18:* A9923-K, 15 Jan. 1980; A9927-L, 19 Dec. 1981. *23:* A9927-A, 8 Feb. 1981; A9927-J, 11 Feb. 1981. *24:* A9926-I, 8 Feb. 1981; A9924-I, 13 Feb. 1981; A9928-C, 14 Dec. 1981. *25:* A9926-K, 8 Feb. 1981. *26:* A9928-H, 14 Dec. 1981. *27:* A9927-F, 8 Feb. 1981; A9927-B, 14 Feb. 1981. *28:* A9924-N, 7 Apr. 1979; A9926-F, 13 July 1979; A9924-C, 17 Jan. 1981. *29:* A9927-D, 14 Feb. 1981. *30:* A9925-A, 28 Jan. 1980. *31:* A9928-I, 18 May 1981. *35:* A9922-A, A9924-G, 9 May 1981. *37:* A9926-H, 11 July 1980; A9925-K, 7 Feb. 1981. *38:* A9923-D, 15 May, 1979; A9923-F, 18 July 1980; A9924-F, 8 Jan. 1981; 355251, 20 Jan. 1981; A9926-G, 5 Feb. 1981; A9923-C, 5–6 Jan. 1981; A9927-H, 16 Jan. 1981; A9923-H, A9926-J, 6 Feb. 1981. *41:* A9925-G, 17 Feb. 1981. *43:* A9924-M, 7 July 1979; A9927-I, 13 July 1979; 355245, A19928-L, 17 Feb. 1981. *45:* A9926-N, 10 Apr. 1981. *48:* A9925-L, 23 Feb. 1981; A9925-J, 5 May 1981. *49:* A9925-F, 23 Feb. 1981. *50:* A9925-C, A9925-I, 1 Sept. 1978. *51:* 355247, A9928-N, 20 Apr. 1979. *52:* A9924-D, 21 Jan. 1981. *56:* A9928-F, 18 Mar. 1981; A9925-B, 19 Mar. 1981. *58:* A9924-A, 17 Jan. 1981. *59:* A9923-J, 19 Jan. 1981. *61:* A9927-M, 18 Jan. 1981. *62:* A9927-N, 15 June 1981. *63:* A9926-C, 8 Jan. 1979. *64:* A9926-B, A9928-D, 31 May 1979; A9928-A, 21 Jan. 1981; A9926-L, 27 Apr. 1981; A9928-G, 30 May 1981. *65:* A9923-A, 22 May 1979; A9924-H, 31 May 1979; 355248, A9923-L, 4 Apr. 1980; A9923-I, 24 July 1980; A9923-J, 1 Oct. 1980. *72:* A9927-E, 19 May 1979. *73:* 355252, A9926-D, 5 Apr. 1980. *74:* A9926-A, 2 May 1979; A9925-H, 12 Apr. 1980. *75:* A9925-M, 4 May 1979. *77:* A9924-E, 16 Jan. 1981.

10. *Mexipyrgus churinceanus. 1:* A9909-H, 23 Mar. 1979; 355230, 15 Sept. 1980; A9907-I, 20 Sept. 1980; A9909-I, 13 Mar. 1981. *5:* A9908-I, 16 May 1980; A9907-H, 24 Sept. 1980; 355231, 5 Oct. 1980. *10:* A9909-C A9911-A, 27 Oct. 1980; 344232, 29 Oct. 1980; A9909-D, 31 Oct. 1980. *11:* 355233, 2 Nov. 1980; A9901, 10 Nov. 1980. *13:* A9911-F, 8 Nov. 1980. *18:* 355220, A9912-I, 12 June 1980; 355216, A9912-E, 12 Aug. 1980; A9911-H, 8 Dec. 1980. *19:* A9911-J, 10 Dec. 1980. *20:* A9910-G, 26 June 1980; 355234, 10 Dec. 1980; A9907-E, 11 Dec. 1980. *21:* 355214, A9912-C, 29 Apr. 1981. *22:* A9907-B, 3 Apr. 1979; 355241, 16 Dec. 1980; A9907-C, 18 Dec. 1980. *30:* A9911-C, 9 Apr. 1979; A9907-F, 20 Aug. 1980; 355222, A9914, 18 Dec. 1980. *37:* 355213, A9912-B, 30 Dec. 1980. *38:* 355236, 1 Jan. 1981; A9910-H, 5–8 Jan. 1981;

A9911-B, A9911-D, A9911-E, 16 Feb. 1981; A9910-J, 21 May 1981. *43:* A9910-J, 7 July 1979; A9911-G, 16 Feb. 1981. *44:* A9911-I, 10 Apr. 1981. *46:* A9908-J, 19 Feb. 1981; 3553212, A9912-A, 20 Feb. 1981. *47:* 355227, 23 Feb. 1981. *48:* A9895, 23 Feb. 1981; 355235, 24 Feb. 1981; A9897, 5 May 1981. *49:* A9898, 23 Feb. 1981; 355242, 24 Feb. 1981; A9907-G, 5 May 1981. *50:* 355218, A9920, 26 Feb. 1981. *53:* A9909-E, 23 Apr. 1979; A9909-F, 6 Mar. 1981. *54:* A9909-G, 8 Mar. 1981. *65:* A9909-A, 31 May 1979. *67:* A9910-E, 4 May 1981; A9910-D, 5 May 1981. *70:* A9910-F, 1 May 1981; A9909-J, 2 May 1981. 71: A9910-A, 4 May 1979; 355240, A9910-B, 25 Mar. 1981; A9905, 5 May 1981; A9910-C, 4 May 1981; A9907-A, 12 Dec. 1981. *73:* 355219, A9912-H, 11 Mar. 1981. *76:* A9906, 16 Mar. 1981; 355239, 17 Mar. 1981. *78:* A9907-D, 9 Apr. 1981; 355215, A9912-D, 29 Apr. 1981. *79:* A9900, 6 May, 1981; 355237, A9908-F, 8 May, 1981. *81:* 355223, A9915, 11 May 1981. *82:* 355226, A9918, 11 May 1981. *83:* A9902, 9 May 1981; 355243, 11 May 1981. *84:* A9908-A, 12 May 1981. *85:* A9908-C, 14 May 1981. *86:* A9896, 14 May 1981. *87:* A9909-B, 16 May 1981. *88:* 344229, A9921, 17 May 1981. 89: A9908-B, 17 May 1981. *90:* 355225, A9917, 17 May 1981. *91:* A9908-G, 18 May 1981. *92:* A9908-H, 20 May 1981. *93:* A9904, 22 May 1981. 94: A9908-E, 22 May 1981. *95:* 355221, A9913, 20 May 1981. *96:* 355218, A9912G, 20 Dec. 1981. *97:* 355224, A9916, 23 May 1981. *98:* A9907-J, 20 Dec. 1981. *99:* 355217, A9912-F, 10 Apr. 1981. *100:* A9908-D, 10 Apr. 1981. *101:* A9903, 28 May 1981.

11. *Orygoceras?* sp. *67:* 355254, A9929-A, 19 Dec. 1981.

12. *Paludiscala caramba. 2:* A9890-D, 13 Dec. 1981. *16:* A9890-K, 11 June 1979; A9890-H, 5 Jan. 1980; A9889-K, 27 Nov. 1980. *17:* A9890-J, 10 Jan. 1979. *18:* A9891-J, 19 Dec. 1981. *23:* A9889-N, A9891-D, 11 Feb. 1981. *24:* A9891-H, 13 Feb. 1981; A9889-M, 14 Dec. 1981. *25:* A9891-K, 11 Feb. 1981. *26:* A9889-G, 14 Dec. 1981. *27:* A9891-L, 11 Feb. 1981. *28:* A9880-B, 17 Jan. 1981. *31:* A9891-E, 11 May 1981. *32:* A9890-L, 18 May 1981. *33:* A9889-A, 21 May 1981. *35:* A9889-I, 9 May 1981. *38:* A9880-H, 16 Jan. 1981; A9890-I, 8 Jan. 1981. *56:* A9889-L, 18 May 1981. *57:* A9890-M, 15 Jan. 1981. *58:* A9889-C, 17 Jan. 1981. *59:* A9889-E, 19 June 1981. *60:* A9889-B, 19 Jan. 1981. *61:* A9890-C, 18 Jan. 1981. *62:* A9890-A, 15 Jan. 1981. *63:* 355207, A98911, 16 Apr. 1981. *64:* A9889-J, 31 May 1979; A9889-F, 26 Apr. 1981; A9890-F, 13 Dec. 1981. *65:* A9889-H, 31 May 1979; 355208, 24 July 1980; A9891-I, 26 Apr. 1981. *66:* A9891-A, 28 Apr. 1981. *67:* A9890-E, 30 Apr. 1981. *68:* A9891-B, 1 May 1981. *69:* A9891-G, 6 May 1981. *72:* A9891-F, 19 May 1979.

APPENDIX 3

Fifty-one characters and their character states are used in this study to distinguish between genera and generic groups of Hydrobiidae. To the right are references to publications in which the various character states have been figured. "H" indicates a figure within this paper.

Shell	
1. Shell shape:	
a) planispiral	H, Figs. 15A–G, I–K
b) trochoid-globose	H, Figs. 4, 9, 27
c) ovate-conic	H, Fig. 37
d) turriform	H, Fig. 33
2. Maximum shell dimension:	
a) <2 mm	—
b) ≥2 mm <5 mm	—
c) ≥5 mm	—
3. Shell sculpture:	
a) collabral ribs	H, Fig. 19
b) spiral cords	H, Fig. 27
c) absent	H, Fig. 32
4. Apical whorl microsculpture:	
a) wrinkled, pitted	H, Fig. 11; Thompson, 1977, fig. 4
b) absent	H, Fig. 39A
5. Shell with periostracal bands	H, Fig. 37
6. Shell aperture flared	Boeters, 1974, figs. 9–11; Bole, 1970, fig. 6
External features	
7. Tentacle ciliation:	
a) absent	H, Fig. 40A
b) *Hydrobia*-like	Davis, 1966, fig. 2; Hershler & Davis, 1980, fig. 1A
c) *Spurwinkia*-like	Davis *et al.*, 1982, fig. 5; H, Figs. 29A, B
8. Animal blind, unpigmented	—
9. Mantle edge papillate	H, Fig. 30A, 31A

10. Osphradium elongate (≥0.30 of the ctenidium length)	Davis & Pons da Silva, 1984, fig. 6
11. Number of operculum whorls:	
a) <4	H, Fig. 35B
b) ≥4	H, Fig. 5B
12. Position of nucleus of operculum along its long axis:	
a) <30%	H, Fig. 35B
b) ≥30%	H, Fig. 5B
Digestive system	
13. Digestive gland with reduced tubercles	H, Fig. 22B
14. Intestine with anterior loop	H, Fig. 17C; Boeters, 1974, fig. 3
15. Caecal chamber extends posterior to stomach	H, Fig. 7A
16. Origin of basal cusps of central tooth of radula:	
a) from face of tooth	H, Fig. 16C; Davis & Pons da Silva, 1984, fig. 14
b) from lateral angles	H, Fig. 28D
17. Number of pairs of basal cusps on central tooth of radula:	
a) 1–2	H, Figs. 12B, C
b) ≥3	H, Fig. 28D
18. Central cusp of lateral tooth:	
a) massive	H, Fig. 6B
b) small	H, Fig. 12A
Male reproductive anatomy	
19: Male gonad morphology:	
a) simple lobes	Davis & Greer, 1980, fig. 9A
b) bush-like	H, Fig. 31A
c) single un-lobed mass	H, Fig. 17C
20. Male gonad extends onto stomach	—
21. Seminal vesicle coiling:	
a) on stomach	H, Fig. 17C
b) posterior to stomach	Davis & Greer, 1980, fig. 9A
22. Position of prostate:	
a) entirely posterior to end of mantle cavity	H, Fig. 17C; Davis *et al.*, 1982, fig. 14A
b) overlaps mantle cavity	H, Fig. 31A
23. Anterior vas deferens:	
a) exits from anterior tip of prostate	H, Fig. 31A
b) exits from posterior portion of prostate	Davis, 1967, pl. 25, fig. 4
24. Penis with slender penial filament	H, Fig. 31B
25. Penis ciliated	H, Figs. 35D, 44B
26. Penis with terminal eversible papilla	H, Figs. 35D, 44A
27. Penial lobe(s):	
a) absent	H, Figs. 25D, 31B
b) bulb-like	H, Figs. 17D, 21C
c) simple	H, Fig. 35D
d) with folds	H, Fig. 8A
28. Gland(s) of penis:	
a) absent	—
b) large, specialized	H, Figs. 17D, 21C, 44A
c) small, in glandular ridges	H, Figs. 8B, C; Boeters, 1974, figs. 6, 7; Thompson, 1968, figs. 42–47
29. Of snails having glandular ridges on the penis:	
a) <4 ridges	H, Figs. 8B, C, 13D; Thompson, 1977, figs. 5A, 7A, 11C, 13
b) ≥4 ridges	Thompson, 1968, figs. 42–47
Female reproductive anatomy	
30. Reproductive mode:	
a) oviparity	—
b) ovoviviparity	—

31. Female gonad morphology:	
a) relatively large, lobed	H, Fig. 41A
b) relatively large, un-lobed	H, Fig. 17B
c) relatively small, a mere thickening at end of oviduct	H, Fig. 30A
32. Female gonad extends onto stomach	H, Fig. 30A
33. Oviduct without coil	H, Fig. 17B
34. Length of pallial oviduct/length of body	
a) <0.30	H, Fig. 17B
b) ≥ 0.30	H, Fig. 30A
35. Posterior pallial oviduct:	
a) without bend	H, Fig. 17B
b) with short bend	H, Figs. 30A, 36A; Davis *et al.*, 1982, fig. 10A
c) with long bend, coiling in >1 plane	H, Fig. 43
36. Albumen gland:	
a) normal sized	H, Fig. 17B
b) reduced in size	H, Fig. 26A
c) reduced to a glandular smear	H, Fig. 36A
37. Of ovoviviparous taxa, capsule gland:	
a) with 1 tissue region	Hershler & Davis, 1980, fig. 2
b) with 2 tissue regions	H, Fig. 17B
c) with 3 tissue regions	H, Fig. 22A
38. Length of bursa/length of pallial oviduct:	
a) <0.20	H, Fig. 30B
b) ≥ 0.20 <0.40	H, Fig. 17B
c) ≥ 0.40	
39. Position of bursa:	
a) posterior to pallial oviduct	H, Figs. 7A, B
b) overlapped by pallial oviduct	H, Fig. 14B
40. Normal seminal receptacle present	H, Fig. 7B
41. Secondary seminal receptacle present	H, Fig. 22A
42. Of snails with a normal seminal receptacle, the length of the seminal receptacle/length of bursa is:	
a) <0.30	H, Fig. 42B
b) ≥ 0.30 <0.50	H, Figs. 26B, C
c) ≥ 0.50	H, Fig. 30B
43. Of snails with a normal seminal receptacle, opening of seminal receptacle into:	
a) oviduct	H, Fig. 30B
b) sperm duct	H, Fig. 42C
44. Of snails with a normal seminal receptacle, the seminal receptacle is:	
a) overlapped by the bursa	H, Fig. 42B
b) lateral to the bursa	H, Fig. 30B
45: Female with:	
a) ciliated ventral channel inside the pallial oviduct	H, Fig, 7B
b) spermathecal duct	H, Fig. 30A
46. Of snails with a ciliated ventral channel:	
a) pallial oviduct opens at anteror tip	H, Fig. 14D
b) pallial oviduct opens laterally	H, Fig. 7E
47. Of snails with a ciliated ventral channel:	
a) bolster weakly developed	H, Fig. 14C
b) bolster well-developed	H, Fig. 7D
48. Of snails with a spermathecal duct, the duct is:	
a) long	H, Fig. 30A
b) short	H, Fig. 26A
49. Of snails with a short spermathecal duct, the duct is:	
a) muscularized	H, Fig. 42B
b) non-muscularized	H, Fig. 26A

50. Of snails with a long spermathecal duct, the openings of the spermathecal duct and pallial oviduct are:
    a) separate — H, Figs. 36F, G
    b) joined — H, Fig. 22F
    c) separate, but with an open channel between them — H, Fig. 30E
51. Of ovoviviparous snails, the anterior pallial oviduct has:
    a) a slight muscular loop — H, Fig. 41B
    b) a well-developed muscular loop — H, Fig. 26E

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THE PELAGIC GENUS *PTEROTRACHEA* (GASTROPODA: HETEROPODA) FROM HAWAIIAN WATERS: A TAXONOMIC REVIEW

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ABSTRACT

The four recognized species of pterotracheid heteropods appear to be present in Hawaiian waters. Specimens that could be assigned to *Pterotrachea hippocampus* Philippi, 1836 and *P. minuta* Bonnevie, 1920, however, were indistinguishable on the basis of visceral nucleus shape, eye morphology, fin and sucker size in males, pedal ganglion location, morphology of the male copulatory apparatus, and radular structure. The morphologies of the visceral nucleus and eye have been considered of particular importance by previous workers in separating the two species. The ratio of length to maximum width of the visceral nucleus was found to be highly variable, however, probably as a result of preservation effects and differences between individuals in fullness of the gut. Eye morphology, on the other hand, appears to be the most conservative and useful taxonomic characteristic. Regression of the ratio of eye length to retinal width against body length resulted in a size-specific relationship, with the eye shape ranging from narrowly triangular in small specimens (as in *P. minuta*) to broadly triangular in large individuals (as in *P. hippocampus*). The present results suggest that *P. minuta* should be considered a junior synonym of *P. hippocampus.*

Key words: *Pterotrachea*; Heteropoda; taxonomy; pelagic; Hawaii.

INTRODUCTION

All four species of *Pterotrachea* that are currently recognized (Van der Spoel, 1976) appear to occur in oceanic waters off Oahu, Hawaii. Two of these species, *P. coronata* Niebuhr (ms. Forskal), 1775, and *P. scutata* Gegenbaur, 1855, possess distinctive taxonomic features. However, *P. hippocampus* Philippi, 1836, and *P. minuta* Bonnevie, 1920, to which the great majority of specimens collected off Hawaii can be assigned, could not be clearly distinguished. Large specimens could be identified as *P. hippocampus,* specimens of intermediate size were closer in appearance to *P. hippocampus,* whereas small individuals were more similar to *P. minuta.* Both species have been reported from the North Pacific Ocean off Baja California (Dales, 1953; McGowan, 1967) and Japan (Okutani, 1957a,b). (Additional records and synonyms of *P. hippocampus* and *P. minuta* from the world's oceans are cited in Van der Spoel (1976).)

To resolve this taxonomic problem, morphometric analyses were performed to allow comparison of the Hawaiian material with descriptions of *P. hippocampus* and *P. minuta* (Bonnevie, 1920; Tesch, 1949; Richter, 1968, 1974; Van der Spoel, 1972, 1976) and with the holotype of *P. minuta. Pterotrachea coronata* and *P. scutata* collected from Hawaiian waters were examined for comparative purposes.

MATERIALS AND METHODS

Samples were collected in oceanic waters (bottom depth between 900 and 2,300 m) at a distance of 6.5 to 14.8 km off the western (leeward) side of the island of Oahu, Hawaii, with a 3-m Isaacs-Kidd midwater trawl (IKMT) that was continuously open and was equipped with a large cod-end bucket to minimize damage to captured animals. Specimens of *Pterotrachea* spp. were obtained from IKMT tows taken between the surface and 800 m (day), and the surface and 200 m (night) in June 1978, May–June 1981, and December 1981, during cruises of the R/V KANA KEOKI, University of Hawaii.

The eyes and visceral nucleus are the principal morphological features used to separate the species of *Pterotrachea* (Bonnevie, 1920; Tesch, 1949; Okutani, 1957a; Richter, 1968; Van der Spoel, 1972, 1976). The shapes of these structures (Figs. 1, 2) were characterized for series of specimens that ranged broadly in size. Within one hr. after capture,

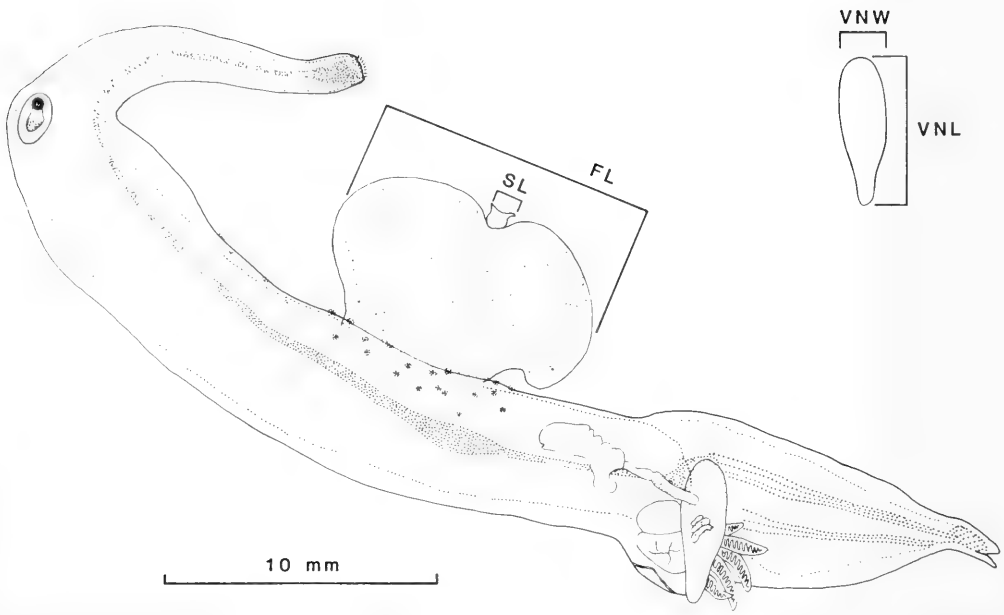

FIG. 1. Natural swimming posture (lateral view) of a 50.2 mm male *Pterotrachea hippocampus* collected from Hawaiian waters in June 1981. Measurements: FL = fin length, SL = sucker length, VNL = visceral nucleus length, VNW = visceral nucleus width.

specimens were preserved in buffered, 10% seawater-formalin solution. For the eyes, the ratio between eye length and retinal width (Fig. 2) was determined using an ocular micrometer scale in a dissection microscope with the eye oriented such that the dorsal surface was at a 90° angle to the plane of view through the microscope. This perspective is important because the eyes are not radially symmetrical around the optical axis. In lateral view, the eye is flattened posterior to the lens and possesses a narrow, strip-like retina (Grenacher, 1886; Hesse, 1900).

The ratio of length to maximal width of the visceral nucleus was obtained while viewing each individual from the right side (Fig. 1). Additionally, the fin and sucker lengths (Fig. 1) and the lens diameter (Fig. 2) of specimens that could be assigned to *P. hippocampus* and *P. minuta* were measured with the ocular micrometer. Body lengths were determined with vernier calipers (measured to the nearest 0.1 mm) in order that the length to width ratios and the lengths of the fins and suckers could each be compared with respect to the size of the animal.

The holotype of *Pterotrachea minuta* Bonnevie, 1920 (ZMUB 23259) was obtained from the Zoologisk Museum, University of Bergen, Norway. Body length was determined with vernier calipers. Lens diameter, eye length, retinal width, visceral nucleus length and width, sucker length, and fin length of the male specimen were measured with the ocular micrometer.

RESULTS

Among the four species of *Pterotrachea* that are currently recognized, two species pairs are immediately apparent on the basis of eye shape (Fig. 3). In broadest outline (dorsal view), the eyes of *P. coronata* and *P. scutata* are approximately rectangular in shape (Figs. 3F & G). In contrast, the eyes of the holotype of *P. minuta* (Fig. 3E) and of *P. hippocampus/minuta* from Hawaii (Figs. 3A to D) have retinas that are broader than the lens, with the result that the eye is roughly triangular. When expressed in terms of the ratio between eye length and retinal width, these differences can be quantified (Fig. 4): at all sizes, the ratio exceeds 1.8 in *P. coronata* and *P. scutata*, and is less than 1.6 in *P. hippocampus/minuta*.

Separation of *P. coronata* from *P. scutata* in the Hawaiian material is not possible on the

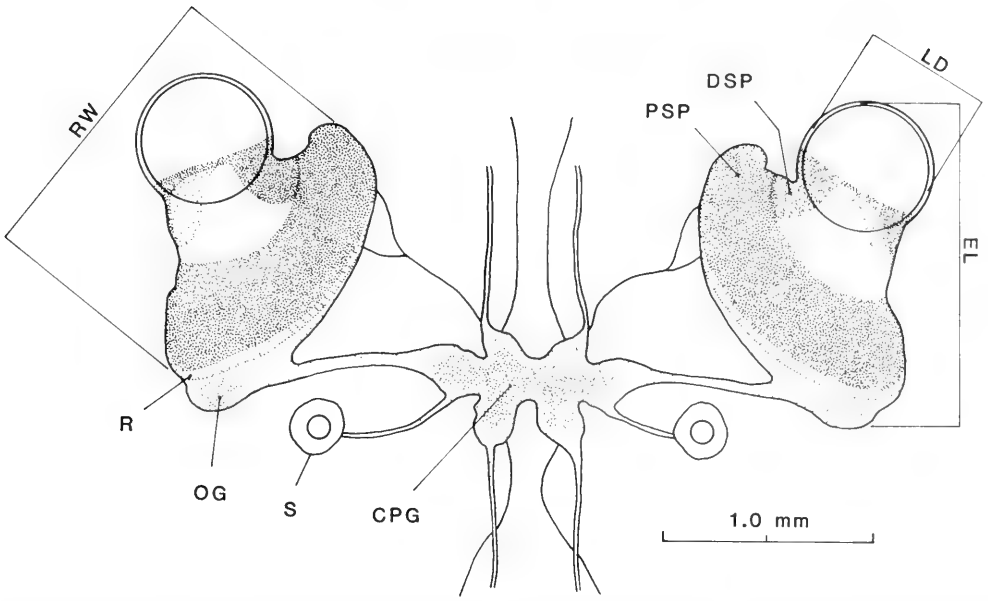

FIG. 2. Eyes and brain of the *Pterotrachea hippocampus* from Fig. 1 drawn in dorsal view. Measurements: EL = eye length, LD = lens diameter, RW = retinal width. Structures: CPG = cerebropleural ganglion, DSP = distal screening pigment, OG = optic ganglion, PSP = proximal screening pigment, R = retina, S = statolith.

basis of eye morphology, since the eye length to retinal width ratios overlap and range from about 1.8 to 2.5 (Fig. 4). The two species are clearly different, however, on the basis of visceral nucleus shape (Fig. 5). Length to width ratios ranged from 4.1 to 7.2 for *P. coronata,* while in *P. scutata* the ratios were less than 4.0 and ranged more narrowly from 2.0 to 3.9.

The Hawaiian specimens assignable to *P. hippocampus* and *P. minuta* could not be distinguished on the basis of either visceral nucleus or eye morphology. The length to width ratio of the visceral nucleus for all sizes averaged 2.9 and decreased with increasing body size (Fig. 5). The visceral nucleus of the *P. minuta* holotype had a length to width ratio of 3.2, which is only slightly greater than the 2.9 average for the Hawaiian specimens and is well within the range of obtained ratios (2.1 to 3.9). The ratio of eye length to retinal width decreased sharply with increasing body length (Fig. 4), from 1.6 in small specimens to nearly 1.0 in large individuals. Variation about the regression line for *P. hippocampus/minuta* in Fig. 4 was reduced substantially by using corrected body length values based on the lens diameter of each individual rather than on the body length measurements taken with vernier calipers. In another heteropod, *Carinaria cristata* forma *japonica* Okutani, 1955, lens diameter was shown (Seapy, 1980) to be an excellent predictor of body length. For *P. hippocampus/minuta,* then, lens diameter and body length were measured for a series of specimens that did not appear to have lengthened or shortened as a result of capture damage or due to preservation. A clearly-defined line of best fit resulted (Fig. 6), which was used subsequently to estimate the body lengths of the *P. hippocampus/minuta* plotted in Figs. 4, 7 and 8. It is noteworthy that little sexual difference could be distinguished in the lens diameter-body length relationship (Fig. 6), which is substantiated by the statistical result that the regressions for the two sexes were not significantly different ($p > .05$; F-test for coincidental regressions).

The eye length to retinal width ratio of the *P. minuta* holotype (Fig. 3E) was 1.4. The animal was extremely stretched and deflated, probably as a result of net damage during capture, and measured 38.8 mm in length. The lens was only 0.33 mm in diameter, however, which would be characteristic of a 20.5 mm animal from Hawaiian waters (Fig. 6). This latter body length is consistent with

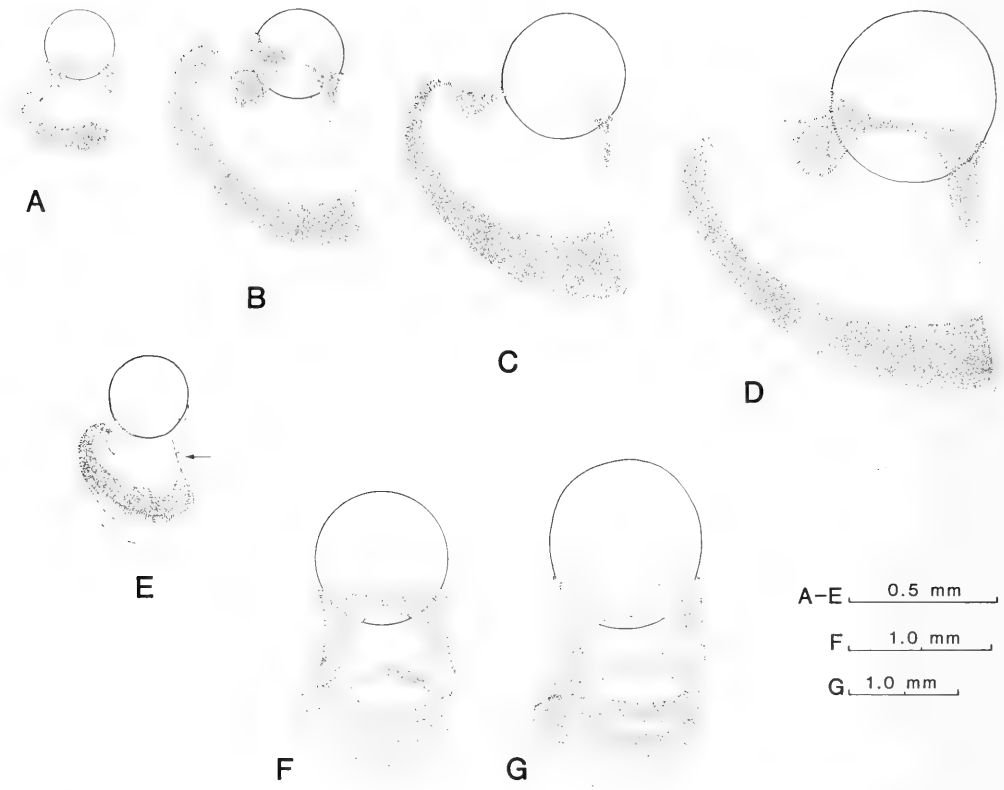

FIG. 3. Right eyes of *Pterotrachea* spp. in dorsal perspective. (A) to (D) *P. hippocampus minuta*: Hawaii, 1981, (A) female, 16.2 mm, (B) male, 39.7 mm, (C) male, 50.2 mm and (D) female, 68.7 mm (SBMNH 33884); (E) *P. minuta*: holotype, male, 38.8 mm (ZMUB 23259); (F) *P. coronata*: female, 78.4 mm, Hawaii, June 1981 (SBMNH 33885); (G) *P. scutata*: male, 60.8 mm, Hawaii, June 1981 (SBMNH 33886). The lens in the right eye of the holotype specimen of *P. minuta* was partially detached, as indicated by the arrow in (E).

the maximal size of 25 mm for *P. minuta* recorded by Tesch (1949) from the "Dana" Expedition material and by Thiriot-Quiévreux (1973) for specimens presumably taken in the general region of the type-locality of the species (29° 8′ N, 25° 16′ W) off West Africa. Specimens reported from waters off Japan by Okutani (1957a) ranged from 16 to 29 mm in length. The eye length to retinal width ratio for the holotype specimen of *P. minuta* falls within the range of ratios obtained for Hawaiian individuals in the 15 to 25 mm size range (Fig. 4).

Two features described by Tesch (1949) and noted later by Van der Spoel (1976) as distinctive for *P. minuta* were the small sizes of the sucker and fin in males. To determine whether *P. hippocampus* males could be distinguished from *P. minuta* males in the Hawaiian fauna, lengths of the suckers and fins were measured for a series of 30 individuals which ranged widely in size and were plotted against body length (Figs. 7 and 8, respectively). Although the relationship shows variability, sucker length increases in an approximately linear fashion as body length increases (Fig. 7). Assuming that the live holotype of *P. minuta* was 20.5 mm in length, the measured sucker length of 0.40 mm agrees well with those of Hawaiian individuals in this size range (Fig. 7). Fin length increases with body length, but not in a linear manner (Fig. 8), with individuals below about 30 mm in body length exhibiting much greater variability than those larger than 30 mm. As in the case of the sucker, the 5.5 mm fin length of the holotype specimen of *P. minuta* falls within the range of measure-

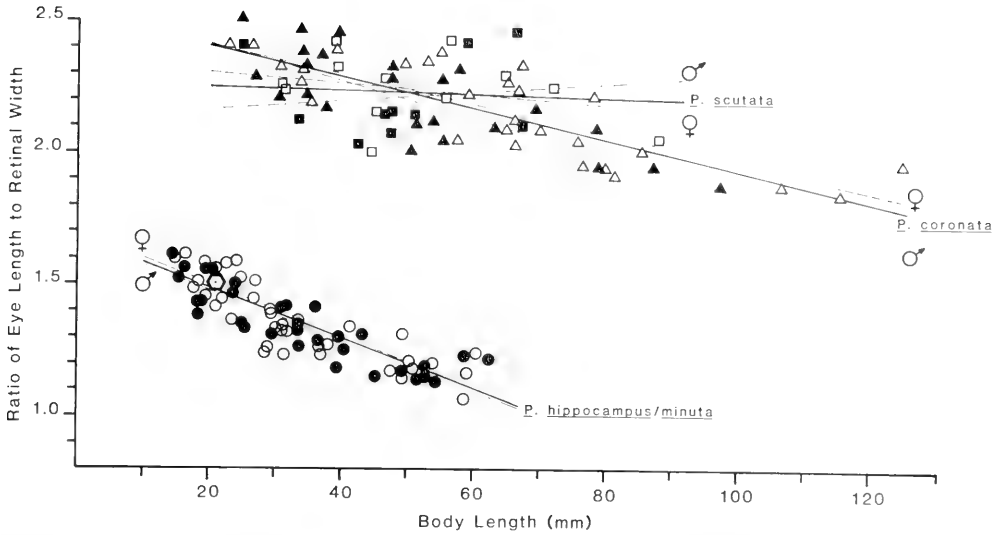

FIG. 4. Comparisons of body length (mm) and the ratio of eye length to retinal width for *Pterotrachea* spp. collected in June 1978 and May–June 1981 from Hawaiian waters. *P. scutata*, squares ($Y = -.0007X + 2.257$, $r = -.07$; $p > .05$; $n = 22$), where solid squares are males ($Y = .0017X + 2.120$; $n = 10$) and open squares are females ($Y = -.0020X + 2.343$; $n = 12$). *P. coronata*, triangles ($Y = -.0058X + 2.518$, $r = -.79$; $p < .001$; $n = 52$), where solid triangles are males ($Y = -.0071X + 2.560$; $n = 24$) and open triangles are females ($Y = -.0057X + 2.524$; $n = 28$). *P. hippocampus/minuta*, circles ($Y = -.0090X + 1.652$, $r = -.85$, $p < .001$; $n = 70$), where solid circles are males ($Y = -.0099X + 1.653$; $n = 32$) and open circles are females ($Y = -.0093X + 1.667$; $n = 38$). The open hexagon denotes the eye length to retinal width ratio for the holotype of *P. minuta* (body length estimated to be 20.5 mm on the basis of lens diameter).

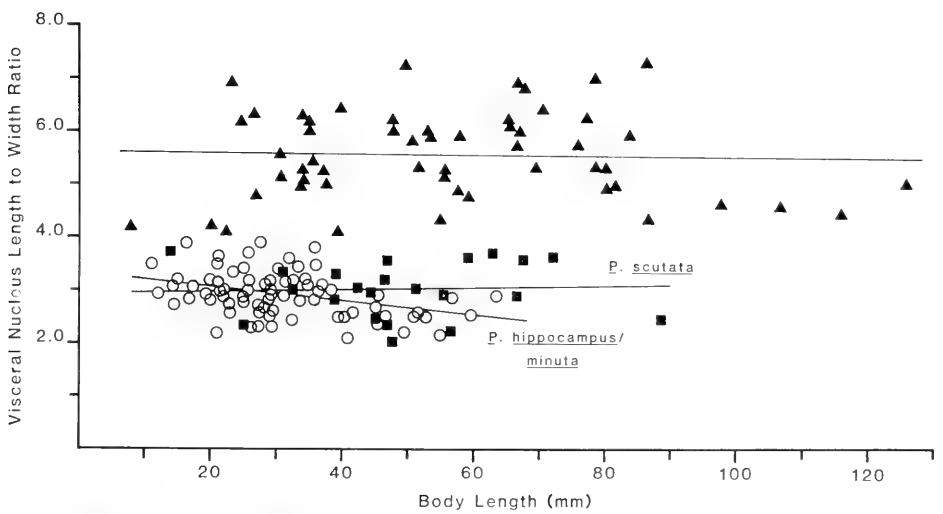

FIG. 5. Comparisons of body length (mm) and length to width ratios of the visceral nucleus of *Pterotrachea* spp. collected in June 1978 and May–June 1981 from Hawaiian waters. *P. coronata*, solid triangles ($Y = .0006X + 5.585$, $r = -.02$, $p > .05$; $n = 55$). *P. scutata*, solid squares ($Y = .0023X + 2.922$, $r = .08$, $p > .05$; $n = 22$). *P. hippocampus/minuta*, open circles ($Y = -.0129X + 3.338$, $r = -.37$, $p < .01$; $n = 74$).

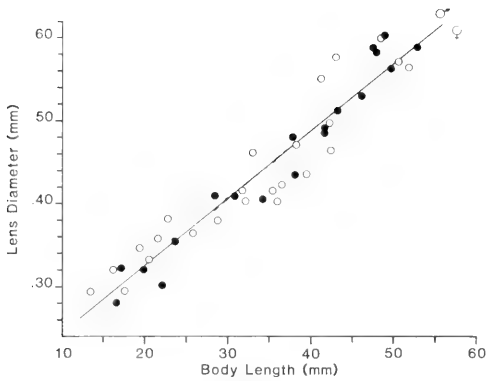

FIG. 6. Relationship between lens diameter (mm) and body length (mm) of *Pterotrachea hippocampus/minuta* ($Y = .0081X + .164$, $r = .96$, $p < .001$; $n = 43$) collected in May–June 1981 from Hawaiian waters. Males (solid circles; $n = 19$) and females (open circles; $n = 24$) are plotted separately, and the lines of best fit ($Y = .0086X + .148$ and $Y = .0075X + .181$, respectively) are indicated as long and short dashed lines.

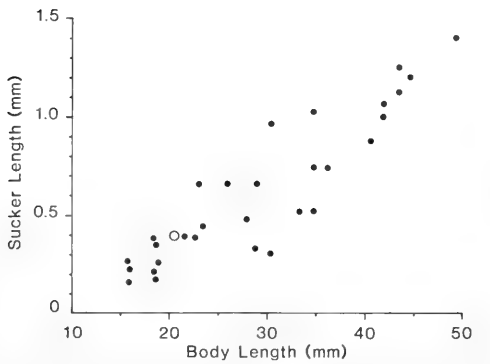

FIG. 7. Relationship between sucker length (mm) and body length (mm) of *Pterotrachea hippocampus/minuta* (solid circles; $Y = .0321X - .318$, $r = .90$, $p < .001$; $n = 30$) collected in May–June 1981 from Hawaiian waters. The open circle denotes the sucker length of the holotype of *P. minuta* (body length estimated to be 20.5 mm on the basis of lens diameter).

ments made on Hawaiian animals of similar body lengths (Fig. 8).

The position of the pair of pedal ganglia relative to the insertion point of the anterior edge of the fin has been used by Tesch (1949) and Okutani (1957a) to distinguish *P. minuta* from *P. hippocampus*. According to these authors, the pedal ganglia are located anterior to the insertion point (base) of the fin

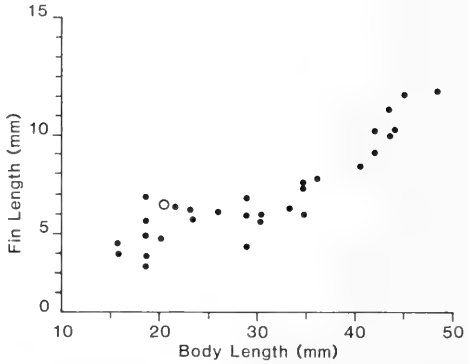

FIG. 8. Relationship between fin length (mm) and body length (mm) of *Pterotrachea hippocampus/minuta* (solid circles; $n = 30$) collected in May–June 1981 from Hawaiian waters. The open circle denotes the fin length of the holotype of *P. minuta* (body length estimated to be 20.5 mm on the basis of lens diameter).

in the former species, while in the latter species the ganglia are positioned just posterior to the insertion point. This difference was also illustrated (Figs. 34a and 49), but not discussed by Bonnevie (1920). A series of 53 specimens of *P. hippocampus/minuta* from Hawaii were examined for position of the pedal ganglia. The ganglia were situated just posterior to the insertion point in a single individual (22 mm in length); directly above the insertion point in 21 specimens (12 to 42 mm); slightly anterior in 20 animals (14 to 37 mm); and distinctly anterior in 12 specimens (21 to 38 mm). Clearly, there was no size-related pattern to the location of the pedal ganglia, and the observed variability in position implies that this is not a valid taxonomic criterion.

A secondary sexual characteristic that potentially distinguishes species is the copulatory apparatus in males. Van der Spoel (1976) stated that such differences could be of taxonomic utility, and he illustrated the penis and associated structures of *P. hippocampus* and *P. minuta,* although he did not discuss how these structures differed in the species diagnoses. Detailed descriptions of the male reproductive system of *P. coronata* and *P. hippocampus* were given earlier by Gabe (1965). Examination of approximately 50 Hawaiian specimens of *P. hippocampus/minuta* demonstrated considerable variability, irrespective of size, in the appearance of the copulatory apparatus. Comparisons were also made between the Hawaiian specimens

and the illustrations of the copulatory apparatus of *P. hippocampus* and *P. minuta* in Van der Spoel (1976: 400, 401) and for *P. hippocampus* in Gabe (1965: 1038, 1039) with the general conclusion that the present material most closely resembles the illustrations of *P. hippocampus* by Gabe. The variability in structure that I observed can probably be attributed to the state of the organ at the time of death and/or to preservation effects. The morphology of the copulatory apparatus, then, would not appear to be a usable taxonomic character.

Radular differences were cited by Bonnevie (1920) to distinguish *P. minuta* from *P. hippocampus*. The heteropods have a taenioglossan radula (one lateral and two marginal teeth on either side of the central or rachidian tooth in each row) characteristic of the mesogastropods (Fretter & Graham, 1962). In the genus *Pterotrachea,* the central tooth is broad and possesses an enlarged median spine with about 5 small spines arranged on both sides (Bonnevie, 1920, textfig. B). Bonnevie considered the central spine to be more pronounced in *P. hippocampus* than in *P. minuta.* Thiriot-Quiévreux (1971) also noted a strongly developed central spine in *P. hippocampus,* but did not state that it was reduced in *P. minuta.* In fact, the central spine in *P. minuta* appeared in Thiriot-Quiévreux's scanning electron micrographs to be of comparable size in the two species. Based on the latter information, I would not consider the median spine size to be of taxonomic value.

The second radular difference reported by Bonnevie (1920) was that the lateral (or intermediate) tooth in *P. minuta* possesses a small secondary spine near its free end. Although Thiriot-Quiévreux (1971) did not describe such a structure, her scanning electron micrograph for *P. minuta* indicates its presence. The lateral teeth of *P. hippocampus* in larval, juvenile, and adult forms were illustrated by Richter (1968: 51) and clearly show a large secondary spine in the larva, its reduction in the juvenile, and its absence in the adult. If *P. minuta* represents the young of *P. hippocampus,* as the present study suggests, then the loss of this secondary spine would explain the apparent species difference described by Bonnevie. Microscopic examinations of the radulae of a series of 12 *P. hippocampus/minuta* between 15 and 40 mm in body length from the Hawaiian material are in close agreement with the observations of *P. hippocampus* by Richter. It is noteworthy, however, that the secondary spine in the Hawaiian specimens was not lost until a body length of 34 mm was attained. This is significant because this body length is somewhat greater than the upper size limit of approximately 25 to 30 mm cited for *P. minuta* by Tesch (1949), Okutani (1957a), and Thiriot-Quiévreux (1973).

Lastly, Thiriot-Quiévreux (1971) observed a peculiar feature that is shown in her scanning electron micrograph of the radula of *P. minuta*: the fusion of the first and second spines as a bifid spine located immediately adjacent to the protruding middle spine on the central tooth. Similar bifid spines were illustrated by Buchmann (1924: 525) for the central teeth of small *P. coronata.* In one specimen of *P. coronata,* a bifid spine was formed by the two spines immediately lateral to the central spine (as shown for *P. minuta* by Thiriot-Quiévreux), while for another small *P. coronata,* a bifid spine was formed by the second and third lateral spines. I did not observe any such fused spines in any of my specimens. Since Thiriot-Quiévreux did not comment on the consistency of this feature on adjacent rows or on the central teeth of other radulae, and similar bifid spines have been reported to occur irregularly in another pterotracheid by Buchmann (1924), I question the utility of this minor feature as a taxonomic characteristic.

DISCUSSION

The morphometric analyses of *Pterotrachea* from Hawaiian waters indicate that two of the species, *P. coronata* and *P. scutata,* are distinctive in possessing rectangular eyes (Fig. 3F, G) with an eye length to retinal width ratio exceeding 1.8 (Fig. 4). Separation of these two species can be made on the basis of differences in the shape of the visceral nucleus, with the length to width ratio exceeding 4.0 in *P. coronata* and being less than 4.0 for *P. scutata* (Fig. 5). Additionally, *P. scutata* can be distinguished by the lateral expansions of the anterior portion of the body that form a gelatinous disc (see plate V in Tesch, 1949, and plate II in Okutani, 1957a).

Separation of the remaining two species of *Pterotrachea, P. minuta* and *P. hippocampus,* from the Hawaiian material was not possible. Bonnevie (1920) and subsequent workers (Tesch, 1949; Richter, 1968; Van der Spoel, 1976) considered *P. minuta* to be intermediate between *P. coronata* and *P. hip-*

pocampus in terms of visceral nucleus and eye shapes. Perhaps because the visceral nucleus is a much larger structure than the eye and can be measured more easily, both Tesch (1949) and Van der Spoel (1976) used the visceral nucleus in their keys to distinguish *P. minuta* (length/width ratio = 3) from *P. hippocampus* (length/width ratio = 2). However, ratio values for the Hawaiian specimens were highly variable and ranged from 2.1 to 3.9 (Fig. 5). This variability could have resulted from differences in gut fullness, preservation effects, and possibly several other factors (see Appendix for elaboration). Nonetheless, it is noteworthy that the length/width ratio decreased with increasing body size (Fig. 5), such that smaller individuals possessed, on the average, a more elongate visceral nucleus than larger animals; e.g., based on the line of best fit in Figure 5, a 20-mm animal would have a ratio value of 3.2, while that of a 70-mm specimen would be 2.4. In view of the observed variability in shape, the visceral nucleus appears to be useful only in distinguishing species whose nuclei differ greatly, as between *P. coronata* and the other species of *Pterotrachea* (Fig. 5).

The eye length to retinal width ratios for a wide size range of Hawaiian *P. hippocampus/minuta* revealed a steeply-sloping, size-specific relationship (Fig. 4), in which the eyes of small individuals (Fig. 3A) strongly resembled the holotype of *P. minuta* (Fig. 3E), and intermediate-to-large specimens (Figs. 3B–D) were comparable to the eyes of *P. hippocampus* photographed by Richter (1968: 370) and illustrated by Bonnevie (1920: 9) and Van der Spoel (1976: 400). Furthermore, the eye length to retinal width ratio for the holotype of *P. minuta* falls within the range of ratios recorded from the Hawaiian specimens (Fig. 4).

Tesch (1949) considered that male *P. minuta* possessed a sucker and fin that were conspicuously smaller than those of *P. hippocampus.* Comparisons of sucker size and fin size with body length (Figs. 7, 8) gave clear, size-specific relationships for the Hawaiian material. The sucker and fin sizes of the holotype of *P. minuta* proved to be well within the range of the Hawaiian animals of comparable size, assuming that the true body length of the holotype was close to 20 mm (based on its lens diameter) and not the measured value of 38.8 mm. The stretched condition of the holotype was almost certainly the result of net damage. In collections off southern California made with 1-m plankton nets and 3-m IKMT nets lacking cod-end devices, I have observed small *P. coronata* and *P. scutata* in a similar state. However, large individuals of these two species do not seem prone to this distortion. For the holotype specimen of *P. minuta,* the sucker and fin would have appeared small in relation to overall body length. Conversely, in large *P. hippocampus,* which may be less easily stretched, the fin and sucker would have appeared proportionately larger. This size difference was probably further exaggerated because the sucker and fin in undistorted males (Figs. 7, 8) appear to increase disproportionately in size with increasing body length. For example, the sucker on a 15-mm specimen is 1.1% of the body length, while it is 2.6% of the body length for a 50-mm individual (Fig. 7). Similarly, a 15-mm animal has a fin length equal to about 17% of its body length, while the fin length of a 50-mm specimen is about 25% of its body length (Fig. 8).

Hypothetically, while the adult morphologies of *P. hippocampus* and *P. minuta* could overlap considerably, there might be larval differences that could serve to distinguish the species. One study (Richter, 1968) investigated the larvae of Mediterranean heteropods and described three types that were distinctively different in shell morphology and which could be assigned to the genus *Pterotrachea.* Richter was able to follow development of the larvae for a maximum of 10 days after metamorphosis. Since the radula, visceral nucleus, and general body morphology had not differentiated to the point that species could be recognized, Richter based his tentative identifications of the three larval types on eye morphology. The first two larvae possessed eyes which were shaped like those of *P. hippocampus* and *P. minuta,* respectively, while the third larva had a more tubular eye like that of *P. coronata* or *P. scutata* and a unique, enlarged anterior lobe of the four-lobed velum. Richter was cautious, however, in assigning species names, and indicated that he would defer to the results of further developmental studies. In her review paper, Thiriot-Quiévreux (1973) agreed with Richter that three distinctive larval types from the Mediterranean Sea could be assigned to *Pterotrachea.* However, the larva that Richter considered closest to *P. minuta* on the basis of eye shape, Thiriot-Quiévreux (1969, 1971) identified as *P. coronata.* She was apparently

following the earlier designation of the species as *P. coronata* by Franc (1948) on the basis of its possession of about 30 transverse grooves on the shell. Larval differences, then, would appear to be of taxonomic utility, but additional developmental studies of the larvae following metamorphosis are obviously required. Nonetheless, an important result of Richter's (1968) and Thiriot-Quiévreux's (1973) studies was that only three larval types could be distinguished in waters that reportedly contained all four species of *Pterotrachea*. The absence of a fourth larval type may be due to there being only three species of *Pterotrachea*.

In conclusion, I was not able to find any evidence supporting the separation of *P. minuta* from *P. hippocampus* on the grounds that the former species has (1) a more elongate visceral nucleus, (2) a triangular eye that is narrower, (3) a conspicuously smaller fin and sucker in males, (4) pedal ganglia positioned anterior to the insertion point of the fin, or (5) a radular morphology that includes a more weakly developed median spine on the central tooth and a secondary spine on the lateral tooth. Eye morphology appears to be the most useful taxonomic characteristic in pterotracheids. However, because the size-specific relationship between eye shape and body length reported here for *P. hippocampus/minuta* (Fig. 4) was not previously noted, earlier investigators possibly mistook small *P. hippocampus* for *P. minuta*. Based on the Hawaiian material, *P. minuta* does not appear to be a valid species and should be treated as a junior synonym of *P. hippocampus*. Studies of the pterotracheid faunas in other regions of the world's oceans are required to substantiate the present results and permit a formal synonymy of *P. minuta* with *P. hippocampus*.

A broad size range of male and female specimens of *P. hippocampus/minuta* from the Hawaiian study collection has been deposited in the National Museum of Natural History (USNM 804410) and the Santa Barbara Museum of Natural History (SBMNH 33887).

The following key incorporates the above information and represents a modification of earlier keys based on eye and visceral nucleus morphology (Tesch, 1949; Okutani, 1957a; Van der Spoel, 1976), with *P. minuta* omitted.

KEY TO SPECIES OF THE GENUS *PTEROTRACHEA*

1a. Eyes rectangular in dorsal view. Eye length greater than 1.8 times the width of the retinal base 2

1b. Eyes narrowly triangular (small individuals) to broadly triangular (large individuals) in dorsal view. Eye length less than 1.6 times the width of the retinal base . . . . . *P. hippocampus*

2a. Visceral nucleus short; length 2 to 4 times the maximal width. Anterior portion of the body expanded laterally as a gelatinous disc . . . . . *P. scutata*

2b. Visceral nucleus elongate; length 4 to 7 times the maximal width. Anterior portion of the body not expanded laterally as a gelatinous disc . . . . . *P. coronata*

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APPENDIX

The length to width ratios for the visceral nucleus were highly variable for the examined species of *Pterotrachea* (Fig. 5), and particularly for *P. coronata.* Hypothetically, this variability is the result of: (1) differences between individual animals in the fullness of the digestive system portion contained within the visceral nucleus at the time of preservation, (2) changes resulting from placing individuals in preservative solution, (3) differences between individuals in the degree of gonadal development, and (4) morphological changes in the basic shape of the nucleus as related to age (size) of the individual.

During a cruise of the R/V KANA KEOKI in December 1981, data were obtained that addressed the first two hypotheses using *P. hippocampus/minuta.* Before proceeding, however, several points should be made relative to the third and fourth hypotheses. Although I have noted qualitatively that the width (thickness) of the visceral nucleus is greater in specimens of *P. hippocampus/minuta* that have a well-developed gonad, this relationship was not quantified. Enlarged gonads are particularly evident in large specimens and could explain the somewhat lower length to width ratios recorded for larger *P. hippocampus/minuta* and, as a result, the slightly negative slope of the regression line in Fig. 5. Because of variations in the shape of the visceral nucleus that appear to result from the first three factors hypothesized above, the fourth factor, change in shape as a function of age of the individual, cannot be addressed at the present time.

The effect of gut fullness on visceral nucleus shape was tested by isolating freshly-collected specimens in finger bowls. The selected individuals appeared healthy and undamaged by trawl capture. The length and width of the nucleus were measured through a dissecting microscope with an ocular micrometer at varied intervals for up to 2.5 to 7.1

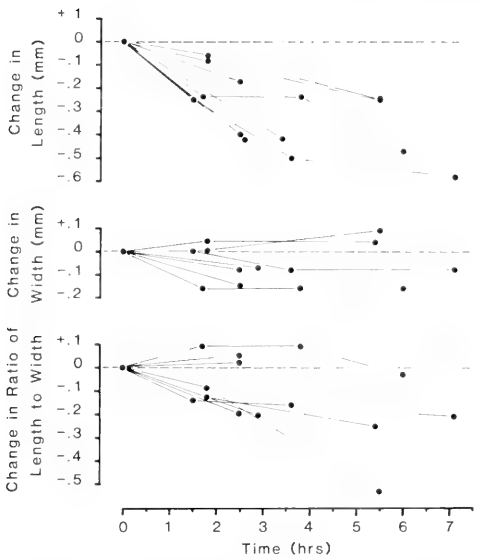

FIG. 9. Change in length (mm), width (mm) and length to width ratio of the visceral nucleus as a function of time in *Pterotrachea hippocampus/minuta* collected from Hawaiian waters in December 1981.

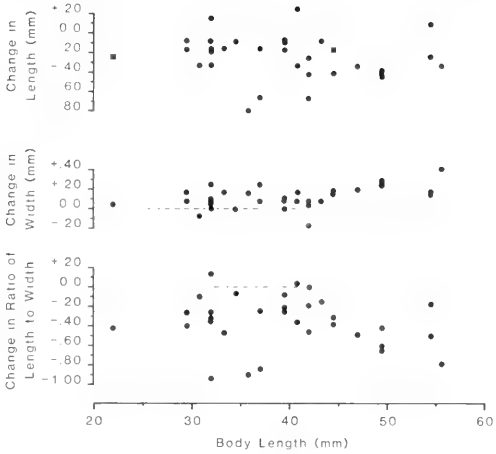

FIG. 10. Change in length (mm), width (mm), and length to width ratio of the visceral nucleus after preservation in *Pterotrachea hippocampus/minuta* collected from Hawaiian waters in December 1981.

hrs. Fecal strings were produced that commonly remained attached to the animal. Thus, it was possible to determine which specimens were eliminating fecal matter. In 8 of 14 animals tested, fecal strings were produced which varied in thickness (not quantified) and in length (from 4 to 18 mm). The results of these 8 trials are summarized in Fig. 9. In all cases, length of the nucleus decreased with time. In 6 of the trials, the width also decreased with time, but to a much lesser degree. The length to width ratio clearly decreased with time in 5 of the 8 trials, a result that can be attributed to the relatively greater decrease in nucleus length. It is noteworthy that the excretion of large amounts of wastes by *P. coronata* was observed on two occasions to result in a dramatic shortening of the nucleus and therefore a substantial reduction in the length to width ratio.

The effect of preservation on shape of the visceral nucleus was examined by measuring the length and width of the nuclei from a series of 32 freshly-captured and apparently healthy specimens (ranging in length from 22.0 to 55.6 mm) before and after preservation in a buffered, 10% seawater-formalin solution (Fig. 10). In 29 of the trials, the length decreased following preservation, while the width increased in 30 cases. With only two exceptions, the length to width ratio decreased after preservation. Preservation resulted in contraction of the nucleus in nearly all of the trials and, because of simultaneous increases in width, the length to width ratio decreased. These results imply that specimens preserved while still alive would exhibit a lower length to width ratio than those that had died before preservation.

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TAXONOMIE EXPÉRIMENTALE DE *BIOMPHALARIA* (GASTROPODA: PLANORBIDAE)—III. MOBILITÉS ENZYMATIQUES CONSIDÉRÉES COMME ÉLÉMENTS DE DIAGNOSTIC POUR LES *BIOMPHALARIA* ANTILLAIS. ÉTUDE DE SEPT SYSTÈMES ENZYMATIQUES

Jens Erik Jelnes[1,2] & Jean-Pierre Pointier[3]

RÉSUMÉ

Une analyse électrophorétique de sept systèmes d'enzymes chez *Biomphalaria glabrata, B. straminea, B. schrammi, B. havanensis* et *Biomphalaria* sp. a nettement révélé des différences de mobilités enzymatiques entre les espèces.

Des caractères ont été considérés comme éléments de diagnostic pour les trois premières espèces originaires de la Guadeloupe et de la Martinique. Les informations obtenues suggèrent d'autre part, que les caractères enzymatiques peuvent également être utiles pour les déterminations spécifiques dans d'autres îles des Antilles.

Mots clés: Les Antilles; *Biomphalaria*; caractères diagnostiques; Hbdh; Pgi; Pgm; Got.

INTRODUCTION

La faune malacologique dulçaquicole des départements français de la Guadeloupe et de la Martinique est actuellement bien connue. En Guadeloupe, des études détaillées ont été entreprises sur la taxonomie et la distribution des Mollusques d'eau douce (Pointier, 1974, 1976), tandis que des recherches similaires étaient réalisées en Martinique (Guyard & Pointier, 1979). Toutes ces études ont été essentiellement motivées par le fait que des espèces appartenant au genre *Biomphalaria* jouent le rôle d'hôte intermédiaire de *Schistosoma mansoni* Sambon, 1907, agent de la schistosomose intestinale dans ces îles.

En Guadeloupe et Martinique, trois espèces seulement de *Biomphalaria* sont actuellement reconnues: *B. glabrata* (Say, 1818—localité type: Guadeloupe), *B. schrammi* (Crosse, 1864—localité type: Guadeloupe) et *B. straminea* (Dunker, 1848—localité type: Amérique du Sud).

Dans les Grandes Antilles, des inventaires malacologiques ont été établis principalement en Haiti (Robart *et al.,* 1977) et à Porto-Rico (Ferguson & Richards, 1963; Harry & Hubendick, 1964).

Dans les Petites Antilles, la répartition de la schistosomose intestinale et de ses hôtes intermédiaires a été revue récemment par Prentice (1980) et dans de nombreux cas, la présence de certaines espèces de *Biomphalaria* n'a pu être établie avec certitude par suite de déterminations douteuses.

Ces problèmes posés par l'identification des espèces de Mollusques hôtes intermédiaires ont amené à rechercher de nouveaux critères de détermination. L'utilisation des méthodes électrophorétiques enzymatiques s'inscrit parfaitement dans le cadre de ces recherches.

Les résultats d'une étude préliminaire réalisée sur huit enzymes chez des espèces de *Biomphalaria* américains, ont déjà montré que les caractéristiques de ces enzymes pouvaient être très utiles pour l'identification et donc la révision de ce genre dans la zone Néotropicale (Jelnes, 1982). Nous présentons dans cet article les résultats obtenus par l'analyse de sept loci enzymatiques concernant 23 échantillons de populations de *Biomphalaria* antillais. Les espèces étudiées sont *B. glabrata, B. schrammi, B. straminea, B. havanensis* (Pfeiffer, 1839—localité type: La Havane, Cuba), et *Biomphalaria* sp. dont l'identification pose un problème mais qui pourrait correspondre à *B. albicans* (Pfeiffer, 1839—localité type: Cuba), *B. pallida* (Adams, 1846—localité type: Jamaique) ou *B. obstructa* Morelet, 1849—localité type: Ile de Carmen, Mexique).

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MATÉRIEL ET MÉTHODES

L'origine des souches étudiées est donnée Tableau 1. Toutes les souches sont considérées comme "sauvages" selon le critère utilisé par Jelnes (1982) exception faite de l'échantillon no. 6 qui correspond à une 2è génération de laboratoire.

Les techniques utilisées pour la préparation des échantillons, l'électrophorèse sur gel d'amidon, et la révélation des enzymes ont déjà été décrites en détail (Henriksen & Jelnes, 1980; Jelnes, 1982). Pour chaque individu de l'échantillon étudié, les enzymes suivants ont été analysés: 3-hydroxybutyrate déshydrogénase (EC 1.1.1.30), phosphoglucose isomérase (EC 5.3.1.9), isocitrate déshydrogénase (EC 1.1.1.41), alpha-glycérophosphate déshydrogénase (EC 1.1.1.8), glutamate-oxaloacétate transaminase (EC 2.6.1.1), mannose-6-phosphate isomérase (EC 5.3.1.8) et phosphoglucomutase (EC 2.7.5.1). Les estérases n'ont pas été étudiées car elles donnent des schémas extrêmement complexes qui se révèlent par conséquent peu utilisables pour les identifications des espèces.

RÉSULTATS

Sept enzymes représentant 7 loci génétiques ont été analysées par électrophorèse sur un total de 269 Mollusques. Les mobilités des différentes bandes obtenues par les différentes enzymes sont présentés Tableau 2. Des analyses supplémentaires, non présentées Tableau 2, ont été effectuées pour comparer la position des bandes dans les cas où il a été nécessaire de savoir si deux enzymes avaient une mobilité identique ou non. Des photographies de zymogrammes représentatifs de *Biomphalaria* ont été présentés dans un précédent article (Henriksen & Jelnes, 1980).

3-hydroxybutyrate déshydrogénase (Hbdh). Un total de six mobilités différentes ont été observées pour cette enzyme. Les deux plus rapides (1,34 et 1,20) caractérisent *B. glabrata* tandis que 1,00 est caractéristique de *B. havanensis,* 0,74 de *Biomphalaria* sp., 0,93 de *B. schrammi* et 0,47 de *B. straminea.* Pour la population no. 2 (*B. glabrata*) une variation d'allozymes est observée et les phénotypes suivants ont été trouvés: Hbdh-1,20

TABLEAU 1. Origine des souches analysées.

No.	Espèces	Iles	Localités	No. de référence à DBL
1	*B. glabrata*	Sainte-Lucie	Marécage à Soufrière	80/257
2	*B. glabrata*	Guadeloupe	Mare des Grands Fonds	80/259 + 81/1
3	*B. glabrata*	Guadeloupe	Mare de Céligny	81/135
4	*B. glabrata*	Guadeloupe	Mare de Céligny	81/201
5	*B. glabrata*	Guadeloupe	Mare de Tombeau	81/202
6	*B. glabrata*	Martinique	Marécage du Quartier Boisneuf	81/248
7	*B. glabrata*	Martinique	Marécage de l'Anse Rivière	81/266
8	*B. glabrata*	Martinique	Rivière de Pointe La Mare	81/268
9	*B. glabrata*	Porto Rico	Humacao	81/234
10	*B. glabrata*	Hispaniola	Santo-Domingo, jardin botanique	81/249
11	*B. straminea*	Martinique	Marécage du Quartier Boisneuf	80/249
12	*B. straminea*	Martinique	Canal de Sainte-Marie	81/134
13	*B. straminea*	Martinique	Rivière Epinette à Trinité	81/170
14	*B. straminea*	Martinique	Canal de Sainte-Marie	81/171
15	*B. straminea*	Martinique	Marécage du Quartier Boisneuf	81/182
16	*B. straminea*	Martinique	Rivière de Pointe La Mare	81/267
17	*B. schrammi*	Guadeloupe	Mare de Tombeau	81/186
18	*B. schrammi*	Guadeloupe	Etang Cocoyer	81/187
19	*B. schrammi*	Guadeloupe	Mare de Céligny	81/200
20	*B. schrammi*	Guadeloupe	Mare de Tombeau	81/253
21	*B. schrammi*	Guadeloupe	Mare de Céligny	81/258
22	*B. havanensis*	Hispaniola	Haiti	81/250
23	*Biomphalaria* sp.	Hispaniola	Santo-Domingo, jardin botanique	81/251

TABLEAU 2. Mobilités enzymatiques observées chez des *Biomphalaria* antillais. Les phénotypes sont exprimés par les valeurs des mobilités enzymatiques par rapport à la souche de référence de *Biomphalaria camerunensis* originaire de Kinshasa, Zaïre. A / la séparation des valeurs de rm indique le polymorphisme génétique. A + la séparation des valeurs de rm indique que les deux bandes sont trouvées chez tous les individus analysés.

No. de souche	Nombre d'individus analysés	Mobilités enzymatiques observées						
		Hbdh	Pgi	Idh	Gpdh	Got	Mpi	Pgm
1	9	1,34	1,18	—	1,08	0,50	0,92	0,71+0,85
2	8	1,20/1,34	1,18	0,92	1,08	0,50	0,92	0,71+0,85
3	14	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
4	7	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
5	2	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
6	8	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
7	12	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
8	24	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
9	40	1,34	1,18	0,97	1,08	0,50	0,92	0,71+0,85
10	7	1,34	1,18	1,01	1,08	0,50	0,92	0,71+0,85
11	17	0,47	1,34	0,88	1,08	0,50	0,92	0,85+1,00
12	11	0,47	1,34	0,88	1,08	0,50	0,92	0,85+1,00
13	19	0,47	1,34	0,88	1,08	0,50	0,92	0,85+1,00
14	16	0,47	1,34	0,88	1,08	0,50	0,92	0,85+1,00
15	3	0,47	1,34	0,88	1,08	0,50	0,92	0,85+1,00
16	17	0,47	1,34	0,88/0,92	1,08	0,50	0,92	0,85+1,00
17	4	0,93	1,00	0,83	0,91	1,00	0,76	0,71+0,85
18	3	0,93	1,00	0,83	0,91	1,00	0,76	0,71+0,85
19	1	0,93	1,00	0,83	0,91	1,00	0,76	0,71+0,85
20	28	0,93	1,00	0,83	0,91	1,00	0,76	0,71+0,85
21	6	0,93	1,00	0,83	0,91	1,00	0,76	0,71+0,85
22	5	1,00	1,18	0,92	1,08	1,00	0,92	0,85+1,00
23	8	0,74	1,18	0,97	1,08	1,00	0,98	1,00+1,13

(4 spécimens) et Hbdh-1,20/1,34 (4 spécimens).

Phosphoglucose isomérase (Pgi). Pour cette enzyme, trois mobilités différentes sont observées: 1,18 chez *Biomphalaria* sp., *B. glabrata* et *B. havanensis,* 1,34 chez *B. straminea* et 1,00 chez *B. schrammi.* Il n'a été noté aucun cas de variation d'allozymes.

Isocitrate déshydrogénase (Idh). Cinq mobilités différentes ont été observées: 1,01 chez une population de *B. glabrata,* 0,97 chez *B. glabrata* et *Biomphalaria* sp., 0,92 chez une population de chacune des trois espèces *B. glabrata, B. straminea* et *B. havanensis.* La mobilité de 0,88 a été trouvée seulement chez *B. straminea* et 0,83 semble caractéristique de *B. schrammi.* Pour la population no. 16 (*B. straminea*), on peut noter une variation d'allozymes représentée par les allèles Idh-0,88 et Idh-0,92. Treize spécimens du phénotype Idh-0,88 et 4 spécimens du phénotype Idh-0,88/0,92 ont été observés.

Alpha-glycérophosphate déshydrogénase (α-Gpdh). Deux mobilités seulement peuvent être révélées: 0,91 chez *B. schrammi* et 1,08 chez les autres espèces étudiées.

Glutamate-oxaloacétate transaminase (Got). Pour cette enzyme deux mobilités ont également été notées: 0,50 chez *B. glabrata* et *B. straminea* et 1,00 chez *Biomphalaria* sp., *B. schrammi* et *B. havanensis.*

Mannose-6-phosphate isomérase (Mpi). Trois mobilités ont été observées. *B. schrammi* parait caractérisé par une mobilité de 0,76, tandis que chez *B. glabrata, B. straminea* et *B. havanensis,* celle-ci est de 0,92. *Biomphalaria* sp. est caractérisé par 0,98.

Phosphoglucomutase (Pgm). A la révélation enzymatique tous les individus présentent deux bandes. Pour Pgm les mobilités de 0,71 et a 0,85 ont été révélées chez *B. glabrata* et *B. schrammi,* tandis qu'elles sont de 0,85 et 1,00 chez *B. straminea* et *B.*

havanensis et de 1,00 et 1,13 chez *Biomphalaria* sp. Aucun cas de variation d'allozymes n'est rapporté.

DISCUSSION

Jelnes (1982) a montré que les modèles enzymatiques d'autres échantillons de *Biomphalaria* américains sont reproductibles et indépendants de l'âge, de la taille, de la nutrition des Mollusques et de la qualité de l'eau, si les méthodes décrites sont employées. Ceci est encore le cas pour cette étude, quoique deux enzymes (Mpi et Idh) se révèlent si faiblement qu'il est quelque peu difficile d'établir leurs phénotypes. Pour cette raison, aucune donnée sur l'enzyme Idh n'a été rapportée pour la population no. 1 et les valeurs de rm quelque peu variables de cette enzyme pour la population no. 10 peuvent être dues à une incertitude dans l'établissement des phénotypes.

Une population de *B. glabrata* originaire de Belo Horizonte, Brésil (Jelnes, 1982), présente une différence sur un seul enzyme (Hbdh-1,20) par rapport aux populations de *B. glabrata* antillais étudiés ici. Cette grande similitude des réponses enzymatiques d'échantillons d'origine très différente peut être l'indice d'une faible variation géographique de l'espèce. Au contraire de *B. glabrata*, *B. straminea* montre un plus grand degré de variation géographique. La population étudiée par Jelnes (1982) et originaire d'Amérique du Sud diffère de la souche martiniquaise par des mobilités différentes des trois enzymes: Pgi, Got et Pgm.

La valeur des caractéristiques enzymatiques comme élément de diagnostic

Des mobilités identiques ne furent trouvées pour aucune enzyme des cinq espèces étudiées (Tableau 2). Le Tableau 3 présente les nombres d'enzymes pour lesquelles les mobilités enzymatiques peuvent être considérées comme éléments de diagnostic. On y voit que le nombre minimum de bandes enzymatiques "diagnostic" est de trois. Toutes les cinq espèces étudiées présentent des mobilités différentes en ce qui concerne l'enzyme Hbdh. Pour Pgi les espèces de Martinique et de Guadeloupe montrent également des mobilités enzymatiques différentes.

Le Tableau 4 présente quelques mobilités enzymatiques qui peuvent être utilisées pour l'identification des espèces de *Biomphalaria* antillais. Comme les techniques utilisées permettent la révélation de deux enzymes à par-

TABLEAU 3. Nombre d'enzymes utilisables comme critère d'identification spécifique pour des combinations deux par deux des espèces de *Biomphalaria*.

	B. glabrata	*B. straminea*	*B. schrammi*	*B. havanensis*
B. straminea	4			
B. schrammi	6	7		
B. havanensis	3	3	6	
Biomphalaria sp.	4	6	6	4

TABLEAU 4. Quelques valeurs rm, utiles pour l'identification de *Biomphalaria* sp. de la région Caraïbe. A / la séparation des valeurs de rm indique le polymorphisme génétique. Pour les allozymes, les mobilités rares sont mises entre parenthèses. A + la séparation des valeurs de rm indique que les deux bandes sont trouvées chez tous les individus analysées.

	Hbdh	Pgi	Pgm	Got	Idh
B. glabrata	1,34/(1,20)	1,18	0,71+0,85	0,50	(1,01)/0,97/(0,92)
B. straminea	0,47	1,34	0,85+1,00	0,50	(0,92)/0,88
B. schrammi	0,93	1,00	0,71+0,85	1,00	0,83
B. havanensis	1,00	1,18	0,85+1,00	1,00	0,92
Biomphalaria sp.	0,74	1,18	1,00+1,13	1,00	0,97

tir d'une seule électrophorèse, l'utilisation des enzymes Pgi et Hbdh sera donc recommandée pour les déterminations spécifiques. L'enzyme Pgi est visible sur la plaque environ cinq à dix minutes après l'application du révélateur tandis que pour Hbdh, la durée de révélation est de une à deux heures. Donc, si l'on considère que la mobilité de l'enzyme Pgi est un critère d'identification valable (comme c'est le cas pour les échantillons guadeloupéens et martiniquais), il est possible de vérifier les déterminations grâce aux valeurs des rm de Hbdh.

Il convient cependant d'émettre deux réserves si l'on veut extrapoler les résultats à d'autres régions que la Guadeloupe et la Martinique. La première est que cinq autres espèces de *Biomphalaria* sont signalées dans les Antilles dont les profiles enzymatiques ne sont pas connus (voir ci-après). La deuxième est qu'il existe une certaine variation géographique des enzymes chez les *Biomphalaria* comme l'ont déjà montré les résultats exposés.

Les mobilités enzymatiques présentées dans le Tableau 2 sont toutes exprimées par rapport à la mobilité de la même enzyme fonctionelle chez *Biomphalaria camerunensis* (Boettger, 1941) originaire de Kinshasa, Zaïre et maintenu en élevage au Laboratoire Danois de Bilharziose. Il est évidemment criticable d'utiliser une espèce africaine de *Biomphalaria* comme témoin pour des études réalisées sur des *Biomphalaria* en Amérique. N'importe quelle souche ou population de *Biomphalaria* américain pourrait en principe servir de matériel de référence. Il est cependant préférable d'utiliser une espèce qui ne présente pas de grande variation géographique et il y a un avantage supplémentaire à choisir une population qui ne révèle pas de variation génétique dans les enzymes étudiées. De toutes les espèces analysées dans ce travail, il semble que *B. glabrata* remplisse le mieux ces conditions. D'autre part cette espèce a l'avantage supplémentaire de pouvoir être facilement maintenue en élevage dans les conditions du laboratoire.

A partir des mobilités enzymatiques exprimées par rapport à *B. camerunensis* il est possible de calculer les mobilités par rapport à toute autre souche qui serait choisie comme référence, à condition que les valeurs de la nouvelle souche de référence soient connues par rapport à celles de *B. camerunensis.* Pratiquement, il suffit de diviser les valeurs de rm de l'échantillon par rapport à *B. camerunensis,* par les valeurs de rm de la nouvelle souche de référence par rapport à *B. camerunensis.* Comme les valeurs de rm varient lorsque la souche de référence change, il est evidemment très important de préciser la souche de référence qui est utilisée. D'autre part il est souhaitable de comparer les mobilités enzymatiques des differentes souches de référence utilisées dans les différentes laboratoires.

En dehors des espèces étudiées dans cet article, d'autres *Biomphalaria* ont été signalés dans les Antilles. Il s'agit de *B. helophila* (d'Orbigny, 1835—localité type: Callao, Pérou), *B. peregrina* (d'Orbigny, 1835—localité type: Patagonie, Argentine), *B. obstructa, B. albicans* et *B. pallida.*

Une des espèces analysées dans ce travail sous la dénomination de *Biomphalaria* sp. pose un problème de détermination. Du point de vue morphologique, elle apparait assez proche de *B. havanensis* bien que la coquille présente quelques différences. Du point de vue enzymatique les deux formes présentent des différences portant sur 4 des 7 enzymes étudiées. Nous considérons donc ces deux formes comme deux espèces distinctes.

Trois espèces déjà décrites des Antilles pourraient correspondre au *Biomphalaria* sp. analysé dans ce travail: *B. albicans, B. pallida* et *B. obstructa.*

Paraense & Ibañez (1964) ont placé *B. albicans* en synonymie avec *B. helophila* tandis que Robart *et al.* (1977) considèrent *B. albicans* comme une espèce distincte. A Porto Rico, Harry & Hubendick (1964) considèrent également cette espèce comme distincte bien que les coquilles présentent des caractéristiques assez voisines de celles de *B. helophila* avec notamment une déviation prononcée du dernier tour de spire vers la gauche. Les spécimens analysés ici du point de vue enzymatique sous la dénomination de *Biomphalaria* sp. ne comportent pas cette déviation vers la gauche du dernier tour de spire bien qu'ils soient adultes.

La deuxième espèce, qui nous paraît mieux correspondre à *Biomphalaria* sp. est *B. pallida.* Décrite originellement de la Jamaique, cette espèce a également été signalée à Porto Rico par Harry et Hubendick (1964) qui la considèrent comme une espèce distincte de *B. havanensis* et *B. albicans.* Les caractéristiques de la coquille apparaissent proches de celles de l'échantillon de *Biomphalaria* sp. analysé dans ce travail. *B. pallida* pourrait donc correspondre à notre espèce.

B. obstructa a été décrit par Morelet de l'île de Carmen (Mexique) et est signalé à Porto Rico et Cuba par PAHO (1968). Cette espèce pourrait correspondre également du point de vue morphologique à *Biomphalaria* sp.

B. helophila n'a été signalé jusqu'ici que dans l'île de la Barbade. Nous avons vu que ce planorbe est caractérisé par une déviation prononcée du dernier tour de spire vers la gauche comme *B. albicans.*

Enfin, la présence de *B. peregrina* semble très douteuse dans les Antilles d'après les révisions présentées par PAHO (1968) et Prentice (1980).

Cette revue des espèces de *Biomphalaria* signalées dans les Antilles fait apparaître les lacunes qui existent encore concernant la systématique de ce genre. Afin d'arriver à une meilleure compréhension de cette taxonomie, il sera nécessaire d'analyser beaucoup plus d'échantillons de populations d'origines très diverses.

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TAXONOMIA EXPERIMENTAL DE *BIOMPHALARIA* (GASTROPODA: PLANORIBIDAE)—III. MOVILIDADES ENZIMÁTICAS COMO CARACTERES DIAGNOSTICOS DE LOS *BIOMPHALARIA* DE LAS ANTILLAS. UN ESTUDIO DE SIETE SISTEMAS ENZIMÁTICOS

Jens Erik Jelnes & Jean-Pierre Pointier

RESUMEN

El análisis electrophoretico de siete sistemas enzimaticos que pertenecen a las especies *Biomphalaria glabrata, B. straminea, B. schrammi, B. havanensis* y *Biomphalaria* sp. reveló unas diferencias distinctas de movilidad entre las citadas especies.

En cuanto a las tres primeras especies, unas propiedades enzimaticas que son útiles para la identificación de la especie, han sido propuestas en material de origen las islas Martinique y Guadeloupe.

Han sido conseguidas informaciónes que demuestran la utilidad de las propiedades enzimaticas en la identificación en otras islas del Caribe.

EXPERIMENTAL TAXONOMY OF *BIOMPHALARIA* (GASTROPODA: PLANORBIDAE)—III. ENZYME MOBILITIES AS DIAGNOSTIC CHARACTERS FOR ANTILLEAN *BIOMPHALARIA*. A STUDY OF SEVEN ENZYME SYSTEMS

Jens Erik Jelnes & Jean-Pierre Pointier

SUMMARY

Electrophoretic analysis of seven enzyme systems of *Biomphalaria glabrata, B. straminea, B. schrammi, B. havanensis* and *Biomphalaria* sp. revealed clear differences in mobility between the species.

For the three first mentioned species enzyme characters useful for identification to species have been suggested for material originating from the islands of Martinique and Guadeloupe.

Data have been obtained that suggest enzyme characters as useful for species identification on other Caribbean islands.

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A NEW PIGMENTATION MUTANT IN *BIOMPHALARIA GLABRATA*

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ABSTRACT

Three alleles of a gene regulating mantle pigmentation in *Biomphalaria glabrata* are described: pigment coalescence, discrete spotting, and absence of black mantle pigment. This gene is apparently located on a different chromosome than the gene with three alleles determining basic pigmentation (wildtype, blackeye, and albino). Combinations of these two pigmentation characters result in nine different pigment phenotypes.

Key words: *Biomphalaria glabrata*; pigmentation; genetics; mutation.

INTRODUCTION

Biomphalaria glabrata (Say) is the major intermediate host for *Schistosoma mansoni* in the Western Hemisphere. It has been demonstrated that genetics plays an important role in the host-parasite relationship, determining variations in susceptibility of *B. glabrata* for infection by *S. mansoni* (Newton, 1953; Richards & Merritt, 1972; Richards, 1973a, 1975, 1977). Pigmentation served as a genetic marker in these studies. Newton (1954) demonstrated that albinism in *B. glabrata* is a simple recessive character. Richards (1967) reported a third allele of the same gene, "blackeye," dominant over albino but recessive to black wildtype pigmentation. In wildtype pigmentation (C), snails have black eyes, black pigment in connective tissue of headfoot and mantle collar and in the mantle epithelium in the form of spots (Richards, 1969). As snails age, the mantle often becomes all black. In blackeye pigmentation (c^b), snails have black eyes and black pigment in the mantle epithelium in the form of spots, but are deficient in black headfoot and mantle collar pigment. Albino snails (c) lack black pigment. Black mantle pigmentation of wildtype and blackeye *B. glabrata* occurs in variable degrees and typically in the form of spots (Richards, 1969, 1973a). Richards (1969) developed relatively "unspotted" *B. glabrata* by selection for minimal spotting through several generations and concluded from crossing experiments that spotting was dominant over lack of spotting.

Although additional morphological characters showing single factor inheritance have been reported (Richards, 1972, 1973b, 1974a, 1974b, 1980), no linkage has been demonstrated with factors determining variation in susceptibility to *S. mansoni* infection. Additional simple morphological characters are therefore needed. Studies on mantle pigmentation reported here add to our knowledge of the complex variations in mantle pigment and provide additional good genetic markers (Richards, 1984).

MATERIALS AND METHODS

Methods of maintaining snails and crossing procedures were described by Richards & Merritt (1972). The origins of the snail lines involved in these studies were indicated in a diagram by Richards (1975). Snails were reared in isolation, reproducing by selfing, and each digit in the numerical identification represents an individual snail in each succeeding generation.

Wildtype Puerto Rican *B. glabrata* lacking black mantle pigment spots were provided by Mr. Walter Stewart (NIAMDD, NIH). He observed snails with and without mantle spotting and demonstrated that spotting was

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dominant over lack of spotting (personal communication). Random segregation of codominant alleles at an autosomal locus of a selfed heterozygote should produce ratios of 1:2:1 among the F_2 progeny. For dominant traits, a ratio of 3:1 is expected. A X^2 statistic was calculated to test the fit of the data to Mendelian expectations and a X^2 test for homogeneity was calculated to determine consistency among families. A contingency X^2 statistic was calculated to test for independent assortment of the two loci contributing to the pigment phenotype.

RESULTS

Mantle pigment variations described in this paper are determined by three alleles of a different gene from that determining albinism. These alleles are designated as follows: S^d = coalesced mantle pigment, S = discrete mantle spotting, s = absence of these mantle pigment expressions (Figs. 1–9). Alleles S^d and S are codominant, and in heterozygous S^dS juvenile or young adult snails (<10 mm), this genotype is indicated by a combination of spots and coalescence. In older S^dS snails, coalescence may obscure the spots. Before the interaction of the mantle pigment alleles was clarified, both S^dS^d and S^dS snails were scored as coalesced (Figs. 10, 11).

Combinations of the two pigment characters (basic pigmentation: C = wildtype, c^b = blackeye, c = albino; mantle pigmentation: S^d = coalescence, S = discrete spotting, S^dS = codominant pigmentation, s = absence of mantle pigmentation) result in nine different phenotypes (Fig. 1–9). Crossing experiments that demonstrated the inheritance follow.

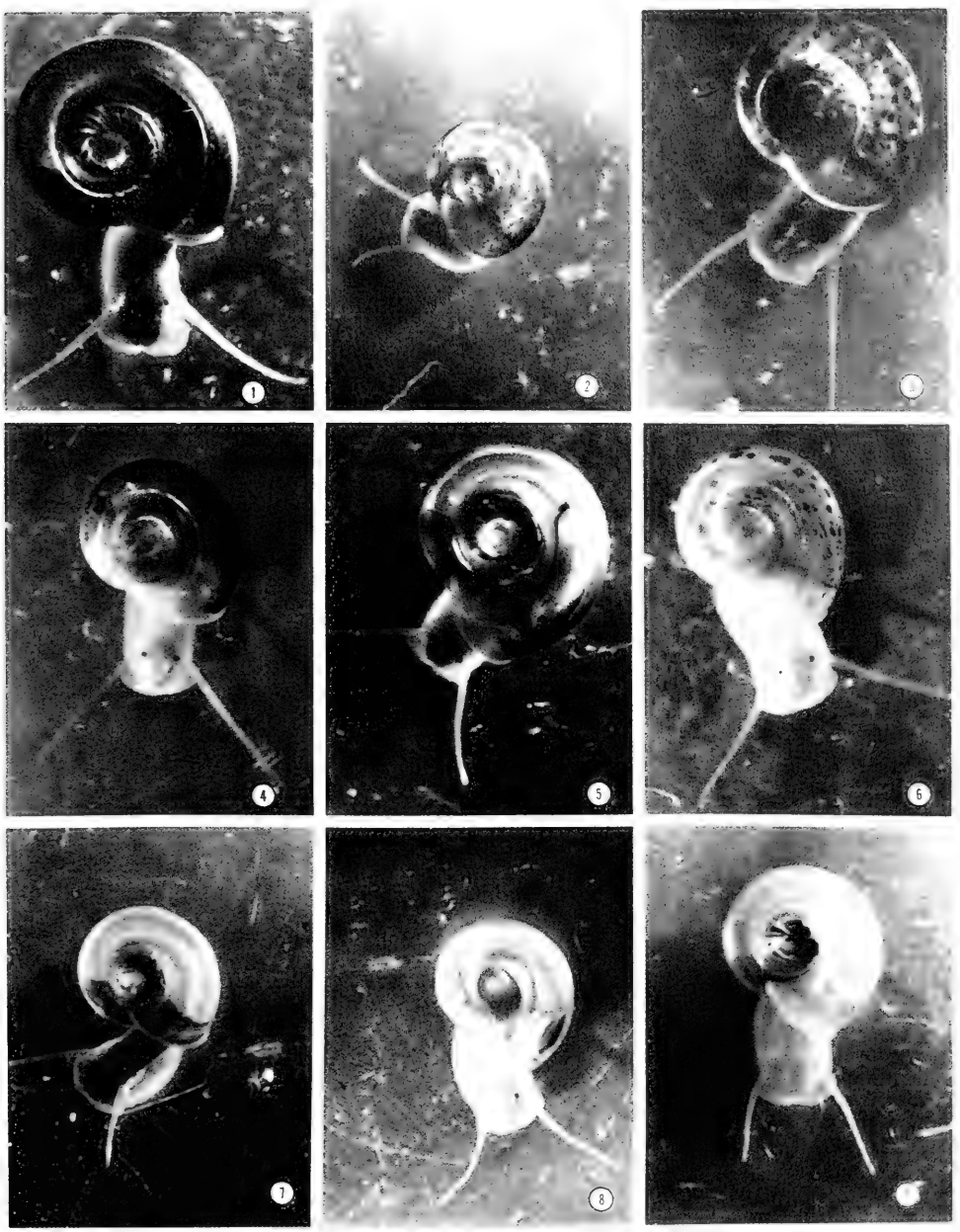
Offspring of spotted blackeye *B. glabrata* 1-3-1-4-2-10-1 (c^bc^bSS) by selfing included 19 (c^bc^bSS) with discrete mantle spotting and 6 ($c^bc^bS^dS$) mutants with coalesced mantle pigment (Fig. 10). The observed progenies of the 19 c^bc^bSS F_1s by selfing totaled 155, all c^bc^bSS. Observed progenies of the 6 $c^bc^bS^dS$ F_1s by selfing totaled 77 $c^bc^b(S^dS^d + S^dS)$: 33 c^bc^bSS (X^2 = 1.168, df = 1, P = 0.20). Several crosses were carried out involving the 6 mutant F_1s or their descendants. Some of these are depicted in Figs. 11 and 12.

Mutant snail 1-3-1-4-2-10-1-4 produced offspring by selfing in the ratio 20 c^bc^b (S^dS^d + S^dS) : 8 c^bc^bSS (X^2 = 0.047, df = 1, P = 0.84) (Fig. 11). This snail was mated with an albino cc(SS), resulting in postcross hybrid offspring in the ratio 12 c^bcS^dS : 12 c^bcSS (X^2 = 0.0042, df = 1, P = 0.84). Eight of the c^bcS^dS F_1s, reared in isolation and reproduced by selfing, produced 134 F_2s: 65 (c^bc^b + c^bc)(S^dS^d + S^dS) : 30 (c^bc^b + c^bc)SS : 39 cc (S^dS^d + S^dS + SS) : (X^2 = 3.262, df = 2, P = 0.04). Four of the c^bcSS F_1s produced by selfing: 55 (c^bc^b + c^bc)SS : 21 cc (SS) (X^2 = 0.155, df = 1, P = 0.64).

An F_2 albino (No. 5) from one of the c^bcS^dS F_1s (No. 7) was first selfed and then mated with a spotted mantle blackeye snail (c^bc^bSS) (Fig. 11). All the offspring of this cross, from both parents, were blackeye snails that developed coalesced mantle pigment (c^bcS^dS), suggesting that albino No. 5 was homozygous for coalesced mantle pigment (ccS^dS^d). One of the offspring of F_2 (No. 5) by precross selfing (7-5-1) was used in the three-way cross illustrated in Fig. 12.

At about the same time the coalesced mantle pigment mutant was observed in our laboratory, Mr. Walter Stewart, working with Dr. Ned Feder (NIAMDD, NIH), observed mantle pigment variation in a wildtype *B. glabrata* stock of Puerto Rican origin. Some snails lacked black mantle pigment, while others displayed discrete mantle spotting. By crosses, they demonstrated that the unspotted character was simple recessive (personal communication). They kindly provided us with some of these unspotted (CCss) snails to determine the relationship between the various types of mantle pigmentation.

CCss snails were used in several crosses with other pigment types in paired matings. The results were consistent with those in Fig. 12, which is of interest as a promiscuous mating of three snails involving six alleles, three for each of two pigment genes. Each of the snails bred true for its pigment pattern by selfing before the matings: a wildtype snail lacking mantle pigment (CCss), a blackeye snail with spotted mantle (c^bc^bSS), and an albino carrying alleles for coalesced mantle pigment (ccS^dS^d No. 7-5-1, Fig. 11). The three snails were maintained together in a 400 ml beaker for 10 days and then reisolated. Resulting offspring demonstrated that each snail had cross-fertilized both of the others. The wildtype CCss snail produced two types of hybrid offspring, Cc^bSs and CcS^ds; the spotted blackeye produced Cc^bSs and c^bcS^dS hybrid offspring; and the albino ccS^dS^d produced CcS^ds and c^bcS^dS hybrid offspring. Hybrid F_1s of all the above types were isolated and progenies by selfing were



FIGS. 1–9. Photomicrographs of nine *Biomphalaria glabrata* ranging 4–7 mm in maximum shell diameter, showing nine different pigment phenotypes (36 possible genotypes): Fig. 1, wildtype with coalesced mantle pigment (CCS^dS^d, CCS^ds, $Cc^bS^dS^d$, Cc^bS^ds, CcS^dS^d, CcS^ds); Fig. 2, wildtype heterozygous for mantle pigment (CCS^dS, Cc^bS^dS, CcS^dS); Fig. 3, wildtype with spotted mantle (CCSS, CCSs, Cc^bSS, Cc^bSs, CcSS, CcSs); Fig. 4, blackeye with coalesced mantle pigment ($c^bc^bS^dS^d$, $c^bc^bS^ds$, $c^bcS^dS^d$, c^bcS^ds); Fig. 5, blackeye heterozygous for mantle pigment ($c^bc^bS^dS$, c^bcS^dS)—poor lighting makes the headfoot of this snail appear dark and obscures the partially coalesced mantle spots; Fig. 6, blackeye with spotted mantle (c^bc^bSS, c^bc^bSs, c^bcSS, c^bcSs); Fig. 7, wildtype with unpigmented mantle (CCss, Cc^bss, Ccss); Fig. 8, blackeye with unpigmented mantle (c^bc^bss, c^bcss); Fig. 9, albino (ccS^dS^d, ccS^dS, ccSS, ccS^ds, ccSs, ccss).

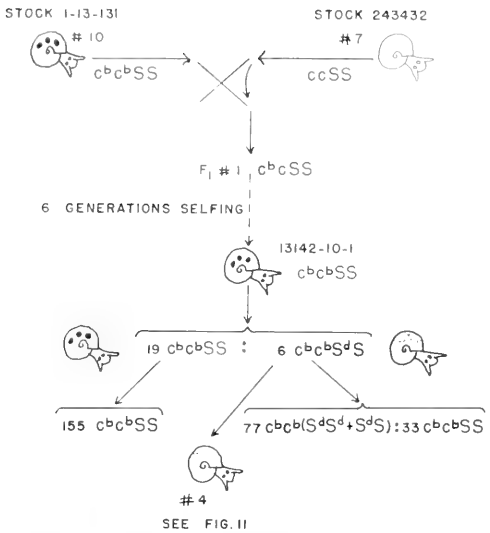


Fig. 10. Diagram illustrating origin of the mantle pigment coalescence mutants.

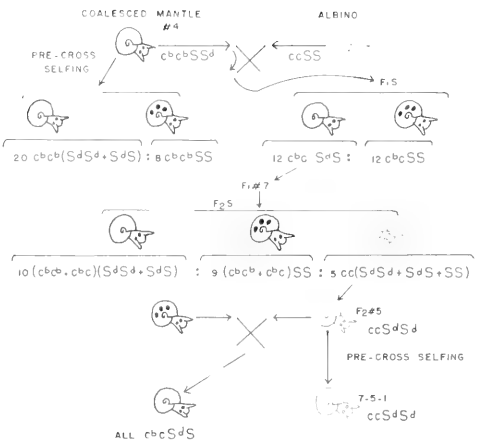


FIG. 11. Diagram of crosses to determine the method of inheritance of the mantle pigment coalescence character.

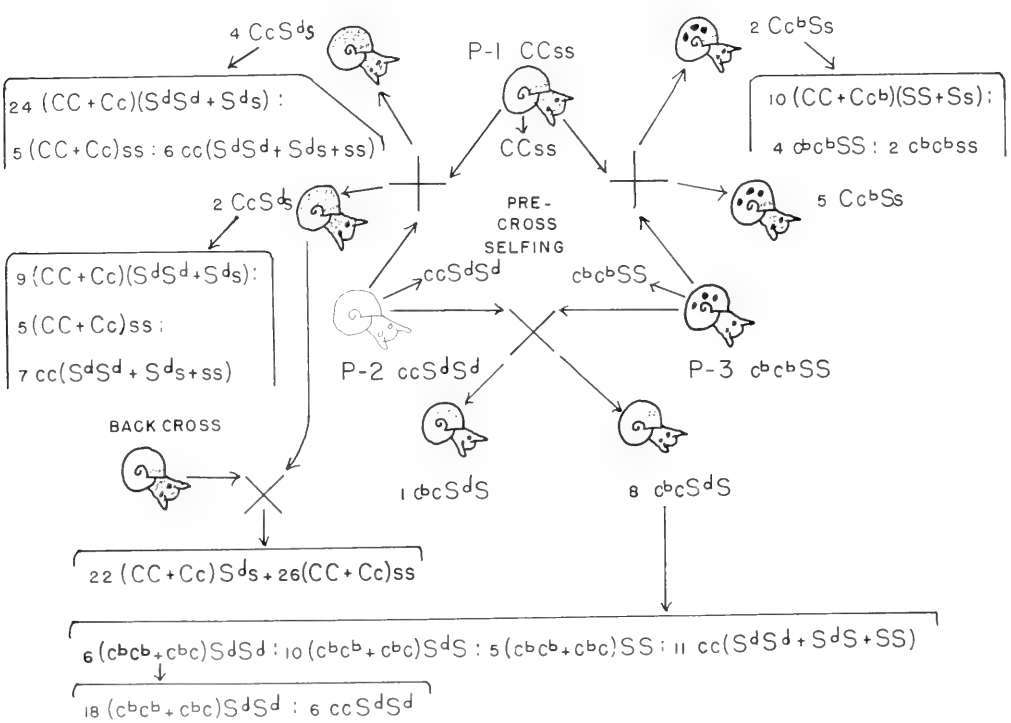


FIG. 12. Diagram showing mating results when three *Biomphalaria glabrata* were maintained together for ten days and then re-isolated. The three snails (CCss, c^bc^bSS, and albino 7-5-1 ccS^dS^d) involved six different pigmentation alleles, and the results demonstrated the inheritance of these pigment factors.

scored. Some snails were followed through additional generations. This and other crosses demonstrated that the coalescence allele S^d was not completely dominant over S. Although coalescence appeared complete in old S^dS snails, young S^dS heterozygotes could be distinguished from either S^dS^d of SS homozygotes by the combination of spots and coalescence. Scored progenies (118) of S^dS heterozygotes by selfing totaled 30 S^dS^d : 58 S^dS : 30SS.

DISCUSSION

The 6 original mutant snails (Fig. 10) all produced mixed progenies in the total ratio 77 c^bc^b (S^dS^d + S^dS) : 33 c^bc^bSS. Backcrosses between two of the snails and albinos produced hybrid progenies in the total ratio 32 c^bcS^dS : 32 c^bcSS. These results suggested that the 6 snails were all heterozygous for mantle pigmentation ($c^bc^bS^dS$). In retrospect, there is no way to be certain of the phenotype and genotype of the parent 1-3-1-4-2-10-1 snail. It is probable that had it developed coalesced mantle pigment, this would have been noted. If its genotype had been S^dS, the 19 SS : 6 S^dS ratio of its progeny would not be expected. If the parent was spotted c^bc^bSS, the 6 mutant progeny, all heterozygotes, could have been the result of a mutation in one cell in the ovotestis giving rise to a group of either sperm or egg gametes.

Crosses with wildtype snails demonstrated that the coalescence allele modified mantle pigment in wildtype as well as blackeye snails, but the trait is masked in the albino snails which lack black pigmentation.

Data presented in Table 1A provide a summary statistical analysis for crosses shown in Figs. 11 and 12 and for additional families

TABLE 1. Segregation and assortment of loci controlling basic pigmentation and mantle pigment pattern in *B. glabrata*. Expected values based on an assumption of Mendelian segregation are shown in parentheses.

A. Genetic segregation at a locus controlling mantle pigment pattern. Offspring are the product of self-fertilization in isolated snails.

Parental phenotype	Genotype	Offspring SS	Offspring SS^d	Offspring S^dS^d	X^2
Discrete Spots	SS	155			
		32			
		18			
		11			
Coalesced Mantle	S^dS^d			16	
				14	
				23	
Spots/Coalesced	SS^d	5(5.25)	5(10.50)	11(5.25)	9.028
		7(7.75)	19(15.50)	5(7.75)	2.117
		3(3.75)	9(7.50)	3(3.75)	0.637
		8(6.50)	11(13.0)	7(6.50)	0.692
Total		23(23.25)	44(46.50)	26(23.25)	0.451
					X^2 hom = 8.577
					DF hetero. = 6
					X^2 N.S. at 0.05

B. Simultaneous segregation and assortment of loci controlling basic pigmentation and mantle pigmentation pattern. Phenotypic expectations based on an assumption of independent assortment are given in parentheses. A X^2 test of goodness of fit for observed values is also shown.

Parental snails	Offspring Phenotype									
	CS	CSS^d	CS^d	Cs	c^bS	c^bS^dS	c^bS^d	c^bs	c	X^2
CcS^ds(selfed)			24(19.688)	3(6.533)					6(8.75)	2.176
CcS^ds(selfed)			9(11.812)	5(3.936)					7(5.250)	1.520
Cc^bSs(selfed)	10(9.00)				4(3.00)			2(4.00)		1.444
c^bcSS^d(selfed)					2(2.026)	4(4.125)	1(2.062)		4(2.75)	1.098
C^bcSS^d(selfed)					5(6.00)	10(12.00)	6(6.00)		11(8.00)	1.625

which were not included in the illustration. Genetic control of mantle pigment pattern is amply demonstrated. Homozygous individuals produce only homozygous offspring and the ratio of progeny of selfed heterozygotes approximates the 1:2:1 ratio expected for codominant alleles at a gene locus.

The occurrence and ratios of three F_2 phenotypes (including albinos) in Fig. 11 and text suggested that the mantle pigment gene was probably on a different chromosome from that determining albinism. Crosses (including selfing) involving segregation at the two loci are summarized in Table 1B. The X^2 statistic was used to test the fit of the data to expectation based on independent assortment of the loci. In all cases, the data are consistent with this hypothesis, and the two pigment loci are assigned to independent linkage groups.

Mating between an F_2 albino (Fig. 11) and a normally spotted blackeye snail indicated one albino No. 7-5 was homozygous (S^dS^d) for pigment coalescence.

B. glabrata has many pigmentation variations and inheritance is complex. In a previous study on mantle pigmentation (Richards, 1969), it was concluded that spotting was dominant over lack of spotting, but that this variation was determined by multiple genetic factors. The "unspotted" snail lines were derived by several generations of selection from stocks with relatively few mantle spots. The spotted and unspotted stocks provided by Mr. Stewart showed single factor inheritance apparently being homogenic for other mantle pigment factors. Results depicted in Fig. 12 demonstrated that mantle pigment coalescence (S^d), discrete spotting (S), and lack of mantle pigment(s) are three alleles of the same gene. The S^d and S alleles are both dominant over the s allele. Young S^dS heterozygotes can usually be distinguished from S^dS^d or SS homozygotes. An S^dS heterozygote reproducing by selfing should produce progeny in the ratio 1 S^dS^d : 2 S^dS : 1 SS. A total of 93 offspring of snails identified as S^dS heterozygotes were scored: 23 S^dS^d : 44 S^dS : 26 SS. Combinations of these mantle pigment factors and the basic pigment factors C, c^b, and c result in 9 phenotypes (Figs. 1-9).

Coalesced mantle pigmentation, determined by the S^d allele, is initiated and can be recognized in very small juvenile snails. The expressivity of "diffuse" pigmentation described by Richards (1969) is apparently influenced by environmental conditions as well as other genotypic factors and develops later in the snail's life. Histologic studies indicate that S^d mantle coalescence involves black pigment in mantle epithelial cells, as with mantle spotting.

With the range of pigment markers now available, planned experiments involving multiple matings and serial matings with varying time intervals could provide information on the dynamics of cross-fertilization in populations of *B. glabrata*.

Six characters in *B. glabrata,* each determined by a single gene pair, have been described (Richards, 1973b, 1980). Mantle pigmentation variation described here constitutes a seventh such character. Studies to date have failed to demonstrate linkage between any of these characters, so they are tentatively considered markers for seven linkage groups.

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The cooperation of Mr. Walter Stewart in providing the *B. glabrata* lacking mantle pigment, and information gained from discussions with him during these studies, are greatly appreciated. Review of the manuscript by Dr. Philip T. LoVerde, Dr. David S. Woodruff, and Dr. Margaret Mulvey, provision of statistical analyses by Dr. Mulvey, and the technical assistance of Mr. Paul C. Shade and Mr. Thomas A. Hallack are gratefully acknowledged. These studies were funded in part by Office of Naval Research Contract N1.N00014-78-C-0081.

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POPULATION ECOLOGICAL ASPECTS OF THE EULIMID GASTROPOD *VITREOBALCIS TEMNOPLEURICOLA*[1]

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ABSTRACT

The parasitic life history of the eulimid gastropod *Vitreobalcis temnopleuricola,* parasitic on the sea urchin *Temnopleurus toreumaticus,* has been studied at five subtidal stations around Mukaishima Island. Rates of infestation vary with the population size of the host and with the season. Annually, the parasites begin to settle on the hosts in the autumn and grow exponentially at periodic intervals until the following summer. There was little relation between the infestation rate and the host size. A marked difference in parasite position according to parasite size was found; individuals of this species settle on the oral side near the peristome at first, subsequently migrating towards the ambitus, and lastly to the tube feet. An analysis of the aggregation pattern reveals that the parasites do not distribute at random among the hosts but in a negative binomial pattern.

INTRODUCTION

The superfamily Eulimacea (or Eulimoidea) is a large group of free-living and parasitic species. Consequently, it is an interesting taxon in which to study adaptive modifications. Seven familial taxa have been recognized in this superfamily, namely: Eulimidae, Stiliferidae, Entoconchidae, Thycidae, Pelseneeriidae, Paedophoropodidae, and Asterophilidae. The three first-mentioned families are closely related (Lützen & Nielsen, 1975; Ponder & Gooding, 1978), but the phyletic interrelations of the other families are still unclear. Grusov (1965), in his histological study of *Asterophila,* proposed uniting the six families, with the exception of Entoconchidae, into a single broad family, Melanellidae (= Eulimidae) s.l. Warén (1980a, b, 1981) supported this concept in his comprehensive taxonomical study of this family.

Lützen and his colleagues have greatly contributed to the knowledge of the taxonomy, anatomy, and histology of this family (Lützen, 1972a, b, 1976; Gooding & Lützen, 1973; Lützen & Nielsen, 1975). Ponder & Gooding (1978) gave a good review of this family. Habe (1952, 1976) studied the classification of this group and reported about 40 species from Japanese waters. Morton (1976, 1979), Elder (1979), and Lützen (1979) contributed ecological studies on species of *Balcis* and *Mucronalia, Thyca,* and *Enteroxenos,* respectively. Nevertheless, compared with the many anatomical and taxonomical discussions, there are very few practical reports on the population ecology of eulimids. The probable reasons for this are that the population sizes of eulimids are usually small; also, the animals are small in size.

The present species, *Vitreobalcis temnopleuricola* Fujioka & Habe (1983), is found in the Seto Inland Sea of Japan exclusively parasitizing the sea urchin *Temnopleurus toreumaticus* (Leske), which inhabits the sheltered subtidal zone around the southwestern Japanese waters. The present contribution is concerned with basic knowledge of the distribution and growth of this species.

Dr. A. Warén (University of Göteborg), on the basis of some morphological characters of shells, considered that there are two species in my samples. However, judging from the continuity of such characters and ecological knowledge, I believe that they belong to a single species. Voucher specimens for the present study are deposited in Mukaishima Marine Biological Station, Hiroshima University (No. 3412013).

[1]Contribution from the Mukaishima Marine Biological Station. No. 218.

SURVEY AREA AND METHODS

The field survey and collection were carried out at five stations around Mukaishima Marine Biological Station (34°22′N, 133°13′E), in the northwest area of Bingo-nada, central part of the Seto Inland Sea of Japan (Fig. 1). St. 1 has sunken rocks with surrounding fine gravel bottom. Water depth (below the mean tidal level) ranges from 4–9 m. St. 2 has sandy bottom at depths less than 7 m and muddy bottom at greater depths. St. 3 is about 4–5 m deep and has spacious sandy to fine gravel bottom. St. 4 and St. 5 are exposed rocky shores, near which currents run strong.

The host sea urchin, *Temnopleurus toreumaticus,* inhabits various kinds of substratum. Larger populations of this species are found on fine gravel bottom at 3–7 m depth or around rocks at 3–15 m depth, but the urchin is rarely distributed in an aggregated pattern. The maximum mean density of the urchins reached about 6 individuals per square meter at St. 3 in 1980. After the spring of 1981, however, population size of the urchins of this station decreased, possibly because of change in the environmental conditions caused by construction of a nearby artificial reef and seawall.

The monthly investigations were made diurnally using scuba for 40 minutes to 2 hours per station, from the end of June 1980 to November 1981. As far as possible, a full-air scuba tank was used for each station, but the number of urchins found was influenced by the population size and the water conditions such as the tidal current, the depth of water, and the turbidity.

After measuring the diameter of the test (horizontal axis) of each sea urchin, the number of parasites and their positions on the host were determined and recorded. From May to September these procedures were done *in situ* with the naked eye or by a ×5 magnifying glass. This method is important for avoiding artificial disturbance of the host population, and I believe no parasite was overlooked with this method in this season. Some parasites were removed from the host and collected in a polyethylene pouch for measurements of shell length, and the rest were left to maintain the parasite population. From October to April, however, there was a slight possibility of overlooking the parasites with this method. For this reason each urchin was collected in a separate polyethylene bag and carefully transported back to the shore laboratory and examined under a binocular microscope to locate small parasites.

The longitudinal shell axis was measured to the nearest 0.1 mm with the aid of a binocular microscope.

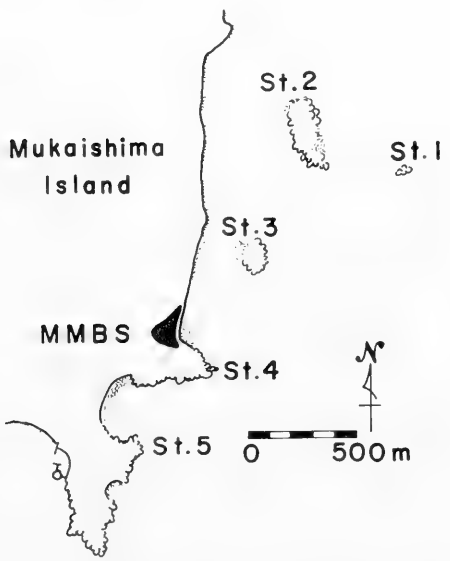

FIG. 1. A sketch map of the environs of Mukaishima Marine Biological Station (MMBS) showing the locations of stations studied.

RESULTS

Seasonal occurrence of parasites

Seasonal fluctuation in the percentage of sea urchins parasitized at each station is shown in Fig. 2. At the end of June 1980, 459 parasites were found from 300 urchins at St. 3 and the infestation rate reached 55%, which trend continued through the next month. There was a very large host population in this station at this time. In August the infestation showed a marked decline and the parasites were rarely found at all stations in September. In this season, a few dead shells were found on the substratum around the urchins at St. 3, but living specimens were never found away from the host throughout the present survey. In November 1980, the parasites appeared on the host again, resulting from new settlement in this season. From November to the next summer the infestation rate at Sts. 1, 2, 4 and 5 ranged from 5.3 to 21.1%. Mating took place on the tube feet from the end of June,

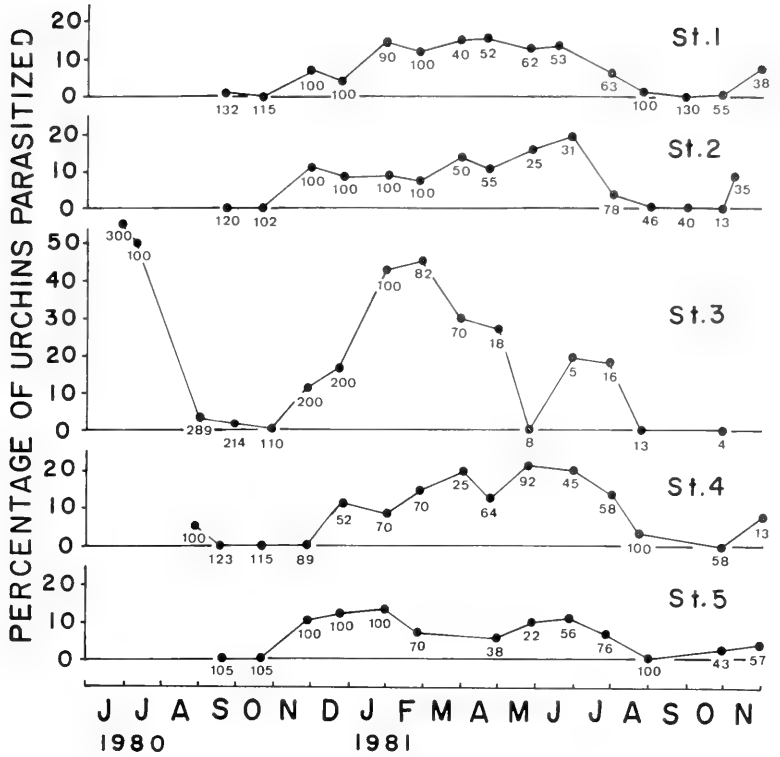

FIG. 2. Seasonal fluctuation in the infestation rate of *Temnopleurus toreumaticus*. The number under each plot indicates the number of urchins examined.

but only five cases were observed in the field and two cases in the laboratory. At St. 3 the rate increased to about 50% in January and February 1981, but after spring the rate decreased and was unstable due to the reduction in the host population.

The differences of the infestation rate from January to February 1981 between every two stations were not significant at Sts. 1, 2, 4, and 5, respectively, but were significant between St. 3 and each other station (Student's t-test, $P < 0.01$).

Growth of Vitreobalcis temnopleuricola

As far as the growth of *Vitreobalcis temnopleuricola* is concerned, there were no remarkable differences between the five stations examined. The combined seasonal change of the size frequency distribution of this species at five stations is shown in Fig. 3. In 1980, they showed gradual growth from June to August. Freshly settled individuals smaller than 1 mm shell length were continually collected in the period from November to early the next spring. They grew slowly in winter, rapidly after spring, and reached a length of 3–5 mm in July to September. The parasitic life on the urchin was terminated within about ten months. There was only one generation per year and the parasitic generations never overlapped each other. Fig. 4 shows the shift of the mean sizes and its standard deviations based on the results of Fig. 3. On the whole, the relationship is expressed by the following exponential formula:

$$SL = a \cdot \exp(0.1362x)$$

where a is the initial estimated size (Oct. 1980, 0.8661) and x is the month. The formula can be expressed as a straight line on a logarithmic scale (a correlation coefficient = 0.9474) and therefore the relative growth rate is constant.

The shell-length frequency in each month does not show a normal distribution and shows high frequencies on some specific values such as 0.9, 1.2, 1.7 mm, and so on (Fig.

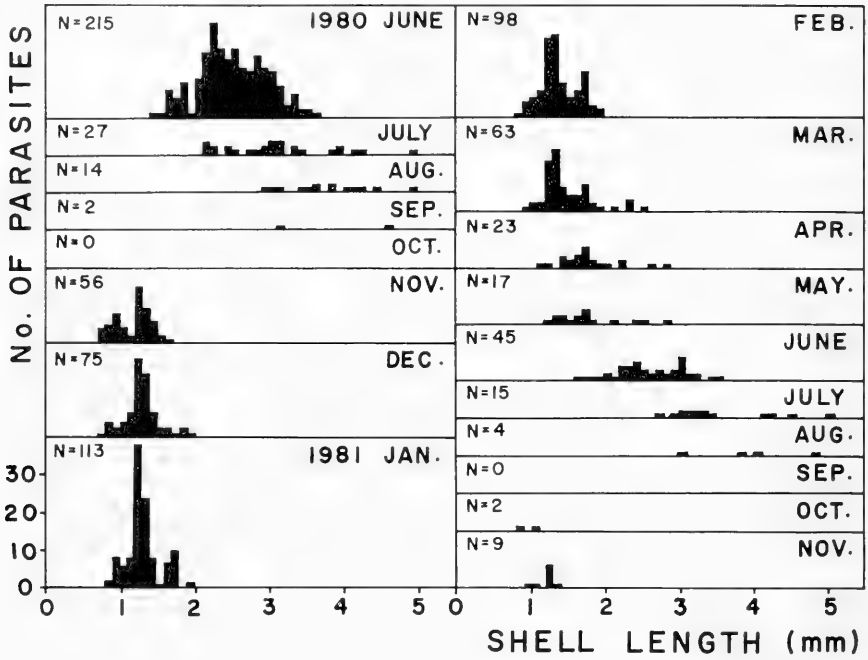

FIG. 3. Seasonal shell-length frequency distribution of *Vitreobalcis temnopleuricola.*

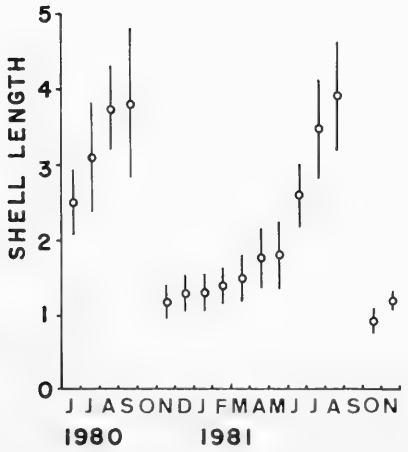

FIG. 4. Growth in mean shell-length of *Vitreobalcis temnopleuricola.* Bars indicate standard deviations. Sample sizes are shown in Fig. 3.

3). However, shell-length frequency according to the whorl number of the teleoconch shows a normal distribution as in Fig. 5. Such a tendency evidently appears in the small classes less than 4, but larger ones more than 5 do not correspond with a normal distribution (*e.g.* no. of teleoconch whorls 5, χ^2-test, $0.05 > P > 0.025$). Since there is no morphologically apparent sexual dimorphism in this species, this abnormality may be due to large variation of the growth pattern.

The first 3–3.5 whorls of this species constitute the larval shell and the succeeding teleoconch whorls consist of almost 7.5 whorls in fully grown specimens. On every whorl of the teleoconch there is a microscopic longitudinal line at nearly an identical position (see Fig. 6) which corresponds with the peak of the normal distribution shown in Fig. 5. These results suggest that the body whorl of the teleoconch is not always formed at a uniform rate but at periodic intervals. In other words, the resting stage of formation is represented by the longitudinal line. In fact, 93% of the specimens collected were in this resting stage.

The relationship between infestation rate and host size

Roughly speaking, this sea urchin breeds in the summer, recruits the population in autumn, reaches 20 mm or so in test diameter in the next summer, and attains adult size (more than 30 mm or so) the following summer.

In Table 1, the infestation rates are com-

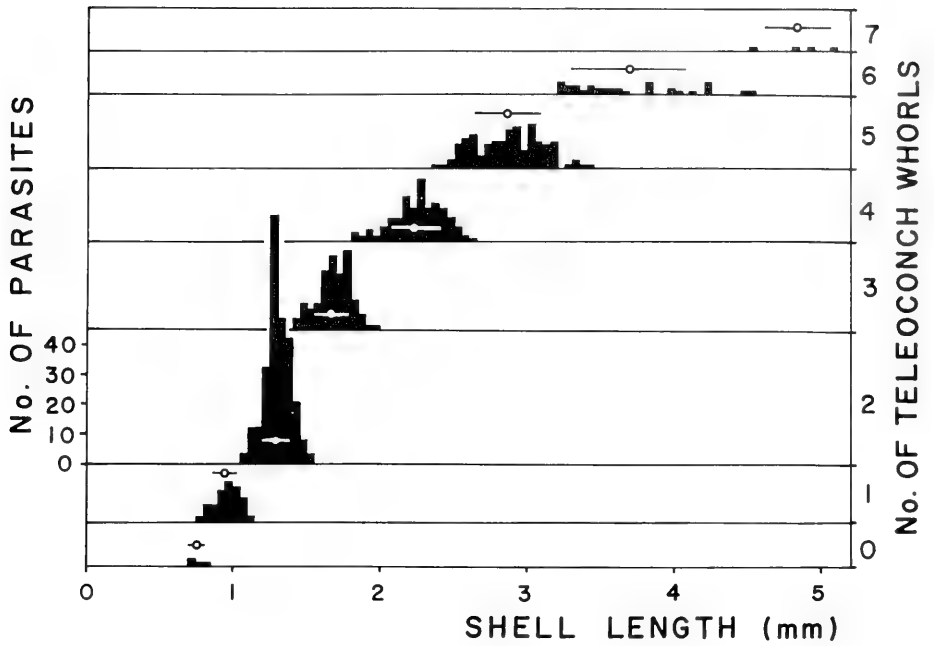

FIG. 5. Shell-length frequency distribution according to the number of teleoconch whorls of *Vitreobalcis temnopleuricola*. Teleoconch whorl 0 means the range from 0≦ to <1 (the same rule applies successively). Open circles indicate mean shell-length. Bars indicate standard deviations.

TABLE 1. Comparison between the infestation rate and the test diameter of *Temnopleurus toreumaticus*.

St. 1+2+4+5				St. 3			
Diameter of urchins (mm)	No. of urchins observed	No. of urchins infested	Infestation rate (%)	Diameter of urchins (mm)	No. of urchins observed	No. of urchins infested	Infestation rate (%)
<10	6	0	0.0	<10	1	0	0.0
10≦, <20	47	5	10.6	10≦, <20	2	0	0.0
20≦, <30	157	19	12.1	20≦, <30	77	32	41.6
30≦, <40	637	59	9.3	30≦, <40	471	193	41.0
40≦	291	29	10.0	40≦	43	19	44.2

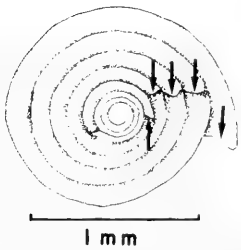

FIG. 6. An apical view of the shell. Arrows indicate the longitudinal lines of teleoconch.

pared according to the test diameter of *Temnopleurus toreumaticus* based on the results excepting August to November, which show the low infestation rate. St. 3 is separated from other stations because the population structure of urchins is somewhat different from that of the other stations. All size groups of urchins were parasitized except for several juveniles up to 10 mm test diameter. The smallest urchin parasitized measured 11.2 mm. There seems to be no available position for the parasites on small urchins up to 10 mm. There was no tendency for the

TABLE 2. Parasitic positions of *Vitreobalcis temnopleuricola* based on the divisions of Fig. 7. Surface areas expressed as a percentage of total surface areas are shown in the bottom row.

No. of teleoconch whorls of parasites	Position on sea urchin (percentages for each whorl class in parentheses)													
	I	II			III			IV			V			VI
		IZ	AZ	TF	IZ	AZ	TF	IZ	AZ	TF	IZ	AZ	TF	
0	0	2 (50.0)	1 (25.0)	0	0	1 (25.0)	0	0	0	0	0	0	0	0
1	0	20 (39.2)	14 (27.5)	2 (3.9)	7 (13.7)	4 (7.8)	0	2 (3.9)	2 (3.9)	0	0	0	0	0
2	0	81 (34.9)	45 (19.4)	10 (4.3)	47 (20.3)	22 (9.5)	8 (3.4)	6 (2.6)	5 (2.2)	1 (0.4)	3 (1.3)	0	4 (1.7)	0
3	0	26 (27.4)	16 (16.8)	8 (8.4)	20 (21.1)	8 (8.4)	8 (8.4)	3 (3.2)	0	4 (4.2)	0	2 (2.1)	0	0
4	0	3 (10.0)	3 (10.0)	4 (13.3)	4 (13.3)	3 (10.0)	8 (26.7)	1 (3.3)	1 (3.3)	1 (3.3)	0	0	2 (6.7)	0
5	0	1 (3.3)	0	4 (13.3)	4 (13.3)	1 (3.3)	17 (56.7)	0	0	2 (6.7)	0	0	1 (3.3)	0
6	0	0	0	4 (19.0)	0	1 (4.8)	12 (57.1)	0	1 (4.8)	1 (4.8)	0	0	2 (9.5)	0
7	0	0	0	1 (25.0)	0	0	3 (75.0)	0	0	0	0	0	0	0
Percentage of total surface area	(3.8)	(7.5)	(3.3)	(1.8)	(13.0)	(5.7)	(3.2)	(12.4)	(5.5)	(3.0)	(23.6)	(10.3)	(5.7)	(1.3)

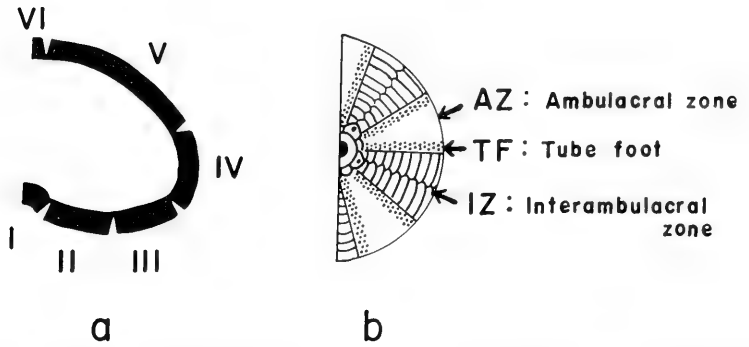

FIG. 7. Diagram showing the divided areas on the urchin in order to examine the placement of *Vitreobalcis temnopleuricola* on its host. a: Longitudinal section of the urchin showing the six horizontal zones used in this study. b: Dorsal view of the urchin showing the longitudinal surface elements.

parasites to select a specific size of urchin larger than 10 mm. Therefore, the infestation frequency is very loosely related to the size of the host.

Parasitic position on host

The foot is well developed and functional for crawling, but plays no role for maintaining the position of this parasite. Instead, the extendible proboscis and the pseudopallium, which is developed around the base of the proboscis, are used for attaching the animal to the various parts of the host, such as spines, the tube feet, the surface of the test, and rarely the stalk of globiferous pedicellariae (only two cases). They were not found attached to the gill or the buccal tube foot.

The proboscis of the parasite did not penetrate the plates of the host and did little damage to the skeletal part of the spines. The epithelial tissue at the parasitized position was very loose, but the apparent tissue removal was not observed.

In order to examine the parasite's position on the host, a longitudinal section of the urchin was divided into six horizontal zones (I–VI) and each zone was divided into three longitudinal elements, the interambulacral zone (IZ), the ambulacral zone excepting the tube foot (AZ), and the tube feet (TF), as shown in Fig. 7. The results are shown in Table 2 for every teleoconch whorl number. The surface area occupied by each division was measured on 10 urchins and the mean percent shown in the bottom row of Table 2. If the parasites distribute randomly on the host, it would be expected that the parasites were collected with the approximate percentage of the surface area of the host. However, the actual distribution never agreed with these percentages. Many parasites were distributed on the oral side of the host. Moreover, the following three tendencies are recognizable: (1) the parasites were never found on the peristome and periproct, (2) as they grew, they were found to migrate radially from the oral side near the peristome toward the ambitus, and (3) as they grew, they migrated from the area overspread by the spine to the tube foot.

To verify statistically tendencies (2) and (3) a χ^2-test was based on the results shown in Table 2. Every adjacent two whorl classes of parasites are combined as shown in Table 3a (for tendency (2)) and Table 4a (for tendency (3)) because this test required that the expected frequencies in each cell should not be too small. In addition, zones IV and V are also combined and zones I and VI are excluded for the same reason. The values of χ^2 were calculated for every combination of whorl classes and given in Table 3b and Table 4b, respectively. In Table 3b, lower values were obtained with neighboring whorl classes except the test between whorl classes 2–3 and 4–5, and higher values with distant whorl classes. Therefore, there is a general trend for the parasites to migrate radially on the host with growth. In Table 4b, the tendency is more clear than in Table 3b. It is evident that the parasites migrate from the ambulacral and the interambulacral zones to the tube foot with growth.

TABLE 3. Differences of parasite position on *V. temnopleuricola* with snail growth. a: Comparison between the whorl classes of parasites and the horizontal zones on the host. b: Results of χ^2-test (DF = 2).

a

Whorl class	II	III	IV + V	Total
0–1	39	12	4	55
2–3	186	113	28	327
4–5	15	37	8	60
6–7	5	16	4	25

b

2–3	4.00		
4–5	24.57*	20.77*	
6–7	18.15*	12.74*	0.29
Whorl class	0–1	2–3	4–5

*Significant difference ($P < 0.005$).

TABLE 4. Differences of parasite position on *V. temnopleuricola* with snail growth. a: Comparison between the whorl classes of parasites and the longitudinal elements on the host. b: Results of χ^2-test (DF = 2).

a

Whorl class	IZ	AZ	TF	Total
0–1	31	22	2	55
2–3	186	98	43	327
4–5	13	8	39	60
6–7	0	2	23	25

b

2–3	5.13		
4–5	47.17**	81.70**	
6–7	62.93**	95.11**	7.60*
Whorl class	0–1	2–3	4–5

*Significant difference ($P < 0.05$).
**Significant difference ($P < 0.001$).

Parasite incidence amongst the hosts

Analysis of the number of individuals of *Vitreobalcis temnopleuricola* per host reveals that large numbers of specimens had no parasites and a small number of specimens had large numbers of parasites, up to 25. In order to judge the parasite incidence amongst the hosts, the observed frequencies and the expected frequencies calculated by negative binomial probabilities and by Poisson probabilities are given in Table 5. They are divided into five categories on the basis of the differences in rate of infestation and season. Bliss' (1956) method was employed for computing the parameter "a common *k*" of the negative binomial distribution (a common *k* is present in all categories, $P < 0.05$). In each of the five categories the observed frequencies agree with the negative binomial probabilities rather than with the Poisson ones, and the χ^2-test shows good agreement between the observed and the negative binomial distributions. Hence, the parasites are not distributed at random, but are clustered. This situation continues regardless of the season.

DISCUSSION

Among the five stations around Mukaishima Island the infestation rate of St. 3 exhibits a higher percentage than that of the four other stations (Fig. 2). Gooding & Lützen (1973) studied *Robillardia cernica* parasitic in the rectum of the sea urchin *Echinometra* and found that the highest rate of infestation occurred at the mid-tide level of the intertidal zone. Elder (1979), in his study of *Thyca crystallina* parasitic on the blue starfish *Linckia laevigata,* determined that the infestation rate varies directly with the degree of water movement. Hoberg *et al.* (1980), in their examination of the endoparasitic gastropod *Asterophila japonica,* treated the parasite-host spatial distribution, but gave no detailed information about the influence of environmental factors. The present species, *Vitreobalcis temnopleuricola,* inhabits the subtidal zone where the influence of vertical components such as tidal level and water depth are not important. Although the water movement seems to be high at Sts. 4 and 5, there is no direct relation between the infestation rate and water movement. At St. 3 the host urchin formed the highest density population until the winter of 1981. *V. temnopleuricola* may have a pelagic larval stage because the parasites are not distributed at an aggregated pattern even in the recruit season. Therefore, it is possible that the larger the population of the host becomes, the more effectively the larva settles. The effect of chemical in-

TABLE 5. Comparison of the observed frequencies of urchin for different numbers of parasites with the expected frequencies calculated from the negative binomial and the Poisson distributions.

St. 1+2+4+5

No. of parasites per host	No. of hosts observed	Expected frequencies negative binomial	Expected frequencies Poisson
XI'80–II'81		(k_c = 0.1208)	
0	1217	1225.5	1161.8
1	97	82.4	176.1
2	25	25.7	13.4
3	6	10.1	0.67
4	2	4.39	0.03
5≦	5	2.98	0.00
		χ^2 = 4.35 < 5.99 (P = .05, DF = 2)	
III'81–V'81		(k_c = 0.3444)	
0	454	453.4	437.3
1	53	54.2	80.0
2	12	12.6	7.31
3	5	3.42	0.45
4≦	1	1.43	0.02
		χ^2 = 0.33 < 3.84 (P = .05, DF = 1)	
VI'81–VIII'81		(k_c = 0.1644)	
0	609	601.1	582.0
1	33	42.8	73.2
2	13	10.8	4.61
3	2	3.38	0.19
4	0	1.16	0.01
5≦	3	0.68	0.00
		χ^2 = 2.81 < 3.84 (P = .05, DF = 1)	

St. 3

No. of parasites per host	No. of hosts observed	Expected frequencies negative binomial	Expected frequencies Poisson
VI'80		(k_c = 0.4669)	
0	135	152.2	65.0
1	70	54.5	99.4
2	33	30.6	76.0
3	25	19.3	38.8
4	10	12.8	14.8
5	10	8.76	4.54
6	3	6.12	1.16
7	3	4.33	0.25
8≦	11	11.45	0.06
		χ^2 = 11.03 < 12.59 (P = .05, DF = 6)	
I'81–II'81		(k_c = 0.5487)	
0	102	103.0	67.0
1	39	36.5	67.0
2	17	18.2	33.5
3	9	10.4	11.2
4	7	5.95	2.79
5	2	3.50	0.56
6	2	2.09	0.00
7	2	1.26	0.00
8≦	2	1.09	0.11
		χ^2 = 0.63 < 7.81 (P = .05, DF = 3)	

teractions in larval settling, if any, is a subject for future study.

Morton (1979) reported that *Mucronalia fulvescens* and *Balcis shaplandi* are both ectoparasitic on the starfish *Archaster typicus,* and that they show a biannual reproductive pattern and leave the host in the late summer. Thus it appears that seasonal factors are necessary to explain the differences of the infestation frequencies. In the present study, parasites were rarely found on or in the vicinity of hosts in September and October. It is clear that there is one generation per year and that growth occurred from autumn to the next summer. Generally speaking, growth of most mollusks corresponds with a sigmoid curve (Wilbur & Owen, 1964), but the present species grows exponentially. Thus the plateau phase toward the end of the life span is not observed. It is suspected that absence of a plateau has been caused by either the death of parasites or migration from the hosts to other substrates until oviposition, although there is no reliable information about the behavior after mating.

The attachment positions of ectoparasitic eulimids are usually specific for each species (*e.g.* Habe, 1952, 1976; Lützen & Nielsen, 1975; Lützen, 1976; Morton, 1976; Warén, 1980a, b, 1981). However, Elder (1979) found that the position changed with growth of *Thyca.* He demonstrated that the juvenile *Thyca* is restricted to the aboral surface of *Linckia* and the adults to the oral surface. Elder also discussed either positive geotactic of negative phototactic behavior of the ju-

veniles. Then the adults migrate to a more favourable site for proboscis penetration.

It was found in the present study that *V. temnopleuricola* also migrates with increasing shell length (Table 2). The species has a tendency to settle on the oral side near the peristome at first, be radially oriented toward the ambitus, and finally to migrate to the tube foot. Since the space associated with the tube feet is large and the surface area of the ambulacral and interambulacral plates of the ambitus are larger than those of the oral side, it is believed that the parasites migrate with growth to larger and more favourable sites for parasitism. In other words, the parasite position is restricted spatially by the external appendages of the host.

In the Aglossa, the radula has degenerated and a long acrembolic proboscis is developed for suctorial feeding. Examples of ectoparasite proboscises perforating the plates or skin of echinoderms have been reported by Fretter (1955) for *Balcis devians* and *B. alba,* by Bacci (1948, cited by Fretter, 1955) for *Melanella comatulicola,* by Baer (1952, *ditto*) for *Mucronalia mittrei*, by Bartsch (1907, cited by Lützen, 1972b) for *Eulima ptilocrinicola,* by Lützen & Nielsen (1975) for *Echineulima,* by Elder (1979) and Warén (1980a) for *Thyca*, and so on. The proboscis of *Balcis peronellicola* Kuroda & Habe also deeply penetrates the perivisceral cavity of the laganid, *Peronella japonica* Mortenson (personal observation). Fretter & Graham (1962: 259) suggested that the proboscis of *Balcis alba* passes into the body of the host, perhaps seeking the gonad. Morton (1976) mentioned that *Mucronalia fulvescens* and *Balcis shaplandi* draws the fluid contents from the tube feet and coelom of host, respectively. *Pulicicochlea (Pseudoretusa) faba* also feeds on the host's body fluids (Ponder & Gooding, 1978). On the other hand, *Pelseneeria* and *Pulicicochlea* s.s. are considered to digest the epithelium of the body of echinoids (Fretter & Graham, 1962: 255; Ponder & Gooding, 1978). The proboscis of the present species, *V. temnopleuricola,* does not penetrate the plates of the hosts, and many specimens were collected from the spines, attaching superficially. Therefore, it is believed that the species is a parasite of the epithelial tissue, and feeds on it slowly. Such a mode of attachment and feeding are considered the most primitive ectoparasitic form.

The analysis of the aggregation pattern revealed that the parasites do not distribute at random amongst the hosts but in a negative binomial pattern (Table 5). Elder (1979) obtained similar results on *Thyca* and mentioned that the pattern was caused by "the accumulation of repetitive waves of random infestation." This same interpretation can be applied to the present results because the recruitment of parasites extends for a long period and the large number of solitary individuals suggest negation of the presence of pheromone among the individuals when they settle onto the host.

The parasites seldom migrate from the host to other substrata or another host. This is evident by reason of the facts that (1) the living specimen was never found off the host, (2) some specimens were collected in the growth stage of shell formation (they do not migrate even in this time), and (3) negative binomial distribution is observed throughout the parasitic life. As there are large numbers of solitary individuals, however, it is possible to migrate from one host to another only for a short period during reproduction.

In the present study we dealt only with the post-larval parasitic period. Examination of the life span from breeding to settlement is a subject for future study.

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QUANTITATIVE ASPECTS OF LOCOMOTION BY THE MUD SNAIL *ILYANASSA OBSOLETA*[1]

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ABSTRACT

The ciliary-powered locomotion of the marine mud snail *Ilyanassa obsoleta* was quantified for animals exhibiting positive phototaxis under controlled conditions. The absolute rate of locomotion was approximately 2 mm/sec for snails of about 18 mm total shell length. The maximum speed attained was 3.3 mm/sec. Although the absolute rate of locomotion was positively correlated with shell length, relative speed (% shell length moved/sec) was markedly negatively correlated with snail size. Morphometric analysis of pedal morphology indicated that pedal surface area, which increases with shell length, was closely associated with the absolute speed of mud snails. Although snail speed was more than 50% faster on glass than on sand, the presence of a conspecific's mucous trail as substratum upon which to crawl had no effect on this gastropod's locomotion. Maintenance of *Ilyanassa* in the laboratory for as few as three weeks from the time of collection resulted in a 50% reduction in speed of locomotion and a significant decrease in this snail's overall activity.

INTRODUCTION

The marine mud snail *Ilyanassa obsoleta* (Say, 1822) is typical of many prosobranchs inhabiting soft substrata (Miller, 1974a; Palmer, 1980) in that it employs cilia as its principal mechanism of locomotion (Copeland, 1919). Although many aspects of gastropod locomotion have been examined, including classification (Miller, 1974b), functional morphology (Miller, 1974a; Gainey, 1976; Lawrenz-Miller, 1977), functional significance (Linsley, 1978; Palmer, 1980), and energetic relationships (Calow, 1974; Denny, 1980; Houlihan & Innes, 1982), little quantitative information on ciliary locomotion exists.

In addition to being responsive to a variety of sensory stimuli (Crisp, 1969; Dimock & Parno, 1981), *I. obsoleta* can detect and subsequently pursue a trail of mucus deposited by a conspecific (Trott & Dimock, 1978). While some understanding of the mechanisms involved in mucous trail-following has been achieved (Bretz & Dimock, 1983), the functional significance of this behavior is not yet known. It may well be that trail-following is related to quantitative or energetic parameters of locomotion, since ciliary locomotion also involves mucus. Thus, the present study was undertaken to quantify the locomotion of *I. obsoleta.* The interrelationships among snail size, morphometry of the foot and the rate of crawling have been examined. In addition to being determined for snails crawling on glass, snail speed has also been quantified for animals traversing sand, both in the presence and absence of a conspecific's mucous trail. The behavioral consequences of prolonged maintenance of mud snails in the laboratory are shown to include significant changes in this gastropod's overall activity and rate of locomotion.

MATERIALS AND METHODS

All *I. obsoleta* were collected from the Newport River marshes at Morehead City, North Carolina, and were held in aquaria (30–32‰ S, 20–22°C) with salt-marsh mud, but were not given additional food. Experiments were performed between six and eight days after the animals were collected, except as described below. The behavioral studies were conducted between 1000 hr and 1800 hr at 22–24°C with animals in 31‰ S artificial sea water (Instant Ocean Sea Salts). Each snail was used in only one experiment and was

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measured to 0.1 mm (total length from anterior edge of aperture to tip of spire) with vernier calipers after it was used. Except as otherwise specified all snails were from 16.6–18.2 mm in total shell length.

The positive phototaxis of *I. obsoleta* (Dimock & Parno, 1981) was employed in an effort to standardize the stimulus conditions under which parameters of locomotion could be examined. Locomotion was monitored in a chamber (130 × 15 × 15 mm) constructed of clear 3 mm lucite into which a glass slide could be placed to form a removeable floor. The chamber was illuminated horizontally from one end with a 1 cm^2 beam of 5×10^{-2} $\mu E/m^2/sec$ intensity of 500 nm light, following the methods of Dimock & Parno (1981).

In a typical trial a light-adapted snail was introduced into the chamber with its anterior toward the light source. Since *Ilyanassa* is strongly positively phototactic in this light regimen, at least 90% of the snails that moved at all moved directly toward the light. In most experiments an animal's rate of locomotion (mm/sec) was calculated by timing with a stopwatch its progression over a 4 or 5 cm path mid-way in the chamber. Snails less than 10 mm were timed over a 2 cm path. Any snail which failed to move or to traverse the prescribed distance within 2 min was scored as no response. The slowest snail that reached the criterion took 95 sec, but most did so in <45 sec.

The absolute rate of locomotion on glass was determined for 64 *I. obsoleta* of $\bar{X}$ shell length = 17.5 mm. The relationships between size and rate of locomotion were determined for an additional 31 snails (shell length = 7–20 mm) which were also tested separately on detergent-cleaned glass slides.

Analyses of the morphometry of the foot of *Ilyanassa* were accomplished using photographic reproductions of the feet of 20 snails (shell length = 9.1 – 18.3 mm). The photographic technique and its effectiveness for determining parameters of pedal morphology have been described (Dimock, 1984). Briefly, the procedure involved photographing the ventral surface of each snail as it crawled vertically up the side of a glass aquarium. Identically enlarged images of the snails' feet and of standard units of area (graph paper) were cut out of the photographs. Linear dimensions of the foot were determined by direct measurement of the respective photographs, with length being the longest anterior-posterior dimension and width being the maximum width exclusive of the antero-lateral horns of the propodium. The area of the foot was calculated by comparing the weight of an image of the foot to the weight of a standard unit of photographic area.

The wet weight in air (including the shell) was determined for all snails used in the size-rate analyses. The weights of 20 additional snails (shell length = 8 – 17 mm) were measured for animals suspended both in air and in sea water (31‰ S) to permit the determination of immersed weight.

The effects of the type of substratum on mud snail locomotion were assessed under three conditions for snails of mean shell length = 17.5 mm. First, a snail was timed crawling on a glass slide in the test chamber. Second, a different animal was timed as it crawled upon the surface of about 5 mm of clean sand (particle size <0.25 mm) that had been layered over a clean slide in the chamber. Finally, another snail was timed as it moved toward the light on the mucous trail that had been deposited by the snail which first traversed the sand. The dimensions of these snails relative to the size of the chamber insured that a snail crawled directly on the surface of the mucous trail.

The influence of the duration of maintenance in the laboratory on the locomotion and overall activity of *I. obsoleta* was assessed by determining the rate of crawling on clean glass for groups of snails that had been held in the laboratory for 1, 2, 3 and 4 weeks. The level of activity of these animals was determined by reference to their tendency to traverse the prescribed distance in the test chamber within the allotted time.

The data have been analyzed by one-way analysis of variance (ANOVA) and Student-Newman-Keuls multiple range test (SNK), as appropriate. Correlation between various parameters was assessed by Pearson's product-moment correlation (Zar, 1974). The data for the correlations of shell length with area of the foot and with the ratio of snail weight to area of the foot were first log transformed. The weight employed in the expression of snail weight/unit area of foot was the estimated immersed weight.

RESULTS

The absolute rate of locomotion of *I. obsoleta* (mean shell length = 17.5 mm) whose speed on glass was determined 6 or 7

days after being collected was 1.96± 0.49 mm/sec ($\bar{X}$ ± SD; N = 64). The maximum rate recorded for this experimental group was 3.3 mm/sec for a snail of 17.3 mm shell length. The absolute rate was positively correlated with shell length ($r = 0.375$, $df = 29$, $P = 0.038$; Fig. 1). However, the relative rate of locomotion (% shell length moved/sec) was significantly negatively correlated with snail size ($r = -0.833$, $df = 29$, $P < 0.001$; Fig. 2).

The analyses of the morphometry of the foot of *I. obsoleta* revealed several associations among shell length and the size and the shape of a snail's foot (Table 1). Not surprisingly, foot length (FL), foot width (FW), and, consequently, foot area (FA) were all positively correlated with shell length. There was no correlation between shell length and the ratio of FL/FW, which remained constant at approximately 2.0 over the range of snail size examined (Table 1). The rate of increase of the area of the foot as a function of shell length (Table 1) was significantly greater than that predicted from scaling relative to the square of shell length (slope of regression of log FA on log SL >2; t-test, $P = 0.027$).

The immersed weight of *Ilyanassa* was 53.0 ± 3.9% ($\bar{X}$ ± SD; N = 20) of a snail's weight in air. There was no correlation between shell length and the percent reduction of snail weight upon immersion.

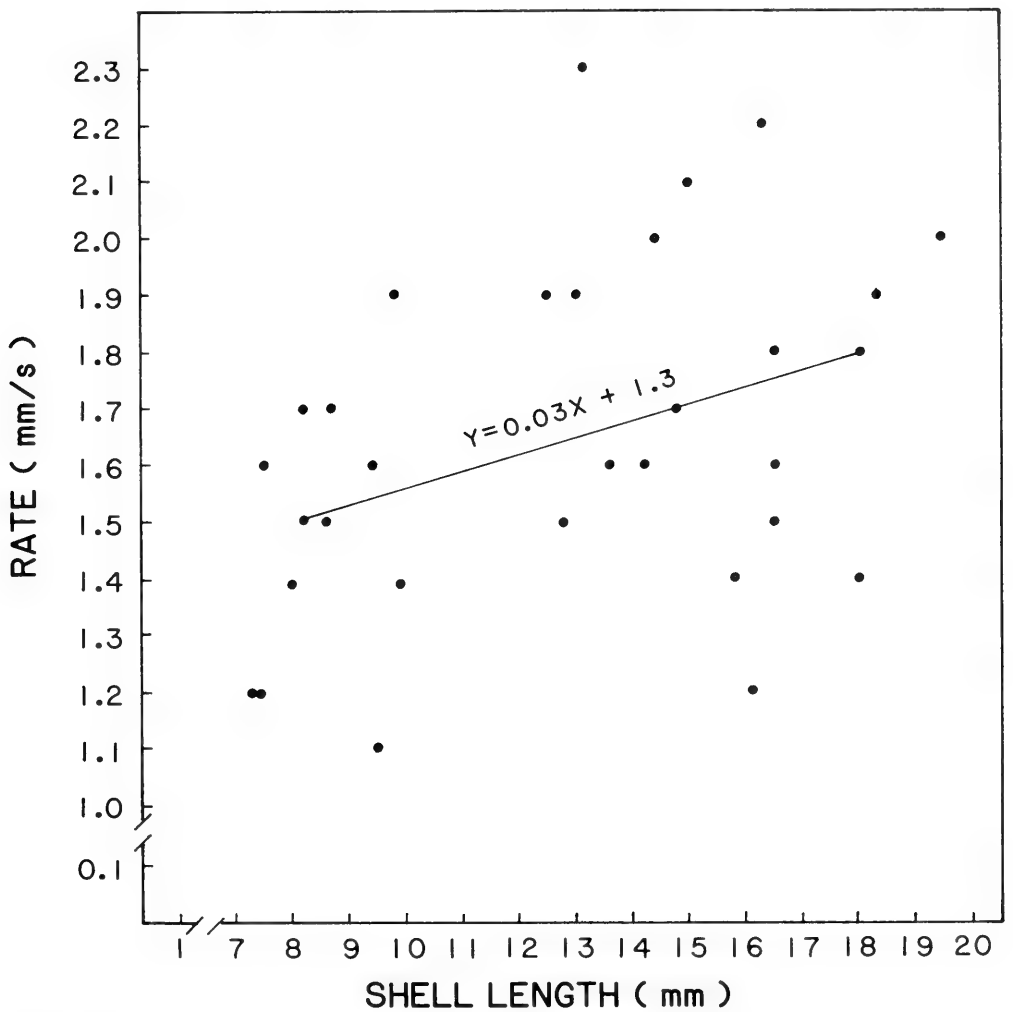

FIG. 1. The absolute rate of locomotion (mm/sec) of *I. obsoleta* as a function of shell length (mm).

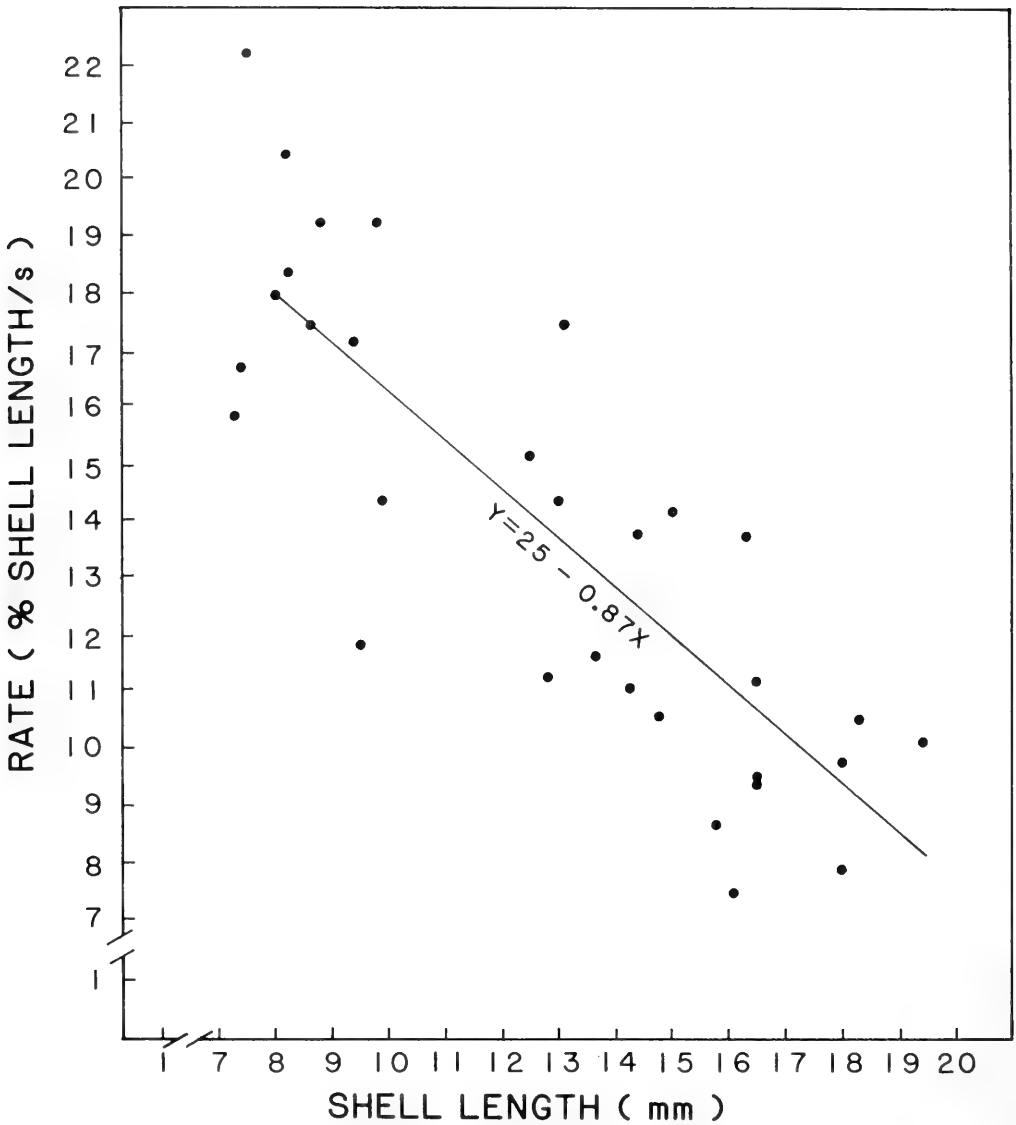

FIG. 2. The relative rate of locomotion (% shell length/sec) of *I. obsoleta* as a function of shell length (mm).

The immersed snail weight (SW)/unit area of foot (A) increased significantly with shell length ($r = 0.857$, $df = 29$, $P < 0.001$; Table 1). However, the slope of the regression of log immersed SW/log A on log SL was significantly smaller than the 3/2 ratio that would be predicted due to scaling (t-test, $P < 0.001$).

Mud snails crawling on glass were approximately 57% faster than snails crawling over clean sand (Table 2). The presence of a conspecific's mucous trail had no significant effect on the rate of a snail's locomotion over sand (Table 2). In fact, the rate of crawling of *I. obsoleta* was not significantly different when snails traversed from 0 to 4 mucous trails overlaid upon one another on sand (Dimock, unpublished observations). The speed of mud snails on glass also is not significantly affected by the presence of a conspecific's mucous trail (L. Styers, unpublished observations).

The rate of locomotion of mud snails de-

TABLE 1. The association of shell length with morphometric parameters of the foot of *Ilyanassa obsoleta*.

Variables	Least-squares regression equation	r^2	df	P
X = SL[1] Y = FL[2]	Y = 0.92X − 1.13	0.925	18	<0.001
X = SL Y = FW[3]	Y = 0.43X − 0.14	0.924	18	<0.001
X = SL Y = FL/FW	Y = 2.04 − 0.002X	0.021	18	n.s.
X = log SL Y = log FA[4]	Y = 2.23X − 0.83	0.957	18	<0.001
X = log SL Y = log SW/log A[5]	Y = 0.50X + 0.31	0.734	18	<0.001

[1]SL = shell length (mm); [2]FL = foot length (mm); [3]FW = foot width (mm); [4]FA area of foot (mm^2); [5]SW/A = immersed snail weight (mg)/area (mm^2) of foot.

TABLE 2. The effects of the type of substratum on locomotion by *Ilyanassa obsoleta*.[1]

	Type of substratum		
	Clean glass	Clean sand	Mucous trail on sand
Rate of locomotion (mm/sec) ($\bar{X}$ ± SD, N = 25)	1.73 ± 0.49	1.10 ± 0.16	1.17 ± 0.25

ANOVA: F = 27.6 df = 2,72 P <0.001

Rates linked by horizontal line are not significantly different (SNK).

[1]Mean shell length = 17.5 mm (range = 16.6 – 18.2 mm). The snails had been in the laboratory 6 days.

TABLE 3. Effects of maintenance in the laboratory on the speed and activity of *Ilyanassa obsoleta*.[1]

	Time in laboratory (weeks)			
	1	2	3	4
Rate of locomotion (mm/sec, $\bar{X}$ ± SD)	2.19 ± 0.49	1.45 ± 0.28	0.99 ± 0.23	1.09 ± 0.34
N	39	41	34	35

ANOVA: F = 88.9 df = 3,145 P <0.001

Rates linked by horizontal line are not significantly different (SNK).

% of snails failing to reach test criterion	9.3%	4.7%	34.6%	32.7%
N	43	43	52	52

[1]Mean shell length = 17.5 mm (range = 16.8 – 18.1).

creased significantly with continued maintenance in the laboratory (Table 3). After three weeks in the laboratory the absolute rate of locomotion of *I. obsoleta* had decreased to about 50% of the rate after one week. This reduction in crawling speed with prolonged maintenance in the laboratory was accompanied by an increased tendency of snails to

be unresponsive to experimental manipulation. Fully ⅓ of the experimental animals failed to reach the response criterion after three or more weeks in the laboratory (Table 3). This decline in locomotory rate and overall activity was not affected by including clams and shrimp, in addition to marsh mud, in the diet of this facultative-scavenger deposit-feeding mud snail (Dimock & Styers, unpublished observations).

DISCUSSION

The published techniques by which the speed of gastropods has been determined do not always facilitate inter- or intraspecific comparisons of parameters of locomotion. Not only may the experimental conditions under which speed is determined be incompletely controlled or inadequately described, but such rates may also be calculated from the total distance moved during time intervals that could include lengthy cessation of locomotion (Calow, 1974; Bertness & Schneider, 1978). The data of the present study apparently comprise the first quantitative analysis of ciliary locomotion by a single species of gastropod in response to a controlled stimulus.

The range of absolute speeds recorded for *Ilyanassa obsoleta* (up to 3.3 mm/sec) are comparable to the few published reports of locomotion by sometimes unspecified species of the family Nassariidae (Copeland, 1919; Miller, 1974a; Palmer, 1980). Linsley (1978) seems to have published the only recent data on a close and sometimes sympatric relative of *I. obsoleta*, *Nassarius vibex* (Say, 1822), but one has to assume, apparently as Palmer (1980) did, that Linsley's data are in mm/sec, since that author never specified units for the 'average speeds' in his paper. In any event, rates of locomotion on the order of a few mm/sec seem to be typical of many small species of gastropods which employ ciliary locomotion (Miller, 1974a).

The effect of size on the speed of ciliary-powered gastropods is not yet well resolved. However, the absolute rate of locomotion is positively correlated with shell length for *I. obsoleta* (Fig. 1). This relationship is similar to that described by Miller (1974a) for an assortment of unspecified gastropods from 15–80 mm long that also employ ciliary locomotion. However, Miller's data also depict similar high rates of locomotion for species (individuals?) both <15 mm and >80 mm in length, with lower rates occurring in intermediate size ciliary movers (Miller, 1974a, table IV). Certainly the fact that *I. obsoleta* can vary the activity of its pedal ciliation and concomitantly its speed (Copeland, 1919) could complicate an assessment of the relationship between size and speed for this or other ciliary-powered gastropods. It is clear, however, from Fig. 2 and the data of Miller (1974a) that small, ciliary-powered snails attain the fastest relative rates (shell length/sec) of all types of gastropod locomotion except leaping.

The pedal surface area of *I. obsoleta* increases significantly more rapidly with increased shell length than is predicted by simple scaling (Table 1). If the density of locomotory cilia/unit area of foot is constant, the effect of this changing pedal area would be the provision of a rapidly increasing locomotory surface as the snails get bigger. This increased locomotory surface would be partly offset by the increasing weight/unit area of foot that also occurs as *I. obsoleta* gets larger. However, the ratio of weight/area of foot increases less quickly with increased shell length than scaling predicts (Table 1). The effective load that pedal locomotory ciliation must bear could be further reduced by the rather routine occurrence of gas bubbles in the mantle cavity and/or the gut of *Ilyanassa* (Kushins & Mangum, 1971); however, the contribution of such gas bubbles to the buoyancy of large and small mud snails has not been determined. Thus, it seems likely that the greater absolute speed of large *versus* small *Ilyanassa* is primarily attributable to the increased pedal surface area that accompanies larger shell size.

Ilyanassa crawls significantly faster on glass than it does on a sandy substratum (Table 2). A similar relationship between the type of substratum and the speed of ciliary-powered gastropods was reported by Miller (1974a) who demonstrated that several species of *Cassis* were 45–70% faster on lucite than on sand. The presence of a conspecific's mucous trail on sand had no detectable effect on the speed of *I. obsoleta* (Table 2). Thus, the significance of the mucous trail-following behavior of this mud snail is not clearly related to locomotion. However, the role of trail mucus *vis-à-vis* locomotion might be quite different under other experimental or environmental conditions of the type of substratum, the exposure of snails to currents, or of the

relative extent of immersion of mud snails in water. It may be that trail-following is more related to the energetic costs of mucus production and of transport (Calow, 1974; Denny, 1980; Houlihan & Innes, 1982). The limited data of Hall (1973) suggesting that *Littorina irrorata* (Say, 1822) crawls faster on than off a mucous trail were interpreted by him as implicating trail mucus as an energy-saving device.

The waning speed and concomitant increase in the percentage of animals exhibiting no response to the stimulus condition with increased duration of maintenance in the laboratory (Table 3) provide quantitative evidence for what heretofore had been subjective personal observation. From the first few days to a week or two after mud snails were brought into the laboratory, they are very active and spend a lot of time on the walls of aquaria. Later, however, their overall activity seems to diminish as they increasingly spend time on or in the substratum, rarely climbing the aquarium walls. It is clear that *Ilyanassa* is behaviorally quite different after extended maintenance in the laboratory. Although the notion that animals become more 'abnormal' with prolonged laboratory maintenance is not new, quantitative behavioral evidence to that effect among marine invertebrates is not widely available.

There is a plethora of evidence that starvation in the laboratory results in a reduction of metabolic activity and the assumption of a standard rate of metabolism by a variety of marine invertebrates, including gastropods (Newell & Roy, 1973; Bayne & Scullard, 1978; Newell & Branch, 1980). The decline in the activity and rate of locomotion of *I. obsoleta* does not appear to be simply a function of the availability of food, although this animal's nutritional requirements may be complex (Curtis, 1979). Significant, rapid changes in the behavior of *I. obsoleta* as a consequence of maintenance in the laboratory should be cautionary to other investigators.

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FUNCTIONAL ASPECTS OF TRAIL FOLLOWING BY THE CARNIVOROUS SNAIL *EUGLANDINA ROSEA*

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ABSTRACT

Euglandina rosea (Férussac) follows the trails of potential mates and of its gastropod prey. Conspecific trail following is decreased after copulation. Prey trail following is decreased after feeding and recovers in about nine days. Feeding also recovers over approximately the same time span. The direction in which *Euglandina* follows both prey and conspecific trails was influenced by the direction of illumination. In even illumination, there is no directionality in prey trail following. Snails discriminate between trails since they do not follow their own trails, nor do they persist in following the trails of less favoured prey.

The elongated lips are used extensively for sampling the substrate and their surgical removal prevents trail following. The ability of the trail to promote trail following persists if it is kept dry for 24 h but a decay in this ability is detectable after soaking the trail in water for 30 min.

Whilst the values of trail following measures are higher than have been reported for other pulmonates, the underlying features of this behaviour are broadly similar, making *Euglandina* a suitable subject for further work on the characterisation of the active factors involved in pulmonate trail following.

Key words: gastropod; behaviour; mucus; feeding; courtship; *Euglandina.*

INTRODUCTION

Trail following in gastropods is widespread (Cook, 1977). It is, however, not a universal feature of gastropod behaviour as attempts to demonstrate it in *Lymnaea* and *Otala,* for example, have failed (Cook, 1977; Chase *et al.,* 1978; Bousefield *et al.,* 1981).

Some species such as *Limax pseudoflavus* Evans (Cook, 1977), *Biomphalaria glabrata* Say (Townsend, 1974; Bousefield *et al.,* 1981) and *Achatina fulica* Bowdich (Chase *et al.,* 1978) tend to follow trails only infrequently and not very far. In these species no clear-cut function for trail following has been demonstrated and yet it is these same species which are being used for the further analysis of this behaviour.

Established functions for trail following include trail blazing through soft substrates (Hall, 1973), aggregation (Lowe & Turner, 1976), prey location (Paine, 1963; Cook, in press) and courtship (Quick, 1946; Cook, in press). Also, many gastropods home and, where this has been examined closely, trail following is frequently involved. (Limpets—Cook *et al.,* 1969; Cook, 1979b; *Onchidium*—McFarland, 1980 and, to a lesser extent, *Limax pseudoflavus*—Cook, 1979a, 1980.)

Preliminary observations on *Euglandina rosea* indicated that, unlike the majority of terrestrial pulmonates, it shows clear-cut responses to the trails of conspecific and prey species and also appears to have well-defined anatomical adaptations for trail following. Therefore, it might be a good model for the characterisation of active factors. Before further work can be undertaken it is necessary to quantify the animal's trail following responses.

MATERIALS AND METHODS

Euglandina rosea with shells of between 3 and 4 cm in length were collected from the Botanic Gardens at the Florida Institute of Technology in Melbourne, Florida and maintained in a laboratory at about 20°C. All observations were conducted between March and August, 1981. Each animal was kept in a separate container with a floor of damp filter paper and was fed individually.

All experiments were conducted on polythene sheets in an open arena approximately 80 × 40 cm. For some of the experiments the laboratory illumination was uneven since the room lights were to the right of the area within

which the experiments were conducted. Most animals tended to move away from the light (i.e. from right to left over the experimental area). Under these circumstances all marker trails (i.e. the trails laid first) were laid from right to left. In some experiments the tracker animal was placed in the centre of the polythene, pointing towards the marker trail and about 1 cm from it. In these experiments, therefore, all tracker animals made a right angle contact with the marker trail and made a choice concerning the direction in which to follow the trail. In other experiments the tracker animal was placed on the end of the marker trail. The experiment was stopped when the tracker left the polythene.

Mucus can be made temporarily visible by breathing on it. This proved to be the best method for observing traces of mucus left by the lips. The trail itself was made visible by immersing the polythene sheet in a suspension of talcum powder.

Two experimental formats were used. The first used polythene sheets of 70 × 20 cm. The marker animal laid a trail along the long axis of the sheet and the tracker animal was then placed near the centre of the trail. This format allowed the measurement of the frequency with which marker and tracker trails were superimposed, the length of that superimposition and the direction in which the marker trail was followed relative to the direction in which the trail was laid. This experimental format is illustrated in Fig. 3. It was used in experiments examining directionality and the effects of feeding and copulation on trail following. *Deroceras laeve* (Müller) (a limacid slug) was mostly used to lay these marker prey trails, but in a short series of experiments conducted in the U.K. the very similar *Deroceras caruanae* (Poll.) was used.

Whilst most experiments were conducted in uneven illumination a short series was conducted in a symmetrical light regime. This was provided by closed arenas of 70 × 50 × 40 cm with matte black walls and white floors and ceilings illuminated by a centrally placed 30 W light 40 cm above the floor.

The second format utilised 20 × 20 cm polythene sheets. Marker trails were laid in the normal way. These smaller trails allowed a greater number of replicates to be performed but at the expense of the accuracy with which the distance followed could be estimated.

To examine the specificity of trail following, three prey species were used to lay 20 cm marker trails. These were *Deroceras laeve*, *Veronicella floridana* Leidy (a systellommatophoran slug), and *Philomycus carolinianus* (Bosc) (an arionid slug). The tracker *Euglandina* was placed on the end of the marker trail and a record was made of whether the trails were subsequently superimposed, the length of the superimposition, and the length of the marker trail.

The effect of the removal of the head appendages was examined in a similar way. The operations were performed with iridectomy scissors on unanaesthetised animals. The two pairs of head tentacles were easily removed but complete extirpation of the lips was impossible. These were, therefore, amputated from the point where the lip left the lateral line of the body. Only one animal was available for each of these operations. After a one week recovery period each animal was tested on 20 cm conspecific trails twice per day for five days. The tracker animals were placed on the end of the marker trails and the proportion of the marker trail followed was measured.

20 cm trails were also used to examine the effect of soaking trails in water. *Deroceras* marker trails were either left dry overnight or soaked for 30 m and then dried overnight. *Euglandina* was placed on the centre of the polythene, pointing towards the tracker trail but about 1 cm away from it. Their behaviour on making contact with the marker trail was noted.

RESULTS

The head of *Euglandina* is highly adapted for chemoreception (Fig. 1). The posterior (optic) tentacles, which in other pulmonates are associated with distance chemoreception, possess an accessory lower lobe beneath the eye. The lips are greatly extended and are extremely mobile. Fig. 2 shows an example of the contacts made by the lips with the substrate, A) during trail following, B) on encountering the end of a followed trail, and C) not trail following. This track illustrates firstly the large area of substrate covered by the lips alone during searching movements and secondly the regular pattern of lip dabbling movements during locomotion. The frequency of lip/substrate contacts was measured during locomotion, before and during trail following, and subsequent to the searching movements seen after the snail had lost

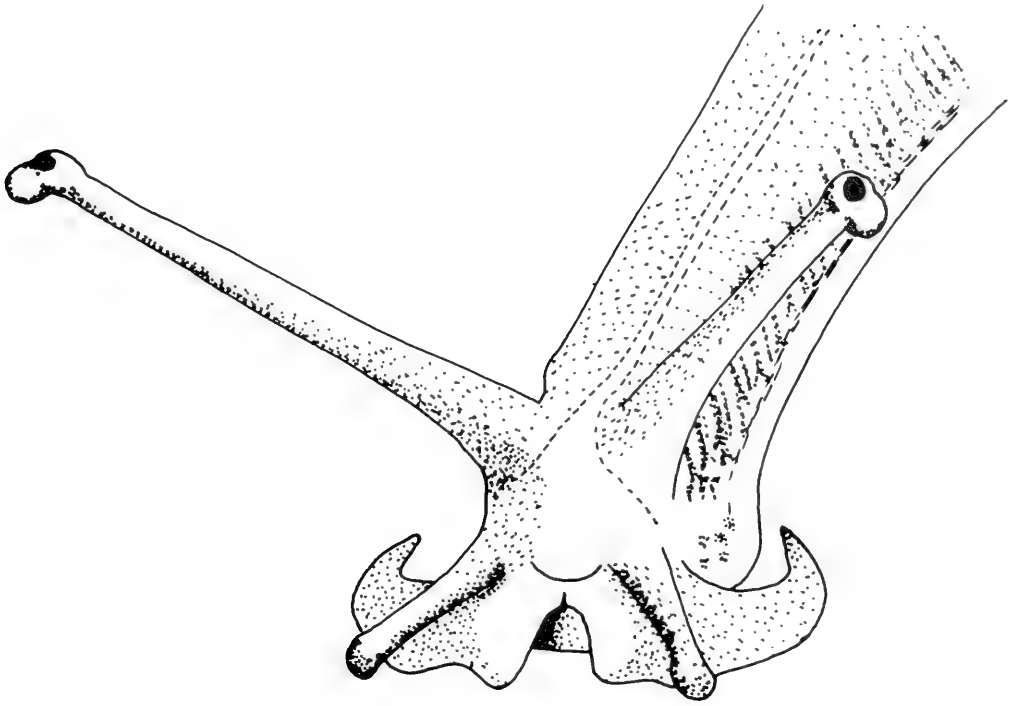

FIG. 1. The head of *Euglandina.* The posterior (optic) tentacles have an accessory lower lobe beneath the eye and the lips are greatly extended.

the trail (e.g. area C in Fig. 2). The distance over which such measurements are possible varies so all data were reduced to the number of contacts per cm and are given in Table 1. Lip/substrate contacts are significantly increased during trail following and during periods of locomotion after losing the trail.

Directionality

Euglandina which had neither mated nor fed for at least 7 days were tested against 70 cm trails of *Deroceras laeve* and other *Euglandina.* Some trails were rotated through 180° after being laid so that they ran from left to right. The proportion of left turns is shown in Table 2 and the experimental format in Fig. 3. It can be seen that for both conspecific and prey trails, turns are generally made to the left rather than relative to the direction in which the trail was laid. That is, the direction of trail following is determined not by trail cues but by other environmental cues; presumably in this case by differential illumination.

As a check that directional responses to the trail were not being masked by an overriding

TABLE 1. The frequency of lip/substrate contacts during locomotion.

	Mean no. of contacts/cm	s.e.	N	t test
Normal movement	4.23	0.23	8	(control)
Trail following	5.47	0.46	9	2.41**
After end of trail	5.87	0.57	9	2.66**

**$p < 0.01$.

TABLE 2. The frequency of left turns taken onto the marker trails shown in uneven illumination. The total number of replicates of each experiment is indicated (N).

	Trail orientation					
	Normal		Rotated 180°			
Marker	%	(N)	%	(N)	χ^2	
Euglandina	84	12	75	12	0	N.S.
Deroceras	77	43	56	25	2.28	N.S.

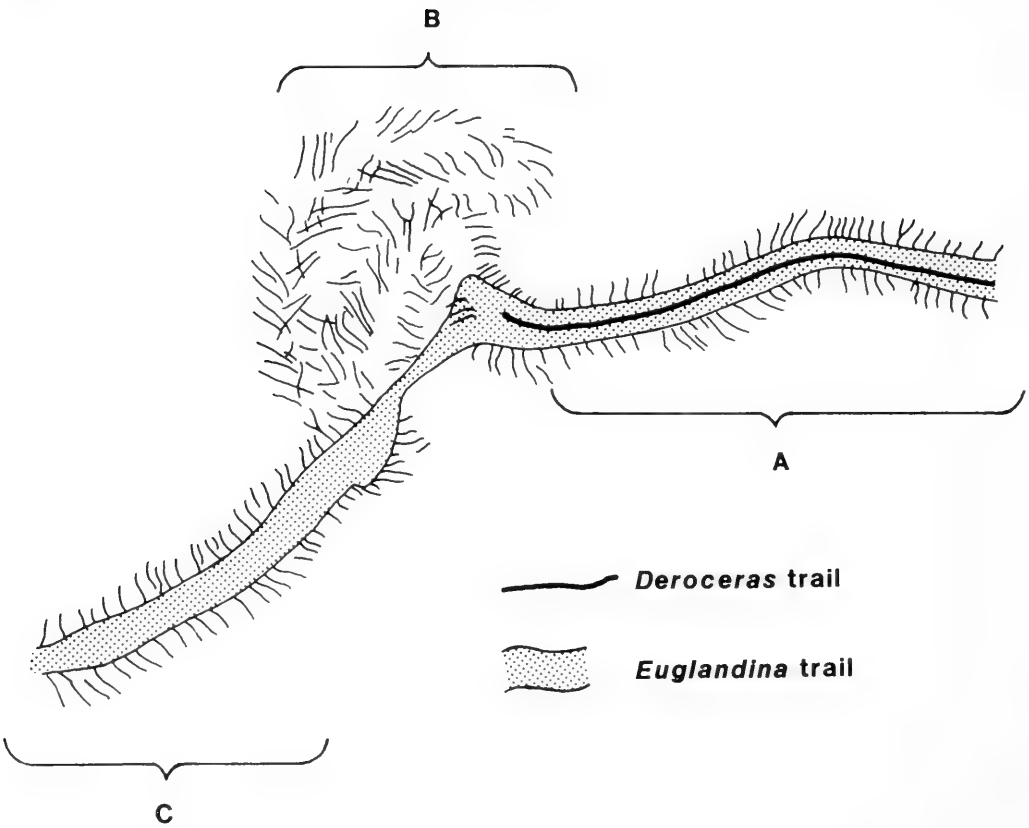

FIG. 2. The use of the lips in *Euglandina*. A) Shows the pattern of lip contacts whilst following the trail of *Deroceras*, B) shows a searching pattern executed by the lips after the *Deroceras* trail ended, and C) shows the lip contacts as the snail moved away.

response to the direction of illumination, an experiment was performed which was similar to that described above but conducted in a symmetrical light regime. *Deroceras caruanae* was used to lay the trails. The results are given in Table 3 and there is no significant indication of the ability to detect the directionality of the marker trail either measured by the direction of turning or by the distance followed.

Specificity

The ability of *Euglandina* to follow trails of various prey species was tested by placing the snail on the end of a 20 cm trail. The marker species were *Deroceras laeve, Philomycus carolinianus*, and *Veronicella floridana*. Individuals were also tested against their own and another *Euglandina* trail using the 70 cm experimental format. All followers had neither fed nor mated for at least 7 days. The results are shown in Table 4.

There is a significant decrease in the frequency with which trails are followed to their ends between *Deroceras* and *Philomycus* ($p < 0.001$). An apparent decrease in the length of the trail followed when responses to *Veronicella* trails are compared to those of *Deroceras* is not significant. An individual follows its trail significantly less than it does that of another *Euglandina* ($p < 0.001$).

Effect of feeding

Animals were starved for a varying length of time and then their trail following ability was tested. A limited number of animals was available for these observations so each animal was used more than once. 73 observations were made in all and divided into six three-day starvation periods; i.e. 1–3 days ($n = 18$

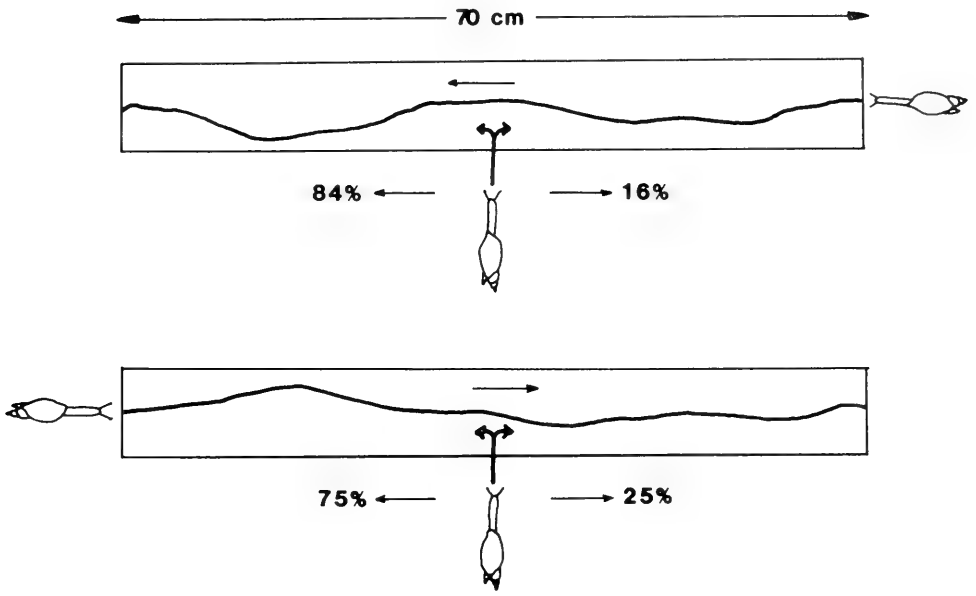

FIG. 3. The 70 cm trail experimental format. A marker trail was laid on a polythene strip 70 cm long and tracker snails placed about halfway along this trail. In this experiment, comparison between the behaviour on normal trails (above) and that on those rotated through 180° (below) shows no significant difference in the frequency of animals turning left.

TABLE 3. The lack of directionality in the following of *Deroceras* trails in even illumination (N = 10).

Direction relative to marker	% following	Distance followed mean ± s.e.
Forwards	50	26.6 ± 4.1
Backwards	50	31.6 ± 1.0

t = 1.16; d.f. = 8 (N.S.)
N.S.—Not Significant.

TABLE 4. Specificity of trail following in *Euglandina*.

Marker	% followed	% followed to end	No. of replicates
Deroceras	88	80	25
Philomycus	53	26***	15
Veronicella	90	70 N.S.	10
A different *Euglandina*	100	100	24
The same *Euglandina*	8	0***	24

***—$p < .001$; N.S. Not Significant.

observations), 4–6 days (n = 14), 7–9 days (n = 12), 9–12 days (n = 9), 13–15 days (n = 11), and longer than 16 days (n = 8). Since each animal contributed to more than one of these groups the experiments were not run concurrently but data were accumulated over a period of several months. All starvation periods commenced with feeding to satiation and ended with the snail being tested for its ability to follow a 70 cm *Deroceras* trail before being offered a slug as prey. The frequency of following, the percentage of the available trail followed and whether the snail subsequently ate a *Deroceras* were recorded. The results are summarised in Fig. 4. Both measures of trail following and feeding activity were decreased following a meal but all animals were back to normal after about nine days.

Quantitative comparisons may be made for starvation periods of up to 12 days (53 observations). Significantly fewer animals fed in the first six days than in the last six. ($\chi^2 = 9.64$; d.f. = 1; $p < .01$.) Significantly more snails followed trails if they subsequently fed than if they did not feed ($\chi^2 = 6.8$; d.f. = 1; $p < .01$). Furthermore, considering only those snails which followed trails (34 observations), significantly more snails followed to the end of the marker trail if they fed than if they did not feed ($\chi^2 = 13.6$; d.f. = 1; $p < 0.001$).

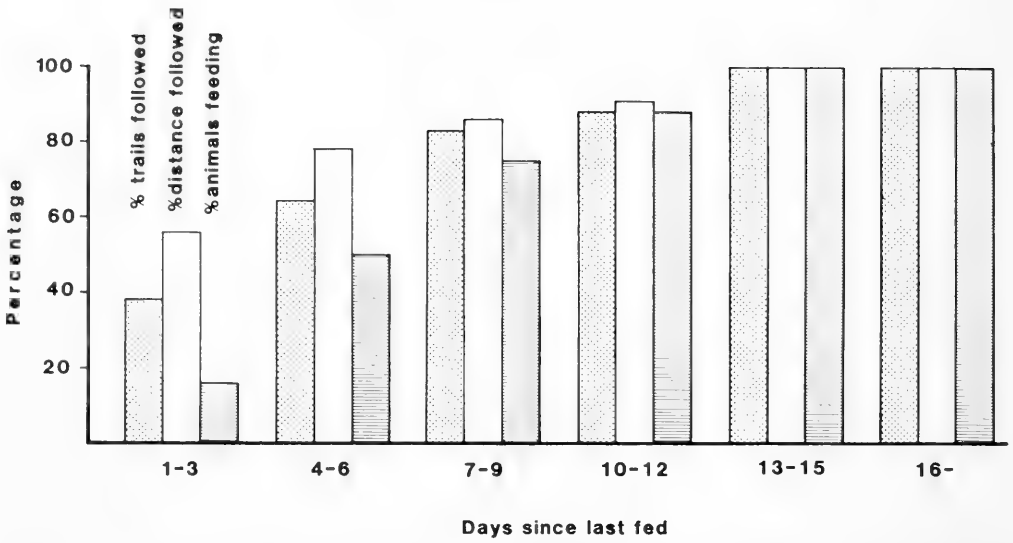

FIG. 4. The effect of food deprivation on trail following. Measures of trail following (percentage of the trails followed and for those that were followed, the percentage of the available trail followed) and the frequency with which snails accepted food all increase with the increasing duration of starvation.

Effect of copulation

Euglandina was kept in isolation and readily mated when two individuals were placed together. Each animal was allowed to lay two 70 cm trails. The first of these trails was used immediately to test the trail following ability of a different snail with which it was subsequently allowed to mate. The next day the second trail was used to test the trail following ability of the mate. In these experiments the followers were placed near the middle of the marker trail so that when they turned to follow the trail approximately half of the marker trail was left unfollowed. This remaining half was tested with an unmated snail to determine whether the trail could still elicit normal trail following behaviour from an unmated individual. Individuals which had mated the previous day were also tested on fresh marker trails laid by *Euglandina* with which they had not mated. The results are shown in Table 5. There is a significant reduction ($p < 0.001$) in the frequency of trail following after copulation as well as a significant reduction ($p < .001$) in the distance followed by those individuals that did follow trails. The decrease in trail following is not specific to the recent copulatory partner, nor is it attributable to a decline in the effectiveness of the trail. Both conspecific trail following and courtship behaviour had returned to normal after about seven days. No animal in these experiments laid eggs.

It would be expected that before courtship the trail following frequency would be as high as it is in the control (unmated) animals. The deficit in Table 5 is attributable to two animals. During courtship one snail follows the trail of the other and then mounts the shell from the rear. The two snails which did not follow trails prior to courtship eventually adopted the passive role of the mounted snail during courtship (i.e. they showed no trail following during courtship either).

Trail persistence

Euglandina was allowed to lay trails on 20 cm square pieces of polythene. Twelve such pieces were soaked for 30 min and a further thirteen left dry. All trails were dry when tested. Test snails were placed halfway along the trail pointing towards it but not in contact with it. Movements were classified as 1) IGNORED (where no examination of the trail with the lips occurred, 2) EXAMINED (where the marker trail had been investigated by the lips of the follower but where no trail following had taken place), 3) FOLLOWED (the trails of the marker and follower were superimposed but not for the whole distance available), and

TABLE 5. The effects of copulation on trail following. Each combination of marker and tracker animal was repeated 24 times. Frequencies are compared using χ^2 and distances using 't' tests. All comparisons are made with the unmated control (top line).

Marker	Tracker	Measure of trail following: Frequency of following %	Measure of trail following: Distance followed cm (± s.e.)
future partner	partner before mating	92	36.9 ± 3.4
future partner	partner after mating	19***	13.6 ± 5.7**
unmated stranger	partner after mating	25***	19.1 ± 4.7**
future partner	unmated stranger	100 N.S.	

***—$p < 0.001$.
**—$p < 0.01$.
N.S.—Not Significant.

TABLE 6. The responses to trails soaked for 30 min in water.

Response	Dry trails (n = 13)	Soaked trails (n = 12)
Ignored	0%	50%
Investigated with lips	16%	0%
Turned onto trail	8%	25%
Followed to end	76%	25%

TABLE 7. The effect of removing the head appendages (one animal for each treatment tested ten times).

Appendage removed	% of trails followed	Proportion of trail followed
None	100	0.85
Posterior tentacles	100	0.94
Anterior tentacles	100	0.88
Lips	40*	0.29**

**—Mann–Whitney U-test; $p < 0.01$.
*—$\chi^2 = 5.9$; $p < 0.05$.

4) FOLLOWED TO END (where the maximum amount of trail following had occurred). These results are shown in Table 6. A χ^2 test performed on these data comparing the frequency of positive responses to the marker trail with the frequency with which the trail was ignored completely showed that there was a significant reduction in these responses after the trail had been soaked ($\chi^2 = 4.58$; d.f. = 1; $p < 0.05$).

The effect of tentacle and lip amputation

Four animals were used in amputation experiments. From one the posterior (optic) tentacles were removed, from another the anterior ones and from a third the lips were amputated. The fourth was left unharmed as a control. All animals had been used in previous experiments and had behaved normally. After amputation the snails were less active than before but fed when a slug was presented to them. Each animal was tested ten times on fresh 20 cm trails laid by normal *Euglandina*. The test animals were placed on the end of the marker trail and the percentage of occasions on which the trail was followed was measured. For those that followed part of the trail, the proportion of the available trail that they followed was also measured. These results are shown in Table 7. Whilst interpretation must be cautious because only one animal was used for each of the treatments, the results suggest that the lips are essential for the successful performance of trail following.

DISCUSSION

Euglandina is well suited for use as an experimental animal for the further analysis of the general features of trail following in pul-

monates. Although this snail is highly adapted for trail following, the features of its trail following behaviour conform to those seen in less specialised pulmonates. Firstly, any directionality exhibited by the tracking animal is not based on the way in which the trail was laid (Tables 2 and 3). This is similar to the pattern seen in other terrestrial pulmonates, e.g. *Limax* and *Achatina* (Cook, 1977; Chase *et al.,* 1978). Directionality has been demonstrated in the trail following of the aquatic pulmonate *Biomphalaria glabrata* (Say) (Townsend, 1974) but it has been suggested that this may be attributed to the decay of the trail in water rather than to any directionality inherent in the way in which the trail was laid (Cook, 1977).

Secondly, the ability of the trail to promote trail following declines in water over a similar time scale to that observed in other pulmonates (Table 6) (e.g. *Biomphalaria*—Townsend, 1974; Bousefield *et al.,* 1981; *Physa*—Wells & Buckley, 1972; *Limax*—Cook, unpublished observations). Thirdly, because of the nature of their diet the hunger and therefore the trail following of the animals is easily controlled (Fig. 4). Finally and most importantly, trail following occurs regularly and at very high frequencies. A suitable *Euglandina* follows trails on over 90% of the occasions that it meets them. *Limax* on the other hand will only follow trails on approximately 30% of such occasions (Cook, 1977). The distances followed are also very high. *Limax* rarely follows trails for more than 20 cm, whereas *Euglandina* rarely follows a suitable trail for less than this distance. In the present experiments when a snail followed a trail it normally followed for the full 35 cm allowed and in preliminary experiments snails followed trails for more than one meter.

Rough comparisons can be made by estimating 'coincidence index' (Townsend, 1974) for *Euglandina.* The data presented in Table 5 (unmated animal following conspecific trails) produce an estimated index of 0.88. This compares with a maximum value of 0.19 for *Biomphalaria* (Townsend, 1974), 0.66 for *Mariaella dussumieri* (Gray) (Ushadevi & Krishnamoorthy, 1980), 0.49 for *Achatina* (Chase *et al.,* 1978), and an estimate of 0.26 for *Limax* (from data in Cook, 1977). In the experiments involving *Achatina, Biomphalaria* and probably *Mariaella* the trackers were placed at the end of, or in contact with, the marker trail. In the experiments with *Euglandina* and *Limax* however, the trackers were placed away from the trail so it might be expected that for these animals the coincidence index has been underestimated. *Euglandina* therefore follows trails far more frequently and for greater distances than any other pulmonate in which this behaviour has been measured.

The regularity of trail following in *Euglandina* is related to its clear function. The frequency with which it follows prey trails and the decrease in the measures of trail following of less favoured prey (Table 4) and after a meal (Fig. 4) are all clear evidence of the close relationship between trail following and feeding. Similarly the high frequency of following conspecific trails but not its own (Table 4), the decrease in the measures of trail following after copulation (Table 5), and the initial contact during courtship being from the rear (Cook, in press) all indicate the integral part that trail following plays in mate finding and courtship.

These changes in behaviour after feeding or copulation in *Euglandina* fall into the general pattern of changes associated with motivational phenomena. Trail following is the initial part of the appetitive behaviour for both events. Once adequate consummatory acts have been performed the snails enter a quiescent phase in which the appetitive behaviour is difficult to elicit. Furthermore the behaviours measured during the feeding observations recover in sequence—the trail following measures recovering faster than feeding itself (Fig. 4). After copulation the decrease in the measures of the appetitive behaviour is not specific to the original partner (Table 5). The Coolidge effect (Michael & Zumpe, 1978) is therefore not apparent in these observations.

Trail following then is an important part of the behavioural repertoire of this snail. This is reflected in structural adaptations for its performance. The organs most closely involved with trail following in other pulmonates are the lower (anterior) pair of tentacles (*Limax*—Cook, unpublished data; *Mariaella*—Ushadevi & Krishnamoorthy, 1980; *Achatina*—Chase & Croll, 1981). In *Euglandina,* however, the lips are greatly elongated and these are used for trail detection (Figs. 1, 2; Tables 1, 7). There appears to be no residual trail sensing ability in either pair of tentacles.

The lack of trail-controlled directionality in following should be a considerable handicap if a snail is to find prey or mates by this means. However, snails are influenced in

their choice of direction by the same factors that influenced the laying of the original trail (at least as far as illumination is concerned) so this need not be such a serious problem as it would first appear.

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THE ORGANISATION OF FEEDING IN THE CARNIVOROUS SNAIL *EUGLANDINA ROSEA*

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ABSTRACT

The types and sequences of behaviour involved in feeding on different types of prey are described. The sequences of behaviour were similar for small snails, large snails and slugs, though they were modified in response to the defensive tactics of the prey. The process of feeding can be conveniently divided into an attack, consumption of the prey and clearing up. The time allocated to each of these phases for each prey type is presented. Small snails eaten whole were consumed most rapidly (1 min). Slugs took longer (1.5 min) since time was allocated to clearing up mucus and other debris (1 min). Snails that were too large to be eaten whole but whose body weight was similar to that of the slugs took a considerable time to be consumed (15 min). Approximately two thirds of this time was taken in grazing over the empty shell after the soft parts had been eaten.

Key words: Gastropoda; feeding; behaviour; *Euglandina*.

INTRODUCTION

Euglandina rosea (Férussac) is a predatory snail native to the SE U.S.A. Its general biology has been studied as part of programmes to introduce the snail into tropical islands as a control measure against large pest species such as *Achatina fulica* Bowdich (Chiu & Chou, 1962; Davis & Butler, 1964; Mead, 1979). Whilst there have been some descriptions of its behaviour (e.g. courtship—Cook, in press a; feeding—Cook, 1984, and trail following—Cook, 1985) these accounts have not included detailed analyses of its feeding behaviours when presented with different prey. The present paper compares in detail the feeding behaviour on a range of prey.

MATERIAL AND METHODS

Sixteen *Euglandina rosea* snails with shells between 3 and 4 cm long were collected from the Botanic Gardens at the Florida Institute of Technology in Melbourne. The prey used was *Deroceras laeve* (Müller), a limacid slug about 2 cm long, *Polygyra septemvolva* Say, a snail with a flat shell about 7 mm in diameter and *Succinea campestris* Say, a bulbous snail with a shell about 10–12 mm long. A representative sample (6) of each prey type was taken, the shells dissolved in 1M HCl and the remaining soft parts dried to constant weight at 60°C. The mean weight of *Deroceras* and *Succinea* were similar (.03 g) but *Polygyra* weighed considerably less (.007 g).

Observations were conducted on an open white surface approximately .4 × .8 m. This surface was covered with clear polythene which was changed between prey items so that the predator was not confused by irrelevant trails. The euglandinas used in these experiments had been starved for at least seven days. The prey was allowed to walk a short distance and then an active *Euglandina* was placed on its trail. All experiments therefore, commenced with trail following. For each observation the sequence of events was recorded using a tape recorder. The tape was subsequently transcribed and the timing of each event noted. Observations were made on the consumption of 33 *Deroceras,* 30 *Polygyra* and 20 *Succinea.*

RESULTS

Classification of predator behaviour

The behaviours recorded were as follows.

1. Trail following (TF).

The predator followed the prey trail which was normally visible as a damp streak. This behaviour is accompanied by increased lip activity.

2. Contact (Con).

The predator made contact with the prey. Normally the initial contact was made by the posterior (optic) tentacles.

3. Eversion (Ev).

The odontophore and upper lip of the snail were everted producing a large white balloon-like structure protruding from an area roughly bounded by the tentacles and the lips (Fig. 2C).

4. Strike (Str).

The everted mouthparts were brought down rapidly onto the prey. A successful strike resulted in penetration by the radular teeth and the pinning of the prey.

5. Swallow (Sw).

The prey or part of it was drawn into the protruded mouthparts.

6. Mop up (Mop).

The everted mouthparts were moved over the substrate or the prey shell after the body of the prey had been consumed.

7. Inversion (Inv).

The mouthparts were inverted.

8. Search (Se).

The anterior of the body of the predator was raised and either waved in the air or moved over the substrate. This body activity was accompanied by increased tentacle and lip activity.

9. Moved off (Off).

The predator moved away from the site at which the prey had been eaten or ceased to trail follow in cases where the prey escaped an attack.

10. Pick up (P.U.)

The prey shell was lifted on the anterior portion of the foot. This action did not necessarily detatch the prey's foot from the substrate.

11. Move down (M.D.)

The lifted prey was moved down the foot and the head of the predator bent over the top of the prey shell (Fig. 3C).

12. Rotate (Rot).

The prey shell was rotated. This is an unsatisfactory behaviour to define since the prey shell was frequently rotated little by little in the performance of other behaviours. Occasionally this shell manipulation occurred by itself.

13. In and out (In, Out).

In the case of larger prey the head of the predator was inserted into the shell of the prey and the flesh eaten from within. Details of the predators' behaviour within the prey shell were not consistently clear.

14. Escape (Esc).

The predator lost physical contact with the prey. Prey that escaped completely did so by leaving the experimental area. Only *Deroceras* escaped once the initial contact had been made.

Behavioural sequences

The sequences of behaviours recorded were cast in transition tables and flow diagrams of the sequences constructed (Fig. 1). By their definitions it is not possible for the behaviours to be randomly organised and therefore no test for non-randomness was conducted.

1. *Succinea. Succinea campestris* responds to being attacked by withdrawing into its shell. Its large foot allows it to adhere firmly to the substrate. These snails are, however, weak compared to *Euglandina* and attachment was never a successful defence. Once attacked, *Succinea* was always eaten. The most frequent sequence of events during feeding was as follows. After the approach from the rear, *Euglandina* moved from right to left over the surface of the prey shell (Fig. 2A). The shell was picked up on the foot and the prey was commonly exposed with its foot attached to the substrate and the shell angled upwards (Fig. 2B). This revealed the columellar muscle of the prey. In these circumstances the first strike was at the columella. The first strike results in the prey detaching from the substrate and withdrawing into the shell if it had not already done so (Fig. 2C). After this withdrawal the prey shell is held between the predator's foot and the ground. Occasional rotations of the prey shell occur on the foot which result in the prey shell being better placed for the predator to enter. The normal position of the prey at first is with its spire to the right with respect to the *Euglandina* (Fig. 2A–C). During the consumption of the exposed part of the prey the prey shell is gradu-

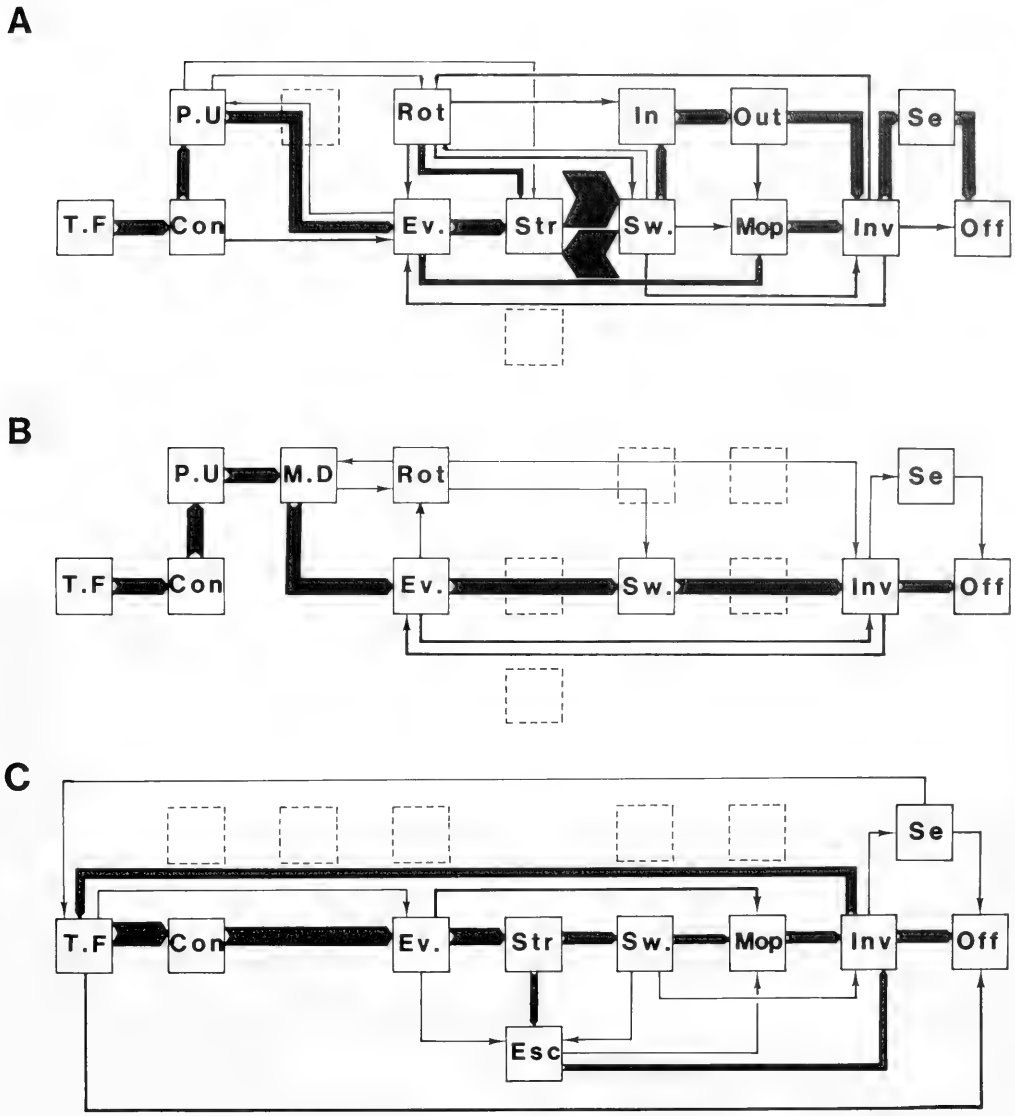

FIG. 1. A flow diagram of the behaviours involved in feeding on A) *Succinea campestris,* B) *Polygyra septemvolva* and C) *Deroceras laeve.* The width of the lines indicates the frequency of transition from one behaviour to another expressed as a percentage of the number of animals involved. The behaviours concerned are: TF—Trail following; Con—Contact; P.U—Pick up; M.D—Move down; Ev.—Evert; Rot—Rotate; Str—Strike; Sw.—Swallow; Mop—Mop up; Inv—Invert; Se—Searching; Off—Moving off; In—Head in prey shell; Out—Head out of prey shell; Esc—prey escapes.

ally turned so that the spire is on the left. When *Euglandina* enters the shell to remove the remnants of the prey it therefore normally enters with its foot over the columella (Fig. 2D). In 35% of attacks an initial strike at the columellar muscle was effective, the prey extracted from its shell whole and eaten outside the shell. Failure to strike at the columellar muscle was a result of the premature withdrawal of the prey into its shell or a poorly directed first strike. After eating the soft parts of the prey the mouthparts were inverted only to be re-everted immediately or after some prey shell manipulation. Re-eversion was fol-

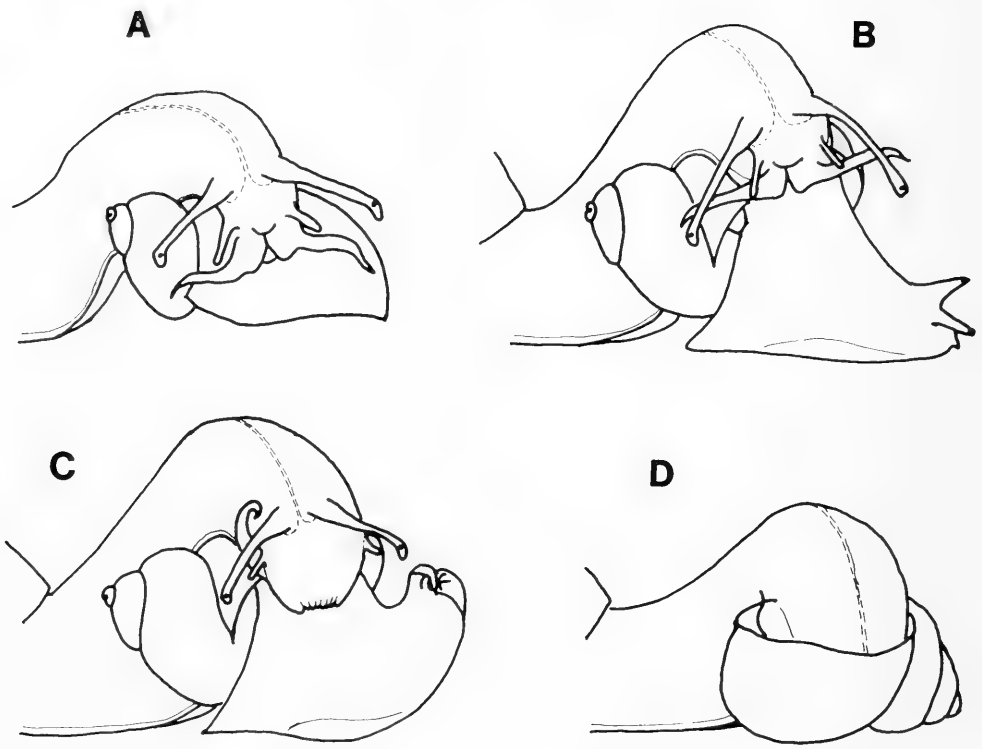

FIG. 2. *Euglandina* feeding on *Succinea*. The predator approaches over the body whorl (A) and lifts the shell (B) before everting its mouthparts and striking in the region of the columella (C). The predator inserts its head into the prey shell to eat the remnants of the prey (D). The *Euglandina* shell length is approximately 3 cm.

lowed by a thorough cleaning of the prey shell both inside and out. Feeding on *Succinea* was normally ended by extensive searching movements prior to moving off.

2. *Polygyra*. The defensive response of *Polygyra septemvolva* is to withdraw deep into the body whorl of its shell. The aperture of the shell is protected by the small tooth. These passive defenses are ineffective, however, against *Euglandina* of the size used and all *Polygyra* attacked were eaten. After the predator made contact with the prey (Fig. 3A) its shell was lifted. Because of the weak foot the whole animal was always lifted from the substrate. The *Euglandina* reared up with the shell attached to its foot (Fig. 3B). The prey shell was then moved down the foot and occasionally rotated (Fig. 3C) before the mouthparts were everted. The mouthparts were moved over the prey and the shell swallowed whole (Fig. 3D, E). There is no strike. Small shelled prey are lifted clear of the substrate and eaten whilst attached to the foot (Fig. 3F, G). Larger prey shells rest on the substrate whilst being eaten. The prey shell can be seen moving back in the 'neck' of the predator. It is digested whole. After swallowing, the mouthparts were inverted and the animal moved off with no mopping up and little searching.

3. *Deroceras*. *Deroceras laeve* is a small slug capable of rapid locomotion. After the initial contact the predator partially overtook the fleeing slug (Fig. 4A) and everted its mouthparts (Fig. 4B). The initial strike was at the tail or the mantle. This strike normally pinned the slug and it was swallowed in one or two pieces (Fig. 4C, D). After the prey had been consumed the predator mopped up the debris (Fig. 4E) before inverting the mouthparts and moving off.

When attacked, *Deroceras* responded with an active defence consisting of rapid locomotion and two specific behaviours. The first and most common was the tail flick in which the rearmost portion of the slug was lifted and

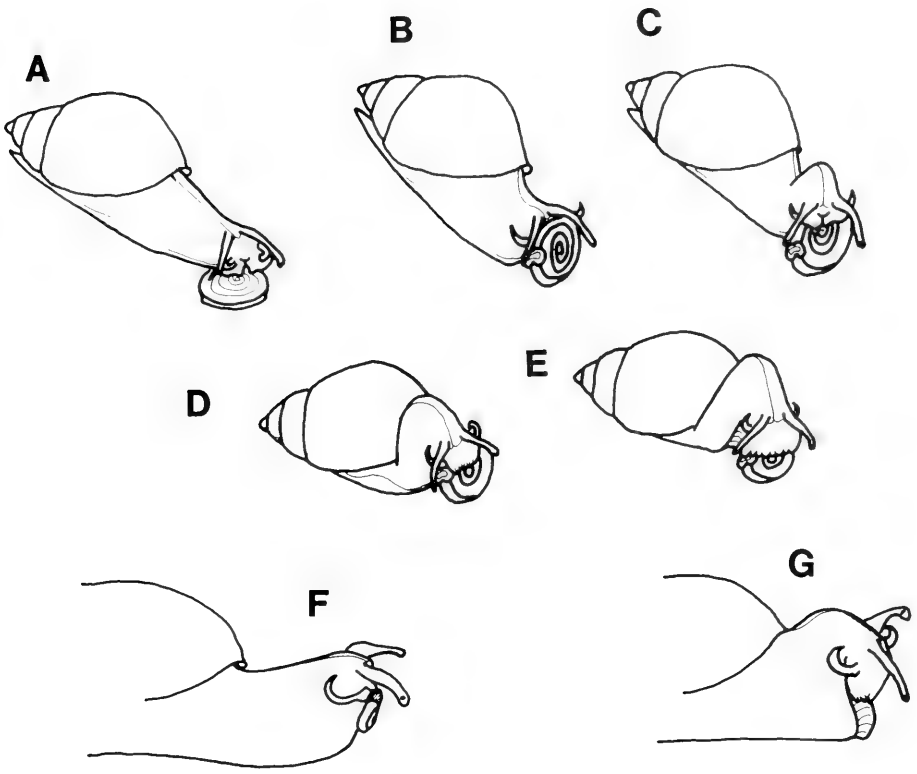

FIG. 3. *Euglandina* feeding on *Polygyra*. Contact is made with the prey shell (A). It is lifted (B) and then moved down the foot (C). The mouthparts are everted (D) and the prey is consumed whole (E). Small prey are lifted clear of the ground (F) and eaten off the foot (G). The *Euglandina* shell length is approximately 3 cm.

flicked rapidly from side to side in a slapping motion. The second was a flaring of the mantle over the head. These two behaviours will be considered together as 'Flick' for the purpose of analysis.

Because the slugs moved rapidly and distracted the predator with these mantle and tail movements the strike sometimes missed or sliced off the rear completely, allowing the prey to escape. Escape was normally followed by the inversion of the mouthparts and the resumption of trail following.

The defensive behaviour of the slug has a considerable effect on the success of the attack (Fig. 5). Forty-two percent of all strikes resulted in the prey escaping. Only 12 of the 33 prey animals flicked their tails or flared their mantles. Of these, 9 escaped once or more and 6 of these escaped completely. Only two animals escaped without flicking or flaring but both these were damaged in an attack and escaped whilst the snail was mopping up mucus and other debris.

Comparison of behaviour patterns—handling times

The total time taken to consume a prey item is made up of various components. Table 1 shows the time taken from the initial contact to eversion of the mouthparts (attack time); the time from that eversion to when the soft parts were completely consumed (eating time), and from that time to when the *Euglandina* moved off (clearing up time). When slugs were the prey, only those attacks in which the prey did not escape are included. These three times represent three distinct phases of an attack which are common to all prey types.

1. Attack time. The time taken to subdue a *Polygyra* was short because they were never

TABLE 1. Handling times of *Euglandina* with different prey species. (Means ± s.e.)

Prey type	Time (sec)			
	Attack time	Eating time	Clearing up time	Total (N)
Succinea	28 ± 3	266 ± 39	631 ± 219	933 ± 216 (20)
Polygyra	11 ± 1	28 ± 6	25 ± 3	67 ± 7 (30)
Deroceras	26 ± 8	15 ± 2	54 ± 9	94 ± 12 (19)

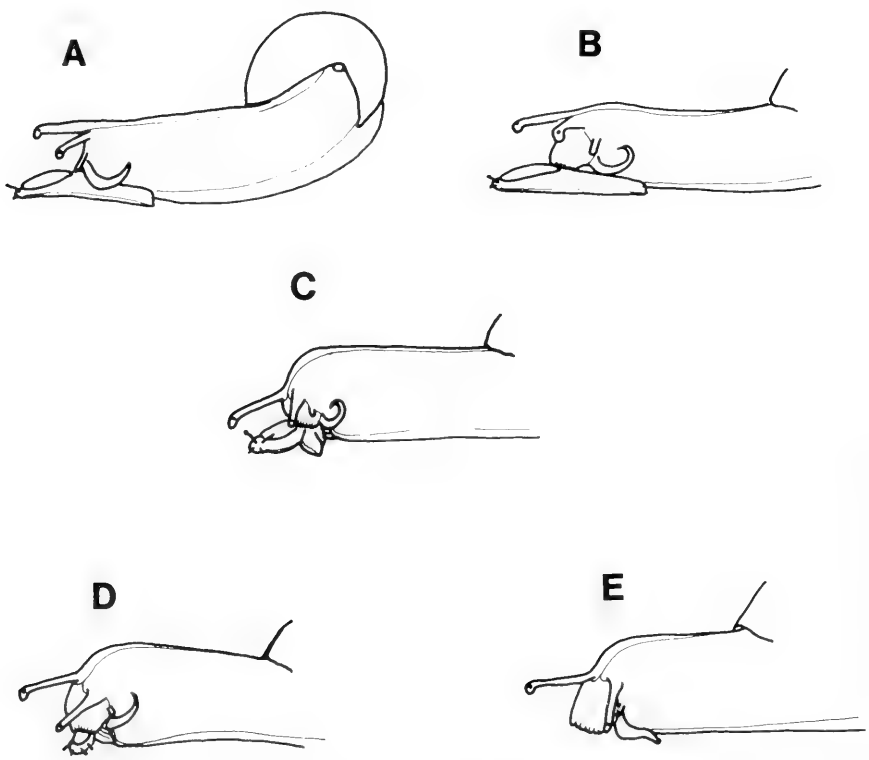

FIG. 4. *Euglandina* feeding on *Deroceras*. The slug is attacked after being overtaken (A). The initial strike is frequently made at the mantle (B) and the prey sucked into the mouth in one or two bites (C–D). After the prey has been swallowed the debris is mopped up (E). The *Euglandina* shell length is approximately 3 cm.

firmly attached to the substrate and were raised clear of it. Subduing a slug took longer because slugs invariably moved rapidly and the predator slowed down to strike. Attacking large snails took the longest time since the predator examined the shell and manoeuvred to make a standard approach across the body whorl from right to left before lifting it.

2. Eating time. The time at which the soft parts of a *Succinea* were completely consumed within the shell was taken as the time at which the clearly visible, dark digestive gland disappeared from the shell apex. The time taken to consume this snail depends upon the site of the initial attack. In seven of the 20 attacks observed the initial strike severed the columellar muscle and the prey was extracted from its shell whole. For all attacks on *Succinea* the mean time (± s.e.) taken to eat the exposed part of the prey was 107 ± 16 sec (n = 20). In cases where the remnants of the digestive gland had to be extracted from the shell a further 213 ± 37 sec (n = 13) were expended before the complete consumption of the prey. The time taken to eat a

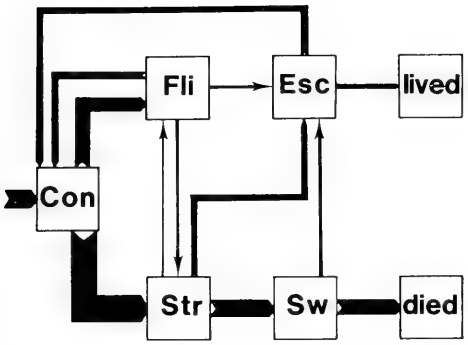

FIG. 5. A flow diagram of the events occurring during feeding on *Deroceras* showing the responses of the slug and its effect on its survival. The conventions are as in Fig. 1. The additional behaviour is: Fli—prey flicks tail or flares mantle.

small prey snail is all taken up by the process of extending the mouthparts over the shell. Slugs were eaten extremely rapidly.

3. Clearing up time. *Euglandina* took the longest time to clear up after eating *Succinea.* Of the final 613 sec, 464 ± 139 was spent moving the everted mouthparts over the surface of the prey shell. The remaining time was spent in similar activity on the substrate and in searching behaviours.

Polygyra was eaten whole and left no debris and no *Euglandina* ever performed mopping up behaviours after eating this species. A mean of 21 ± 3 sec of the final 25 sec was spent after the inversion of the mouthparts and before moving off. There was little searching behaviour and this static time allowed the passage of the uncomfortably large prey to the stomach.

Slugs left a considerable amount of debris since the initial strike normally splits the digestive gland which spills out. Also, slugs produced a copious mucus as a response to being attacked. Of the final 54 sec 43 ± 8 sec was therefore spent with the mouthparts everted.

DISCUSSION

Most pulmonates are herbivores and in some instances their feeding behaviours and their physiological bases have been described in detail (e.g. *Helisoma*—Kater, 1974; *Lymnaea*—Rose & Benjamin, 1979). In general they have cyclical feeding patterns involving repeated odontophore movements integrated with regular swings of the head from side to side (Dawkins, 1974). Some pulmonates are facultative predators consuming annelids and fellow gastropods as the opportunity arises (Cooke, 1895). In these cases the regular feeding behaviour is supplemented by other behaviours. Rollo & Wellington (1979) describe aggressive encounters between slugs. Some of their descriptions of the behaviour of *Limax maximus* L. bear a striking resemblance to the behaviour of *Euglandina,* e.g. "Following this initial 'tasting' the aggressor suddenly bit the victim, striking simultaneously with its everted radula and jaw." A "rear and lunge" behaviour is also described. The structures used by slugs to bite in these encounters appear to be the same as those used by *Euglandina,* though the latter has much longer, slicing, radular teeth (Solem, 1974) and the everted mouthparts are much larger. These behaviours in *Limax maximus* may not be primarily predatory since they occur seasonally, coinciding with breeding and may be better viewed as territorial behaviours (Rollo & Wellington, 1979). Nevertheless the predatory behaviour of *Euglandina* is clearly a specialised version of behaviours which appear in non-predatory pulmonates.

There are few descriptions available of the feeding behaviour of other specialised predatory pulmonates with which to compare that of *Euglandina.* Solem (1974) states that pulmonates with specialised stabbing radulae (e.g. *Testacella*) 'harpoon' their prey, whilst a New Zealand predatory snail, *Paryphanta,* apparently smothers its prey by enveloping it in the folds of its foot and dragging it into the body whorl.

Most gastropods are passive prey and, once caught, play no active part in determining the behaviour of the predator. Some potential prey items have passive defences such as distasteful mucus or an extremely tough integument (Cook, in press a). The active defence behaviours of *Deroceras laeve* are those seen commonly in the limacid slugs (Rollo & Wellington, 1979). Tail flicking, mantle flaring and rapid movement often lead to misdirected strikes and the frequent escape of the slug. In the present experiments approximately 25% of slugs escaped completely. In the field the proportion of escaping slugs is likely to be higher since the slug can avoid further attacks by hiding in cracks too

small to accommodate the shell of *Euglandina.*

The handling times of *Succinea,* which was the largest snail used in the present work (Table 1) seem out of proportion to the benefit gained from its consumption since its soft parts are about the same size as those of the *Deroceras.* The greatest gain in energy per unit time actually spent feeding therefore is probably derived from *Deroceras* but the precise relationships between handling times and prey preferences are problematical. The prey of *Euglandina* normally lives aggregated and at high densities (Cook, 1984). It would seem to be, therefore, of no advantage to take up so much time in the *complete* consumption of prey tissues as is seen with *Succinea.* There have been no experimental studies of prey preferences in *Euglandina,* however, and therefore there is no sound basis for the interpretation of the extended handling time of larger prey.

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CAUSES OF LIFE HISTORY VARIATION IN THE FRESHWATER SNAIL *LYMNAEA ELODES*

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ABSTRACT

Intraspecific life history variation in the pond snail *Lymnaea elodes* Say was studied in three ponds in northeastern Indiana. Population densities fluctuated more dramatically in the temporary ponds due to high juvenile mortality caused by unpredictable drying times. Shell growth rates were greater in a more productive, permanent pond. In a reciprocal transfer experiment, snails in the most productive pond, regardless of origin, grew roughly twice as fast, and laid eight to nine times as many eggs as snails in the less productive, temporary ponds. However, snails originating from the most vernal pond always had slower growth rates, smaller shell lengths at maturity, and higher fecundities than other snails reared in the same pond. Although proximal factors like habitat productivity therefore explain much of the intraspecific life history variation, genetic divergence among populations is still discernible. The lower productivity and uncertain nature of vernal ponds apparently favor early maturity and high fecundity in this freshwater snail.

Key words: *Lymnaea*; life history variation; proximal and evolutionary causes.

INTRODUCTION

Intraspecific life history variation is often considered as the result of natural selection producing local adaptations in life history tactics. Stearns (1976) summarized the proposed selection forces, as well as how they are predicted to produce covariation in life history traits. However, there are other explanations for intraspecific life history variation. Variation may be the result of environmentally induced phenotypic changes such as developmental plasticity, or physiological acclimation (Stearns, 1980). Second, variation (or the lack of it) may be due to phylogenetic constraints caused by past evolutionary history. For example, Calow (1978) suggests that egg size may be phylogenetically limited in fresh-water prosobranchs. Finally, intraspecific variation may indeed be due to differing selection regimes among habitats.

It is therefore necessary to determine the degree of genetic basis to intraspecific life history variation before we can rule out any of these alternatives. One technique is to use quantitative genetic methods to determine the heritabilities of and genetic correlations among life history traits. However, this approach is often difficult under field conditions. Another technique is to perform reciprocal transplant experiments. That is, if individuals are transferred among habitats, and compared to residents, the relative importance of genetic and environmental factors can be discerned. For example, Berven (1982) was able to show that water temperature was the most important factor explaining intraspecific life history variation in wood frogs from different elevations. However, genetic variation did occur in some life history traits, often opposing the environmentally induced variation ("countergradient selection"). Thus both proximal and evolutionary factors may be important in explaining life history variation, and more studies need to be done to extend our knowledge of the relative importance of each.

The objective of the current study is to perform such an analysis for the freshwater pulmonate snail *Lymnaea elodes* (= *palustris*) Say. We present sampling data on food resource levels, population dynamics, and shell growth rates in three pond populations in northeastern Indiana. We then reciprocally transplant snails among all three populations in a field rearing experiment, and compare growth rates, shell size at maturity, fecundities, and egg weight and caloric content among ponds and populations. We also rear snails from all three populations under con-

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stant conditions in the laboratory to determine if contrasts remain among populations and thus have a genetic basis.

Lymnaea elodes is a good candidate for such a study. Populations vary in productivity, length of life cycle, and fecundity, possibly because of differences in habitat productivity (Hunter, 1975). On the other hand, Forbes & Crampton (1942) reported considerable intraspecific variation in life histories, which they considered to be genetic, since variation remained after several generations of laboratory rearing. Finally, the density of adult snails may also determine fecundity in *L. elodes* (Eisenberg, 1966, 1970).

The species is fairly common in ephemeral habitats in the northern United States and Canada (Harman & Berg, 1971; Brown, 1979). It has an annual life cycle, with breeding in late spring to early summer. Juveniles overwinter in temporary ponds by burrowing into the soil, and adults can survive for a second year by forming epiphragms (Eisenberg, 1966; Jokinen, 1978). Populations are bivoltine in extremely productive habitats (Hunter, 1975) and univoltine in temporary ponds (Brown, 1979). *Lymnaea elodes* feeds primarily on periphyton, although it also utilizes carrion (Brown, 1982).

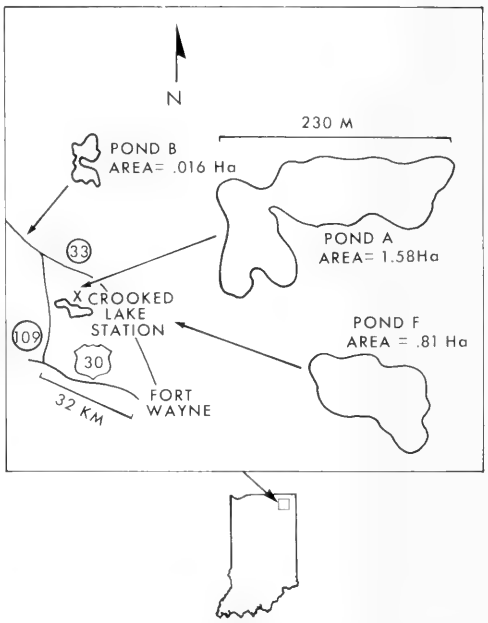

FIG. 1. Location and surface area of the three ponds sampled. Ponds A and B are temporary; pond F is permanent.

METHODS

Sampling of habitats

The three ponds are within 30 km of the Crooked Lake Biological Station, 33 km NW of Fort Wayne, Indiana. The ponds are drawn to scale in Fig. 1. Surface areas of the temporary ponds (A, B) vary, but the areas shown are representative for the breeding period of *L. elodes*. Pond A is the most vernal, drying by mid July. It, like the other ponds, has a muck substrate (a mixture of clay and organic detritus). Food resources for snails are both allochthonous (leaf litter, decaying grasses) and autochthonous (periphyton composed mostly of diatoms and blue-green algae). Pond B dries by late July or early August. Food resources are decaying terrestrial grasses that invade the pond during the dry season, and periphyton. Pond F is permanent, and food resources for snails are mostly autochthonous (periphyton, duckweed, submerged plants). The ponds have similar levels for water temperature, pH, and dissolved oxygen (Brown, 1982). Pond B has harder water, and lower dissolved phosphate levels (19 $\mu g/\ell$ vs. 44 $\mu g/\ell$ in pond A and F).

Since nutrient levels differ among ponds, we test whether periphyton biomass also varies among ponds. Periphyton biomass accumulation was determined with a Wildco periphyton sampler. From 3 to 8 replicate, preweighed, slides were removed at each date during a study of biomass accumulation. Slides were dried overnight at 60°C in a drying oven, and weighed on an analytical balance (sensitivity = .1 mg). Values reported are dry biomass per slide ± s.e.

Ponds were quantitatively sampled at biweekly intervals during the field season, starting in 1978 in A and B, and 1979 in F. Sampling areas were allocated randomly throughout the pond, but no area was sampled twice on the same day. An Ekman dredge (sampling area = .05 m^2) removed vegetation and the first few cm of substrate. Hauls were pooled in groups of four to form a sample. Preliminary sampling was done early in the spring to determine the number of replicate samples needed at each date. Early spring was chosen because snail densities were lowest then, and sampling variances highest. In general, enough samples are required in benthic studies to reduce the ratio of

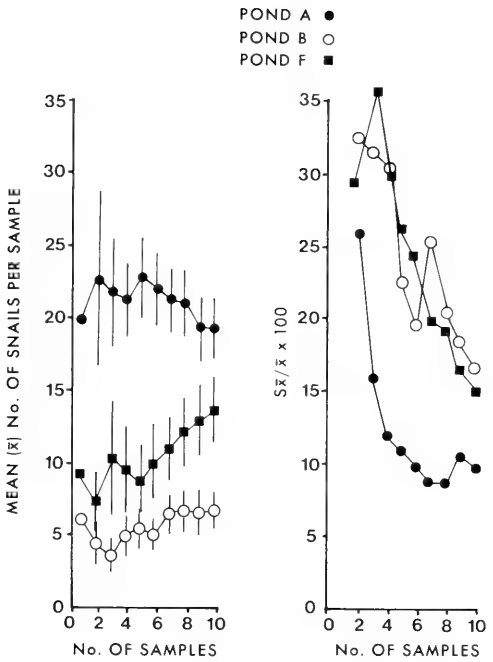

FIG. 2. Sampling variation for preliminary Ekman dredge sampling of the three ponds, plotted against the number of replicate samples.

the standard error to the mean to less than 20%, or to a point where the mean density ceases to fluctuate (Green, 1979). After 10 replicate samples (40 dredge hauls), Fig. 2, standard errors were less than 20% of mean densities in all 3 ponds, and mean densities were stable in ponds A and B. Ten replicate samples were therefore taken at each date.

Adult snails were removed first from samples, and the remaining vegetation was hand sorted, and juveniles and egg cases removed. Samples containing mud were washed through a series of sieves. A sample of 50 egg cases was counted at each date and the mean number of eggs per case was multiplied by the number of cases per sample to estimate egg abundances. Egg, juvenile, and adult counts were converted to a m^2 basis to determine changes in population dynamics.

Growth in shell length was estimated by following cohorts in the ponds. The age of the cohort (symbolized X) was the time since the peak of egg production in the appropriate breeding season. Examination of shell length histograms indicated a sigmoid relationship between shell length and age: little growth in the first fall and winter, rapid growth the next spring, and slowing of growth after the onset of reproduction. Differences in shell growth rates among populations were therefore estimated by fitting growth curves to a sigmoid function:

$$L = Cl \cdot (1 + \exp(C2 - C3 \cdot X))^{-1}.$$

The parameter Cl is equivalent to the final shell length, the parameter C3 is proportional to shell growth rates, and the parameter C2 has no simple biological analogue. Values of parameters were fit with an iterative non-linear technique (Conway *et al.,* 1970), and non-linear 90% confidence intervals were used to determine whether parameter constants differed among populations.

Experimental methods

The effect of resource abundance was determined by rearing snails in each of the three ponds. The effect of snail density was determined by rearing snails at densities of two and four snails per container. We tested for genetic differences in tactics by rearing two populations in each pond. Snails from the most temporary pond (Pond A) were reared in each pond. Comparison populations in ponds B and F were resident snails, the pond F snails were used in pond A. The design was completely randomized with a factorial arrangement of treatments (3 ponds × 2 populations per pond × 2 densities per population).

We used 1 ℓ plastic containers, with openings covered by aluminum screens to minimize fouling (Brown, 1979, 1982). Aluminum ions were not released into solution as the pH of the ponds seldom dropped below 6 (Brown, 1982). Containers were attached to floats to allow air space for the air breathing pulmonates. There were 15 replicate containers in each of the 12 treatments. Two to four immature snails (4–7 mm) were introduced to the containers on May 9–12, 1980. Each week containers were removed and the snails measured, 5 g of fresh pond vegetation added, and all egg cases removed. The experiment was terminated on July 17, as pond A was drying. Analysis of covariance was used to remove variation (due to shell growth before the start of the experiment) in fecundity per snail, shell length at maturity, egg weight in mg, and growth increments in shell length. Fecundity data were also log-transformed to remove a mean-variance correlation.

Three replicate egg samples from each treatment were combusted in a Phillipson microbomb calorimeter to determine caloric investment in eggs. Separate samples were ashed overnight at 550°C in a muffle furnace to convert caloric data into calories per mg ash free dry weight (A.F.D.W.). To test the hypothesis that caloric expenditures in eggs were independent of pond productivity, population, and density, all data were fit to a common regression line of calories against A.F.D.W. of sample.

We also tested whether population contrasts in life history traits would remain under common conditions with a laboratory rearing experiment. Snails were reared in controlled temperature cabinets maintained at 21°C ± 1°C (an average temperature during growth periods in the pond) and a 12:12 light cycle. Pairs of immature snails were placed in aerated 3 ℓ aquaria, with 15 aquaria from each population. Fresh water and 10 g of fresh pond vegetation were added bi-weekly. Pond vegetation in all experiments was a mixture of grasses and leaves from the ponds, dried to kill eggs and small snails. It provided both a raw food resource and a substrate for periphyton colonization in the aquaria and containers. No supplements were added since they may artificially bias growth and fecundity results (Eisenberg, 1970). Shell length and egg laying rates were monitored weekly.

RESULTS

Field sampling

The accumulation of periphyton biomass on submerged slides differed among the three ponds (Fig. 3). Pond B, with lower dissolved phosphate levels, had the lowest periphyton biomass. Biomass accumulation was similar in the other two ponds; production was somewhat higher in pond A up until 10 days, but increased no further. Accumulations in pond F continued to increase (Fig. 3). The two temporary ponds dried at this point, but biomass in F was still greater after 30 days. Thus, in terms of standing crop of periphyton, the three ponds would be ranked F ≥ A > B.

Shell growth was related to differences in periphyton biomass among ponds (Table 1). The value of parameter 3 (proportional to growth rate) was highest in F, somewhat lower in A, and significantly lower in pond B. Non-linear confidence intervals for parameter 3 overlapped slightly in ponds A and F, but not with pond B. The model predicted snails in pond F to reach shell lengths of 20.6 mm during their first year, 19.8 mm in pond A, and only 14.1 mm in pond B. The smallest reproductive snails are about 14 mm in *L. elodes* (Brown, 1979) and so snails may not reach reproductive size in their first year in the least productive pond.

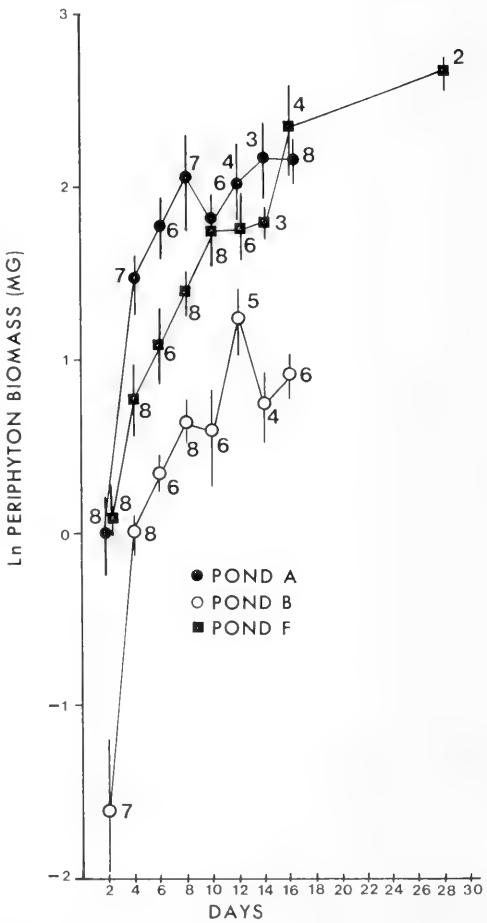

FIG. 3. Natural logarithms of periphyton biomass accumulation (X ± s.e., n given in each case) on submerged slides in each pond.

However, final adult shell lengths in the three populations (parameter 1) overlap broadly (Table 1), because of a difference in life cycle length. Two year old snails could seldom be found in pond F, but cohorts could be followed in the two temporary, less productive ponds for two or three seasons. Snails in the temporary ponds therefore reach

TABLE 1. Parameter estimates for fit of shell growth to a sigmoid function and 90% non-linear confidence intervals.

Pond	Parameter	Value	Non-linear 90% confidence interval	Ratio of explained to total sum of squares
A	C1	27.6	23.2–32.1	96%
	C3	.026	.024–.030	
B	C1	28.9	26.1 – 31.8	97%
	C3	.005	.004–.006	
F	C1	26.3	22.9–29.8	99%
	C3	.031	.029–.035	

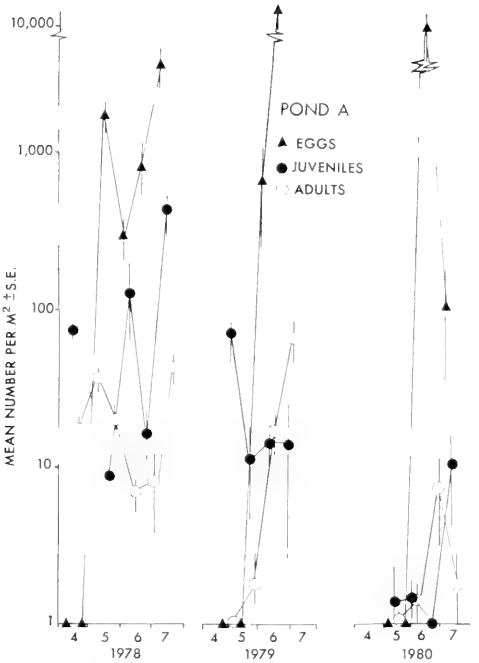

FIG. 4. Semilogarithmic plots of densities of adults, juveniles, and eggs through time in Pond A. Values are means ± s.e. (n = 10).

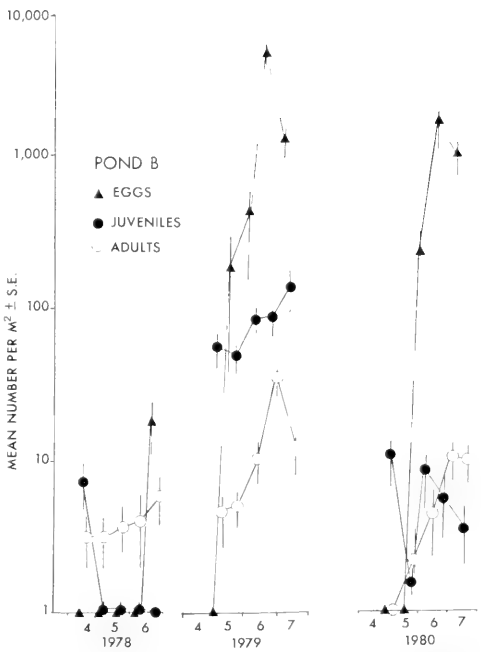

FIG. 5. Semilogarithmic plots of densities of adults, juveniles, and eggs through time in Pond B. Values are means ± s.e. (n = 10).

the same final shell lengths, but at a later age due to their slower growth rates.

The abundances of eggs, juveniles, and adults fluctuated dramatically through time, especially in the two temporary ponds (Figs. 4, 5). Egg abundances were usually highest in pond A, often near 10,000 per m^2. Juvenile densities were usually near 100 per m^2 in the most vernal pond, while adult densities were near 20 per m^2. A crude estimate of survivorship to maturity (simply adult density divided by egg density) would be near 0.2%. Inspection of seasonal trends indicates that egg laying usually peaked in early June, and that no eggs survived over the winter (Fig. 4). Juvenile and adult densities increased during the season, due both to recruitment and the shrinking of the pond. Adults apparently overwintered much more poorly than juveniles. The 1979 field season was very short with the pond drying by mid June, and even juvenile densities were much lower in 1980, indicating that early pond drying significantly lowers juvenile survivorship.

Egg densities in pond B were somewhat lower, usually peaking around 5,000 per m^2

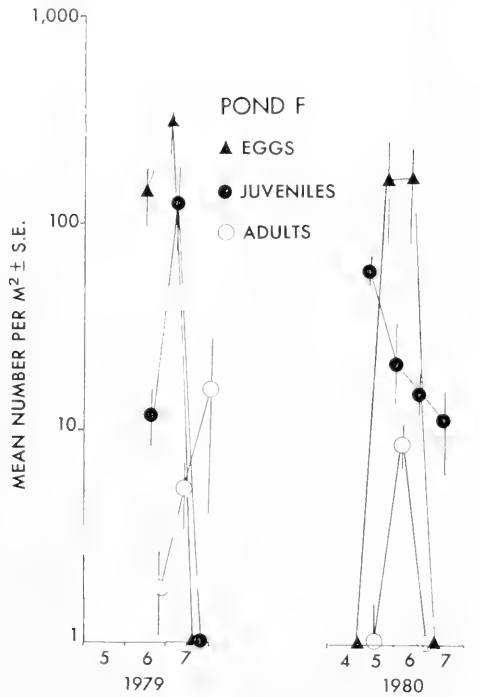

FIG. 6. Semilogarithmic plots of densities of adults, juveniles, and eggs through time in Pond F. Values are means ± s.e. (n = 10).

(Fig. 5). Juvenile densities varied dramatically from year to year, but an average would be around 10 per m^2, similar to the adult density. Thus an estimate for survivorship to maturity would again be near 0.2%. Adult survivorship was fairly high over the winter of 1978–1979, but was very poor after the short field season of 1979. Again as in the most vernal pond, densities of juveniles and eggs increased during the season due to recruitment and shrinking of the pond.

In contrast, egg densities never reached above 500 m^2 in the permanent pond (Fig. 6). Juvenile densities averaged around 30 per m^2, and adult densities around 10 per m^2. A crude estimate for survivorship in the permanent pond would then be approximately 2.0%. Of course, this survivorship estimate could be biased upwards if eggs or juveniles were being preyed upon at a higher rate in the permanent pond.

Experiments

In the field rearing experiment, snails grew rapidly in the most productive pond (Fig. 7), and growth increments decreased linearly in the other two ponds. Pond productivity had a highly significant effect on shell growth, as did snail density (Table 2). However, snails from the most vernal pond still had significantly lower growth rates than comparison populations, at each site and density (Fig. 7). The significant pond A × density interaction term (Table 2) indicates pond A snails suffered greater decreases in growth rates at the higher densities. In summary, judging from the relative magnitude of F ratios (Table 2) habitat productivity had the greatest effect on growth increments, followed by population contrasts, and density. The average coefficient of variation for growth increments, over all treatments, was 31.9%.

Habitat productivity had even more dramatic effects on snail fecundity (Fig. 7). Snails in pond F grew roughly twice as large, but laid on the average eight times as many eggs. Initial shell size also had a significant effect on fecundity, unlike growth increments (Table 2). Density also had a highly significant effect on fecundity.

Population contrasts in fecundities were also significant (Table 2). Snails from the

TABLE 2. Table of F values from ANCOVA of 1980 field container experiment. An asterisk indicates significance at .05 level, two at .01 or more.

Source of variation	Growth increment (mm)	Shell length at maturity (mm)	Fecundity per snail	Egg weight
Initial size (covariate)	.58	7.93**	64.34**	3.47
Pond	63.32**	28.70**	81.65**	4.38*
Pond A snails vs. others	29.84**	68.63**	6.46*	3.04
Density	14.60**	13.54**	23.48**	<1
Pond × pond A snails	2.85	2.79	<1	<1
Pond × Density	<1	1.50	<1	4.73*
Pond A × density	5.09*	<1	<1	<1
Pond A × density × pond	1.84	1.75	<1	<1

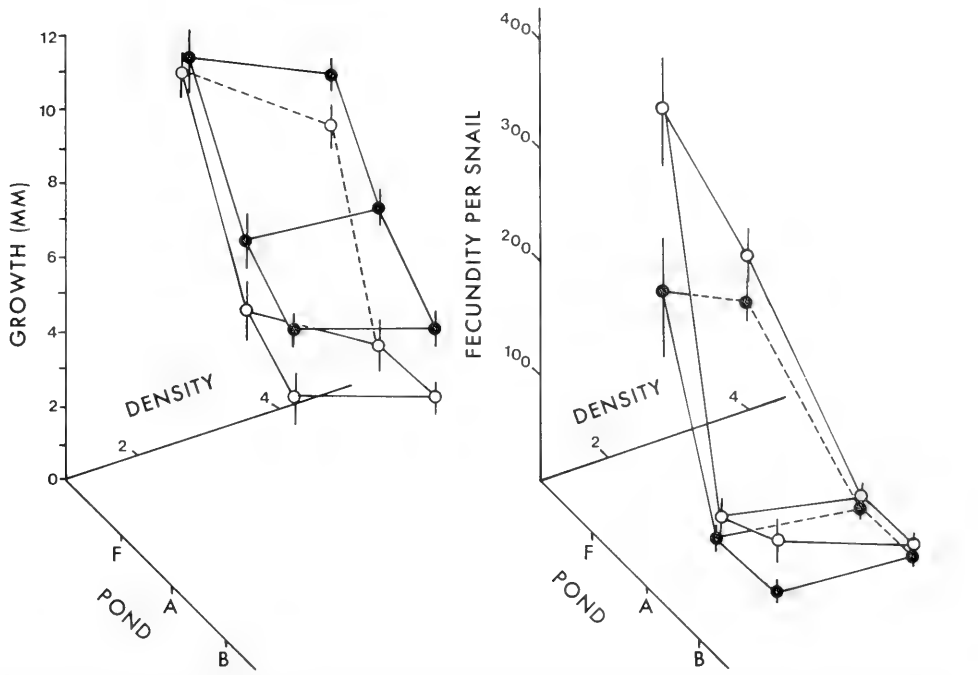

FIG. 7. Response surfaces for shell growth increments (left) and fecundity (right) for snails reared in 3 different ponds, and at two snail densities per container. Ponds are arranged from left to right in order of declining productivity. Response surfaces for pond A snails have open circles, comparison populations are solid circles. Dotted lines indicate where response surfaces lie below each other. Data are means ± s.e. for each treatment (n = 15).

most vernal pond had significantly higher fecundities than comparison populations, regardless of rearing site or density. Judging from the value of F ratios, habitat productivity had the greatest effect on fecundity, followed in order by initial shell length, density, and population contrasts. Over all treatments, the average coefficient of variation for fecundity was three times higher than for growth rates, (106.5%).

Shell length at maturity was also a function of habitat productivity (Fig. 8, Table 2). Snails matured at smaller sizes in the less productive ponds. Snails from the most vernal pond also reproduced at significantly smaller shell lengths. Finally, both density and initial shell length had significant effects on size at maturity.

In contrast, mean dry egg weights varied extensively within treatments (Fig. 8), and only the pond main effect and the pond-density interaction were significant in the analysis of covariance (Table 2). There was a general trend of decreasing egg weight with declining habitat productivity, although not marked. The pond-density interaction occurred because mean egg weight decreased with density in pond A, but remained the same or increased in the other two ponds. Although in most cases pond A snails laid less massive eggs (Fig. 8), the high variation within treatments (coefficient of variation = 136%) kept contrasts from being significant (Table 2).

Finally, neither habitat productivity, source population, nor snail density affected caloric content per mg A.F.D.W. of eggs (Fig. 9). Over all treatments, the coefficient of variation in calories per mg A.F.D.W. was only 12.8%. In a separate analysis of variance the effects of habitat productivity ($p = .27$), population contrast ($p = .42$), and density ($p = .34$) were all insignificant.

Although snails from the temporary pond tended to lay more eggs in the laboratory rearing experiments, no significant differences occurred due to high variances within populations (Fig. 10). A one way analysis of covariance (with initial shell length) indicated that neither shell growth increments ($p = .54$),

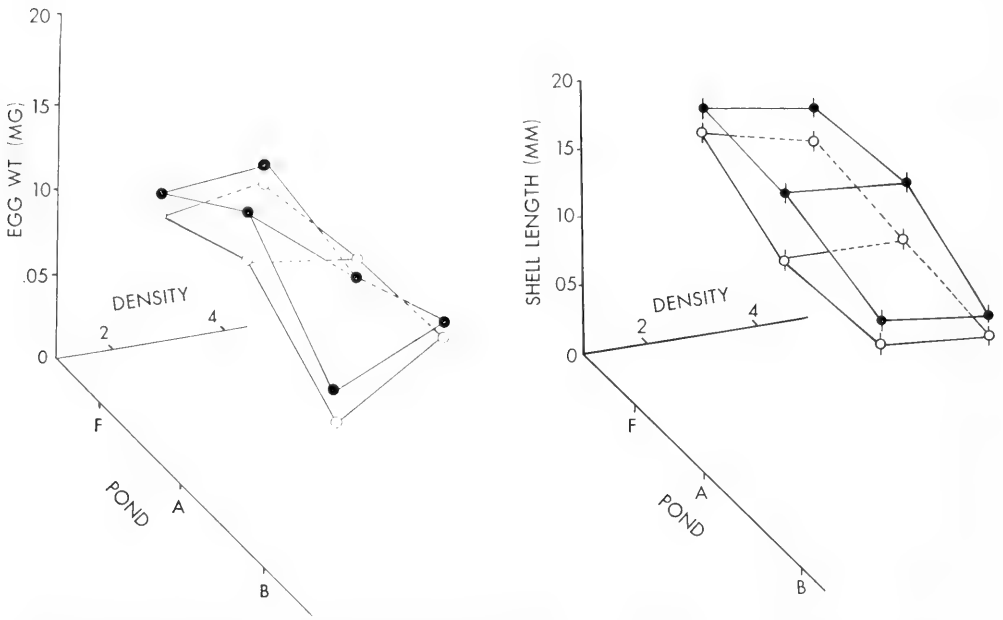

FIG. 8. Response surface for mean dry egg weights (left) and shell length at maturity (right) for snails reared at 2 densities in each of the ponds. Symbols as in Fig. 7. Standard errors are not included for egg weights due to the high coefficient of variation (see text).

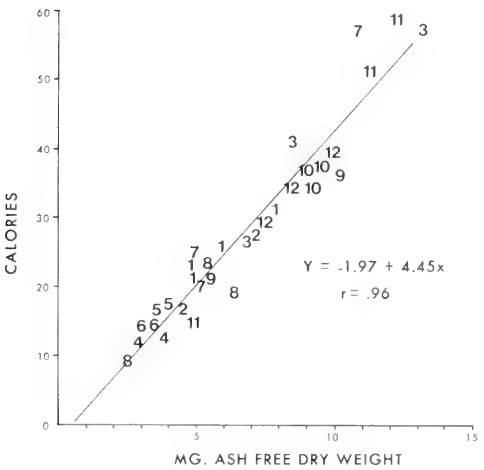

FIG. 9. Regression of caloric content in eggs against mg A.F.D.W. of sample. Numbers 1–4 are from snails reared in pond A, 5–8 in B, and 9–12 in F. In each pond the first 2 numbers are resident snails at 2, and 4 snails per container, and the latter 2 are transferred snails.

final shell length ($p = .08$), nor fecundity per snail ($p = .33$) differed significantly among populations. Again, coefficients of variation for fecundity (169%) were much higher than for growth rates (30%).

DISCUSSION

Habitat productivity explained a considerable proportion of intraspecific variation in life history tactics of *L. elodes*. In the field rearing experiment, snails grew roughly 1.6 times larger in pond F than in pond A, and 1.8 times larger than in the least productive pond B. Fecundities were 8.1 times greater, averaged over both source populations, in pond F than in pond A, and 9.2 times greater than in pond B. Habitat productivity also increased shell size at maturity, but did not have as large an effect on mean weight of eggs, or caloric content in eggs. Shell growth data collected from field populations also indicated that snails grew faster in the more productive ponds and had shorter life cycles.

Overall, resource availability is an important proximal determinant of life history variation in snails. Hunter (1975) also found variation in productivity and voltinism to be a function of habitat poductivity in *L. elodes*, and Browne (1978) found the same for the freshwater prosobranch *Viviparus georgianus*. The length of time resources are available during the life cycle may also be important (Aldridge, 1982).

Snail density also had significant effects on

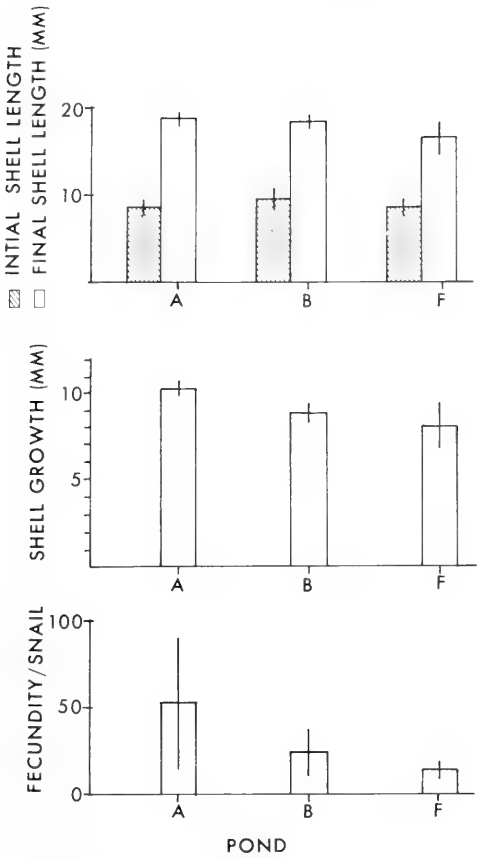

FIG. 10. Life history variation for snails reared in a common laboratory environment. Initial and final shell lengths are at the top, growth increments in the center, and fecundity per snail at the bottom. Data are means ± s.e. (n = 15).

growth rates and fecundities. Effects on fecundity were especially large, with increments in density reducing fecundities, averaged over both source populations, 63.2% in pond F, 82.4% in pond A, and 79.3% in pond B. Eisenberg (1966, 1970) suggested that adult densities regulate fecundity in this species. Our data indicate this to be more the case in temporary ponds, where periphyton biomasses were lower.

Genetic divergence among populations explained a comparatively small proportion of the variation in life histories. However, snails from the vernal pond always grew more slowly, matured at a smaller shell length, and had higher fecundities than other populations. These smaller differences may still be important over evolutionary time scales; little is known, for example, about the amount of gene flow among snail populations. Interestingly, Berven (1982) also found a large proximal component to intraspecific life history variation in ranid frogs, caused by temperature variation among ponds at different altitudes. Frogs from higher altitudes still grew more rapidly than low altitude frogs in the same pond, suggesting genetic adaptation produced by "counter-gradient selection." Hence the pattern was similar to this study: most variation explained by proximal factors, but still a discernible genetic component.

The selection forces responsible for such intraspecific genetic variation in most cases are still unknown. Calow (1981) noted that *Lymnaea peregra* from exposed sites matured earlier and had higher reproductive effort. Differences also remained in the laboratory, suggesting genetic origin. Calow considered that exposed populations were r-selected due to greater density independent mortality. However, other studies of life history variation in molluscs do not report traits covarying as expected from r- and K-theory (McCleod *et al.,* 1981, Hart & Begon, 1982).

In the current study, proximal factors explain most of the variation in life histories among populations, although genetic differences are still important. In temporary ponds, low resource levels reduce growth rates and fecundities. The combination of low productivity and unpredictable drying dates have favored the evolution of high reproductive rates in these vernal ponds, as well as early ages and sizes at maturity.

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SCANNING ELECTRON MICROSCOPY OF THE BODY SURFACES OF *BIOMPHALARIA GLABRATA*

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ABSTRACT

The body surfaces of small and large snails of the Puerto Rican strain of *Biomphalaria glabrata* were studied by scanning electron microscopy. The whole surface of the snail is covered by cilia except the epidermal surface of the anterior part of the head and the mantle surface covering the visceral mass.

On large snails, the dorsal surface of the head away from the bases of the tentacles is irregularly folded and has a spongy appearance, while it is smooth with regular folds on small snails.

The ventral and dorsal surfaces of the mantle collar of large and small snails are covered by dense cilia and microvilli. In addition, a basal layer on the external surface of the mantle collar, covered by microvilli, was only observed on large snails.

Bulbous structures with an opening to the exterior were found on the surface of the pneumostome. Their function is unknown.

Several globular structures are extended from the surface of the sole of the foot, probably representing mucous secretions from the subepidermal tissue. These structures were more pronounced in large snails. The surface of the sole of the foot is densely covered by cilia which may play a role in distributing the slime secretions over the surface of the foot.

Key words: *Biomphalaria glabrata*; scanning electron microscopy; body surfaces.

INTRODUCTION

The anatomy and general histology of freshwater Pulmonata have been studied by several authors (Pan, 1958; Bolognani Fantin & Vigo, 1967a, b). Most of this research has used the light microscope, and very little has been done on the ultrastructure of these snails—especially dealing with the epidermal surfaces. It is known that the epidermis of freshwater gastropods has several functions such as respiration (Zaaijer & Wolvekamp, 1958; Jones,1961), osmoregulation (Greenaway, 1970, 1971), and perception (Jager, 1971). Zylstra (1972) studied the epidermis and the associated subepidermal gland cells of the freshwater snail *Biomphalaria* by means of histochemical and electron microscope techniques. He reported that the single-cell-layered epidermis is composed of general epidermal cells, cilia, and a few scattered goblet cells. Sullivan *et al.* (1974) studied the ultrastructure of the rectal ridge of *Biomphalaria glabrata.* The structure and function of the mantle cavity of *Biomphalaria glabrata* were studied by Sullivan & Cheng (1974); they found that the histology of the rectal ridge (a single layer of columnar epithelium) supports its role in the uptake and elimination of substances by the snail. They also reported that the pseudobranch has no respiratory function, but acts as a valve to close the chambers of the mantle cavity on chemical insult.

The purpose of our study was to investigate the body surface structure by scanning electron microscopy (SEM) of young and old *Biomphalaria glabrata* (Say), the intermediate host of *Schistosoma mansoni,* with the hope of adding information to some aspects of the host-parasite relationships of schistosome miracidia and their intermediate hosts.

MATERIALS AND METHODS

Snails of the Puerto Rican strain of *Biomphalaria glabrata* were maintained in an aerated aquarium in spring water and were fed fresh lettuce. The diameter of the shell was

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measured and taken as an indicator of age. To measure the diameter, we started from the lateral edge of the upper margin of the aperture, across the shell to the other side, on the dorsal surface of the snail. The diameter range of snails studied was 1–11 mm.

In preparation for SEM, a snail was put in a small petri dish with a little spring water, and the diameter was measured. Then, the shell was crushed by application of pressure by another, smaller petri dish. The shell fragments were removed and the snail was fixed in 2% buffered glutaraldehyde overnight. Then it was washed for 3 changes in spring water, 10 minutes each.

The remaining steps in specimen preparation for the SEM were carried out following Voge *et al.* (1978). Snails were scanned over their whole surface, including the exposed part of the foot, head, tentacles and the mantle collar. The size of the snail was recorded on each photograph.

For light microscopy, snails were fixed in 10% formalin or Carnoy's fixative, embedded in paraffin and sectioned at 4–6 μm. Slides were stained with haematoxylin and eosin or Barbeito-Lopez trichrome stain.

OBSERVATIONS

As the whole surface of the snail is covered by cilia, the distribution of these cilia, their density on different parts of the snail body, and the presence of microvilli in both small and large snails were studied.

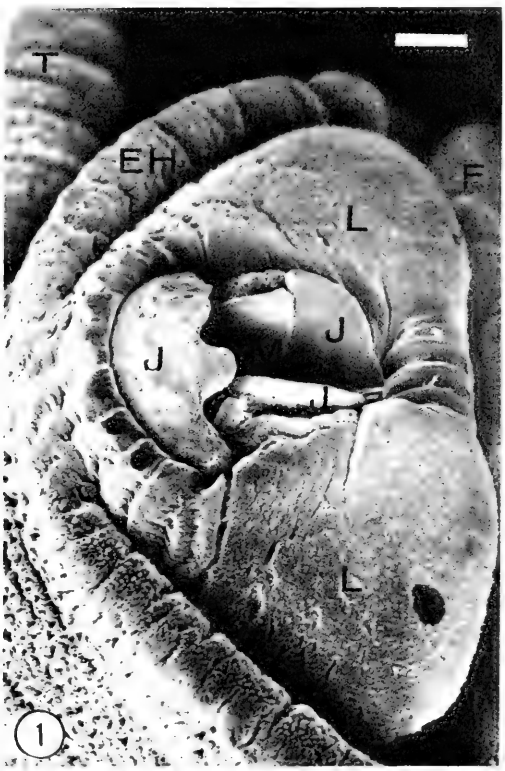
The body of *Biomphalaria glabrata* is divided into head, foot, mantle region and visceral mass. The head is not well demarcated from the body; it bears the tentacles, eyes, mouth, lips and jaws. The epidermal surface covering the two lips and the jaws is smooth. The mouth has a dorsal appendage, or protrusion, which has a surface like the jaws, and is presumably the median jaw (Fig. 1).

The dorsal surface of the head shows a variable distribution of ciliated epidermal cells. In some parts it is densely covered by long cilia, as at the edge of the head of large and small snails (Fig. 2); the cilia gradually become reduced to little patches around the base of a tentacle (Fig. 3). The epidermal surface covering the rest of the dorsum of the head of large snails is nearly devoid of cilia except for widely separated tufts of short cilia. The surface appears spongy, with irregular folds and many pores (Fig. 4). On the other hand, the dorsal surface of the head of small snails has regularly-spaced folds with a few tufts of short cilia (Fig. 5). The undersurface of the head is similar to that of the head near the base of the tentacle. A tentacle is a gradually tapering cylinder, attached to the dorsolateral surface of the head. Its whole surface is covered by epidermal cells having long cilia (Fig. 6) that are very dense at the tip and the upper third (Fig. 7), while decreasing in density toward the base of the tentacle where much of the epidermal surface is devoid of cilia (Figs. 8, 9). The distribution and density of the cilia are the same in small and large snail tentacles. The mantle is composed of a single layer of epithelial cells which were seen in the section obtained for histology. It embraces the neck of the snail and covers the pallial region and visceral mass. The enlarged glandular part of this mantle, often visible along the rim of the shell aperture, is known as the mantle edge or mantle collar.

An accessory structure of many folds situated externally at the junction of the head with the mantle collar, known as the pseudobranch, is suggested to have a respiratory function (Malek & Cheng, 1974). The surface of the pseudobranch is covered by heavy, long cilia.

The short siphon-like pneumostome is located near the pseudobranch. The surface of this structure is covered with short cilia and microvilli. Globular structures which possess openings suggestive of gland cells are scattered between the cilia (Fig. 18). In small snails (1.0–3.0 mm), the mantle collar is flattened over the surface of the body while in large snails, it is deflected dorsally, exposing its ventral surface. The ultrastructure of the dorsal surface of the mantle collar of small and large snails shows slight differences.

FIGS. 1–5. Head of *Biomphalaria glabrata*. 1. SEM of head anterior of 9.5 mm snail, showing the edge of the head (EH), foot (F), jaws (J), lips (L), mouth (M), and tentacle (T). Scale bar 100 μm. 2. SEM of edge of head of 11 mm snail, showing dense cilia. 3. SEM of surface of head of 5.5 mm snail around base of tentacle, showing tufts of cilia. 4. SEM of head surface of 8 mm snail away from base of tentacle, showing irregular folds with spongy appearance. 5. SEM of the same area as Fig. 4 of 2 mm snail, showing few tufts of cilia and smooth regular folds of surface. Scale bar for Figs. 2–5 3 μm.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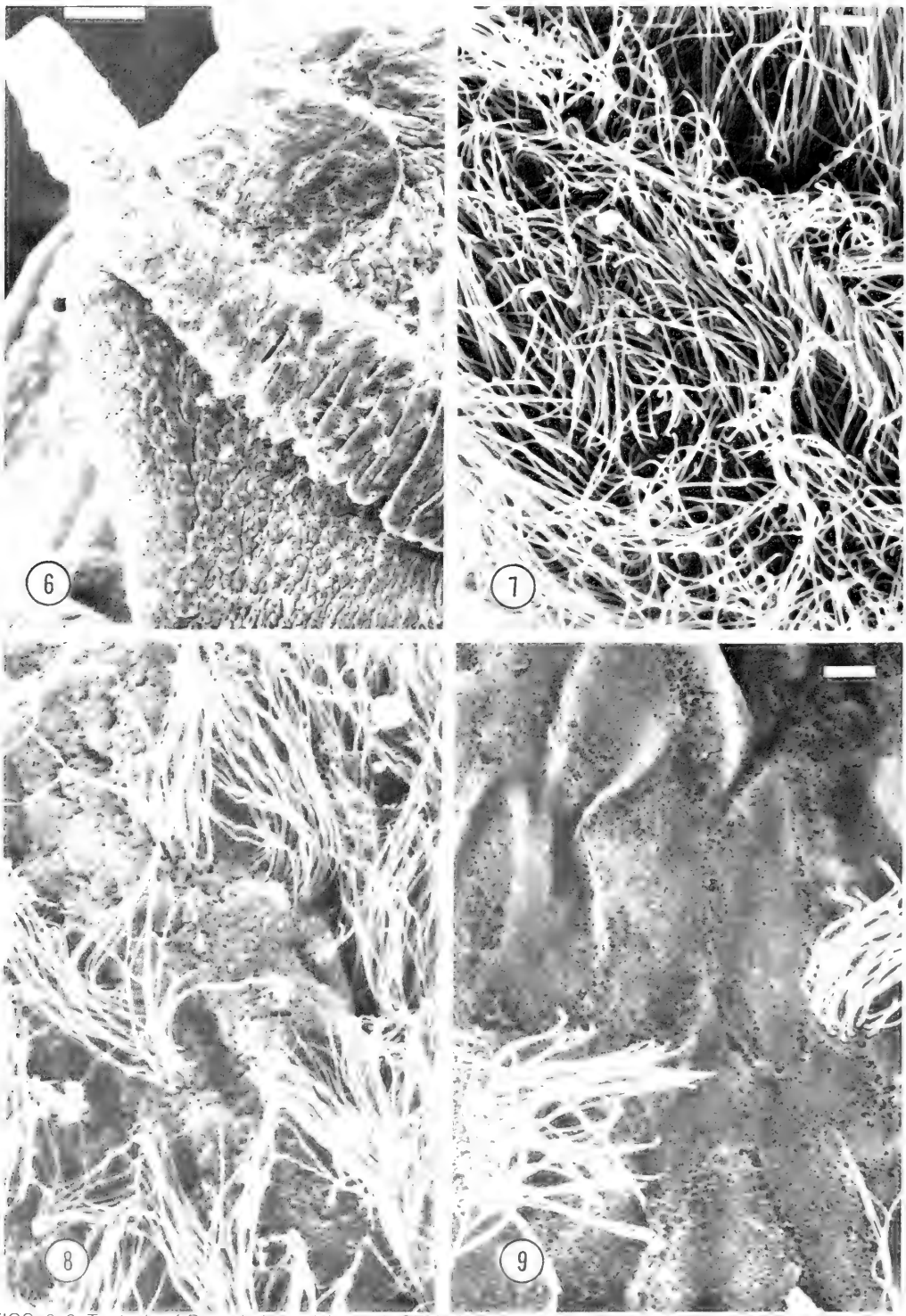
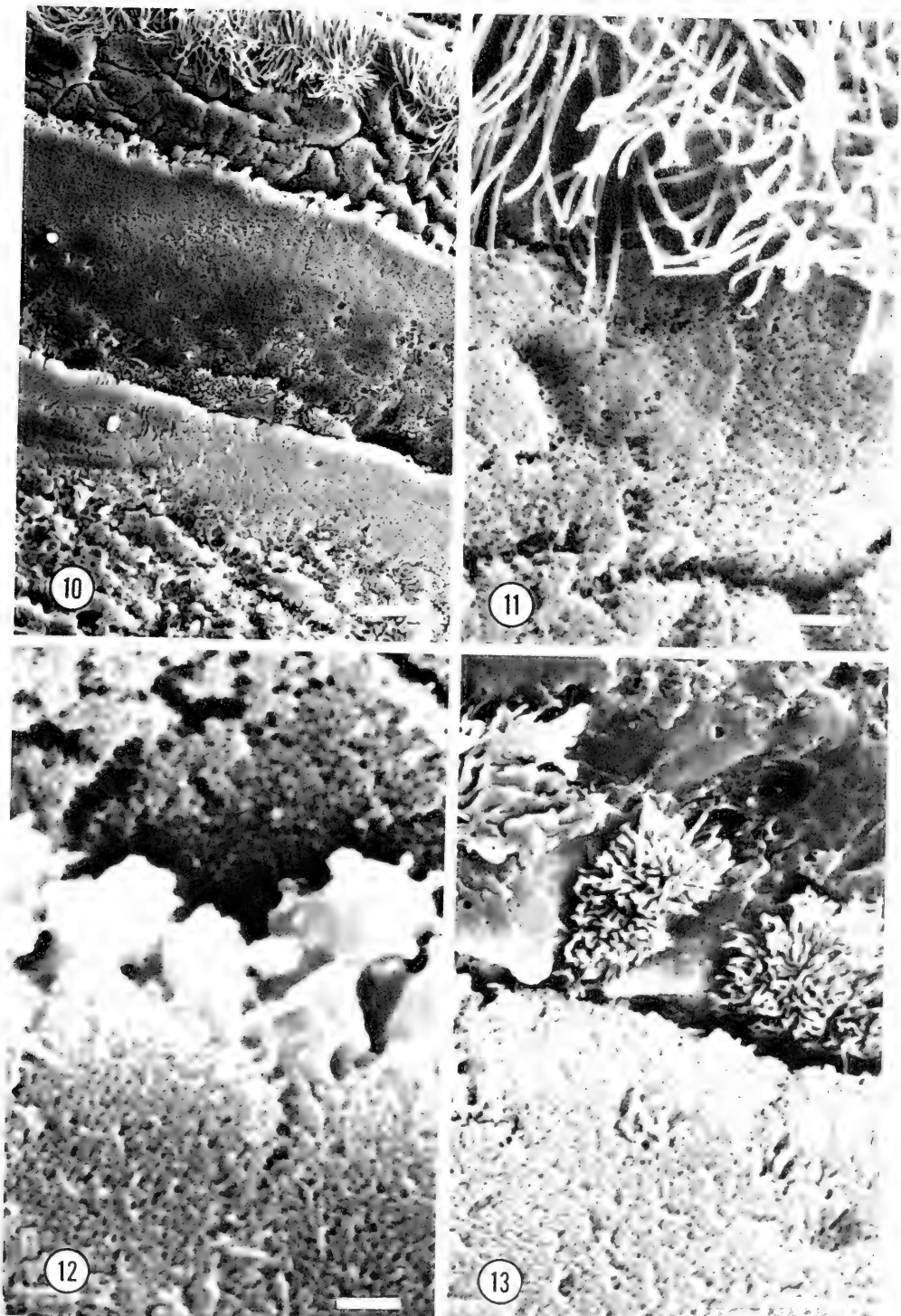
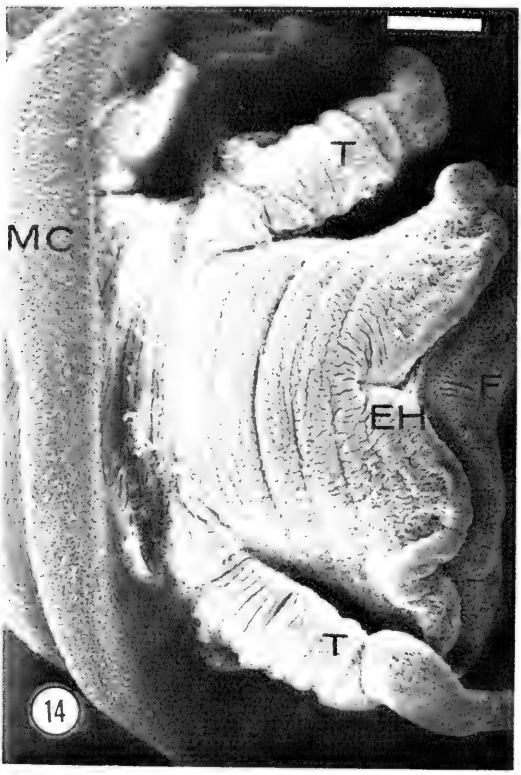


FIGS. 6–9. Tentacle of *Biomphalaria glabrata*. 6. SEM of whole tentacle of 3 mm snail, showing distribution of cilia. Scale bar 50 μm. 7. SEM of tip and upper third of tentacle of 3 mm snail, showing dense long cilia. 8. SEM of middle third of tentacle of 3 mm snail, showing scattered groups of long cilia. 9. SEM of lower third of tentacle of 1.5 mm snail, showing very few cilia. Scale bar for Figs. 7–9 3 μm.



FIGS 10–13. Mantle collar of 2 mm *Biomphalaria glabrata*. 10. SEM of dorsal surface of mantle collar showing three different structures. Scale bar 10 μm. 11. Edge of collar, showing dense long cilia. Scale bar is 2 μm. 12. Second area of the collar showing microvilli and a row of bulbous structures. Scale bar 1 μm. 13. Third area covered by microvilli. Scale bar 2 μm.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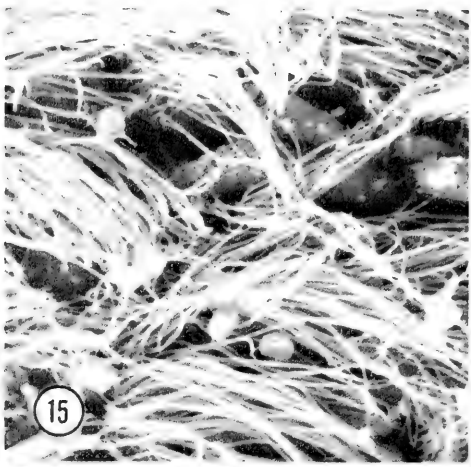
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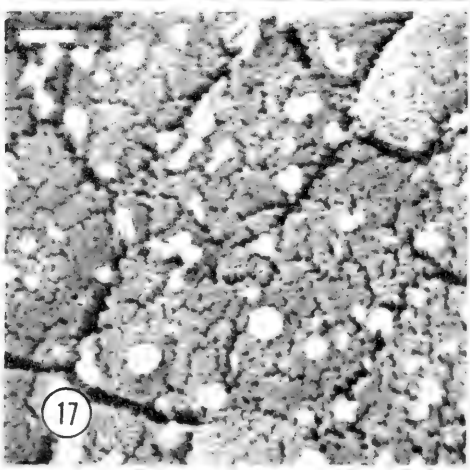
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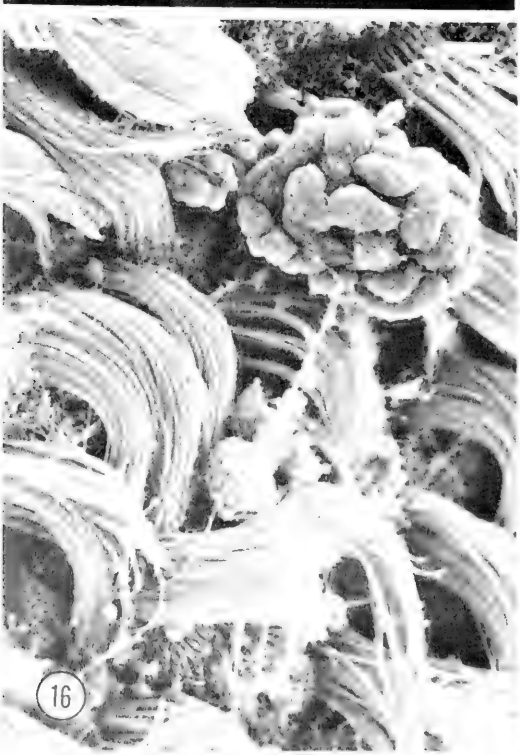
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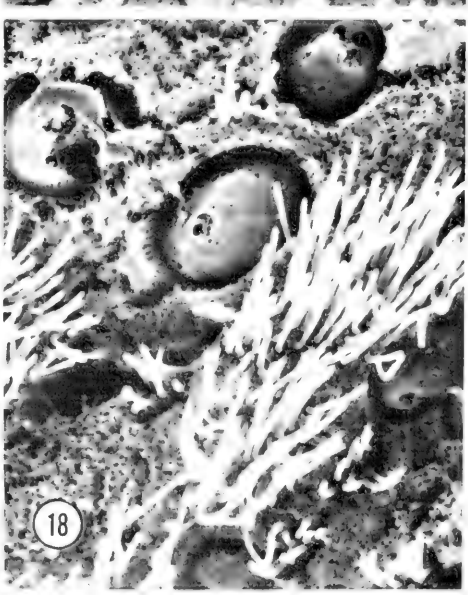
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In small snails, the dorsal surface shows three different areas with different types of epidermal cells (Fig. 10). The epidermal surface at the edge of the collar has a rim of heavy cilia (4 μm in length), followed by a folded, bare surface which has microvilli on it (Fig. 11). The edge of the second area is bulbous, while the rest of the area is covered by evenly distributed microvilli (Fig. 12). The junction of this area with the third area shows big patches of short cilia with smooth surfaces in between. The surface of the third area is completely covered by microvilli followed by an aggregation of many blebs covering the whole surface (Fig. 13). In large snails (Fig. 14), the edge of the mantle collar is covered with very dense, long cilia. The ventral surface of the mantle collar near the edge has many long cilia scattered between the folded surface (Fig. 15). The basal surface of the mantle collar near its junction with the head is completely covered with microvilli and many tufts of long cilia. Globular structures, probably secretions from goblet cells, are seen in this area (Fig. 16).

In large snails, the dorsal surface of the mantle collar, folded backward, has structures similar to those of the small ones, except for the presence of a basal layer on the external surface of the mantle collar This layer contains short microvilli arranged to show the outline of the underlying cells. Many globular secretions are observed in this area (Fig. 17). The mantle covering the rest of the body has a smooth surface and is covered by very short, thin microvilli.

The whole of the ventral surface of the snail is composed of the foot, which forms the typical creeping sole. As the sole of the foot is the part of the body on which the snail depends for its movement, its surface is usually covered by an excess of mucous secretions appearing as a mesh of entangled filaments. In addition to the very densely ciliated epidermal cells covering this surface (Fig. 19), the sole surface of large snails also shows many widely distributed lobular structures which seem to arise from deeper sites and protrude in between the cilia. These structures have different shapes and their surfaces vary from smooth to rough with reticular appearance (Fig. 20). They probably are secretions extruded from the underlying cells. In small snails, there are only small lobular structures probably representing subepidermal glands. The density of the cilia decreases toward the dorsum of the foot.

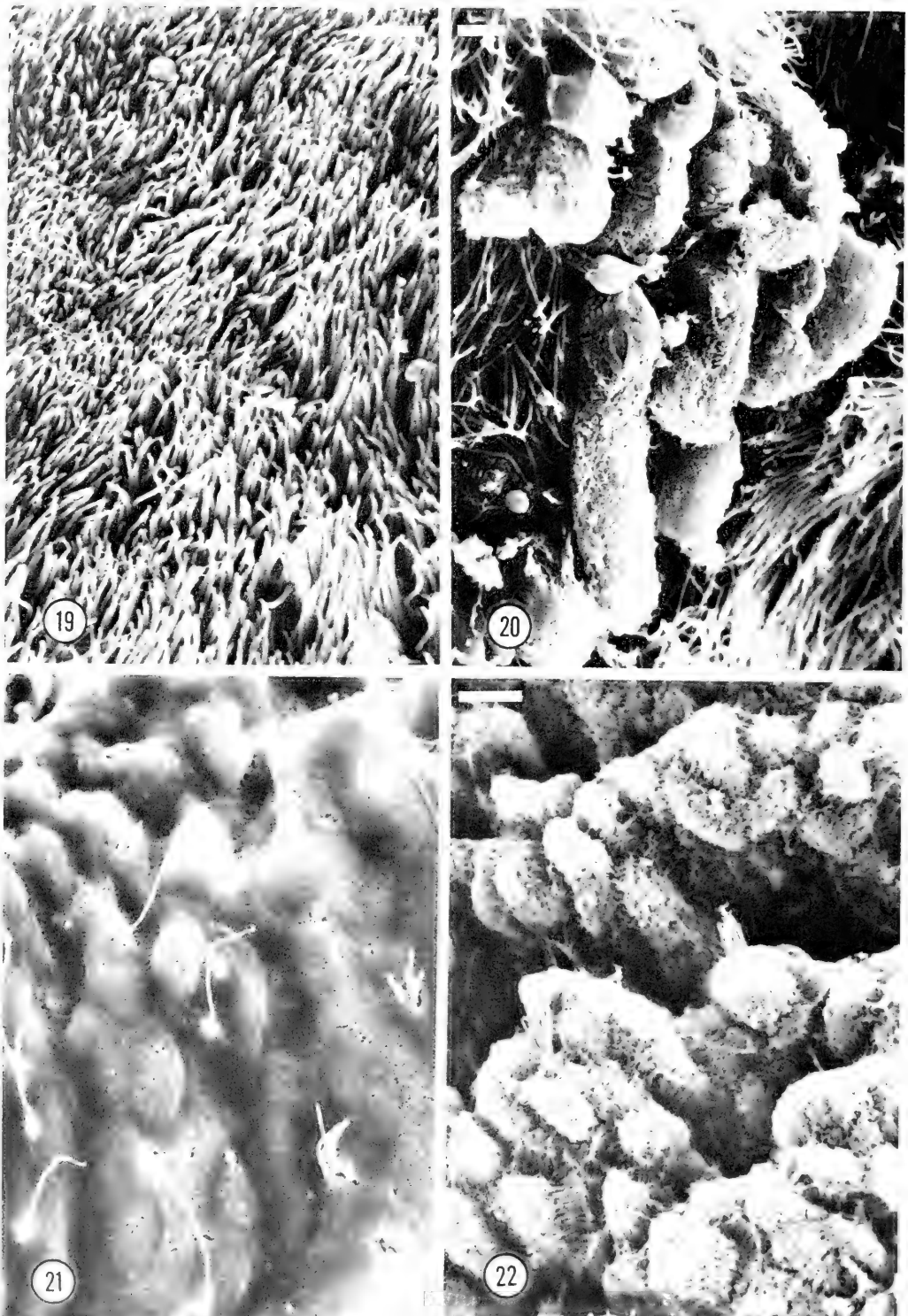
On the dorsal surface of the foot of small snails, long single cilia are scattered among tufts of short cilia. The surface is slightly folded and smooth in appearance (Fig. 21). On the other hand, many irregular folds having tufts of short cilia are observed on the dorsal surface of the foot of large snails (Fig. 22).

Cytological studies of the surface confirmed the light microscope observations on the structure of the epidermis. However, not all the epithelial cells covering the surface are ciliated, particularly those cells covering the head and dorsum of the foot. The epidermis of *B. glabrata* is one layer thick, consisting mainly of four cell types: columnar cells, ciliated columnar cells, pigment cells and goblet cells. The columnar and the ciliated columnar epithelial cells have oval or elongated nuclei with many chromatoidal bodies. The cytoplasm of these cells is granular and basophilic. The ciliated columnar epithelium covering the sole of the foot is more densely stained with Barbeito-Lopez than any other part of the body (Fig. 23).

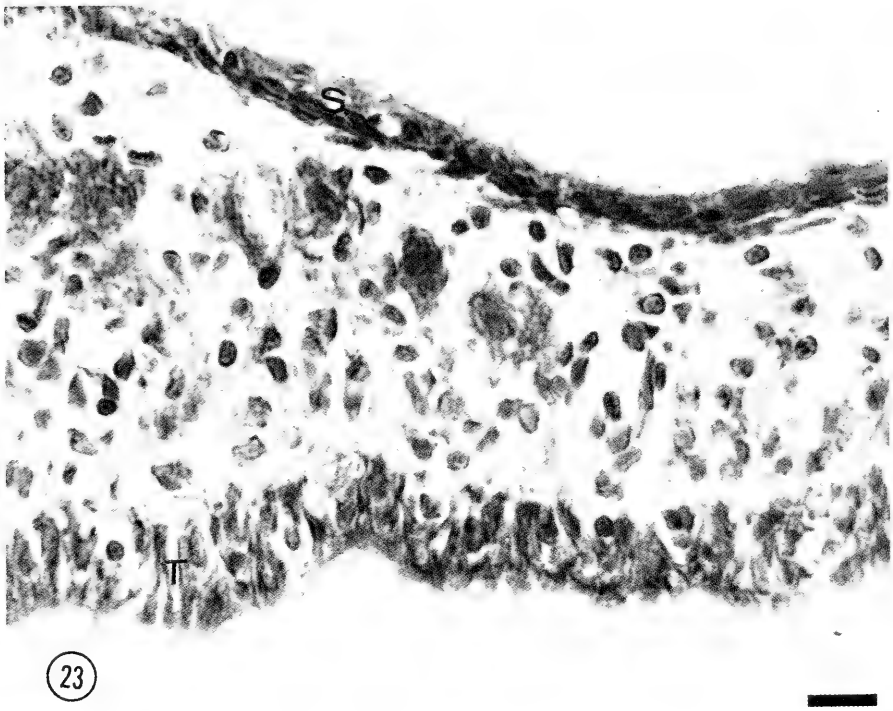
A thick layer of cuticle covers much of the mouth epidermis and buccal cavity, which is thickened into the jaws (Fig. 24). The lips surrounding the mouth are also covered with the same cuticular layer that appears smooth by SEM. The epidermal cells of the tentacle are short columnar and ciliated. This observation was also made by Pan (1958). As he also showed, the cilia are more dense at the tip and the upper third than on other parts.

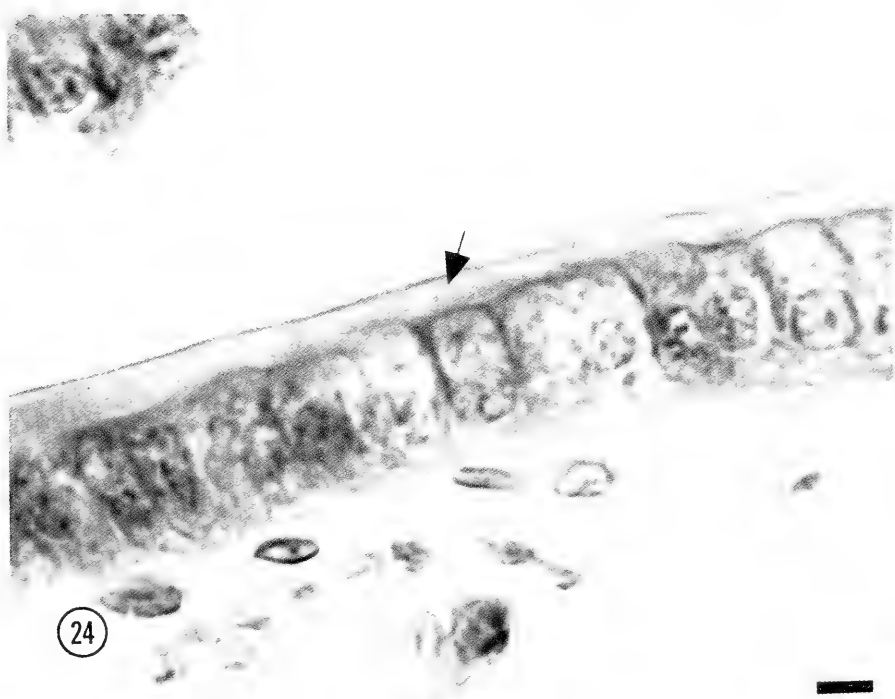
The surface of the mantle collar is composed of pseudostratified epithelial cells in some regions, and simple squamous epithelial cells in others, as already observed by Pan (1958).

FIGS. 14–18. 14. SEM of dorsum of head of 8.0 mm snail, showing edge of head (EH), foot (F), deflected mantle collar (MC), and tentacles (T). Scale bar 200 μm. 15. Surface of edge of mantle collar showing dense and long cilia. 16. Basal part of mantle collar facing the head of 9 mm snail, showing microvilli and tufts of long cilia covering surface. 17. Dorsal surface of mantle collar of 9 mm snail, showing microvilli covering whole surface. Scale bar for Figs. 15–17 3 μm. 18. Pneumostome surface of 5.5 mm snail, showing globular structures, suggesting goblet cells. Scale bar 2 μm.



FIGS. 19–22. Foot surface of *Biomphalaria glabrata*. 19. Sole of foot of 1 mm snail, showing dense cilia. Scale bar 2 μm. 20. Sole of foot of 5.5 mm snail, showing lobular structures in between dense long cilia. Scale bar is 2 μm. 21. SEM of dorsal surface of foot of 1.5 mm snail, showing few tufts of short and long cilia. Scale bar 3 μm. 22. SEM of dorsum of foot of 5.5 mm snail, showing many irregular folds. Scale bar 2 μm.





FIGS. 23–24. Light microscope photographs of sections of *B. glabrata*, 1 mm in diameter. 23. Section of foot showing deeply stained, tall ciliated columnar epithelial cells (T) covering sole as well as short columnar cells covering dorsum (S). Note dense cilia on sole compared to those on dorsum (Barbeito-Lopez trichrome stain). Scale bar 10 μm. Fig. 24. Section of surface of mouth, showing smooth homogeneous layer (arrow) covering epidermis (Barbeito-Lopez trichrome stain). Scale bar is 5 μm.

DISCUSSION

The body surfaces of the snail have many functions. In addition to being a protective boundary, it has a role in osmoregulation, respiration, locomotion, perception, and shell formation and regeneration (Hess, 1964). Therefore, a knowledge of the histology, histochemistry and surface structure of gastropods is fundamental for an appreciation of the host-parasite relationship. A number of reports are available on the detailed structure and function of snail tissues, including the epidermal structure (Pan, 1958, for *Australorbis glabratus*; Hyman, 1967, for Pulmonata in general; Zylstra, 1972, for *Biomphalaria pfeifferi,* and Sullivan & Cheng, 1974, for *Biomphalaria glabrata*).

In the present study, scanning electron microscopy revealed that the exposed surfaces of the snail body are provided with cilia, confirming the light microscope observations (Pan, 1958). The distribution and density of cilia vary in different parts of the body, and even within a given part. For example, while the whole tentacle is covered by cilia, these are dense at the tip and sparse at the base. Perhaps the differences in distribution of cilia are in some way related to the sensory function of the tentacle.

The foot is the main organ of locomotion for a snail. Its surface is modified to fulfill this function. The sole of the foot is covered by long cilia; a slimy secretion which has a reticular appearance is spread over the surface of the sole of the foot. The presence of the long cilia and mucous secretions facilitate the movement of the foot (Zylstra, 1972) in water and on the surface of containers. Hyman (1967) described the slimy secretion as originating from the mucocytes which are gland cells located in the subepidermal tissue. Also, Zylstra (1972) mentioned one type of subepidermal gland cell, located in the ventral region of the foot, extending up to 400 μm from the surface. These cells are grouped together and their necks form a bundle while the cell body has a reticular appearance. This is suggested by the present study, where we observed groups of secretions extending from the ventral surface of the foot of large snails.

Zylstra (1972) stated that the dorsal surface of the foot of *Biomphalaria pfeifferi* is similar to the head epidermis where cilia were only found scattered between the epidermal cells. Our observations have confirmed this. The presence of the few tufts of cilia may bring the slime secretions from the pedal gland cells to the front and ventral surfaces of the foot (Zylstra, 1972).

It is obvious from the present work that the surface of the mantle collar differs from the mantle covering the part of the body hidden by the shell. The mantle under the shell is smooth, while in the mantle collar, it is glandular in Pulmonata (Hyman, 1967). Zylstra (1972) added that the surface of the mantle collar of *Biomphalaria pfeifferi* is nearly covered by short microvilli. Similar microvilli were observed in our work on *Biomphalaria glabrata.* Hubendick (1958) suggested that these microvilli are important for adhesion to the shell, although Zylstra (1972) reported that there is very little structural basis for adhesion to the shell, unless adhesion occurs by suction.

The ultrastructure of the surface of the rectal ridge epithelium of *Biomphalaria glabrata* described by Sullivan *et al.* (1974) is similar to what we found on the ventral surface of the mantle collar which bears microvilli and bundles of cilia. Also, Malek (1980) mentioned that the mantle collar of Pulmonata is covered by irregular, occasionally branched microvilli, protruding into the fluid-filled spaces between the mantle and the shell.

As to the light microscope observations, the cuticle lining the mouth which appeared as a smooth surface by SEM, and also as a homogeneous layer by light microscopy, was also seen in *Lymnaea stagnalis* by Zylstra (1972).

Wilson *et al.* (1971), working with *Lymnaea truncatula,* found that the ground cytoplasm of the columnar epithelial cells contains many mitochondria and vesicles. Malek (1980) mentioned that the brush border of the epithelial cells seen with the light microscope is revealed by the electron microscope to be microvilli on the surface of each epithelial cell covering the surface of *Lymnaea truncatula.* In the present study, microvilli and cilia were found on the surface of *B. glabrata* by SEM.

The ultrastructure of the body of small and large snails revealed some differences. For example, the cells covering the head in the area between the bases of the tentacles had different shapes in the two groups of snails. Also, in small snails, the glands on the sole of the foot are less developed.

From all these observations, it is clear that the snail surface has a complex structure and that it provides a basis for further study on the

functions of the snail epidermis, as well as on the site and snail age preferences by schistosome miracidia.

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ECOLOGY OF THE TERRESTRIAL SNAIL *BREPHULOPSIS BIDENS* (PULMONATA: ENIDAE): MORTALITY, BURROWING AND MIGRATORY ACTIVITY

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ABSTRACT

This paper summarizes studies on the mortality, burrowing and migratory behaviour of the pulmonate snail *Brephulopsis bidens* performed at various sites within the population area (Crimea, USSR) during 1975–1977. The annual survival rate of adult snails was about 0.222–0.252. During the active period mortality of the snails reached a maximum in July. This was followed by the onset of burrowing, which led to a decrease of mortality. Intercolonial migration was extremely limited during the reproductive period (1.4%–2.4% per 10-day period), but increased considerably towards the end of summer, reaching about 20% per 10-day period. The activity radius of individual snails was approximately 3 m. Statistically significant correlations were found between all of the following ecological parameters: mortality, burrowing, migration and population density.

Key words: population; mortality; burrowing; migratory activity; correlations.

INTRODUCTION

Various studies have shown that physical features of the environment (Peake, 1978), or the quantity and accessibility of food (Butler, 1976) may exert a considerable influence on land snail population density. These factors alone, however, could hardly explain the marked variations observed by us (Livshits, 1983) in the spatial and temporal density of populations of the snail *Brephulopsis bidens*. Oosterhoff (1977), in her study of the ecology of the snail *Cepaea nemoralis,* suggested that under constant abiotic conditions population density may be regulated by changes in growth and emigration of the snails.

In the present work, the mortality, burrowing and migration patterns of *B. bidens* were investigated for their correlation with, and influence on, the population density of this snail within naturally changeable environments.

MATERIALS AND METHODS

Description of the Investigated Snail

Brephulopsis bidens (Krynicki, 1833) (synonym *Chondrus bidens* (Kryn., 1833); *Buliminus bidens* (Kryn., 1833)) (Pulmonata: Enidae = Buliminidae) is an endemic mollusc of the Crimean (USSR) fauna and is found in steppes and open glades of foothills (Puzanov, 1925, 1926; Likharev & Rammel'meier, 1952). The shell of this moderately-sized snail (height 15–20 mm and width 4–6 mm) is elongate-ovate, and is white often patterned with black radial bands. The lifespan of *B. bidens* is about two years with an active period of 7–8 months annually, from April to November (Livshits & Shileiko, 1978). For the remainder of the year the snails hibernate beneath the surface, clumped in groups of 3 to 15 individuals. During the active period the snails often climb on grass and aggregate in more or less discrete groups or colonies of different numbers (mean size of a colony in 1975 was 46.6 ± 14.4 individuals) (Livshits, 1983). The space occupied by a colony ranged between 0.039 m^2 and 0.500 m^2; the mean distance between colonies was 0.36 m. The mean number of colonies per random 100 m^2 area in 1975 was 291.4 ± 34.7. During exceptionally hot and dry weather (July–August) the snails descend and burrow into the ground to a depth of 1–5 cm, remaining there for days or even weeks. Copulation and oviposition take place generally in April–May.

The investigation was carried out over a period of three years (1975–1977) on the Internal Cuesta of Crimea (USSR). The studied snail population occupied an area approximately 360 m × 70 m. This area, throughout which the snails were encountered in numer-

ous discrete colonies, was arbitrarily divided into 12 sites of roughly 30 m in length, designated by the letters A through N. Livshits (1981, 1983) described the location of the study site in detail.

Mortality and Burrowing

To determine the winter mortality, 9 plywood boxes were used (12 × 20 ×6 cm) with floors overlaid with soil and leaf litter. The sexually mature snails, removed from the natural habitat in October, were placed in these boxes (3 boxes in 1975/1976, n = 192 individuals; 6 in 1976/1977, n = 500 individuals) which were then covered with gauze and left for the winter (until April) under natural conditions. Each box was fitted into a slight depression in the ground so that its floor was on a level with the surrounding terrain.

The determination of snail mortality and burrowing during May–September was carried out in enclosures. Sections of 1.2 × 3 m within the population area were cleared of live and dead snails of all age groups and were made inaccessible to migrants by a high 75 cm gauze fence. After 12–14 days, ensuring that no snails had appeared in them, test snails were introduced into each enclosure. The values of their mortality and burrowing rates were calculated from the equations: Mo = n/N; Br = N − n − m/N where Mo represents mortality, Br the proportion of burrowing individuals, n the number of snails dying per unit time, m the number of molluscs living on the grass, and N the total number of snails in the enclosure. Four such enclosures located at sites B, E, K and M were used in 1975 (duration of experiment June–September, initial number of snails in enclosures were 439, 361, 733 and 496 individuals respectively). In 1976 a similar experiment in a single enclosure continued from May to October. At this time the initial number of snails was 546 individuals. Enclosures like these (4 × 1 m) were also used to study the comparative mortality on all 12 sites of the population area. The sections, cleared in advance of all snails, were juxtaposed to the areas where the population density was measured (Livshits, 1983). Three hundred adult molluscs were placed in each enclosure in April. Monthly readings were taken in these sections of the number of dead snails.

After each observation in all described experiments, the dead specimens were removed from the enclosures.

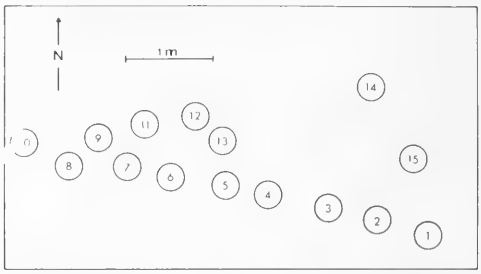


FIG. 1. Distribution pattern of 15 colonies selected for the study of *B. bidens* migration in 1975.

Investigation of the Snail Migration Patterns

The aim of our study of snail migration was threefold: 1. To estimate the radius of individual snail mobility; 2. To determine the direction and extent of snail migration; 3. To determine the migration intensity of the sexually immature individuals.

For these purposes we used the technique of Sheppard (1951) and Cain & Currey (1968) whereby the snails are marked with spots of indelible nitroenamel. Marking was carried out in the field to leave intact the native structure of the colonies. All adult snails were marked in 12 colonies located at sites A, B, D, G, K, and M (2 colonies per site) in 1975 and in 12 colonies at all 12 sites (1 colony per site) in 1976. Subsequently, the decreased numbers of marked snails in the colony between observations were used to estimate emigration. During this period the burrowing and mortality of snails were also considered. Digging out over a radius of 0.5 m from the center of the colony enabled us to count the number of buried individuals. After each observation, all adult snails in the colony (both marked and unmarked) were re-marked by a colour corresponding to a given colony.

To estimate the range of individual activity and emigration directions of the snails, fifteen colonies extending over an area of about 15 m^2 at site D (Fig. 1) in 1975 and twelve colonies distributed over an area 2.5 × 2.5 m at site F in 1977, were marked and assigned different colours. Afterwards, the emigrated snails were searched for in concentric circles having radii 1, 2, 3 and 4 m from the center of each colony. Both in 1975 and 1977 it was impossible to estimate the number of snails returning to their original colonies ("homing") because nonmigrating and returning speci-

mens could not be distinguished from one another.

A similar experiment was carried out in 1976 with sexually immature snails, using select members of 32 colonies distributed over an area of 4 × 8 m.

All statistical procedures were after Sokal & Rohlf (1969).

RESULTS

Snail Mortality and Burrowing

Adult mortality was determined in enclosures under natural conditions during June–September of 1975 and May–September of 1976. The overall mortality rate was 0.591 (N = 535) in 1975 and 0.631 (N = 776) in 1976; the difference between these two values is not significant because the duration of observations was longer by a month in 1976 than in 1975. Actually the death rate per 10-day period during the active season was 0.048 and 0.047 respectively ($t = 0.05$, $p >> 0.05$, test on equality of two percentages).

In plywood boxes with nearly natural conditions the adult snail mortality during hibernation (October–April) was 0.366 (N = 194) in 1975/1976 and 0.312 (N = 500) in 1976/1977. Per 10-day period, the winter mortality was 0.024 and 0.021 respectively, and this difference is also not statistically significant ($t = 0.57$, $p > 0.05$). Also, there were no significant differences between banded and unbanded shell morphs in winter mortality, albeit in summer months the differences were highly significant (Livshits, 1978).

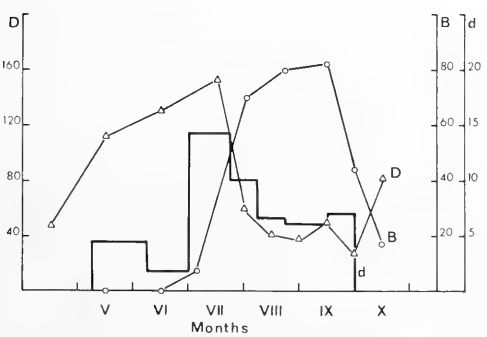


FIG. 2. Relationship between seasonal changes in population density (D), mortality (d) and burrowing (B) of *B. bidens* during 1976. Snails used for mortality and burrowing determination numbered 635 individuals. D is expressed in number of snails per m^2, d and B in percentages.

Fig. 2 presents curves of seasonal changes in mortality and burrowing in relation to changes in the population density during 1976. Between May and June, while the population density increased, the mortality remained at a comparatively low level (0.014–0.048 per 10-day period) and burrowing activity was nil. Subsequently, however, there was a sharp increase in mortality (0.148 per 10-days) which led to a sudden decline in the population density. Following this, burrowing commenced and there was a decrease in the population density to a constant low level. Population dwindling was due not only to mortality, which in fact at that time (August–September) diminished to a mean of 0.066 per 10 days, but also to the burrowing of adolescent snails. Our findings thus suggest that the diminished snail mortality is associated with considerable increase in the proportion of buried snails.

A similar pattern was observed during the summer of 1975 when, along with a decrease in the population density (mid-July), there was a substantial rise in mortality (from 0.025 to 0.185 within 10 days) and a sharp increase in the number of buried snails (from 0 to 0.491). Subsequently (from July 16 to August 20), there was considerable diminution of snail mortality (0.064 per 10 days) and the proportion of buried snails was 0.400. The observed decrease in the population density on the surface was not in fact reflected in the population size, which was actually much larger than apparent when buried snails were taken into account. Indeed, the steep increase in the population density observed for about two weeks each year in the fall (Fig. 2 and see also Livshits, 1983) supports this conclusion.

Mortality varied considerably not only during different seasons, but also at different sites of the population area—along a density gradient (Table 1). Data showing this were obtained by investigating the snail mortality in enclosures of 1 × 4 m which were placed in each site during May–October of 1975, and the results indicate that as the number of individuals per m^2 decreased from site B to site N, the mortality increased in the same direction. There was a good inverse correlation between the mortality and density at the various sites ($r = -0.66$, $p < 0.01$, data from Table 1).

Burrowing also showed a significant spatial fluctuation. Observations on burrowing and

TABLE 1. Data on several ecological parameters collected at different sites of the *B. bidens* population area during 1975. Density and mortality values are averages for May–September, migration for August–September. Snails used for mortality determination numbered 300 individuals at each site.

Parameters	Sites of population area											
	A	B	C	D	E	F	G	H	K	L	M	N
Density individuals/m^2	113.5	226.4	218.7	161.6	179.3	177.2	113.8	105.6	60.3	72.5	103.0	97.1
Mortality per 20-day period	0.031	0.019	0.026	0.034	0.031	0.024	0.042	0.036	0.071	0.055	0.103	0.102
Migration per 20-day period	0.298	0.262	—	0.182	—	0.310	—	—	0.534	—	0.644	—
Sample sizes	492	565	—	834	—	604	—	—	246	—	179	—

TABLE 2. Seasonal dynamics of *B. bidens* migration per 10-day period in 1975.

Variable	Dates of observation							
	8.4 –	28.4 – 8.5	– 29.5	20.6 – 7.7	31.7 – 10.8	– 20.8	– 18.9	– 30.9
Number of marked snails	250	253	243	915	795	690	567	224
Proportion of migrants	0.014	0.024	0.082	0.135	0.162	0.198	0.212	0.155

TABLE 3. The movement of marked *B. bidens* over various distances during selected periods of 1975.

Date	Number of immigrant snails				
	0 m	1 m	2 m	3 m	4 m
21.5–10.6	181	41	17	6	0
10.6–21.6	20	44	13	9	0
27.6–10.7	26	50	16	7	0
10.7–22.8	127	74	0	0	0
22.8–30.9	55	12	11	11	0
Average and standard error	81.8 ± 34.9	44.2 ± 11.1	11.4 ± 3.4	6.6 ± 2.1	0 ± 0

mortality were carried out in four separate enclosures at sites B, E, K and M during 1975. During May, snails collected at the four sites were placed in enclosures as follows: 526 individuals at site B, 684 individuals at site E, 651 individuals at site K and 267 individuals at site M. Two months later the number of dead and buried snails at each site was determined. As illustrated in Fig. 3, there was a gradual increase in burrowing activity proceeding from site B to site M and this was coupled with an increase in snail mortality and a decrease in population density.

There were significant differences between the sites with respect to mortality ($\chi^2 = 100.2$, $p < 0.001$) and burrowing ($\chi^2 = 64.4$, $p < 0.001$).

Snail Migratory Behaviour and Pattern

Migration of *B. bidens* was studied in the field by observing individuals marked with different nitroenamel colours. In the course of the study, 2919 adult and 1795 juvenile snails were thus marked. Data on migrations for the entire field study period of 1975 are summarized in Table 2. As can be seen from the Table, a rather low level of migration (1.4% per 10 days) was observed in April when mating took place, but from early May there was a rapid increase in migration activities which reached a peak (21% per 10 days) between late August and mid-September.

Fig. 4 and Table 3 present data on the radius of activity of individual snails. This radius was 3 m and although snails can traverse such a distance within 20 days there were hardly any migrations into the zone extending 4 m from the center. During the entire period of investigation, May–September

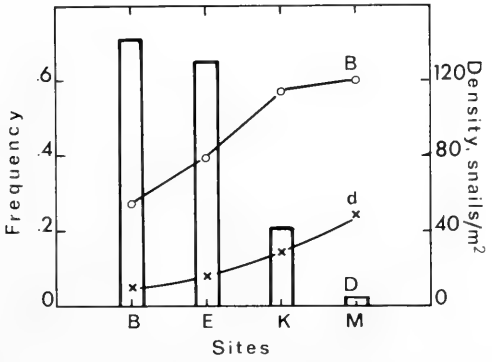


FIG. 3. Relationship between the spatial variability in population density (D), mortality (d) and burrowing (B) of *B. bidens* in July 1975. Snails used for the mortality and burrowing determination numbered 2128 individuals. B and d are given as frequency of buried and dead snails in the enclosure.

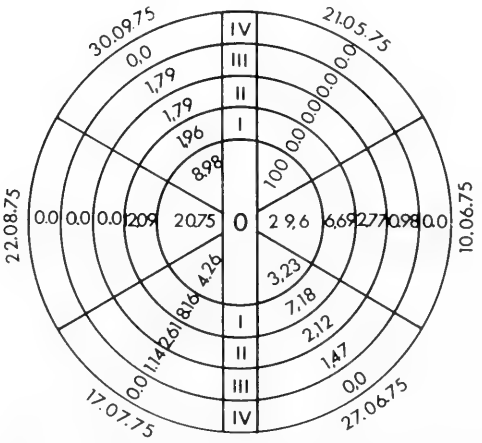


FIG. 4. Radius of individual migration activity of *B. bidens*. I, II, III and IV are concentric zones of distances of 1, 2, 3 and 4 m, respectively, from a marked colony. Numbers in each zone sector shown as percentages of marked individuals found. Total number of snails marked on 21.5.75 was 612 individuals.

TABLE 4. Directions of adult *B. bidens* migrations in 1975 and 1977.

Direction	Numbers of snails migrating during the following periods									
	1975				Wilcoxon's test	1977			Wilcoxon's test
	21.5 –	10.6 – 17.7	– 26.8	– 30.9		18.5 –	20.6 – 20.8	– 14.9	
North	12	15	2	3		44	13	13	
South	4	8	3	5		25	11	12	
West	51	67	50	22		23	39	23	
East	28	32	10	27		9	16	39	
χ^{2*}	6.61	8.5	26.6	0.34		24.5	25.7	22.3	
P	<0.01	<0.01	<0.001	<0.5		<0.001	<0.001	<0.001	
Detected in original colony	37	165	35	15		196	125	120	
Total detected	132	287	100	72		297	264	207	
Total marked	632	632	632	632		350	350	350	

*In 1975 only data for migrations in West-East directions were tested by χ^2.

1975, the average number of snails moving into a 0, 1, 2, 3 and 4 meter range was, respectively, 81.8, 44.2, 11.4, 6.6 and 0.0 individuals (see Table 3). The significant differences of these values were examined by two-way analyses of variance without replication (Sokal & Rohlf, 1969). Since the main concern in this study was the distance factor, only data on this aspect are presented here. The variance (V) of the number of individuals reflecting the influence of distance was 5839.0 (df = 4), and V error was 1086.0 (df = 16). Hence, differences in mean snail numbers according to distance are highly significant, yielding a value of $F = 5.37$, $p < 0.01$. It seems that despite the ability of individual *B. bidens* snails to traverse distances greater than 3 m, most of the adult snails displayed limited mobility and rarely travelled beyond a radius of 1 m.

Data were collected also on the migratory direction of the adults as well as juveniles. To determine the direction of snail migration during the summer of 1976 (15 colonies) and 1977 (12 colonies), all the molluscs in a given area were marked with different colours in accordance with their colony of origin. Subsequently the colonies were observed for migration into areas of individuals marked with "foreign" colours. The observational results are given in Table 4, and relate to an area extending between sites D and E.

Significant preferential movement of snails to the W was already observed in the first 20 days. This tendency persisted through the summer months to the end of August. Beginning in September, however, the picture abruptly reversed, with about half of the migrant snails moving to the E, and 15.4% moving N or S. It should be noted that the sample colonies were mainly located in a W-E direction (Fig. 1) so that information on migration in other directions was not meaningful.

A changing migration pattern was also noticed in 1977 when the analysed colonies were uniformly distributed spatially. In June a tendency for movement to the north was clearly evident, but towards the end of the summer this changed to a westerly direction; in September, the snails commenced moving eastwards again (Table 4).

Wilcoxon's signed-rank paired-sample test (Sokal & Rohlf, 1969) has been used to compare the extent of migration in different directions during the completed seasons of investigation (i.e., May–September 1975 and also May–September 1977). By this test, each two directions of snail migration constitutes a pair, and the various proportions of migrants that migrate in a single direction comprise a series of pairs. During the active seasons of 1975 and 1977 there were no significant overall differences between the different directions of migration ($p > 0.05$).

Thus, during our different study periods or seasons of the year, the incidence of migrations in various directions differed significantly. However, the overall migration in-

cidence for all the active periods as a whole is more or less equal in all directions. It seems that the snails move only around or near their own colony. In fact, near the end of the experiment in August, there was a sudden increase in marked individuals within the starting circle (0) and circle No. 1 (Fig. 4). This phenomenon reflects the apparent remigration of snails to their original colony site.

To assess migration of the preadolescent snails, 1795 individuals with 5–7 shell whorls, deriving from 32 colonies on an 8 × 4 m area, were marked in July 1976. Only 212 (11.7%) of these could be detected in May 1977, most of them (179 individuals or 84.1%) still within their respective colonies and only 15.9% having migrated out of their colonies (Table 5), albeit in groups.

Phenological observations indicated several seasonal cycles of migration and burrowing activity, with the only difference between the two being the somewhat later commencement (at about the end of June) and earlier termination of burrowing. In fact, a correlation was observed between adult snail emigration and the proportion of snails burrowing during the seasonal field observations of 1975 and 1976 (Fig. 5, $r = 0.75$, $p < 0.01$).

Migration as well as burrowing varied considerably in space, and also correlated inversely with the adult snail density at the sites (Fig. 6, $r = 0.56$, $p < 0.001$). This was established by investigating snail emigration during July–September 1975 from colonies at sites A, B, D, K and M, and again in 1976 at each site. A very high coefficient of correlation was found also between mean rates of mortality and migration at the same sites during the summer months of 1975 ($r = 0.93$, $p < 0.05$, data from Table 1).

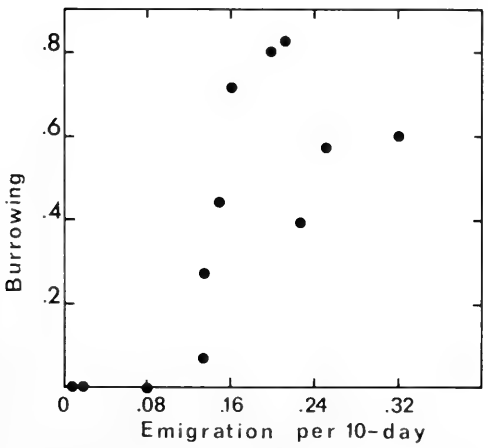


FIG. 5. Burrowing of *B. bidens* as a function of migration.

TABLE 5. Migration of preadolescent *B. bidens* between 26.7.1976 and 19.5.1977.

Colony no.	Total marked 29.7.76	Total detected 19.5.77
1	54	12
2	62	7
3	34	0
4	97	16
5	82	0
6	112	27
7	70	10
8	40	0
9	60	6
10	32	0
11	50	0
12	70	14
13	30	0
14	50	0
15	30	7
16	40	0
17	60	0
18	60	11
19	40	0
20	67	24
21	48	0
22	40	0
23	26	0
24	54	0
25	70	8
26	30	4
27	80	17
28	41	0
29	60	0
30	103	19
31	62	8
32	41	0
Total in colonies	1795	179
Between colonies	—	33
Sum	1795	212

DISCUSSION

Ecological Variables

Different ecological variables were studied separately on discrete colonies of *B. bidens* at various sites within their population area. The annual adult snail survival rate was about 0.222–0.252, which was substantially lower than for other terrestrial snails, e.g. 0.50–0.75 for *Cepaea nemoralis* (Oosterhoff, 1977; Wil-

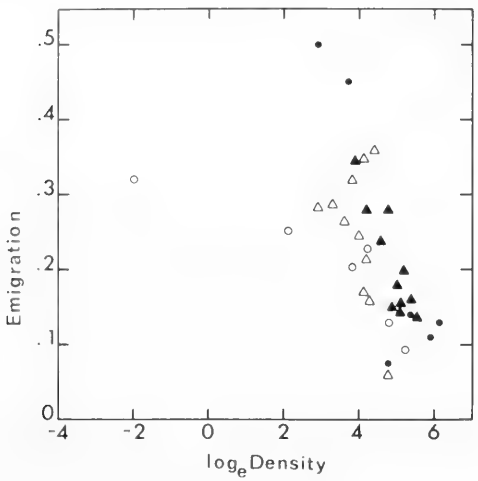


FIG. 6. Correlation between migration of *B. bidens* and the adult population density. ○ and ● August and September of 1975, △ and ▲ July and August of 1976.

liamson *et al.,* 1977) or 0.70 for *Sphincterochila zonata* (Shachak *et al.,* 1975). However, the latter species have a life span of 4–6 years (Cain & Currey, 1968; Shachak *et al.,* 1975; Williamson, 1976) and reproduce repeatedly, whereas the life span of *B. bidens* is only two years and the snails reproduce only once (Livshits and Shileiko, 1978). It is possible, therefore, that biological differences between *B. bidens* and the other two species account for the different annual survival.

Mortality rates of *B. bidens* varied significantly within the population area and also during different months of the active season. In the case of *C. nemoralis* such spatial (Cain & Currey, 1968) or seasonal (Richardson, 1975) variability was not observed.

Seasonal burrowing is typical for many species of terrestrial pulmonates and has an important adaptive value (Wolda, 1963; Shileiko, 1978). For species dwelling in arid zones this behaviour is crucial for survival during the hot and dry season (Yom-Tov, 1971; Shachak & Chaptan, 1976; Smith, 1976).

The present study shows that burrowing can be a means for correcting the population density by lowering the mortality rate during adverse or deteriorating ecological conditions. Previously it was found that the different activities displayed by different *B. bidens* shell morphs was mainly responsible for the maintenance of mean morph frequencies in the populations during the summer (Livshits, 1978).

As for the chronology of the migration pattern of *B. bidens,* this is briefly as follows: during the reproductive period (April–May), the adult snails aggregate and mate in discrete colonies, with snail migrations during this stage ranging between 1.4%–2.4% per 10-day period. This rather low migratory rate agrees with the obtained maximal value of the ratio of the variance S^2 to the mean value of the population density D (Livshits, 1983) as well as with the maximal genetic heterogeneity observed during the reproductive period (Livshits, 1978). The young snails emerging from the eggs remain largely in place but some (approximately 15.9% per year) migrate. Sizeable migrations occur only after the snails mature and mate, at which time the migration attains 20% per 10-day period (July–August). However, even in these months the distance traversed by the snails may be no greater than 3 m, the dispersion intensity diminishing sharply with each additional meter (Fig. 4). In other snail species the rate of migration decreases with increasing age (Cain & Currey, 1968; Pollard, 1975). The activity radius of individual *C. nemoralis* is up to 10 m/year (Greenwood, 1974; Cameron & Williamson, 1977), while for *Helix pomatia* it can be about 4 m/week (Pollard, 1975). Activity of adult *H. aspersa, H. pomatia* and *C. nemoralis* reaches maximum levels in late spring and early summer (Bailey, 1975; Pollard, 1975; Cameron & Williamson, 1977), which according to the last-mentioned authors is not unexpected considering that this is the height of the mating season.

Correlations between Ecological Parameters and Population Density

There are numerous publications on terrestrial pulmonates relating variations in population density to other ecologic parameters. Some of these correlations are summarized in Table 6. As is evident from this table, negative correlations between density and growth rate, density and level of reproduction were discerned in various snail species. Correlation between density and migration, however, may be positive or negative, if any.

On the basis of data obtained in the laboratory as well as under field conditions, Oosterhoff (1977) proposed a causal scheme to explain the regulation of molluscan population density. Her scheme proposes the existence of a negative correlation between snail population density and the growth rate and positive dependence of emigration on density.

TABLE 6. Correlations between population density and other ecological variables in several terrestrial snails. + and − are positive and negative correlations respectively. 0—unaffected by population density.

N	Variables	Nature of correlation	Species	Source
1	Migration	+ +	*Cepaea nemoralis*	Cain & Currey, 1968 Oosterhoff, 1977
2	Migration	0	*Cepaea nemoralis*	Cameron & Williamson, 1977
3	Migration		*Helicella virgata*	Butler, 1976
4	Mean adult size		*C. nemoralis*	Wolda, 1969
5	Growth rate of shell	−	*C. nemoralis*	Oosterhoff, 1977
6	Growth rate of shell		*H. virgata*	Pomeroy, 1969
7	Growth rate of shell		*Trochoidea seetzeni*	Yom-Tov, 1972
8	Production of eggs or young		*C. nemoralis*	Wolda & Kreulen, 1973
9	Production of eggs or young		*T. seetzeni*	Yom-Tov, 1972
10	Production of eggs or young		*H. virgata*	Butler, 1976
11	Survival		*H. virgata*	Butler, 1976

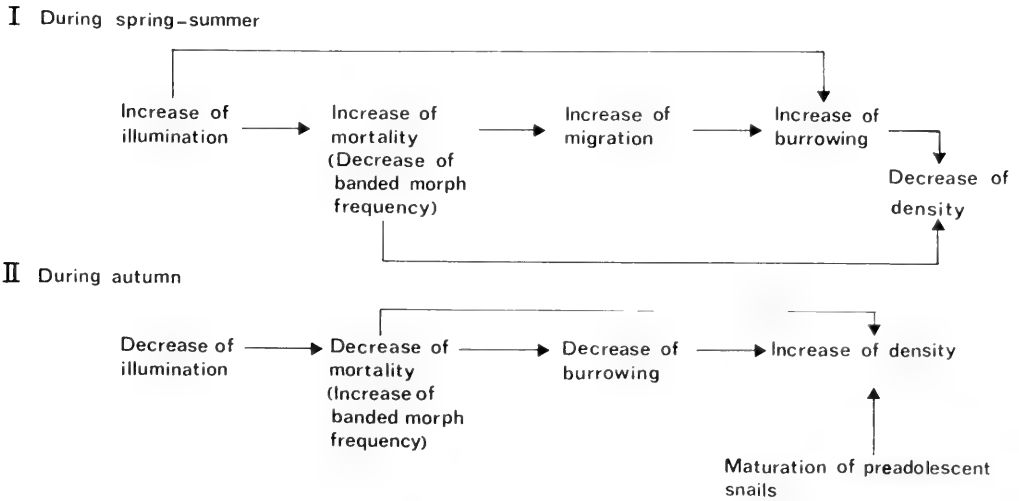
TABLE 7. Correlations between various ecological parameters in the studied *B. bidens* population.

	Density (D)	Migrations (m)	Mortality (d)	Burrowing (B)	Illumination	Source
m	−0.56	—	—	—	—	Present study
d	−0.66	0.93	—	—	—	Present study
B	−0.82	0.75	0.88	—	—	Present study
Frequency of banded morph	−0.56	*	0.88	0.73	−0.85	Livshits (1981)

*It was found that migration activity of the banded morphs was significantly higher than in unbanded ones.

However, these correlations were obtained in snails maintained on a limited food supply and under laboratory conditions.

Correlations between different ecological parameters were also observed for the population studied (Table 7). Our own data suggest the following scheme of density regulation:



In the previous investigation (Livshits, 1981) it was shown that during the hot and dry period of summer the mean frequency of buried snails among the banded morphs was significantly higher than among the unbanded snails ($p < 0.001$). Burrowing of the snails was concomitant with a considerable decline of the total mortality of animals and particularly of the banded morphs. For example, during July 1976 (before burrowing) mortality of banded snails was 13% vs. 4.2% per 10 days during August 1976. Simultaneously the relative mortality of unbanded morphs in the population increased (4.6% vs. 8.4%). As a result of this in September–October, the increase in frequency of the banded morph was parallel to a decrease in mortality.

Analysis of the thermotolerance of shell pattern morphs showed that the resistance of the unbanded morph was significantly higher than that of the banded one (Livshits, 1981). Reversible correlation between banded morph frequency and illumination was also discerned, which suggests that illumination (and/or temperature) may be an important determinant of snail mortality. Indeed, maximal fluctuations of density (= maximal coefficient of variation) were observed at sites M and N, where the frequency of the banded morph was also maximal. Increase of illumination led to the increase not only of mortality but also of migratory activity. Additional experiments revealed that the banded morph preferred shaded areas (Livshits, 1981) and that the morphs may migrate in different directions actively searching for appropriate microhabitats. However, the paucity of suitable microbiotopes and the limited activity radius of the individual snails lead to mass burrowing during the summer. There is consequently a decrease in the population density on the surface, in spite of the concurrent decrease in snail mortality (Fig. 2). In the autumn, the diminution of insolation leads to decreased mortality and the reemergence of buried snails. Restoration of the adult population density is effected also by maturation of pubescent snails. A histogram of age structure (Livshits, 1983) clearly indicates an increase in the proportion of adult snails within the population by autumn.

The data presented herein enable the formulations of several possible mechanisms to explain the seasonal and spatial fluctuation in the population density of *B. bidens*.

ACKNOWLEDGMENTS

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SEASONAL CHANGES IN THE REPRODUCTIVE GROSS ANATOMY OF THE LAND SNAIL *TRIODOPSIS TRIDENTATA TRIDENTATA* (PULMONATA: POLYGYRIDAE)

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ABSTRACT

Triodopsis tridentata tridentata (Say, 1816) is a seasonally protandric hermaphrodite. Three statistically independent stages in its reproductive cycle, named *mating readiness, egg production,* and *allosperm absence*, were detected by principal components analysis of the relative volumes of six reproductive organs from March through July. The range and temporal sequence of volume changes in each of these organs are shown in graphs and in illustrations of the entire reproductive systems of individuals with extreme principal component scores. The illustrations indicate especially well that lengths, widths, and volumes of some reproductive organs should be considered suspect as phylogenetic or taxonomic characters in this and probably many other land snail groups.

Key words: snail; Pulmonata; reproductive anatomy; principal components; protandry; hermaphrodite.

INTRODUCTION

The hermaphroditic reproductive system of pulmonate gastropods is commonly used as a source of characters for phylogenetic inference and systematics. Despite this, intraspecific variation in reproductive characters is poorly understood. Most studies on intraspecific variation in the pulmonate reproductive system (e.g., Krahelska, 1912–1913; Holm, 1946; Laviolette, 1950; Lusis, 1961, 1966; Rigby, 1963, 1965; Galangau, 1964; Kugler, 1965; Luchtel, 1972 [a review]; Duncan, 1975 [a review]) are histological in approach, are restricted to one organ or a limited set of organs, and ignore gross morphology. Several papers (e.g., McLauchlan, 1951; Walter, 1968, 1969; Webb, 1970) contain illustrations of some intraspecific variation, but do not put the variation into its seasonal context and do not attempt to depict the full range of variation. A few papers discuss complete seasonal cycles in the size or weight of pulmonate reproductive organs (e.g., Berrie, 1966; Smith, 1966; Runham & Laryea, 1968) but give no illustrations of gross morphology. To my knowledge, only one paper to date (Solem, 1981, fig. 53) actually illustrates the full range of seasonal variation in a pulmonate's reproductive organs. Such baseline data are essential for the phylogeneticist and systematist, who must compare specimens collected at different seasons or in different climatic regimes.

This paper illustrates the range of temporal variation in the gross morphology of reproductive organs in the polygyrid land snail *Triodopsis tridentata tridentata* (Say, 1816).

The Polygyridae are an endemic and dominant land snail family of North America (Pilsbry, 1940). Polygyridae are increasingly used for studies in physiology (Reeder & Rogers, 1983), ecology (Solem, 1955; Blinn, 1963; Randolph, 1973; McCracken, 1976, 1980; Vail, 1978; Emberton, 1981), and ecological genetics (Fairbanks, 1979; McCracken, 1980; McCracken & Brussard, 1980). Some polygyrids may soon be of considerable economic importance as a source of anti-A agglutinin for typing human blood (Miles & Beck, 1983).

Triodopsis t. tridentata (Fig. 1) belongs to the subfamily Triodopsinae (Pilsbry, 1940; Webb, 1959) and to the subgenus *Triodopsis, sensu stricto* (Pilsbry, 1940; Webb, 1954; Vagvolgyi, 1968); it is the nominate member of the *tridentata* species complex (Vagvolgyi, 1968). *Triodopsis t. tridentata* is a common snail of woodlands and waste ground, ranging from southern Ontario, Michigan, and New England to middle Alabama and Georgia (Hubricht, unpublished range map). This snail lives from near sea level in New York and

New Jersey to between 1200 and 1500 m in the Roan Mountains of Tennessee. The shell varies considerably in size and, to a lesser extent, in shape over its geographical range (Vagvolgyi, 1968).

Triodopsis t. tridentata lives under leaf litter, logs, stones, and trash. It hibernates during the winter and is active through the spring, summer, and fall (Grimm, 1975). During the active season, the snail may enter a short-term quiescent state, with the formation of the same type of thin epiphragm of dried mucus as used in hibernation, when the weather is unseasonably cold (Ingram, 1941) or dry (personal observation).

Courtship is brief. Intromission is either reciprocal or one-sided and lasts 5 to 15 minutes (Webb, 1947, 1959). Small clutches of eggs are laid in loose soil under some cover, usually in early spring. The eggs are 2.0 to 2.1 mm in diameter and are deeply indented when first laid (Ingram, 1944), but later appear "leathery . . . bounded by an outer clear viscid membrane beneath which is a white crystalline layer" (Kingston, 1966). In the laboratory the eggs hatch in two to three weeks and the hatchlings reach maturity in six to eight months. Some laboratory hatchlings mature by fall and others overwinter as young to complete growth the next spring. Adults live two to four years and lay eggs every three weeks to six months in the laboratory. They have not been observed to self-fertilize, and allegedly do not oviposit unless inseminated by another individual, no matter whether of the same or different species (Grimm, 1975). Self-fertilization does occur very rarely and with low fertility in the congeneric *Triodopsis albolabris* (McCracken, 1980).

The reproductive system of *Triodopsis t. tridentata* is shown diagrammatically in Fig. 1. The functions of its organs, as inferred from the literature on other pulmonates, are as follows. Italicized names in the following text are as labeled in Fig. 1. The *ovotestis* first produces sperm, then ova (Pennypacker, 1930; Lusis, 1961; Runham & Laryea, 1968; but see Rigby, 1963, 1965). Sperm are stored in the *hermaphroditic duct* (Duncan, 1975; Solem, 1981). During mating, the *penis* is everted through the genital opening, and sperm travel from the *hermaphroditic duct,*

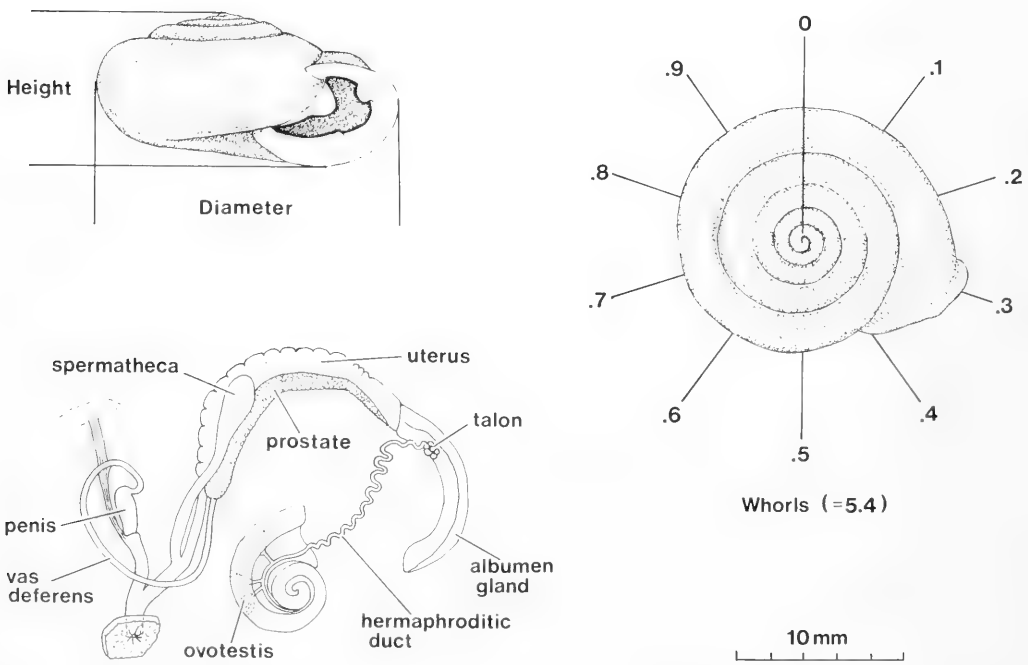

FIG. 1. *Triodopsis t. tridentata* (Say): shell and diagrammatic reproductive system. Shell measurements are indicated. Rank measurements were taken of the spermatheca, prostate, uterus, talon, albumen gland, and hermaphroditic duct.

down a ciliated channel adjacent to the *prostate* (which adds secretions), then out the tip of the everted *penis*. The mate's *penis* inserts through the partially everted vagina and discharges a sperm mass into the duct of the *spermatheca*, which is actually more thick-walled than depicted in Fig. 1 (Webb, 1948, 1959; Duncan, 1975). Much of the received sperm mass is probably digested by proteolytic enzymes in the *spermatheca* (= bursa copulatrix) (e.g., Nemeth & Kovacs, 1972; Reeder & Rogers, 1979; Rogers *et al.*, 1980), but a few sperm escape down the spermathecal duct to swim up through the oviduct and the *uterus* to reach the *talon* (= receptaculum seminis = fertilization pouch = ciliated hood), where they are stored and perhaps nurtured (Rigby, 1965; Bayne, 1973; Lind, 1973; Reeder & Rogers, 1983).

Ova move through the *hermaphroditic duct*, are fertilized by allosperm from the *talon*, receive a yolk from the *albumen gland*, then pass into the *uterus*, where the egg shell is added. Clutches of completed eggs travel down the oviduct and out the genital opening (Duncan, 1975).

METHODS AND MATERIALS

Snails were collected at approximately weekly intervals from 24 March to 29 July, 1979, at six different sites around Dow Lake, Strouds Run State Park, Athens County, Ohio, U.S.A. (Fig. 2). The six sites were chosen as relatively undisturbed, second-growth deciduous hill slopes with occasional sandstone outcrops. For each of the 16 collections, the site was chosen by random number table such that all six sites would be sampled before repeating any site. Collecting was done each afternoon for approximately three hours. All collections are summarized in Table 1. Collections were designed for the dual purpose of detecting seasonal changes in reproductive structures of *Triodopsis t. tridentata* and of assessing microgeographical variation in land snail communities.

Each sample of *Triodopsis t. tridentata* with reflected shell lips was split into two groups. One group was drowned overnight in tap water laced with chloral hydrate, a relaxant, fixed in 95% ethanol, then stored in 70% ethanol for gross anatomy. The second group was likewise drowned in tap water with chloral hydrate, but was then placed directly into Bouins solution for later histological examination. Only the 57 specimens preserved in ethanol are considered here (Table 1, column 6).

The following data were recorded for each specimen: shell diameter and height in mm (Fig. 1); whorl count to the nearest 0.1 whorl

TABLE 1. Collections of *Triodopsis t. tridentata* from Strouds Run State Park, Athens, Ohio.

Date (1979)	Site (see Fig. 2)	Hours spent collecting	Number live collected		Number adults dissected	FMNH catalogue number	Notes
			Adult	Juvenile			
24 March	IV	3:50	10	0	6	209209	
1 April	III	4:30	3	0	2	209232	
8 April	II	3:00	6	1	3	209237	
14 April	I	2:30	7	2	4	209279	
22 April	V	3:00	4	2	2	209294	
28 April	VI	3:00	8	2	4	209333	
5 May	IV	3:00	5	0	3	209348	
14 May	III	3:00	8	4	3	209375	
21 May	II	4:00	12	2	6	209387	
26 May	I	3:00	4	1	4	209420	eggs first seen
3 June	V	3:00	2	1	2	209453	eggs in uterus of specimen
16 June	IV	3:00	9	0	5	209502	
27 June	III	3:10	5	2	3	209536	juveniles are hatchlings
2 July	II	3:00	6	0	3	209554	
25 July	VI	3:00	1	8	1	209571	juveniles are hatchlings
29 July	IV	2:30	6	0	6	209584	

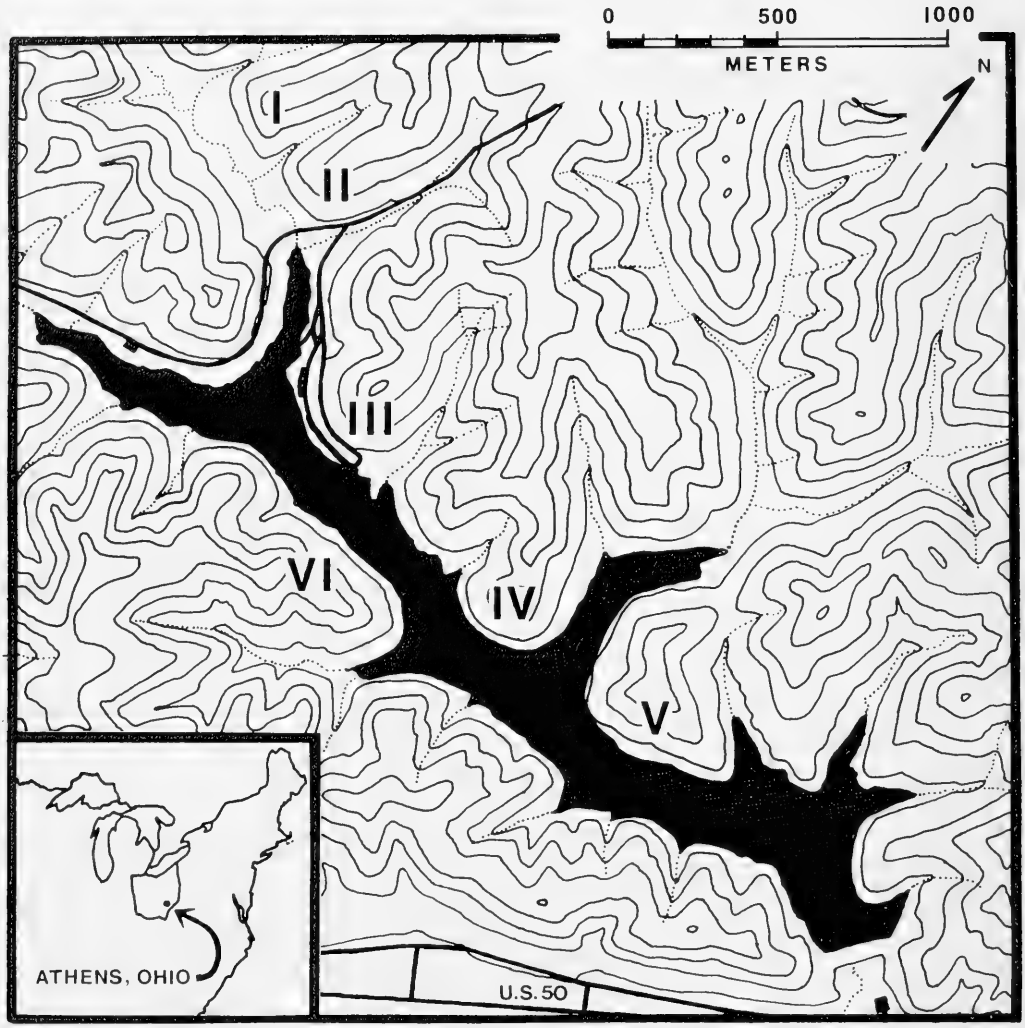

FIG. 2. Six collecting sites (I–VI) at Strouds Run State Park, Athens, Athens County, Ohio. Dow Lake is in black. Contour interval is 60 ft.

(Fig. 1); degree of protrusion of the animal from its shell (ranks 0 to 4); albumen gland relative volume (ranks 1 to 9); relative thickness of edge (ranks 1 to 6), and darkness of color (ranks 1 to 6), cream to dark brown); hermaphroditic duct volume (ranks 1 to 9); talon volume (ranks 1 to 6); uterus volume (ranks 1 to 6); spermatheca volume (ranks 1 to 7); and prostate volume (ranks 1 to 6).

Rank measurements were taken as follows. All 57 vials, each containing the complete reproductive system of one snail, were placed in a shallow, ethanol-filled dissecting tray, and the specimens were rank-ordered for each of the 9 variables in turn. For the albumen gland, for example, the specimens were ordered from small to large glands, with the number of size categories (ranks) determined by my ability to distinguish these from pairwise comparisons. For all organs, the sorting criterion was estimated volume rather than length, width, or area. The penis could not be ranked in like manner because there were varying degrees of extrusion and coverage by the sheath and the retentor muscle. The ovotestis could not be ranked be-

cause it was encased by the digestive gland and thus could not be observed directly.

During the rankings, an effort was made to maintain approximately normal distributions of ranks. This was in no way difficult or unnatural, as the size distributions of the organs had strong central tendencies. Ranks can be used in parametric uni- and multivariate analyses if their distributions are approximately normal (Paul Sampson, personal communication). In fact, the term "rank" is somewhat misleading in this case because the difference between adjacent ranks is close to a constant (the least detectable difference). Thus, the ranks approach being metric variables.

For all statistical analyses I used BMDP-79 programs (Dixon & Brown, 1979) on the Amdahl computer at the University of Chicago. Histograms, as well as normal and detrended normal plots (P5D), were used to test assumptions of normality of all measured variables. Stepwise linear regression (P2R) and canonical correlation (P6M) were used to determine whether shell size or degree of animal extrusion from its shell could explain the size of the prostate, the uterus, or the albumen gland (the three largest and most deformable reproductive organs). Polynomial regression (P5R) with a two degree maximum was used to detect significant linear or unimodal changes in each of the measured variables over time.

For multivariate analysis, only one measure was used for the albumen gland, namely volume. This was done to restrict variables to six because of the small number of snails (57). Clustering algorithms were used to detect natural groupings into blocks of affinity (P3M) by the 57 individual snails (P1M), by the six reproductive organs (P3M), and by both snails and reproductive organs. Principal components analysis (P4M) detected groups of the six reproductive organs that varied together as independent units. For deriving principal components, the covariance matrix was used in order to weight each reproductive organ according to its detectable variation. Snails having extreme values for each principal component were chosen to show the biological significance of the components and to illustrate the full range of size variation of each reproductive organ. Finally, each snail was classified according to its stage in the reproductive cycle, with the stages determined by the aforementioned analysis.

RESULTS

The distributions of all measured variables were not significantly different from normal.

Significant seasonal variation was found in 7 of the 9 measurements of reproductive organs (Fig. 3). The prostate (that is to say a generalized prostate for all dissected snails from all collections) steadily decreased in volume from March through July. Likewise, the hermaphroditic duct steadily decreased in volume from its peak in March. The uterus volume rose to a peak in late May, then decreased. The albumen gland increased slightly to a peak volume in late May, then decreased. The albumen gland increased in granularity of texture to a peak also in late May, then decreased. The albumen gland became darker in color until late April; thereafter, it became lighter. Neither the talon volume nor the edge thickness of the albumen gland underwent any significant seasonal change.

Considerable variability occurred in all reproductive characters at every collection, as is obvious from the scatter of points in Fig. 3. Thus, for any given date and site, individual snails of the same population (on a single hill slope) differed widely in their reproductive development.

Some of the great variation in uterus and albumen gland volumes was apparently a result of preservation: stepwise regression indicated that 25% and 18%, respectively, of their variation was explained by how far the drowning animal had retracted into its shell. These relationships were also evident in the canonical correlation analysis (not shown here for brevity's sake.) The effect of animal extrusion on organ size was not statistically removed from the data prior to subsequent analyses; doing so would not seem to have affected the results.

Clustering the reproductive organs by a standard, minimum distance, single linkage algorithm based on the absolute correlation matrix yielded the dendrogram shown in Fig. 4. Two major clusters are readily apparent. The upper cluster represents the tendency for snails with a large uterus to also have a large albumen gland and a small talon. The lower cluster represents the tendency for snails with a large spermatheca to also have a large prostate and, to a lesser extent, a large hermaphroditic duct. Clustering the individual snails yielded a finely dissected, indecipher-

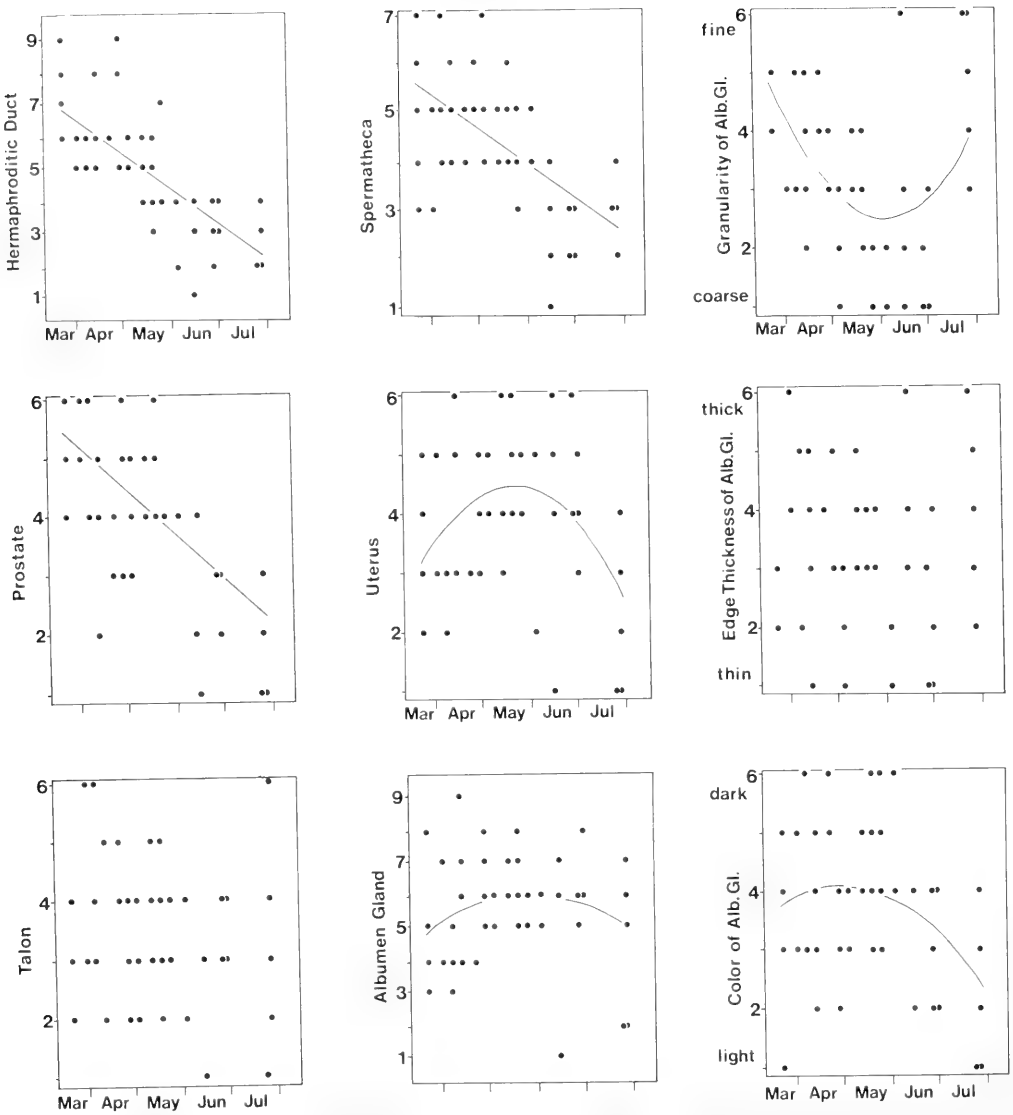

FIG. 3. Ranked sizes and characteristics of reproductive organs plotted against time. Least squares regression lines, either linear or quadratic, are superimposed. Significance levels of the regressions are as follows: hermaphroditic duct, $p < .005$; prostate, $p < .005$; talon, $p = .47$; spermatheca, $p < .005$; uterus, $p < .005$; albumen gland, $p = .08$; granularity of albumen gland, $p < .005$; color of albumen gland, $p = .01$ (linear) or .06 (quadratic, as shown).

able dendrogram. Simultaneously clustering both organs and snails produced an equally confusing array of small blocks of affinity.

Results of principal components analysis are given in Table 2. Three independent components (factors) accounted for 84% of the total size variation in the six reproductive organs. These factors point out the same pattern that appeared in the cluster analysis, only in finer detail. The first factor is a linear function of the tendency for the hermaphroditic duct, the prostate, and the spermatheca to simultaneously increase and decrease in volume. This factor is a new, combined variable which may be called *mating readiness:* the hermaphroditic duct is large and charged with sperm, the prostate is large and full of stored seminal secretion, and the sperma-

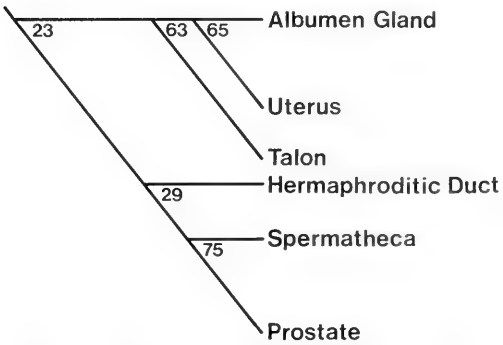

FIG. 4. Cluster tree of reproductive organs. Numbers at branch-points indicate the distance or similarity when the link was formed. Algorithm was a minimum distance (single linkage) method based on the absolute correlation matrix.

TABLE 2. Structures of three independent factors extracted from rank-measurements of six reproductive organs. Names of the factors are based on their strongest contributors, which are underscored. The total explained variance is 83.6%.

	Mating readiness	Egg production	Allosperm absence
Hermaphroditic duct	1.69	0.00	0.40
Spermatheca	1.13	0.17	–0.37
Prostate	1.12	0.17	–0.36
Albumen gland	0.14	1.53	–0.11
Uterus	0.10	1.15	–0.22
Talon	0.14	0.19	–1.00
Percentage of variance explained	45.4%	28.3%	9.9%

theca is enlarged either preparatory to or as a result of receiving the sperm mass of a copulatory partner. *Mating readiness* comprises about half the total variation of the six organs.

The second factor is a linear function of the tendency for the uterus and albumen gland volumes to covary. This factor should be called *egg production:* the albumen gland is enlarged and full of yolk, and the uterus is also engorged with egg shell-producing secretions. *Egg production* comprises about one-fouth of the total variation of the six rank-measured organs. The variable *egg production* is independent of the variable *mating readiness* because they were derived as orthogonal principal components.

The third factor is predominantly a function of talon size. This factor may be called *allosperm absence,* because large values of the factor correspond to small talons presumably having little or no stored foreign sperm. *Allosperm absence* comprises about one-tenth of the total variation of the organs; it is independent of both *mating readiness* and *egg production,* because of the orthogonality of principal components.

In order to check the biological relevance of the three factors, as well as to view the extreme sizes of each organ, I examined the three or four snails with highest and lowest values for each factor. One obvious juvenile (undeveloped genitalia despite the reflected lip of its shell) had the lowest value for both *mating readiness* and *egg production.* Another snail which was transitional between juvenile and adult had the second smallest value for *mating readiness* and the third smallest value for *egg production.* Excluding those two juveniles, I prepared drawings, all to the same scale, of the reproductive systems having extreme values of each of the three factors (Figs. 5 to 7).

The snail with the highest value for *mating readiness* (Fig. 5, top) had a hermaphroditic duct fully charged with sperm, a prostate swollen with secretion, and a huge spermatheca with an enclosed mass, presumably sperm received in a recent mating. This snail was from the first collection in March, and had probably recently emerged from hibernation. The snail with the lowest value for *mating readiness* (Fig. 5, bottom) had a minute hermaphroditic duct, prostate, and spermatheca.

The snail with the highest value for *egg production* (Fig. 6, top) had a large, plump albumen gland and uterus for egg-making. Also, its hermaphroditic duct appeared emptied at its distal end. The snail with the lowest value for *egg production* (Fig. 6, bottom) was apparently a subadult, with all organs small and incompletely developed. This animal could not have been manufacturing eggs.

The snail with the highest value for *allosperm absence* (Fig. 7, top) had a very small talon. This snail seems to have had both male and female systems charged and active. The snail with the lowest value for *allosperm absence* (Fig. 7, bottom) had the largest talon of all the dissected snails. Because this snail was collected at the end of July and had an extremely eroded shell, it could be interpreted

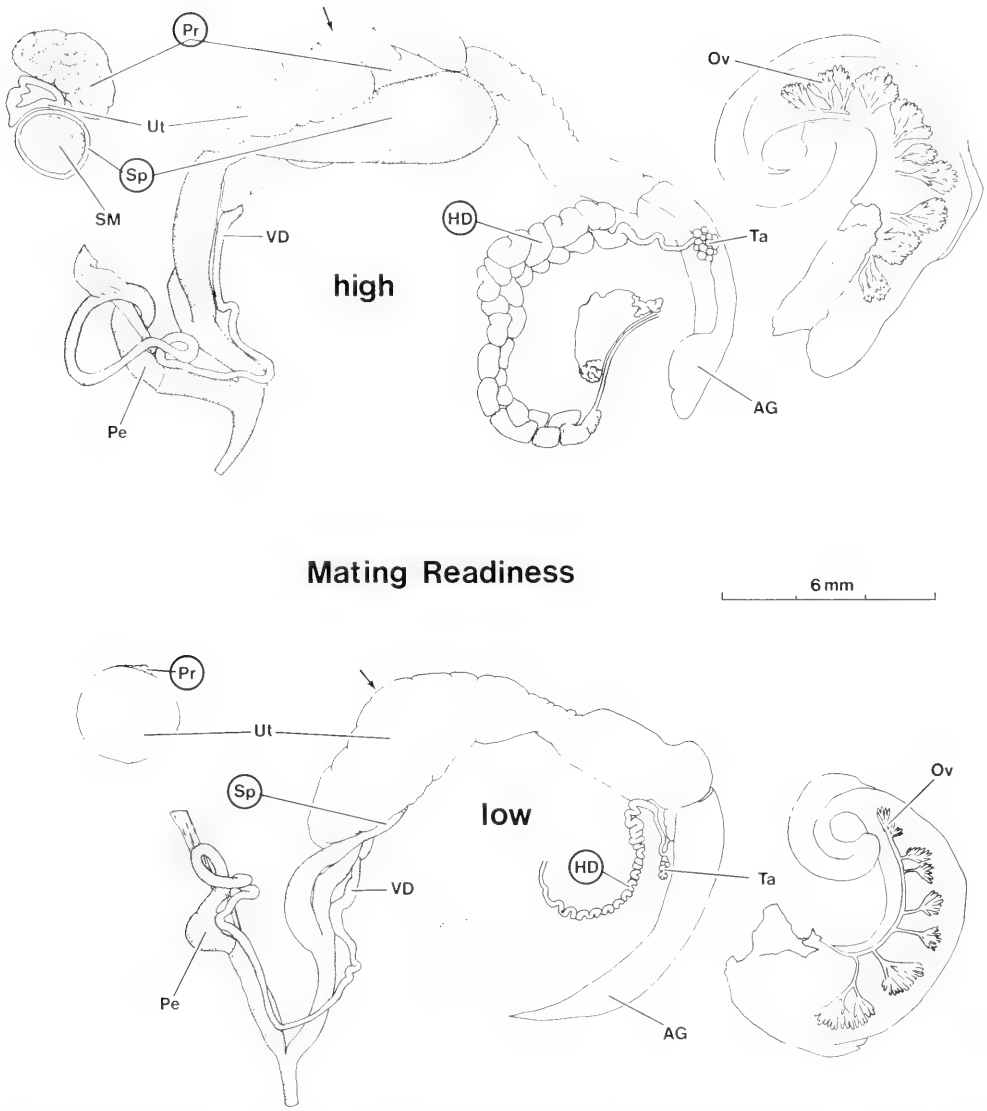

FIG. 5. *Triodopsis t. tridentata:* reproductive anatomies having extreme values for the factor *mating readiness.* AG = albumen gland, HD = hermaphroditic duct, Ov = ovotestis, Pe = penis, Pr = prostate, Sp = spermatheca, SM = sperm mass, Ta = talon, Ut = uterus, VD = vas deferens. The arrow indicates the position of the cross section figured in the upper left. The organs with circled labels are those which make up the factor (see Table 2). "High" was collected 14 April (specimen D) and "low" was collected 29 July (specimen A).

as post-reproductive. Thus, the uterus appeared as though folded down to a compact size, and the albumen gland appeared spent and flaccid.

I next classified each snail according to its stage in the reproductive cycle by studying the 57 reproductive systems, removed from their vials, in random order. There were only two reproductive systems which totally confused me and which I could not place into any category. Repeating the classification process the next day, again in random order, I got a repeatability of 88%. The major difficulty was deciding between early egg production and late post-reproduction. With final decisions made, sometimes aided by reexamin-

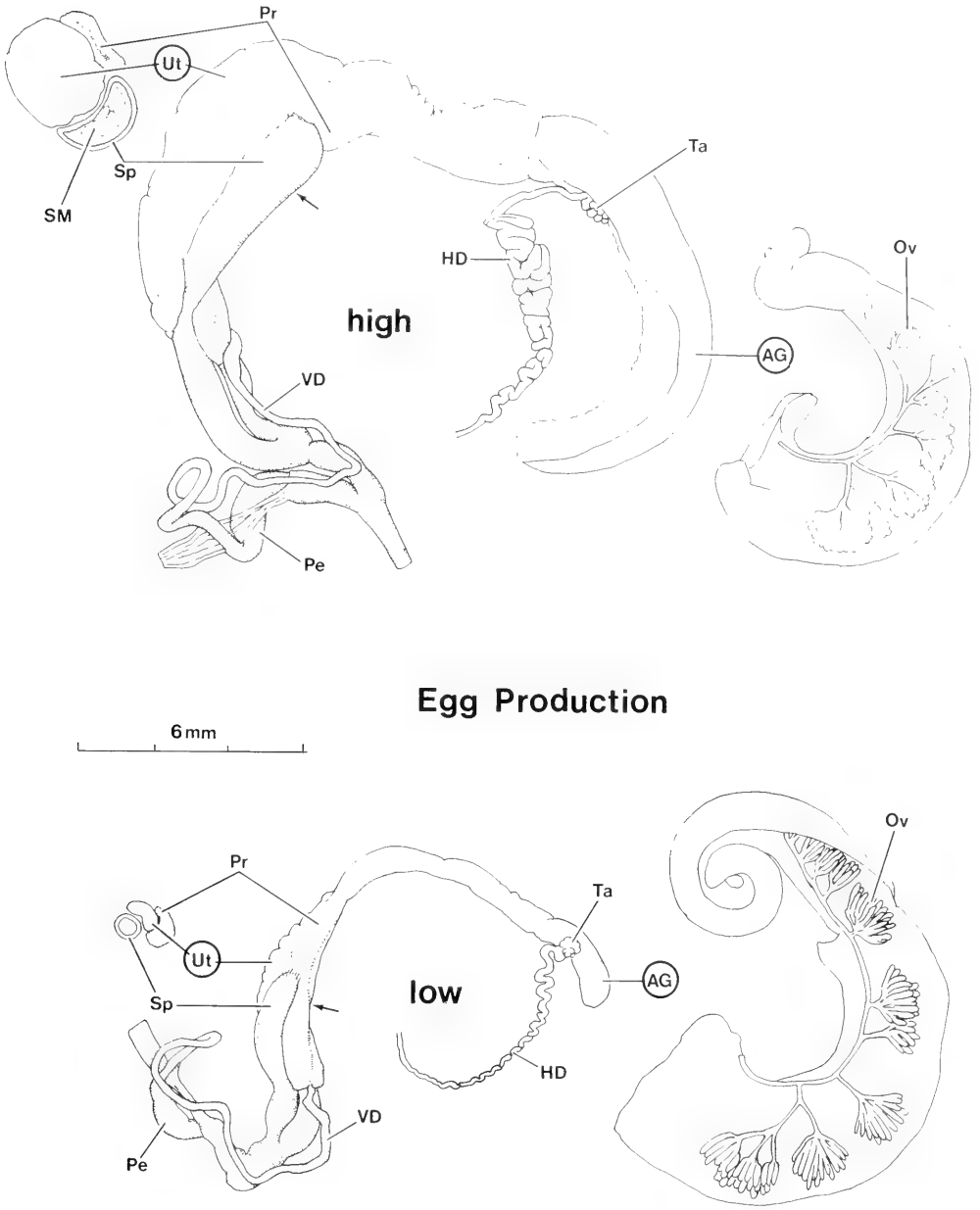

FIG. 6. *Triodopsis t. tridentata:* reproductive anatomies having extreme values for the factor *egg production.* Labels are as in Fig. 5. The organs with circled labels are those which make up the factor (see Table 2). "High" was collected 14 April (specimen C) and "low" was collected 29 July (specimen C).

ing the shell, the results were graphed against time (Fig. 8). Included in the graph were the juveniles and hatchlings which had not been dissected.

From Fig. 8 it is evident that two age groups overwintered: adults began mating upon emergence in March, started laying eggs in May, and their offspring were crawling at least by the end of June; juveniles that had overwintered were approaching sexual maturity by the end of July.

It can be seen from Fig. 8 that the dissected

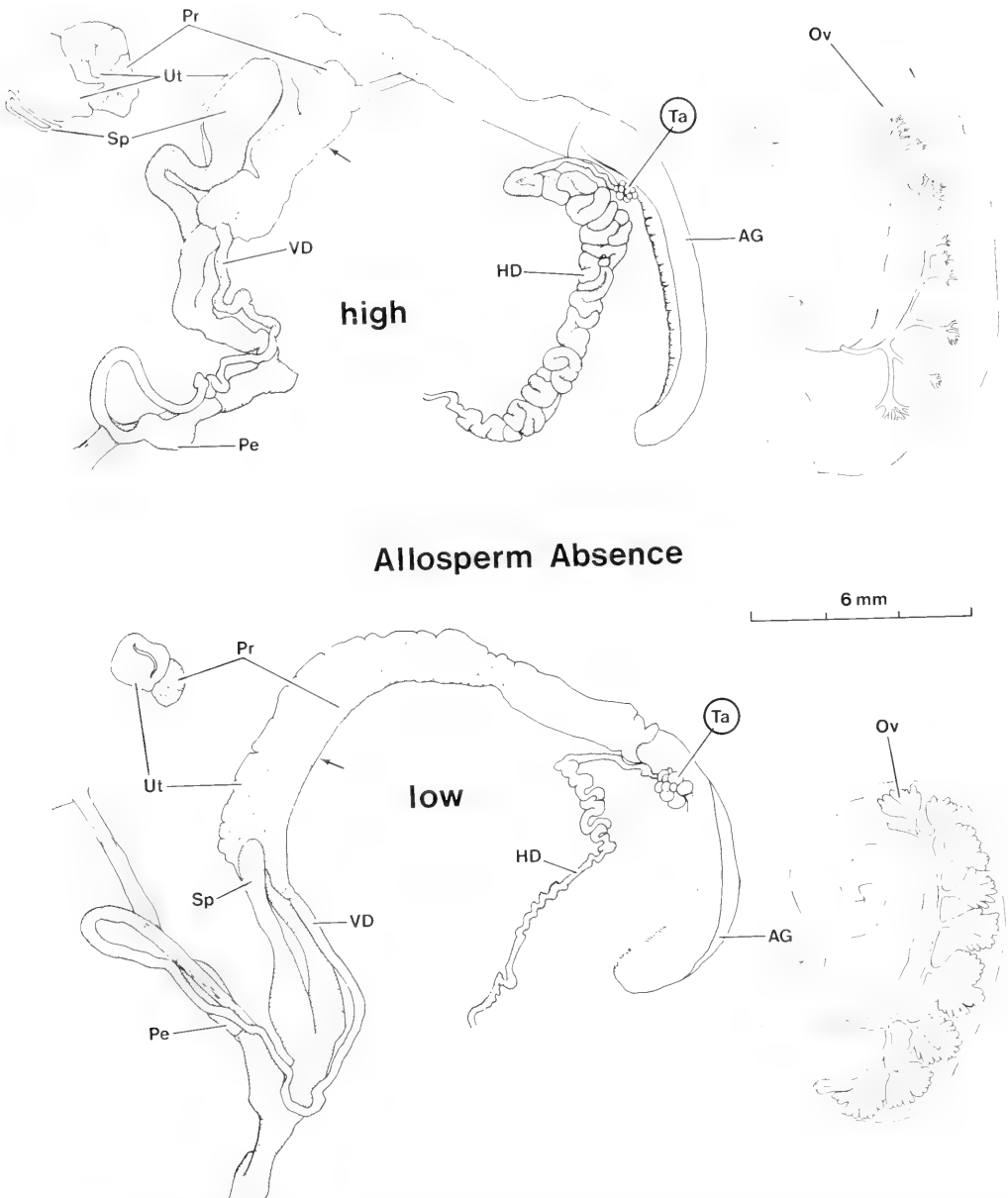

FIG. 7. *Triodopsis t. tridentata:* reproductive anatomies having extreme values for the factor *allsoperm absence*. Labels are as in Fig. 5. The organ with the circled label (the talon) makes up the bulk of the factor, and the organs with cross-marked labels are secondary contributors to the factor (see Table 2). "High" was collected 24 March (specimen A) and "low" was collected 16 June (specimen C).

snails included a cohort of late-maturing juveniles. This cohort, then, is present in the seasonal graphs of organ volumes (Fig. 3) as a "contaminant" of the later stage of each graph.

DISCUSSION AND CONCLUSIONS

Methods used

Rank-ordering reproductive organs by visual estimation of volume is inferior in many

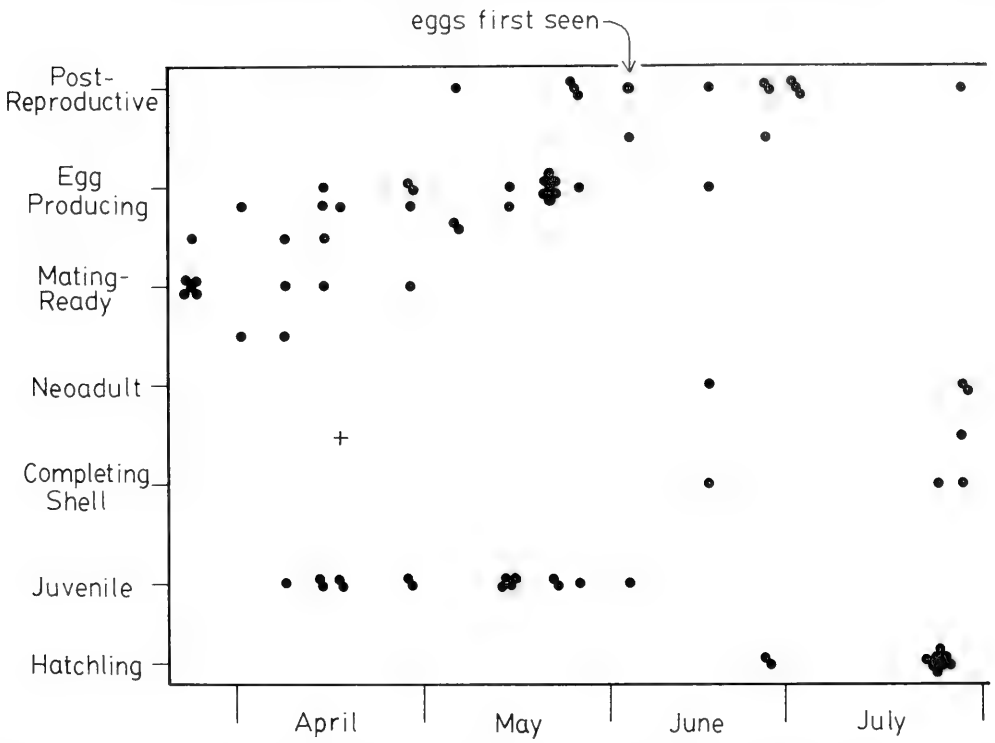

FIG. 8. Reproductive state of individual snails plotted against time. The cross represents an individual with a stunted reproductive system due to a massive parasitic infestation of the digestive gland.

ways to calculating volume from serial sections (Lusis, 1961) or cutting apart and weighing the individual organs (Smith, 1966; Runham & Laryea, 1968). Points in favor of my ranking method are that it requires minimal time and equipment, leaves the reproductive system intact, and permits separate measurements of the uterus, prostate, and talon.

Sample sites were dispersed over several square kilometers (Fig. 2) and included the range of variation in slope, aspect, depth of litter layer, and other parameters of forest microhabitat that were available in the region. Because so many sources of variation were included in the study, the seasonal trends which appeared in the data (Figs. 3 and 8) can parsimoniously be attributed to underlying biological properties of the snails rather than to biased sampling.

Protandric cycle

The regression lines in Fig. 3 and the curve in Fig. 8 represent the anatomical changes of an individual snail from March through mid-June (beyond mid-June the data are "contaminated" by the addition of a cohort of neoadults: see Fig. 8). The adult snail comes out of hibernation in late March ready for copulation, with stores of sperm and prostatic secretion, and a spermatheca enlarged or capable of enlarging. The female organs for egg production develop soon after sperm exchange. Egg-laying begins in early June.

The cause of spermathecal enlargement remains undetermined. The spermatheca may remain relatively small until it is expanded by copulation and receipt of a sperm mass. Alternatively, digestion of excess autosperm and other internal wastes (R. Reeder, personal communication), or response to hormonal changes, may enlarge the spermatheca prior to copulation. Support for the hypothesis of pre-copulatory enlargement is afforded by two apparently hibernating *Triodopsis t. tridentata* (FMNH 209162) collected 3 March near Site I, Strouds Run State Park (Fig. 2). These two snails had enlarged spermathecae much as in the upper anatomy

of Fig. 5. The two were deep under an icy decayed log, had well-formed epiphragms, and presumably had been dormant all winter. During the previous two days, however, minimum and maximum air temperatures at a nearby station had risen to 40 and 54 degrees Fahrenheit, so it is possible that these snails had reactivated and copulated before being collected.

Significant color changes in the albumen gland (Fig. 3) may have been caused either by histological changes associated with yolk production and release, or by staining, under preservation, by the closely adjacent stomach, the color of which may have matched seasonal changes in diet. Therefore, changes in albumen gland color cannot be firmly indicated as a part of the protandric reproductive cycle.

Significant changes in the granularity of the albumen gland (Fig. 3) are readily explained by the gland's development during the reproductive cycle. Overwintered adults had albumen glands spent and granular-looking from last year's egg laying. As these glands began producing yolk, their cells filled, and they looked less granular. After egg production, the albumen glands once again had empty cells, and appeared granular.

The wide variation in reproductive state found in each collection (Figs. 3 and 8) probably resulted from a mixture of three effects. First, each hillside from which a collection was taken doubtless contained a number of different microclimates which would have differentially affected the growth rates of resident snails. Second, with as many as four year classes coexisting (overlapping generations), and with two cohorts (early spring and late summer) per year class, a wide variation in reproductive state was to be expected. Third, it is highly likely that hatchlings from the same clutch of eggs grow at markedly different rates. This phenomenon has been documented in field populations of the confamilial *Mesodon thyroidus* (Blinn, 1961) and of the congeneric *Triodopsis albolabris* (McCracken, 1976), as well as in controlled laboratory rearings of *T. albolabris* (McCracken, 1976; Vail, 1978). In view of these three of possibly many sources of variation in reproductive state, it is not surprising to see the scatter of points underlying the seasonal trends in Figs. 3 and 8. This scatter can be predicted to increase in more and more southerly populations from increasingly milder climates. It would also be interesting to determine how the variance in reproductive state affects the effective population size (see, e.g., Roughgarden, 1979), which is important in ecological genetics.

Factors and life stages

An important point needs to be made about the three factors extracted from the data (Table 2; Figs. 5–7). These factors correspond very closely to Smith's (1966) three phases of the underlying ontogenetic sequence in the arionid slug *Arion ater.* Smith determined these three phases from histological series, and found them, by elegant experimentation, to be independent of both environmental change and body weight. The first phase, "differentiation of male gonads early in the spring," is the equivalent of *mating readiness.* Smith's second phase, "copulation and differentiation of the female glands," corresponds to *egg production.* Smith called the transition between his first and second phases the "critical point," which occurs at or near the act of copulation and sperm exchange. Finally, Smith's third phase, "fertilization, egg-laying, and the onset of atrophy," is associated in a loose way with *allosperm absence.* Fertilization uses allosperm and therefore increases the value of *allosperm absence.* No snails in this study were unequivocally in a state of atrophy, so the correspondence on that aspect is unclear, unless Smith's (1966) term "atrophy" is equivalent to my term "sexual dormancy."

The correspondence between this study and Smith's (1966) suggests that a general mechanism underlies the reproductive cycles of both an arionid slug and a polygyrid snail. Clearly, the results of both studies are consistent with the hypothesis of a sequential release of separate male and female hormones (see Boer & Joosse, 1975), with the release of the latter triggered by some event around the time of copulation. This event is Smith's (1966) "critical point," which Runham & Laryea (1968: 104) equated with Laviolette's (1950) "transitional stage." Along the same line, Solem (1981) hypothesized that development of the female system in the camaenid land snail *Amplirhagada burnerensis burnerensis* was triggered by sperm exchange, because unmated snails, which had been kept quiescent for several months past normal mating time, when killed and dissected, had a hypertrophied male system with an undeveloped female system.

Comparable studies

Male-acting and female-acting anatomies have been illustrated for the camaenid land pulmonates *Meridolum jervisense* (McLauchlan, 1951) and *Polydontes lima* (Webb, 1970). Both these species show suites of mating organs and of egg-producing organs enlarged as in the "high" anatomies in Figs. 5 and 6 of this paper.

Solem (1981, fig. 53) presented a chart showing the gross morphologies of the ovotestis, hermaphroditic duct, prostate, spermathecal head and contents, and coiled section of the vas deferens, for each of six to ten collections over a year from dry season to wet to dry for the Australian camaenid *Amplirhagada b. burnerensis.* The prostate and hermaphroditic duct volumes were greatest at the beginning of the wet season and decreased remarkably during the wet season, exactly paralleling the prostate and hermaphroditic duct volumes of *Triodopsis t. tridentata* from early through late spring. Likewise, the uterus volume of *Amplirhagada b. burnerensis* was smallest in the early wet season, increasing to a maximum in mid-season, then decreasing through the remaining wet and dry seasons. Seasonal changes in the spermathecal volume of *Amplirhagada b. burnerensis* were slight compared to those of *Triodopsis t. tridentata,* but the greatest volume apparently occurred early in the wet season. The configuration of the distal vas deferens, which was not looked at in this study, proved a useful indicator of mating state in *Amplirhagada b. burnerensis:* unmated snails from the dry season had highly convoluted vasa deferentia, whereas mated snails had relatively straightened vasa deferentia. In summary, the Australian camaenid *Amplirhagada b. burnerensis* showed the same seasonal pattern of changes in the reproductive system as the American [illegible]gyrid *Triodopsis t. tridentata,* with the start of the Australian wet season corresponding to the North American spring.

Significance for phylogenetics

Caution must be exercised when using size differences among reproductive organs as taxonomic criteria. When Lutz (1950), for example, described the new subspecies *Triodopsis hopetonensis claibornensis,* he used as a distinguishing character a very long, enlarged spermatheca. Clearly, this character is capable of extreme intraspecific variation (see Fig. 5), and hence is of little value as a taxonomic character. Likewise, the relative size or shape of any pulmonate reproductive organ should be used as a taxonomic character only with caution and a full knowledge of its range of intraspecific variation. Even with this restriction, the pulmonate reproductive system provides a rich source of phylogenetically and taxonomically useful characters, including the internal (or everted) structure of the penis (e.g., Webb, 1947; Solem, 1976, 1981) and vagina (e.g., Solem, 1981), and the presence and disposition of various muscles (e.g., Pilsbry, 1940) and glands (e.g., Shileyko, 1978).

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MALACOLOGIA, 1985, 26(1–2): 241–251

FUNCTIONAL MORPHOLOGY OF "EYESPOTS" OF MANTLE FLAPS OF *LAMPSILIS* (BIVALVIA: UNIONACEA): EVIDENCE FOR THEIR ROLE AS EFFECTORS, AND BASIS FOR HYPOTHESIS REGARDING PIGMENT DISTRIBUTION IN BIVALVE MANTLE TISSUES

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ABSTRACT

Serially sectioned tissues of *Lampsilis ventricosa* "eyespots" reveal that this structure consists of (1) an epithelium with elongated, heavily pigmented cells resting on a consistent basement membrane and thrown into distinct folds; (2) an underlying series of muscles coursing parallel to the postero-anterior length of the flap; (3) connective tissue trabeculae which extend from the basement membrane down into the flap interior, separating the aforementioned muscles into bundles; (4) pigment granule clusters which accompany the connective tissue trabeculae and are distributed from the basement membrane of the "eyespot" epithelial cells into the central flap tissues; (5) a prominent muscle in the center of the flap which is longitudinally displayed in transverse sections and which has a "vertical" orientation in the flapping mussel; and (6) an extensive nerve supply apparently associated almost exclusively with the "eyespot" muscle tissues.

The role of the "eyespot" tissues in the mantle flap during the mussel's flapping behavior makes it seem unlikely that the "eyespots" have a sensory function. This conclusion comports well with findings from previous extensive behavior studies. Evaluation of (1) the nerve and muscle distribution in the "eyespot" region of the flap; (2) the obvious relationship of the pigment granule clusters to the pigmented "eyespot" epithelial cells; and (3) the pigment clusters' evident sequential path of distribution through the flap tissues indicate that the "eyespots" are more likely effectors than sensors. Rather than "eyespots," they should more correctly be termed "pigmented effector spots." Indeed it seems possible that localized mantle movements (e.g., of *Lampsilis* mantle flaps) or even more generalized mantle movements may serve to produce patterned distribution of pigment in bivalve mantle tissues.

Key words: "eyespot" function; mantle pigment; pigment pattern.

INTRODUCTION

The present study is an examination of the structure and an evaluation of the putative function(s) of the "eyespots" of the mantle flaps of *Lampsilis* (Bivalvia: Unionacea). Mantle flaps of this genus have recently figured prominently in systematic treatments of freshwater mussels (Burch, 1975; Johnson, 1980), and in the analysis of systematic genetics (Davis & Fuller, 1981). Behavioral aspects of mantle flap movements and *Lampsilis* mantle flap movements themselves have been involved in reproduction and spawning (Kraemer, 1970).

In recent years the molluscan bivalve mantle has been the subject of physiological studies (e.g., Sick & Siegfried, 1980; Sorenson *et al.*, 1980; Zaba, 1981; Zaba & Davies, 1981). Some histochemistry has been carried out by Mane & Patil (1980), Wheeler (1979), Gilloteaux (1979) and Counts & Prezant (1979). Sensitivity and control of the scallop mantle edge and of the file clam mantle edge were studied by Stephens (1978a, 1978b). In the literature reviewed for this study, however, there were no reports of investigation of mantle structures known to be involved in reproductive behavior of bivalved mollusks; and no further published studies of the structure and function of the mantle flaps of the fresh-water mussel *Lampsilis.*

BACKGROUND

An earlier study (Kraemer, 1970) revealed the presence of complex spawning behavior associated with mantle flapping, which causes some species of *Lampsilis* to resemble small swimming fish. Kraemer (1970) found that mantle flaps develop only in ma-

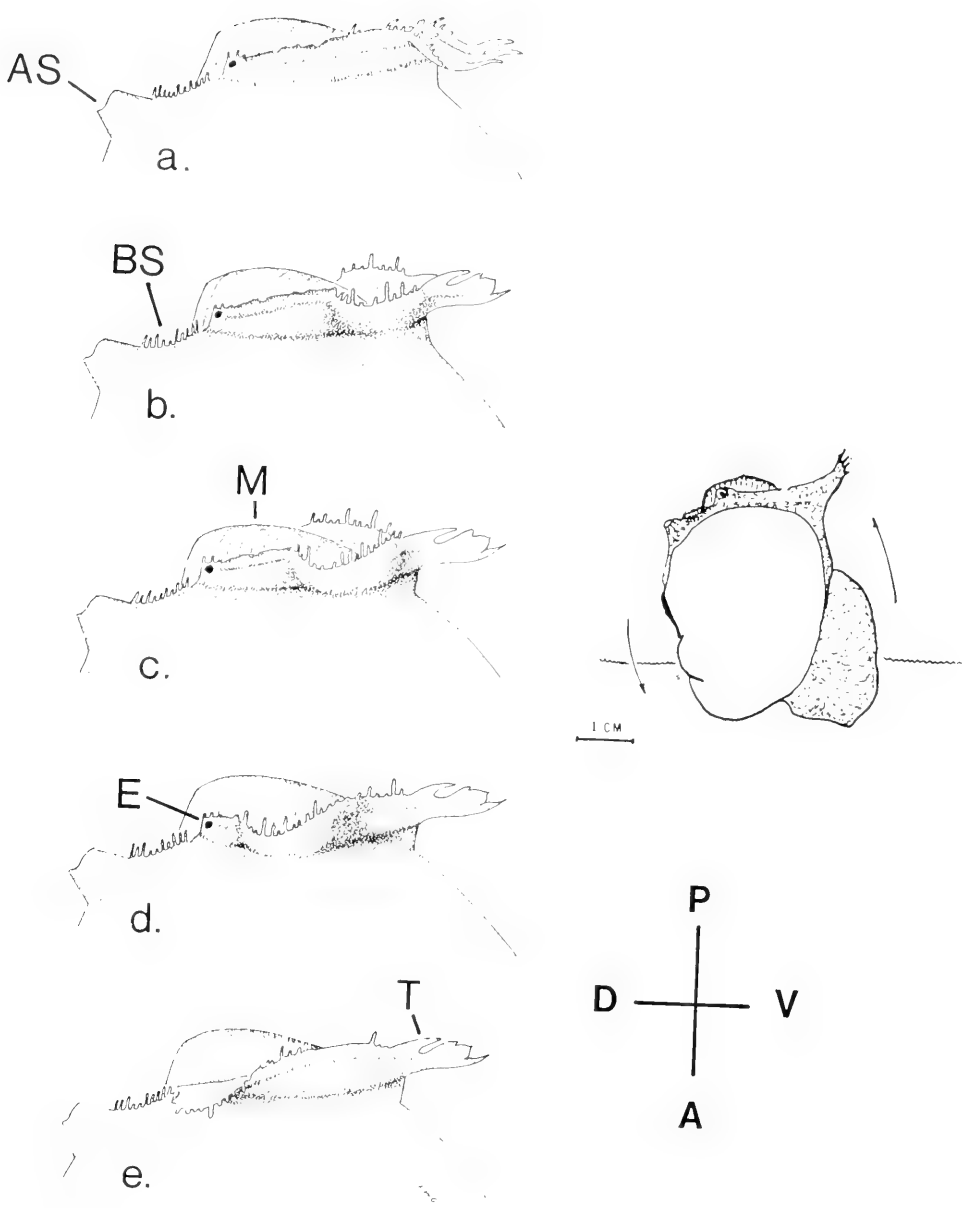

FIG. 1. Sequence of paired pulsing movements of mantle flaps in spawning female *Lampsilis ventricosa*, viewed from the left side (modified from Kraemer, 1970). Pulsing movements may be as rapid as 3 sec. a, end of recovery phase, ("tails" out, horizontally); b, beginning of pulse (initiated at base of mantle flap "tails"); c, pulse moves along flap, causing lateral bulge; d, pulse nears "eyespot" ends of mantle flaps; e, pulse is at flap ends, which are moved down and out, horizontally, exposing the glochidia-charged water tubes of the marsupial gill(s). Insert: sketch showing characteristic "headstand" position of *L. ventricosa* during flapping behavior. A, anterior; AS, excurrent siphon; BS, incurrent siphon; D, dorsal; E, "eyespot" of mantle flap; M, gravid outer gill, serving as marsupium; P, posterior; T, "tail" of mantle flap.

ture females, and that each flap is an extension of the inner lobe of the mantle edge, immediately anteroventrad to the branchial siphon. Each flap is a permanent part of the mantle, and characteristically possesses an "eyespot" (a raised, pigmented patch of epithelium) at its posterior end, and a "tail" anteriorly. The flaps function as part of a flapping behavior complex of gravid females before and during spawning, when the glochidia (parasitic larvae which must effect contact with a specific fish host) are shed into the water. The flaps move in rhythmic, paired pulses which are initiated near the "tail" ends of the flaps and move to the "eyespot" ends (Fig. 1). Near the tails of the flaps where the pulses originate, accessory mantle ganglia have been found, and may be involved as pacemakers for the flap movements (Kraemer, 1968, 1969).

A previous behavior study (Kraemer, 1970) did not imply that the "eyespots" on the flaps are photosensors, although Kraemer (*ibid.*) produced experimental evidence indicating that the *rate* of mantle flap movements will increase or decrease in response to increments and decrements of light, respectively, *at low light intensities.* These findings considerably modified and amplified the earlier conclusion of Welsh (1933) that there is "photic stimulation of the rhythmic contractions of the mantle flaps."

An earlier anatomical study (Kraemer, 1970) showed that there is considerable interspecific variation in the appearance and location of "eyespots" on the mantle flaps. For example, in *Lampsilis ventricosa,* a conspicuous "eyespot" is typically visible only on the outer surface of the flap, while in *L. fasciola,* an "eyespot" is evident on both outer and inner flap surfaces. The present study was carried out on the "eyespots" of *L. ventricosa* to determine the histological characteristics of the structure, and to explain the possible role of the "eyespot" in the mantle flap movements and flapping behavior complex of the spawning mussel.

MATERIALS AND METHODS

Specimens of *Lampsilis ventricosa* (Barnes) used in the histological study were all gravid females collected in July, 1965 from the Raisin River in Washtenaw County, Michigan, from Lee Creek in Crawford County, Arkansas in June, 1964, and from War Eagle Creek in Madison County, Arkansas in July, 1976. Five "eyespots" were removed from the mantle flaps of relaxed specimens, embedded in paraffin blocks, sectioned transversely or frontally at 6–10 μm, and stained with an aniline blue variation of Mallory's triple stain (Schmitz, 1967). One complete series of transverse sections was especially helpful.

Living specimens for SEM preparation were collected from King's River, Arkansas in February, 1982. Excision of "eyespot" tissue from the mantle flap of a relaxed specimen had to be done swiftly, since the membranous mantle flap retains its contractility even in a heavily sedated mussel. Once removed, the tissue was fixed in 2.0% glutaraldehyde, and processed for SEM examination. Samples were viewed with an ISI-60 Scanning Electron Microscope at 30KV with a working distance of 15 nm.

RESULTS

When viewed in cross-section under low magnification (Fig. 2), the "eyespot" region of the mantle flap shows a conspicuous pigmented epithelial layer on the outer surface. Corresponding to the "eyespot" region on the outer surface of the mantle flap, the inner surface epithelium is not pigmented or otherwise modified. Underlying the inner epithelial surface one sees only a few muscles interspersed with loose connective tissue. In the following paragraphs, histological description of the "eyespot" region will focus on the tissues occupying the *outer* surface (where the "eyespot" region is visible), and extending into the mid-interior of the mantle flap (Fig. 2).

Muscle tissue in and near the "eyespot" region of the mantle flap

Beneath the basement membrane of the outer surface ("eyespot") epithelium of the mantle flap are bundles of muscles which course the length of the mantle flap. These muscles are separated into groups of four or more by connective tissue trabeculae which extend at right angles from the basement membrane under the surface epithelium to the interior of the flap (Fig. 2, CT). The bundles of transversely-sectioned outer muscles crowd against the outer epithelial surface of the flap, especially in the areas distally and proximally adjacent to the pigmented epithe-

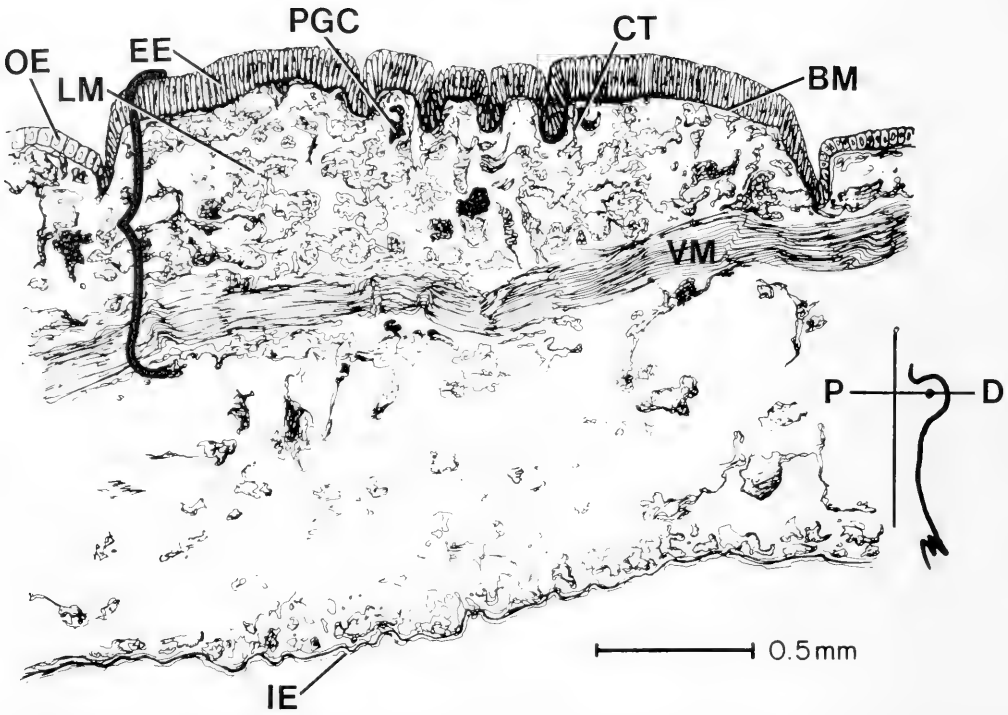

FIG. 2. Semi-diagrammatic cross-section of the *Lampsilis ventricosa* mantle flap "eyespot." Histological boundary of the "eyespot" is indicated by the bracket. Insert: orientation of mantle flap showing plane in which tissue was sectioned. BM, basement membrane; CT, connective tissue trabecula; D, distal; EE, "eyespot" epithelium on outer surface of mantle flap; IE, epithelium on inner surface of mantle flap; LM, transversely sectioned, longitudinally arranged muscle; OE, outer epithelial layer (other than "eyespot" epithelium) of mantle flap; P, proximal; PGC, pigment granule cluster; VM, longitudinally sectioned, vertically arranged muscle.

lial cells of the "eyespot" itself (Fig. 3, LM). Internal to the bundles of transversely sectioned outer muscles are several conspicuous longitudinally-sectioned, inner "vertical" muscles located near the center of the mantle flap interior (Fig. 2, VM). Small nerves course along the inner muscles, thread through the outer muscle bundles, and extend from one muscle to another (Fig. 4A, N). Small nerves often accompany the muscle-separating trabeculae of connective tissue. Occasionally one can see a nerve terminating bluntly in connective tissue (Fig. 4B, N).

Basement membrane and connective tissues in and adjacent to the "eyespot"

A distinct basement membrane occurs between the base of the flap's outer surface epithelium and the underlying connective tissue. In the area adjacent and distal to the "eyespot" epithelium, the basement membrane is greatly thickened, often lying over large, pale-staining, rounded cells (Fig. 5, PC). In the area adjacent and proximal to the "eyespot," the basement membrane is distinct but not thickened (Fig. 3, BM), and is seldom associated with underlying pale, round cells.

Layers of the distinct basement membrane upon which the "eyespot" epithelium stands may separate (1) to form trabeculae which dip between the underlying muscular tissues (described above); or (2) simply to surround small cavities containing pigment granules (Fig. 6A, PG).

Epithelium in and adjacent to the "eyespot"

Proximal to the "eyespot" epithelium, the outer epithelial layer of the mantle flap is comprised of simple, low columnar to cuboidal epithelial cells. Distal to the "eyespot"

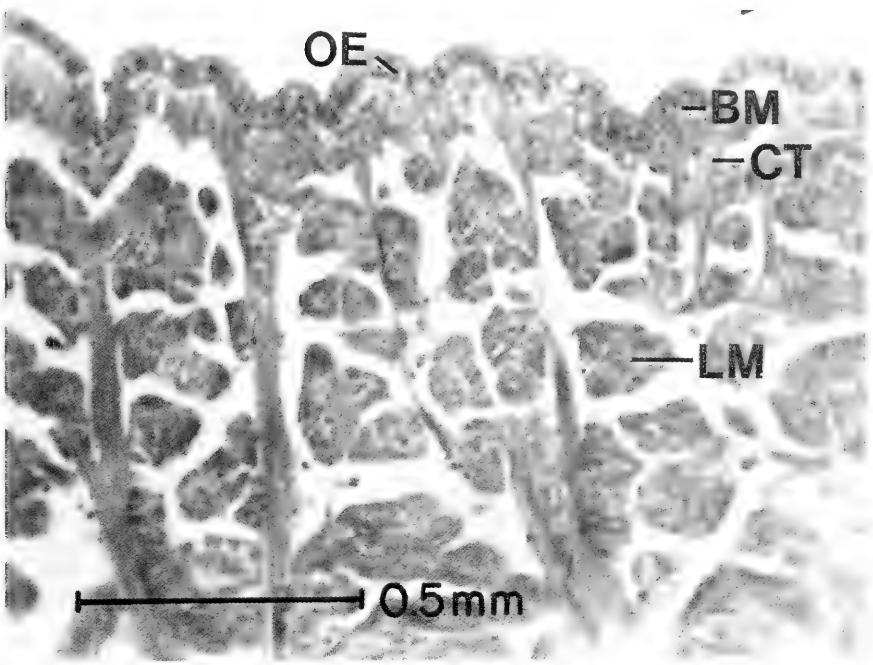

FIG. 3. Photomicrograph of transverse section of outer surface of *Lampsilis ventricosa* mantle flap, immediately adjacent and proximal to the "eyespot" region. Note low columnar to cuboidal epithelium, and absence of pigment granules. BM, basement membrane; CT, connective tissue trabecula; LM, transversely sectioned muscle; OE, outer epithelial layer (other than "eyespot" epithelium) of mantle flap.

epithelium, the outer epithelial layer of the mantle flap contains cells of similar shape and smaller size. The "eyespot" epithelium itself manifests large, very elongate cells which taper to an attenuated base and often appear to be attached to the basement membrane by a threadlike stalk (Fig. 6, EE). The exposed surface of the "eyespot" epithelium shows an evident brush border of microvilli (Fig. 6, 7A). Viewed with the SEM, the "eyespot" epithelial cell surfaces are devoid of cilia (Fig. 7A), and show apical borders with well-developed microvilli (Fig. 7B). Petit *et al.* (1978) did not note comparable features on the mantle of *Amblema* (Unionidae).

It is *only* the great length of the epithelial cells themselves (60–70 μm) which raises the "eyespot" above the surrounding surface of the flap (Fig. 2). "Eyespot" epithelial cells are uninucleate, and the nuclei of neighboring cells form a row, in register, along the base of the cells. Distal to the nucleus, more than a third of most "eyespot" epithelial cells are crowded with brown, granular pigment. In some cells the pigment fills the whole cell above the nucleus. In other "eyespot" epithelial cells the distal cell half is devoid of pigment and tapers to its free surface, sometimes to form what appears to be a pore. In yet other instances, the entire cell distal to the "eyespot" epithelial nucleus may be expanded, without pigment granules.

Unlike the epithelium on the inner surface of the flap, or much of the outer flap surface, the "eyespot" epithelium is thrown into distinct folds (Fig. 2). In the series of transverse sections examined for this study, sections near the outer edges of the "eyespot" typically showed three or four folds, and sections of the center of the "eyespot" displayed as many as eight. Almost invariably, the base of the epithelial folds was associated with one or more clusters of extracellular pigment granules, as described below.

Extracellular pigment granules

In the hundreds of serial sections examined for this study, extracellular pigment granules were found frequently, and were *always*

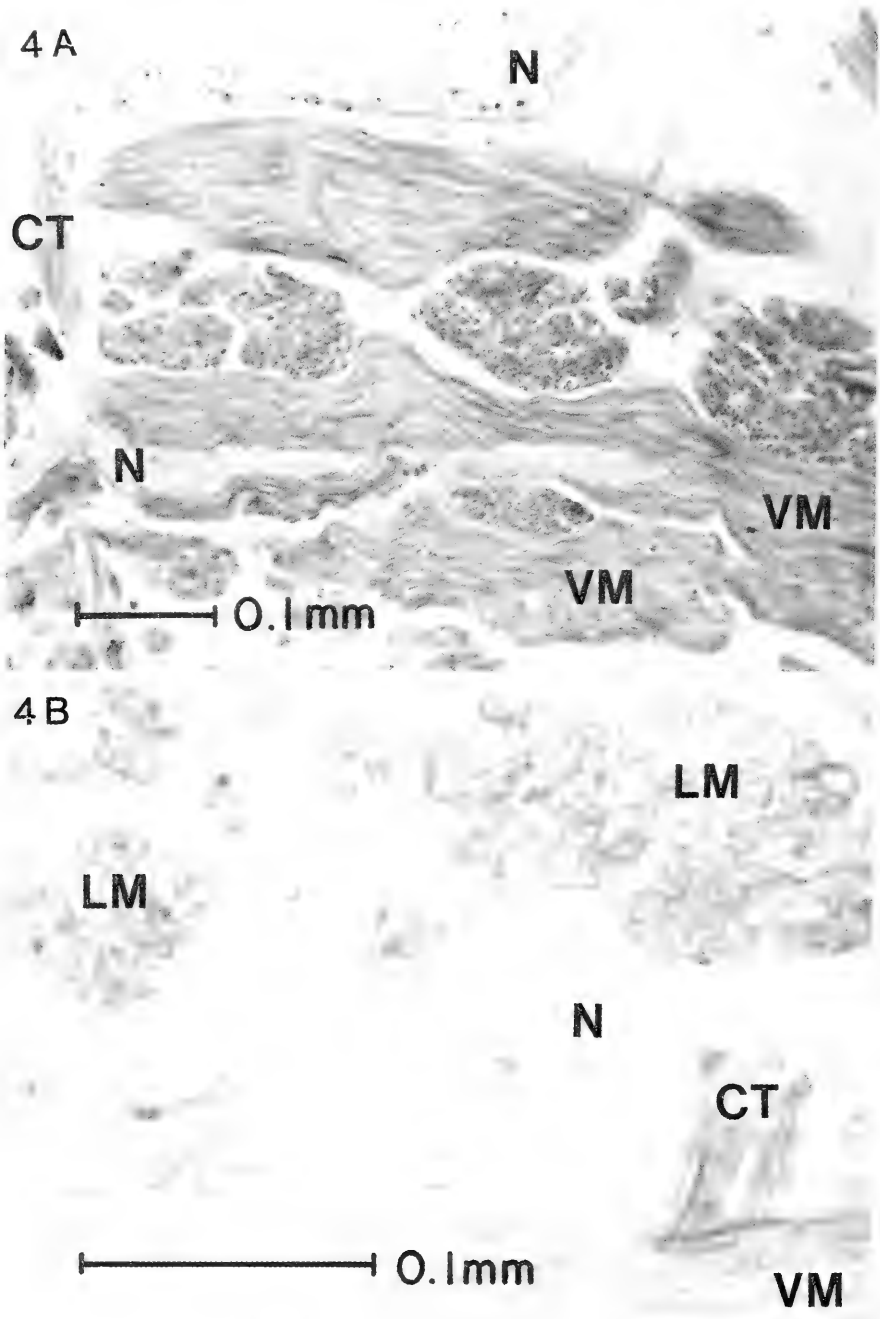

FIG. 4. Nerves associated with "eyespot" region of mantle flap. A, photomicrograph showing nerves associated with longitudinal and vertical muscles and with connective tissue of flap; B, photomicrograph showing nerve abutting connective tissues; CT, connective tissue trabecula; LM, transversely sectioned, longitudinally arranged muscle; N, nerve; VM, longitudinally sectioned, vertically arranged muscle in the mantle flap.

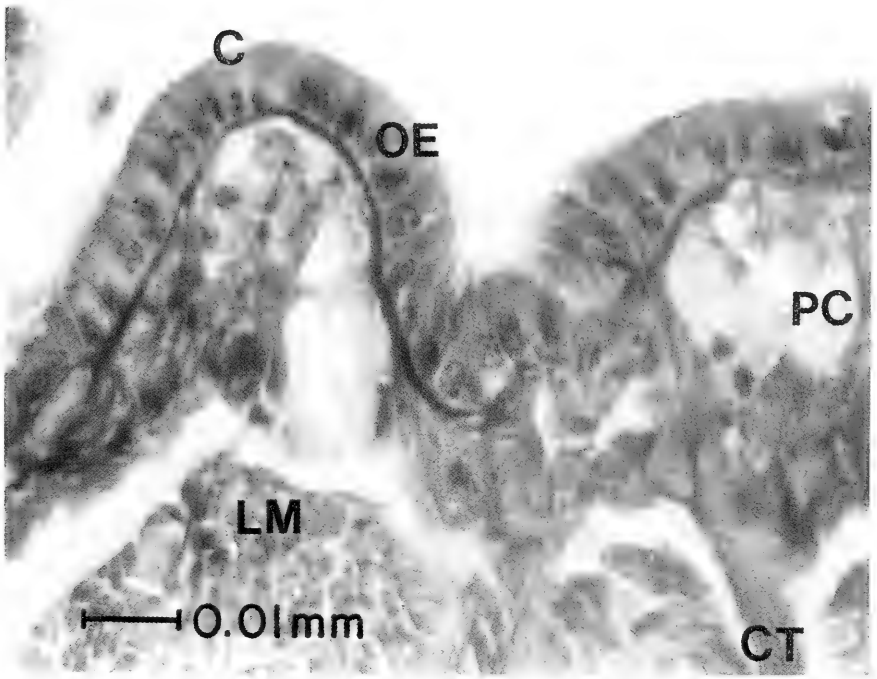

FIG. 5. Photomicrograph of transverse section of region adjacent and distal to mantle flap "eyespot" with ciliated epithelium and underlying pale cells. C, cilia; CT, connective tissue trabecula; LM, transversely sectioned, longitudinally arranged muscle in the mantle flap; OE, outer epithelium (other than "eyespot" epithelium) of the mantle flap; PC, pale cells.

associated with the "eyespot" epithelial cells as follows:

1) At the base of two or three epithelial cells, typically within an epithelial fold, clusters of 3–12 large, round pigment granules occurred.

2) Small clusters of pigment granules were frequently seen surrounded by a fold of basement membrane, adjacent to the base of the epithelium, and often accompanied by a nucleus or other apparent remnants of an epithelial cell (Fig. 6).

3) Pigment granule clusters, encased in a connective tissue membrane, occurred just beneath the epithelial basement membrane (Fig. 6).

4) Large encased clusters, formed from apparent fusion of several smaller clusters, were frequently seen attached to connective tissue trabeculae, the latter extending at right angles from the basement membrane to the interior of the flap (Figs. 2, 6B).

5) The largest pigment clusters, surrounded by connective tissue membranes, were invariably found butted up against the vertical muscles (shown longitudinally sectioned in Figs. 1, 4A) near the center of the flap.

6) Not infrequently, very small longitudinally arranged muscles (shown transversely sectioned) were found enclosed within the pigment clusters (Fig. 6B).

7) Rarely, extracellular pigment granules were seen between or distal to the long axis of the "eyespot" epithelial cells.

We conclude from extended examination of tissues that pigment production by the "eyespot" epithelial cells is followed by packaging of pigment into the conspicuous granules. The pigment granules then appear to undergo extracellular transport and distribution among the tissues in the interior of the mantle flap. Pigment transport seems to occur in the sequence indicated by items 1–5 above.

It also seems likely that pigment transport is effected during the rapid, rhythmic movements of the mantle flaps (Fig. 1). The flap movements are apparently produced by the well-innervated longitudinally-arranged muscles (accounting for the moving "pulse") and

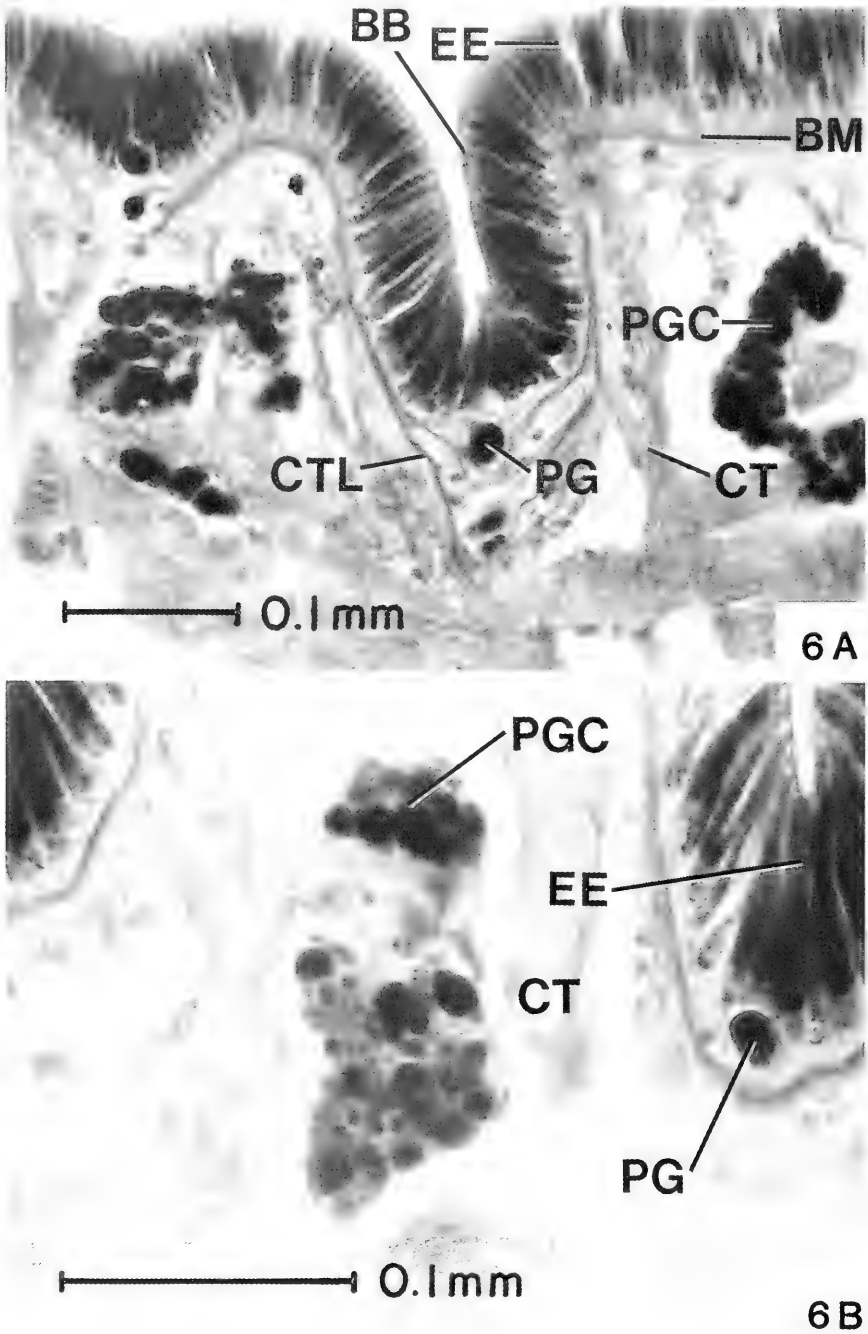

FIG. 6. Photomicrograph of transverse section of *Lampsilis ventricosa* mantle flap "eyespot" region detailing pigmented epithelium, pigment granules and pigment clusters. A, note separation of basement membrane laminae at base of fold of "eyespot" epithelium; B, formation of pigment granula cluster in association with connective tissue trabecula; BB, brush border of epithelium; BM, basement membrane; CT, connective tissue trabecula; CTL, connective tissue lamina, separated from basement membrane; EE, "eyespot" epithelium on outer surface of mantle flap; PG, pigment granule; PGC, pigment granule cluster.

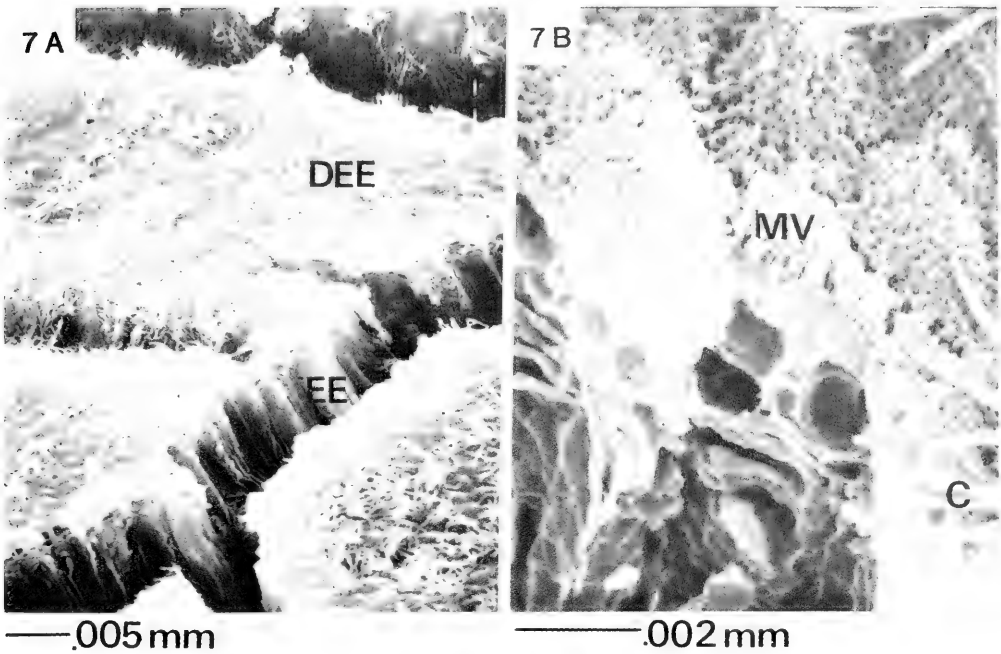

FIG. 7. SEM micrographs of "eyespot" epithelial surface. A, showing columnar structure of epithelial cells visible along a crack which was made during the preparation of the tissue; B, detail showing distinct microvilli on surface of epithelial cells; C, cilia; DEE, distal surface of "eyespot" epithelial cell; EE, "eyespot" epithelium on outer surface of mantle flap; MV, microvilli.

by the vertically-arranged muscles (accounting for the downward and outward jerk of the flaps at the end of each pulse, Fig. 1e). Directional movement of pigment granule clusters to the interior of the flap could be aided by the connective tissue trabeculae in the "eyespot" region of the flap.

SUMMARY AND DISCUSSION

From evidence examined for the present study, the "eyespot" of the *Lampsilis* mantle flap apparently does function in pigment production and transport. It is also clear that the "eyespot" does *not* manifest the histological characteristics of a specialized sensor found elsewhere in freshwater bivalves: (1) there is no conspicuous thinning of the basement membrane underlying the "eyespot" epithelium; (2) there is no evidence of extensive innervation of the "eyespot" epithelium. Both of these phenomena are characteristic of the osphradial epithelia in certain freshwater bivalves (Kraemer, 1981). One might argue that the microvillar surface on the "eyespot" epithelial cells constitutes evidence of a sensory function, as certainly microvilli are components of rhabdomes which constitute the photoreceptor organelles in many mollusks. However, in this case we do not think so, for the foregoing reasons and because the microvilli do not have the appearance of rhabdomeric membranes. Of course, neither the techniques of TEM or histochemistry were used in this study. Hence, it is possible to argue that TEM might turn up rhabdomes, or that histochemical experiments might delineate the kind of secretory activity occurring in the "eyespot" epithelial cells.

However, in addition to the evidence presented here, extensive studies of the behavior of the mantle flaps (Kraemer, 1969, 1970) do not indicate a sensory function for the "eyespots." To the contrary, in the present study, the "eyespot" epithelium and the complex, extensively innervated *muscle* components of the "eyespot" region of the mantle flap, are more likely to be effectors than sensors. Indeed, an effector function comports well with the whole function of mantle flap

movements in the spawning behavior complex of *Lampsilis* (Fig. 1).

As mentioned above, earlier experimental evidence (Welsh, 1933; Kraemer, 1970) did indicate that in certain but not all species of *Lampsilis* the rate of mantle flap movement may be affected by changes in light intensity. The present study was undertaken on one of the light-sensitive species, *Lampsilis ventricosa,* in part to determine whether mantle flap "eyespots" are sensors. The results indicate that *L. ventricosa* "eyespots" are probably not sense organs.

How, then, does one account for the apparent photic response of the mantle flaps mentioned above? Specialized photoreceptors have not been identified in fresh-water mussels (Unionidae), despite the siphons of many species being "light" or more accurately "shadow" sensitive (skioptic). In at least one gastropod, *Lymnaea,* Stoll (1972) demonstrated a non-ocular light sensor. Kennedy (1960) demonstrated photoreceptive activity for the pallial nerve of the marine bivalve *Spisula,* although he was unable to identify pertinent photoreceptor pigments there. Conly-Dillon (1965), in his work on the spectral sensitivity of *Pecten,* notes that his findings do not exclude the possibility that there may be light-sensitive structures within the bivalve nervous system itself. The present study indicates that for *Lampsilis,* too, photosensors will probably have to be sought elsewhere than the "eyespot" of the mantle flap.

As noted earlier (Kraemer, 1970: 241), "because no photoreceptive function has yet been demonstrated for the 'eyespot,' the term is inappropriate." "Eyespot" was used throughout that study, however, because the term was established in the literature, and because many lampsilids possess numerous other (i.e., non-raised) pigment spots. The present study provides abundant histological evidence of a "non-eye" function for the "eyespot."

Perhaps *Lampsilis* "eyespots" could more appropriately be termed "pigment effectors" or "pigment effector" spots. There is compelling evidence from this study of (1) the production of pigment granules by the "eyespot" epithelial cells; and (2) intercellular transport of pigment granule clusters from the base of the epithelial cells to the interior of the flap. Is this phenomenon a direct or indirect effect of the pulsing movements of the mantle flaps? Whatever the answer, it seems likely that a function of the "eyespot" and associated muscles, nerves, and connective tissue trabeculae described here in the mantle flaps of *Lampsilis* is the patterned distribution of pigment within the tissues of the mantle flaps.

While the pulsing movements of *Lampsilis* are confined to specific regions of the mature female mantle, spontaneous rhythmic movements of the bivalve mantle have been known for years (e.g., Redfield, 1917; Barnes, 1955; Bullock & Horridge, 1965). In the context of the present study, it seems logical to hypothesize that one effect of either localized mantle movements (e.g., in *Lampsilis* mantle flaps) or of more generalized, spontaneous mantle movements might be the distribution of pigment in characteristic patterns through the mantle tissue.

ACKNOWLEDGMENTS

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A NEW MUSSEL (BIVALVIA, MYTILIDAE) FROM HYDROTHERMAL VENTS IN THE GALAPAGOS RIFT ZONE

Vida Carmen Kenk[1] & Barry R. Wilson[2]

ABSTRACT

A new subfamily, Bathymodiolinae, and new genus and species, *Bathymodiolus thermophilus,* are described from material collected by the 1977 and 1979 expeditions to the hydrothermal vents in the Galapagos Rift Zone. This large modioliform mussel has very unusual anatomy, exhibiting extreme mantle fusion which restricts the incurrent aperture to a short byssal-pedal gape in the ventral midregion. The gills lack food grooves ventrally; the free edges of the gills fit axial ridges on the visceral mass and mantle lobes, thereby isolating the dorsal excurrent chambers from the rest of the mantle cavity. The gut is short and different from that of other mytilids in lacking a recurrent loop, the stomach is simple and lacks a deep sorting caecum, dorsal hood and left pouch, and there are but three pairs of digestive ducts opening into the stomach. The auricles of the heart have a broad connection to the longitudinal vein laterally between the branches of the divided posterior retractor muscles in addition to the normal connection anterior to these muscles. The kidney is very small.

Feeding is discussed in light of high densities of chemoautotrophic sulphur-oxidizing bacteria in the environment and the possibility of a symbiotic relationship between the mussels and bacteria.

INTRODUCTION

The discovery in 1977 of biological communities surrounding hydrothermal vents in the Galapagos Rift Zone at latitude 00.47°N (Corliss & Ballard, 1977; Lonsdale, 1977; Corliss *et al.,* 1979; Enright *et al.,* 1981; Edmond, 1982) led to the Galapagos Rift Biology Expedition in 1979 (Ballard & Grassle, 1979; Galápagos Biology Expedition Participants, 1979). Since the initial discovery, additional submarine hydrothermal communities have been described at 21°N (Rise Project Group, 1980) and 11–13°N (Desbruyères *et al.,* 1982). The majority of specimens collected on these expeditions are unusual organisms differing from known relatives at generic or higher levels (Newman, 1979; Williams, 1980; Burreson, 1981; Fretter *et al.,* 1981; Jones, 1981; Krantz, 1981; McLean, 1981; Desbruyères & Laubier,1982; Williams & Chace, 1982).

One of the most abundant and conspicuous organisms collected at some of these hydrothermal vents is a large modioliform mussel. Although the shell form is like that of the mytilid genus *Modiolus,* anatomical study of preserved specimens has revealed many distinctive features. This animal is described here as a new genus and species and a new subfamily is erected for it. The mussels were abundant at several vent sites in the Galapagos Rift Zone. The species is also present, though apparently less abundantly, at the 11–13°N site, but was not collected or observed at the vents at 21°N.

MATERIALS AND METHODS

All of the specimens examined in this study were from the Galapagos Rift Zone vents, viz.:

a) 79 specimens preserved in ethanol (size range 0.3 to 14.38 cm in length) collected during the 1977 expedition at Clambake 1, Oyster Bed, and Garden of Eden vent sites (dive stations 713, 723, 727, 728 and 733) and forwarded to the authors by Dr. Jack Corliss.

b) 11 specimens preserved in ethanol (size range 7.9 to 16.2 cm) collected during the 1979 expedition at Rose Garden and Mussel Bed vent sites (dive stations 879, 880, 894 and 896) and forwarded to the authors by Dr. Fred Grassle, and 153 juveniles

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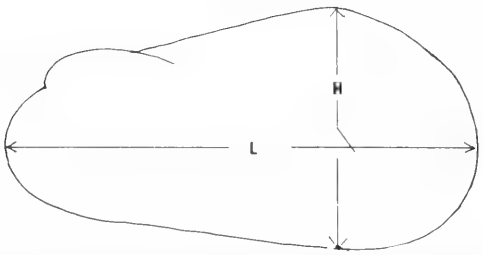

FIG. 1. Diagram indicating the measurement taken for length and height. Width is greatest dimension through both valves.

(size range 0.3 to 9.8 mm) collected from washing of mussels from dives 880 and 884, loaned by Dr. Howard Sanders.

c) 75 dried shells deposited at the U.S.N.M., lot registration number 81331600-3380-P30000.

Seven preserved specimens from sample a) were dissected under a binocular microscope. Anatomical drawings were done freehand; shells were drawn with the aid of a camera lucida. Measurements taken are illustrated in Fig. 1.

The holotype and a large series of paratypes are lodged at the U.S.N.M. Paratypes are also lodged at the following museums: California Academy of Sciences, San Francisco; Museum of Comparative Zoology, Harvard University; Los Angeles County Museum; British Museum of Natural History, London; Museum National d'Histoire Naturelle, Paris; Museum of Victoria, Melbourne; Zoologisk Museum, University of Copenhagen; Academy of Natural Sciences, Philadelphia; Scripps Institute of Oceanography.

KEY TO ABBREVIATIONS IN FIGURES

AA, aa	anterior adductor muscle
a art	anterior artery
aff v	afferent vein
a l	ascending lamella
an	anus
AR, ar	anterior retractor muscle
au	auricle
b	byssus
bg	byssal gland
b-p g	byssal-pedal gape
b s	branchial septum
cb	connecting bar
d c d s	dorsal cul de sac
d d	digestive duct
d l	descending lamella
eff v	efferent vein
ex s	excurrent siphon
f	foot
f g	food groove
g ax	gill axis
g s	gastric shield position
gen ap	genital aperture
gen d	anterior genital duct
i	intestine
i ch	incurrent chamber
i d	internal diaphragm
i dem	inner demibranch
i ex ch	inner excurrent chamber
i g	intestinal groove
i m f	inner mantle fold
k	kidney
l p	labial palp
LPM	labial palp muscle
l v	longitudinal vein
m t	major typhlosole
mth	mouth
o	oesophagus
o dem	outer demibranch
o ex ch	outer excurrent chamber
o g	oral groove
o m f	outer mantle fold
p	papilla
PA, pa	posterior adductor muscle
pbr(a), APR	posterior byssal retractor muscle (anterior)
pbr(p), PPR	posterior byssal retractor muscle (posterior)
per	pericardium
PL	pallial line
pm	pallial muscles
ppr	posterior pedal retractor muscle
r	rectum
r ap	renal aperture
rg	ridge for gill attachment
r p c	reno-pericardial channel
s	septum of principal filament
SR, sr	siphonal retractor muscles
st	stomach
st a	stomach anterior chamber
st p	stomach posterior chamber
s v-au p	secondary venous-auricular passage
v	ventricle
v s m	valvular siphonal membrane

TAXONOMY

Family MYTILIDAE

BATHYMODIOLINAE
Kenk & Wilson, subfam. nov.

Type-genus: *Bathymodiolus*
Kenk & Wilson, gen. nov.

BATHYMODIOLUS
Kenk & Wilson gen. nov.[3]

Type-species: *Bathymodiolus thermophilus*
Kenk & Wilson, sp. nov.[3]

Diagnosis of the subfamily and genus:

Shell smooth, modioliform, with subterminal umbones; hinge edentulous; periostracum hirsute; posterior retractor muscle divided, retractor scars separate; pallial muscles including siphonal retractors strong; excurrent siphon short, extensible, with internal diaphragm; inner folds of the mantle lobes enlarged, extensible postero-ventrally, fused in the mid-line antero-ventrally and postero-ventrally; auricles enlarged, fused posteriorly; gills heterorhabdic, eleutherorhabdic, with short, fleshy filaments, lacking food grooves at ventral edges of demibranchs; with tubular connections present posteriorly between free edges of ascending lamellae and gill axes; labial palps small; stomach without a deep sorting caecum or left pouch; intestine short, lacking a recurrent loop.

Bathymodiolus thermophilus
Kenk & Wilson, sp. nov.

Type-locality: lat. 00°47′.89″N; long. 086°09′.21″W. Depth 2495 m. R/V *Alvin* Dive 879, at "Mussel Bed" geothermal vent, Galapagos Rift. *Holotype* (Fig. 2.): USNM 803661, preserved in 70% ethanol. Collected 20 January 1979 by Ellis and Ballard on *Alvin*. Measurements: length 14.95 cm, height 6.30 cm, width 5.83 cm (Fig. 2).

[3]In a paper on a similar or identical mussel from the East Pacific Rise at 11°–13°N, Le Pennec *et al.* (1984: 70) introduced *Bathymodiolus* as a *nomen nudum*. Also, the generic name has been used repeatedly in a popular article by Laubier & Desbruyères (1984); the manuscript species name *B. thermophilis* [sic] appeared on p.1510. Information in the former paper unfortunately was not considered by the authors of the present paper. ED.

DESCRIPTION

Shell morphology. Modioliform, solid, elliptical in juveniles and subadults, arcuate in old specimens, equivalve. Anterior end rounded; dorsal margin slightly convex; postero-dorsal corner rounded in adults, angular in juveniles; posterior end rounded; ventral margin nearly straight in specimens less than 10 cm length, slightly concave in larger specimens (Fig. 3). Umbones subterminal, prosogyrate.

External surface smooth, sculpture lacking, dull white beneath periostracum. Interior white, nacreous. Periostracum straw-yellow, yellow-brown antero-ventrally, often stained dark brown in large specimens. In young specimens less than 0.8 cm in length periostracum smooth, larger juveniles develop periostracal hairs (of byssal origin, see Bottjer & Carter, 1980; Ockelmann, 1983) on posterior slope; specimens more than 2 cm long have hairs on most of shell exterior; hairs broad, flat. In addition to their own hairs many shells bear byssal end-plates of other mussels which had been attached to them, distinguishable by oval shape and central slender strand.

Ligament opisthodetic, parivincular, strong, extending most of length of dorsal margin; resilial ridge (as defined by Soot-Ryen, 1955: 7) deep, chalky and rather soft, not pitted; sub-ligamental shell ridge strong and angular, with deep groove between it and ligament anteriorly, becoming obsolete below midpoint of ligament. Hinge edentulous except for strong backward pointing projection of anterior hinge margin beneath anterior end of ligament; post-ligamental denticles lacking.

Muscle scars (Fig. 4). Anterior adductor muscle scar half-moon shaped, located below umbo, distant from anterior margin (Fig. 4); young specimens may show small round scar of labial palp support muscles just behind anterior adductor; anterior byssal-pedal retractor scar oval, located high within umbonal cavity behind umbo; posterior adductor scar rounded-rectangular; posterior byssal-pedal retractor muscles form two separate scars with large gap between them, anterior one elongate-elliptical, located high, and close to posterior end of ligament, second one elliptical and located antero-dorsally to but contiguous with posterior adductor scar to form a joint comma-shaped scar. Pallial line distinct, extending ventrally from anterior adductor to posterior adductor, curving upwards to form

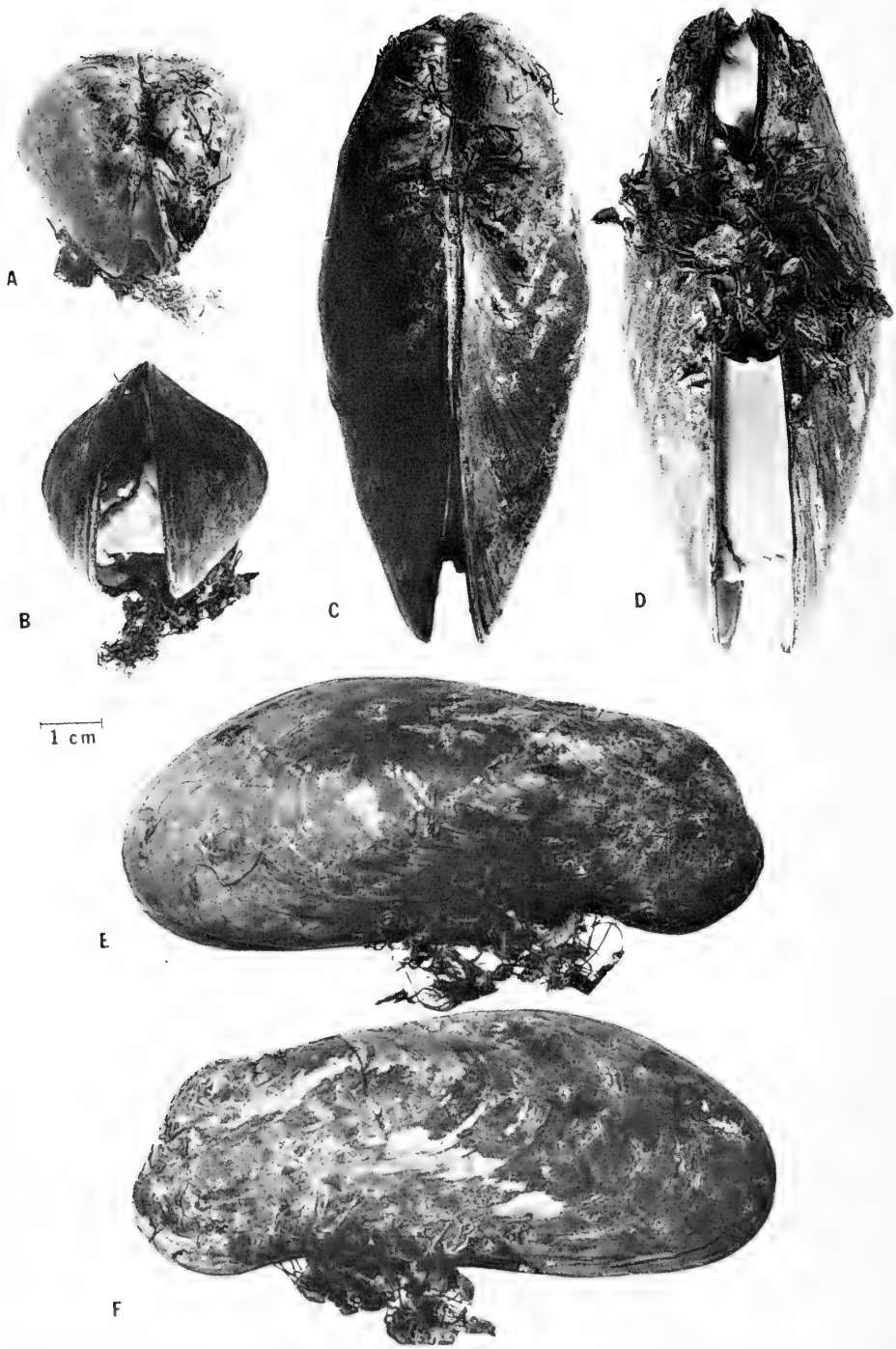

FIG. 2. *Bathymodiolus thermophilus,* holotype, USNM 803661. A, anterior view; B, posterior view; C, dorsal view; D, ventral view; E, lateral view, right valve; F, lateral view, left valve.

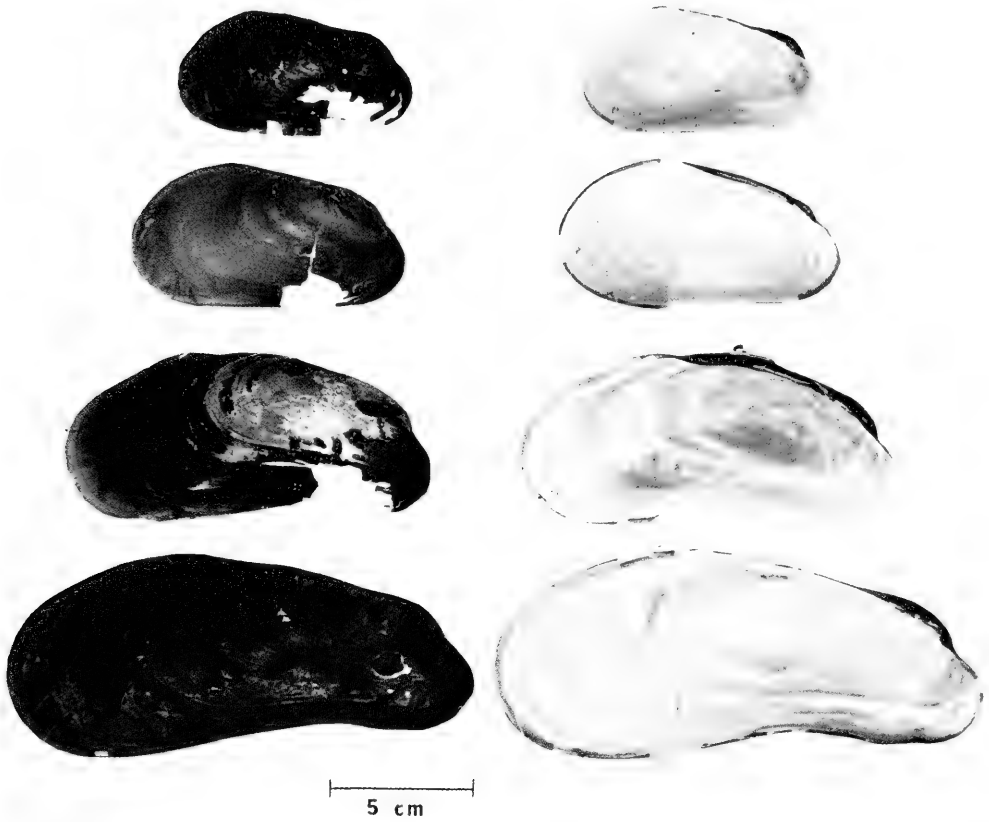

FIG. 3. Growth series of shells illustrating change in form (paratypes, USNM 813316).

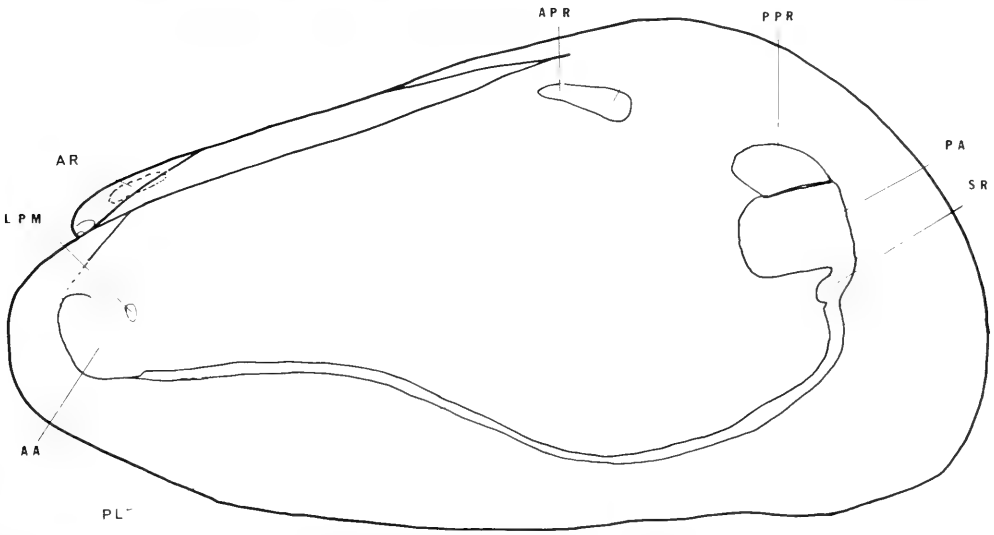

FIG. 4. Diagram of shell illustrating muscle scars.

concavity in byssal region about ⅓ of the distance from anterior end; small siphonal retractor scar present adjacent to posterior adductor scar at posterior end of pallial line.

Measurements. Rhoads *et al.* (1982) reported the largest shell in their series as 18.4 cm long. Maximum shell length observed in this study 16.3 cm. Length, height and width proportions of preserved specimens (paratype series, N = 79):

mean height/length 0.568; range 0.514 to 0.604
mean width/length 0.362; range 0.323 to 0.438

Anatomy

Musculature (Fig. 5). Main features of musculature evident from previous description of muscle scars. Posterior byssal retractors in two roughly equal main bundles arising together at base of byssus but diverge and attach separately to shell. Posterior pedal retractors thick, arising from base of foot anterior to origin of posterior byssal retractors, passing dorsally lateral to anterior retractors and inserting dorsally on both inner and outer sides of most anterior bundles of posterior retractors.

Pallial muscles unusually well developed; strong siphonal retractors present, formed of amalgamated strands originating in inner mantle folds in region of excurrent siphon. Slender strand of anterior pedal retractor muscle extends anteriorly and attaches to shell behind anterior adductor, providing support for labial palps. Posterior adductor large and divided into "quick" and "catch" parts; anterior adductor elongate, half-moon shaped.

Foot and byssus (Fig. 5). Foot thick, flattened, terminally swollen; byssal groove running along ventral surface almost to tip. Byssus profuse, usually emerging as separate strands from orifice, strands thick and strong; byssal gland yellow, extends down centre of foot behind groove, without extension dorsal to anterior retractor muscles.

Mantle. Mantle lobes thin dorsally but become unusually thickened and muscular near posterior and ventral edges (Fig. 6). Free edges of mantle lobes have three folds as in other mytilids (Yonge, 1957); inner folds fuse

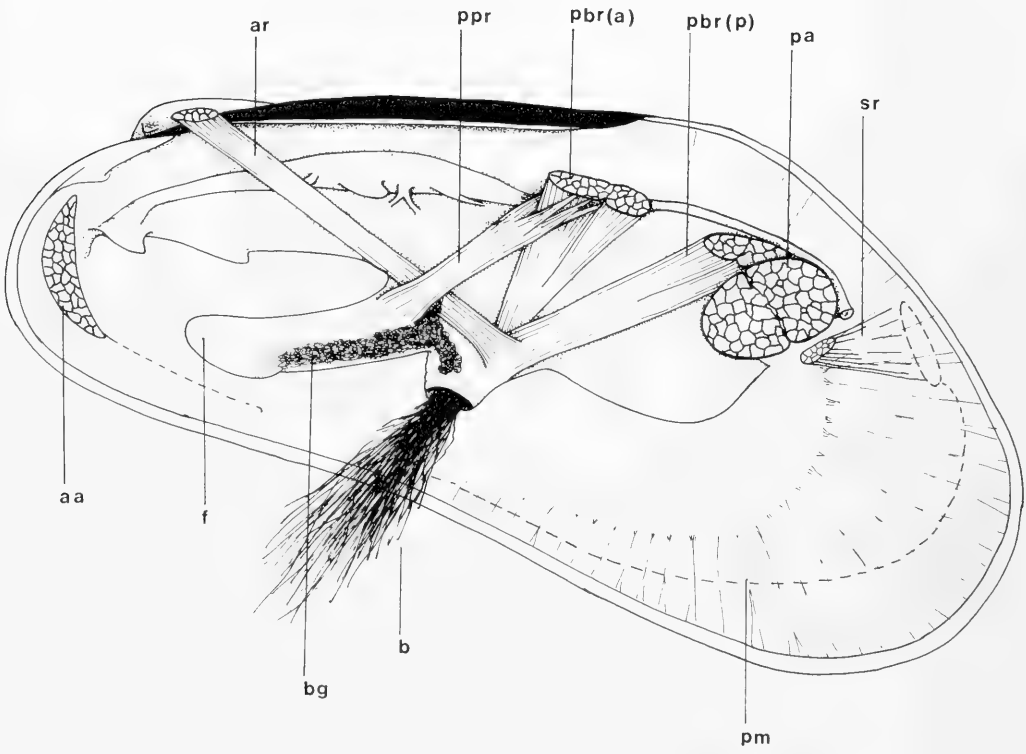

FIG. 5. Musculature; left valve, mantle lobe and ctenidia removed.

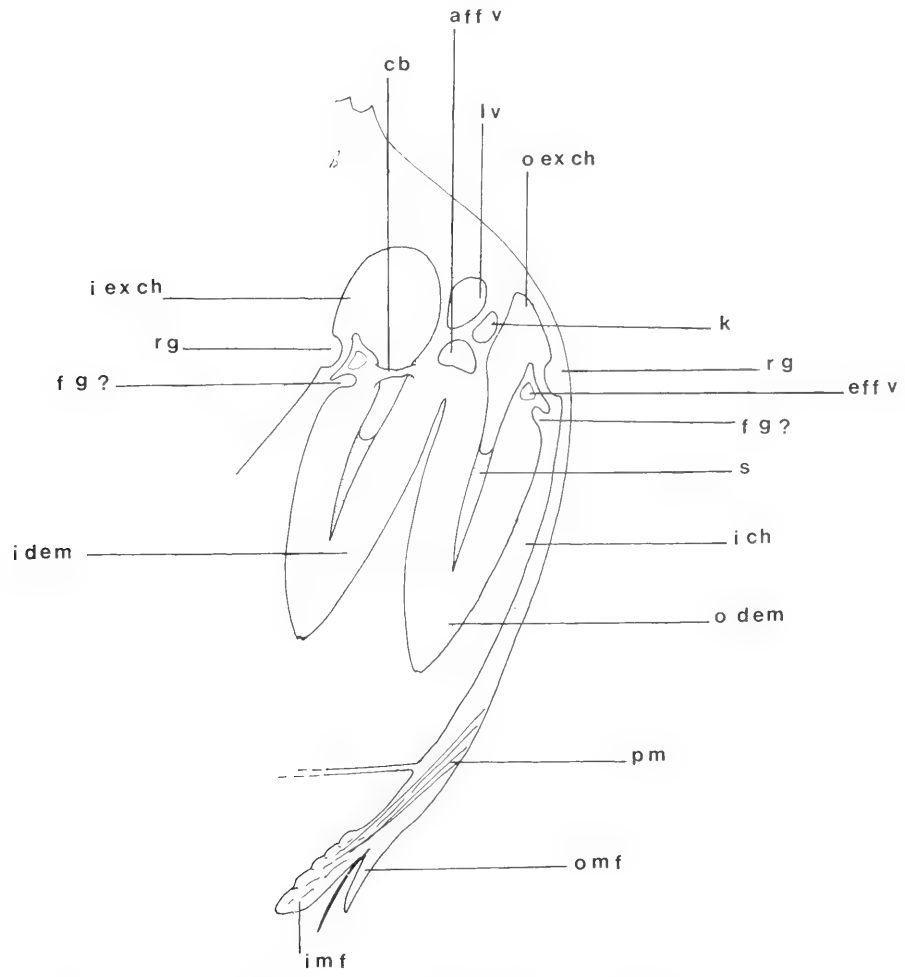

FIG. 6. Transverse section of the right gills, through primary filaments and with one of several connecting bars between the free edge of the inner ascending lamella and the gill axis.

mesially along the entire postero-dorsal slope and ventrally enclosing the mantle cavity to an unusual extent.

Excurrent siphon formed posteriorly between inner folds, capable of moderate extension but shown in retracted position in Fig. 7; thin internal diaphragm with narrow horizontal aperture partly occludes inner end of excurrent siphon.

Fusion of inner mantle folds immediately below excurrent siphon forms horizontal shelf, the branchial septum, with an inner part reaching forward to ventral side of posterior adductor (Fig. 7). Branchial septum separates incurrent and excurrent chambers posteriorly; posterior ends of gill axes attach to its ventral surface. Ventral development of branchial septum forms an oblique, transverse partition, the valvular siphonal membrane (terminology of Yonge, 1955), joining left and right lobes postero-ventrally thus enclosing incurrent mantle cavity in that region; inner folds also fused in mid-line antero-ventrally; incurrent aperture thus confined to a short ventral pedal-byssal gape (Figs. 7, 8).

Rim of gape bordered by another muscular fold which may regulate aperture size by muscular contraction. A small papilla present at posterior end of gape (Fig. 8).

Free edges of inner folds form wide extensible frills postero-ventrally shown partly extended in Fig. 7. Fig. 12 shows them fully

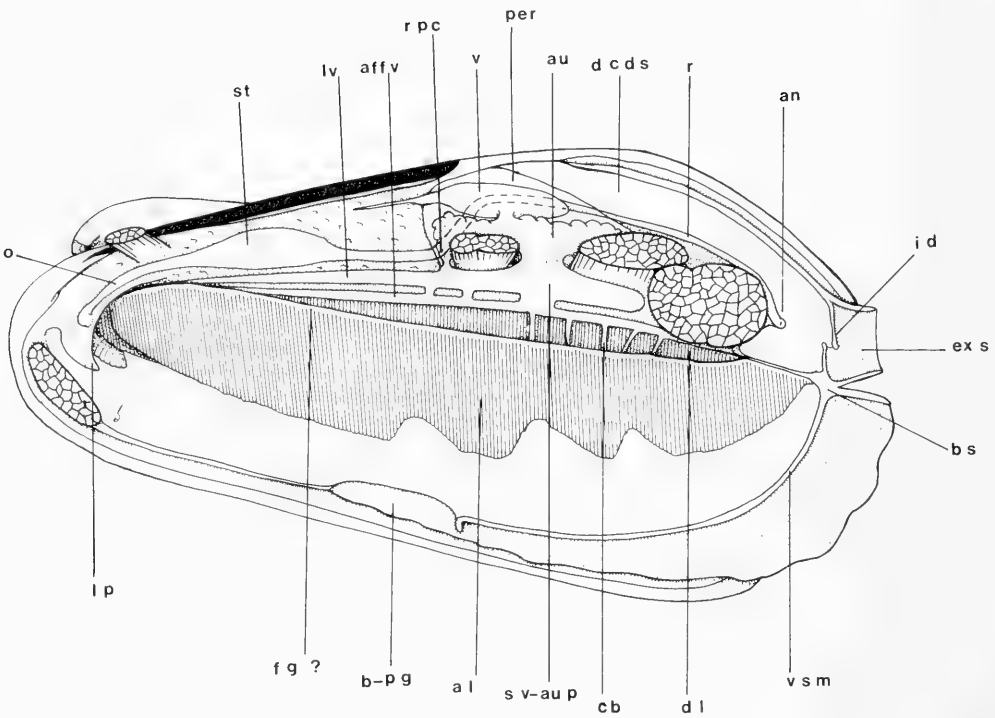

FIG. 7. Vascular and alimentary systems and siphonal structure; left valve and mantle lobe removed; fused inner mantle folds cut down the mid-line (in sagittal section). Outer demibranch deformed.

extended in life. This structure forms functional, though not tubular, incurrent siphon by apposition of edges ventrally. Excurrent and incurrent siphons separate as in *Botula* (Wilson & Tate, 1984).

Mantle cavity. Mantle cavity divided by ctenidia laterally and branchial septum posteriorly into ventral incurrent and dorsal excurrent chambers. Edges of ascending lamellae flanged and fitted to muscular longitudinal ridges on surfaces of mantle lobes and visceral mass thus completely separating incurrent and excurrent chambers in life (Fig. 6). In this way four tunnel-like, longitudinal excurrent chambers are formed along roof of mantle cavity, two on each side; chambers meet posteriorly at entrance of excurrent siphon above branchial septum.

A *cul de sac* of excurrent chamber passes posterodorsally above rectum and posterior adductor, reaching forward as far as posterior wall of pericardium (Fig. 7); thin pericardial wall separates pericardial fluids from sea water in excurrent mantle cavity.

Ctenidia. Paired ctenidia consist of inner and outer demibranchs each with descending and ascending lamellae forming W-shaped gill typical of mytilids (Fig. 6); demibranchs approximately equal-sized, inner demibranchs extend slightly further anteriorly, outer demibranchs slightly deeper; both demibranchs end abruptly anteriorly (Fig. 9). Ctenidia filibranchiate, heterorhabdic and eleutherorhabdic; interlamellar junctions lacking but every third to seventh filament is "principal filament" (see type B(1b) of Atkins, 1937, text fig. 4) with septum or "baffle" rising to more than half the height of gill (Fig. 6). Demibranchs rather short, filaments wide and fleshy; ventral edges lack food grooves though minute indentations present. Deep folds on outer surface of ascending lamellae just below free edges might function as food grooves; anteriorly folds continue in a loop as grooves on mantle wall and terminate in deep oral groove between labial palps leading into mouth.

Inter-lamellar tissue junctions lacking; series of about four large tubular connections present in posterior area between free edges and gill axes (Figs. 6, 10) appearing to connect efferent veins with either afferent or

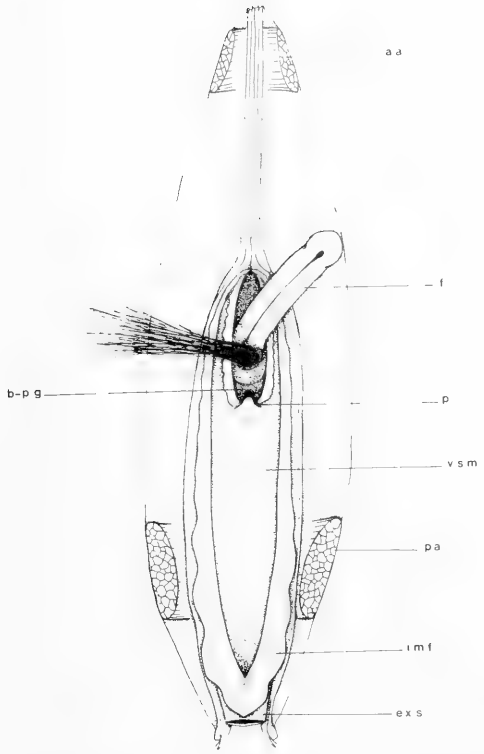

Fig. 8. External view from the ventral side (shell valves removed) showing extensive mid-line fusion of the inner mantle folds, the extended valvular siphonal membrane of the branchial septum and the small byssal-pedal gape.

longitudinal veins. Filaments sometimes thickened, shortened, deformed, particularly posteriorly, possibly due to activities of polychaetes.

Labial palps. Paired labial palps short, broad, flat, triangular; usually strongly plicate on their inner surfaces (Fig. 9); outer palps larger than inner palps and placed farther posteriorly, markedly so in some large specimens (e.g. Fig. 5). In very large specimens palps sometimes smooth, lacking plications on either surface.

Alimentary system. Digestive tract short, more or less straight, direct. Mouth transverse, slit-like; esophagus enters anterior end of stomach which lies superficially in visceral mass below ligament.

Stomach (Fig. 11; nomenclature of parts follows Reid, 1965) small elongate, divided into anterior and posterior chambers; backward-pointing pouch on left dorsal side of anterior chamber; posterior chamber swollen on right side, left pouch lacking; gastric shield small, located in normal position on anterodorsal wall on left side of posterior chamber. Three pairs of digestive ducts enter stomach laterally (Fig. 11) one pair on left and right sides of anterior chamber and two pairs on left and right side of posterior chamber; on right side two ducts open into posterior chamber close together; on left side two openings spaced apart with posterior one much the larger. Major typhlosole straight except for an elbow close to its entry into intestine, passes along floor of posterior chamber in mid-line and terminates in centre of anterior chamber; minor typhlosole branches on right side at elbow and passes up right side of posterior chamber (not traced further). Intestinal groove originates in opening of posterior digestive duct of left side and passes forward along floor of stomach to left of major typhlosole, passes around tip of that typhlosole in the anterior chamber, and returns down right side to enter intestine posteriorly. Surface of major typhlosole transversely plicate. Minor intestinal groove runs along anterior side of minor typhlosole on right side of posterior chamber but its origin not located. Hood groove not observed but this may have been a consequence of the poor preservation.

Style sac and intestine conjoined; crystalline style present in some preserved specimens. Intestine leaves posterior end of stomach and traverses short distance posteriorly down mid-line; rectum turns upwards to enter pericardium and ventricle from below; thence passes posteriorly through ventricle and directly down the mid-line to anus on posterior side of posterior adductor muscle; recurrent loop of intestine lacking.

Vascular system. Pericardium in usual position dorsally between posterior retractor muscles (Figs. 7, 12); broad reno-pericardial canal on each side passes laterally around most anterior of posterior retractor muscles, then ventrally to gill axis; canals superficial and easily seen when shell removed. Heart three-chambered (Fig. 12); medial ventricle thick, muscular, rhomboidal, traversed for much of its length by rectum; anteriorly ventral surface of ventricle fused to floor of pericardium. Paired anterior arteries arise from aortic bulb and pass forward over visceral mass; large ventral artery leads downwards through pericardial floor.

Two auricles unusually large, fused together posteriorly (Fig. 12); each has an anterior arm

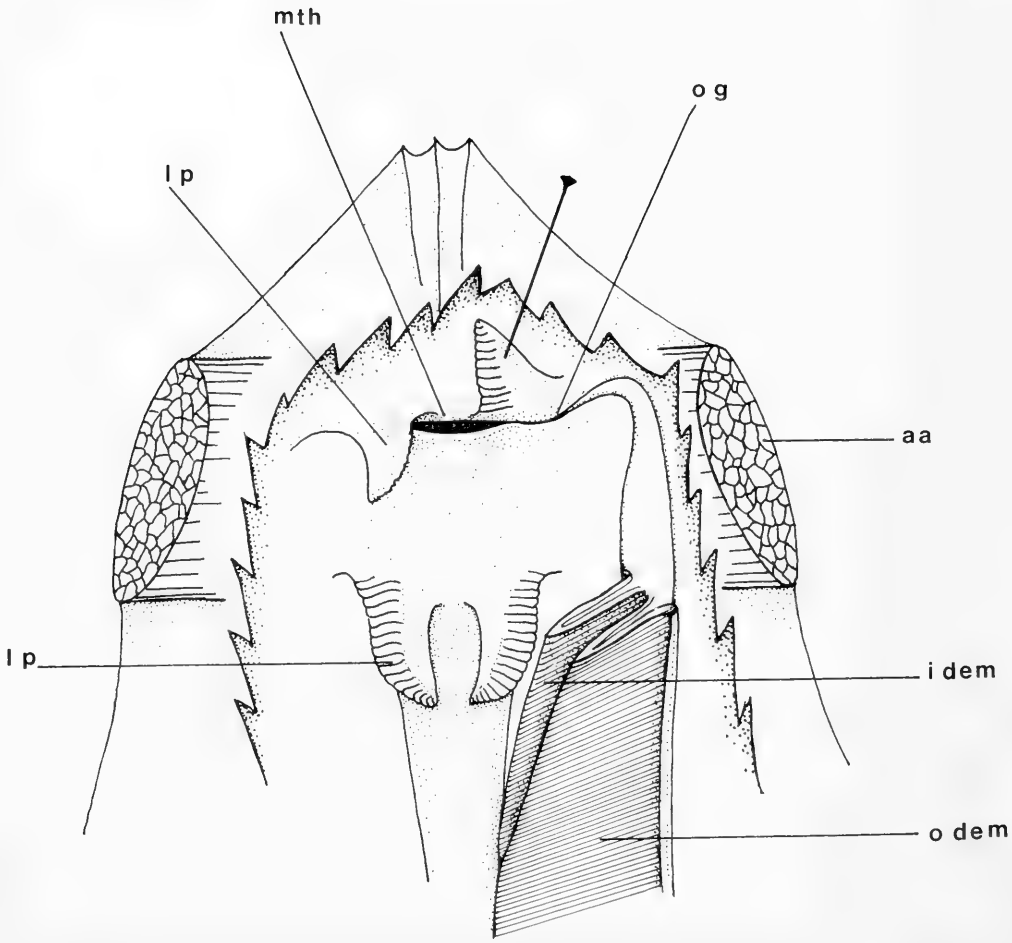

FIG. 9. Ventral view of the labial palps with the mantle and anterior adductor cut away; the inner palp of the left side is pinned back to expose the oral grooves.

curving laterally and downward within reno-pericardial canal but connection via oblique vein to longitudinal vein (see White, 1937, for description in *Mytilus*) not observed. Each auricle has wide latero-ventral flap protruding between bundles of posterior retractor muscles, with a wide foramen opening directly into longitudinal vein; valvular mechanism in that opening appears to be lacking.

Efferent veins in free edges of demibranchs, and afferent and longitudinal veins immediately above gill axis readily observable in hand-cut sections; longitudinal veins large and spacious in zone between reno-pericardial canal and posterior adductor. In dissections it appeared that there are several foramina between afferent and longitudinal veins in this zone; largest of these located directly below wide space connecting longitudinal veins and latero-ventral flaps of auricles.

Plicate membranes lacking (see White, 1937 for details of these structures in *Mytilus* between visceral mass and gill axes and mantle lobes and gill axes).

Tubular junctions between free edges of demibranches and gill axes already noted; whether these are vascular connections needs to be determined.

Nervous system. Paired ganglia situated in normal positions, cerebral ganglia between anterior retractor muscles near attachment of inner labial palps; paired pedal ganglia medially just above region where anterior and posterior retractor muscles meet foot; paired visceral ganglia on ventral surface of posterior adductor muscle.

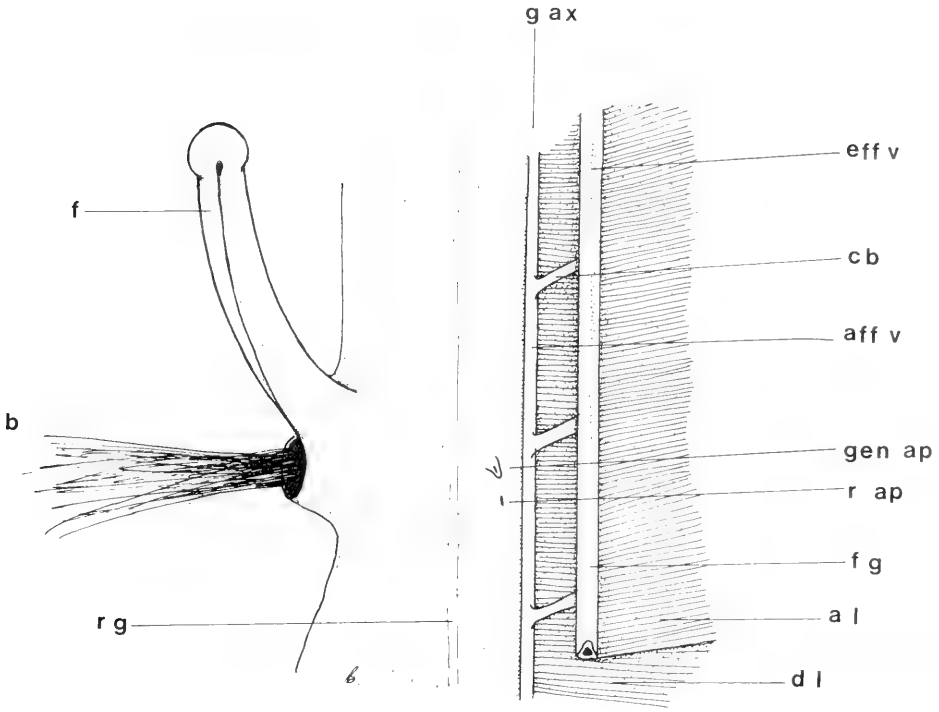

FIG. 10. Location of the genital and renal apertures, and showing the connecting bars between the gill axis and the free edge of the ascending lamella of the inner demibranch.

Reproductive system. In all large specimens examined gonad tubules were in regressed condition; confined to mesosoma and visceral mass over and behind digestive gland and below pericardium, lacking in mantle lobes. Genital apertures located at tips of very small conical papillae in roof of inner excurrent chambers at a point adjacent to byssus (Fig. 10).

Excretory system. In transverse hand-cut section taken through body below pericardium, a small axial duct closely associated with longitudinal vein was tentatively identified as kidney; duct very thin-walled and impossible to dissect out under the microscope, longitudinal extent of it, and whether or not it is recurved, could not be determined.

Renal apertures extremely small slits on slight protuberances in roof of inner excurrent chambers, just behind genital apertures (Fig. 10).

BIOLOGY

Life history and dispersal. The relatively ephemeral nature of a given active vent site and the distance between sites require an effective mechanism for dispersal if a species is to survive after a vent site becomes inactive. Lutz *et al.* (1980) examined the larval shell by scanning electron microscopy and found that prodissoconch I is small relative to the size of prodissoconch II. Comparisons they made with the larval shells of other mytilids such as *Mytilus edulis* and *Modiolus modiolus* suggest that *Bathymodiolus thermophilus* has long-lived planktonic larvae which could be transported from one vent site to another. The small size of prodissoconch I suggests very high fecundity. Lutz *et al.* (1980) proposed that these larvae may be induced to settle and undergo metamorphosis by encountering elevated temperatures at vent areas and might even delay metamorphosis in the absence of this stimulus.

Growth rates. Rhoads *et al.* (1981, 1982) derived growth rates from marked and recovered mussels at the "Mussel Bed" and "Rose Garden" sites and from recently settled young, which are among the highest recorded for deep-sea species. Mature mussels have mean growth rates of about 1 cm per year.

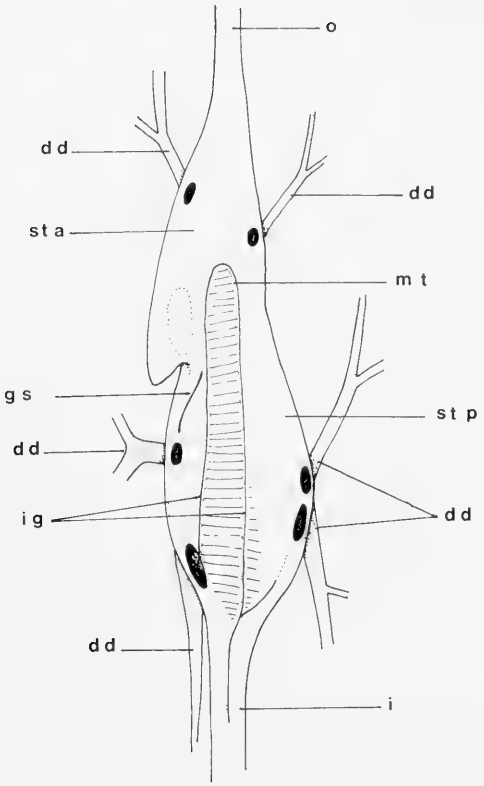

FIG. 11. Dorsal view of stomach with the dorsal wall removed, showing the openings of digestive ducts and the major typhlosole and intestinal groove.

Juveniles may reach a length of 27 mm in 294 days or less. Growth rates appear to be influenced mainly by food concentration. Mussels in dense populations close to the vents where there was a high density of microbial food grew two or three times faster than those in peripheral locations where density of microbial food was less.

Growth rings in different individuals could be tentatively correlated at the "Rose Garden" site, indicating synchronous change in mussel growth, possibly in response to change in temperature and nutrient conditions. If such thermal pulsing occurs, it may serve as a cue for gonad development or spawning. As noted above, all of the mussels examined in our study had gonads in regressed condition, which also implies synchrony of the gametogenic cycle.

Interactions with other species. TV tape recorded the brachyuran crab, *Bathograea thermydron* (Williams, 1980) crawling over mussels and occasionally probing them. Rhoads *et al.* (1982) considered these crabs to be the most likely predators on the mussels. They found that shells often show repaired damage, especially in the region of the byssal notch. They suggested that mussels shorter than 2.0 cm are usually consumed if attacked. Unsuccessful predator attacks appear to be most frequent in mussels of shell lengths between 2.0 and 5.5 cm while larger mussels appear to be ignored.

About one third of the preserved mussels examined contained the polynoid polychaete *Branchipolynoe symmytilida* (Pettibone, 1984). The worms occurred in the mantle cavity, usually in the posterior region. The gills of specimens with these polychaetes were often thickened and uneven, possibly due to disturbance by the worms. Not all mussels with deformed gills had worms at the time of collections, nor were the gills deformed in all specimens with worms inside. *In situ* TV tape photography shows a live red polychaete leaving a mussel and swimming out of view as the mussel was being collected by R/V *Alvin*'s mechanical arm. Other polychaetes of apparently the same kind are seen swimming freely and crawling over the exterior of the mussels.

Krantz (1981) has described a new and unusual species of predatory mite recovered from detritus associated with a sample of mussels.

DISCUSSION

Shell form and structure of *Bathymodiolus thermophilus* are typical of the Mytilidae, most closely resembling *Modiolus.* The anatomy also conforms generally with that of the Mytilidae but there are several features, though common within the family, which are not found in *Modiolus,* and others that are unique. In the former category are the internal diaphragm within the excurrent siphon (as in *Mytilus* White, 1937; *Lithophaga* and *Leiosolenus* Wilson, 1979, *Botula* Wilson & Tait, 1984) and the divided incurrent and excurrent siphons (as in *Botula* Wilson & Tait, 1984).

The enlarged auricles with a second opening into the longitudinal vein between the two bundles of the posterior byssal retractor muscles and the very small, tubular kidney are unique features of the anatomy.

The most obvious and remarkable feature

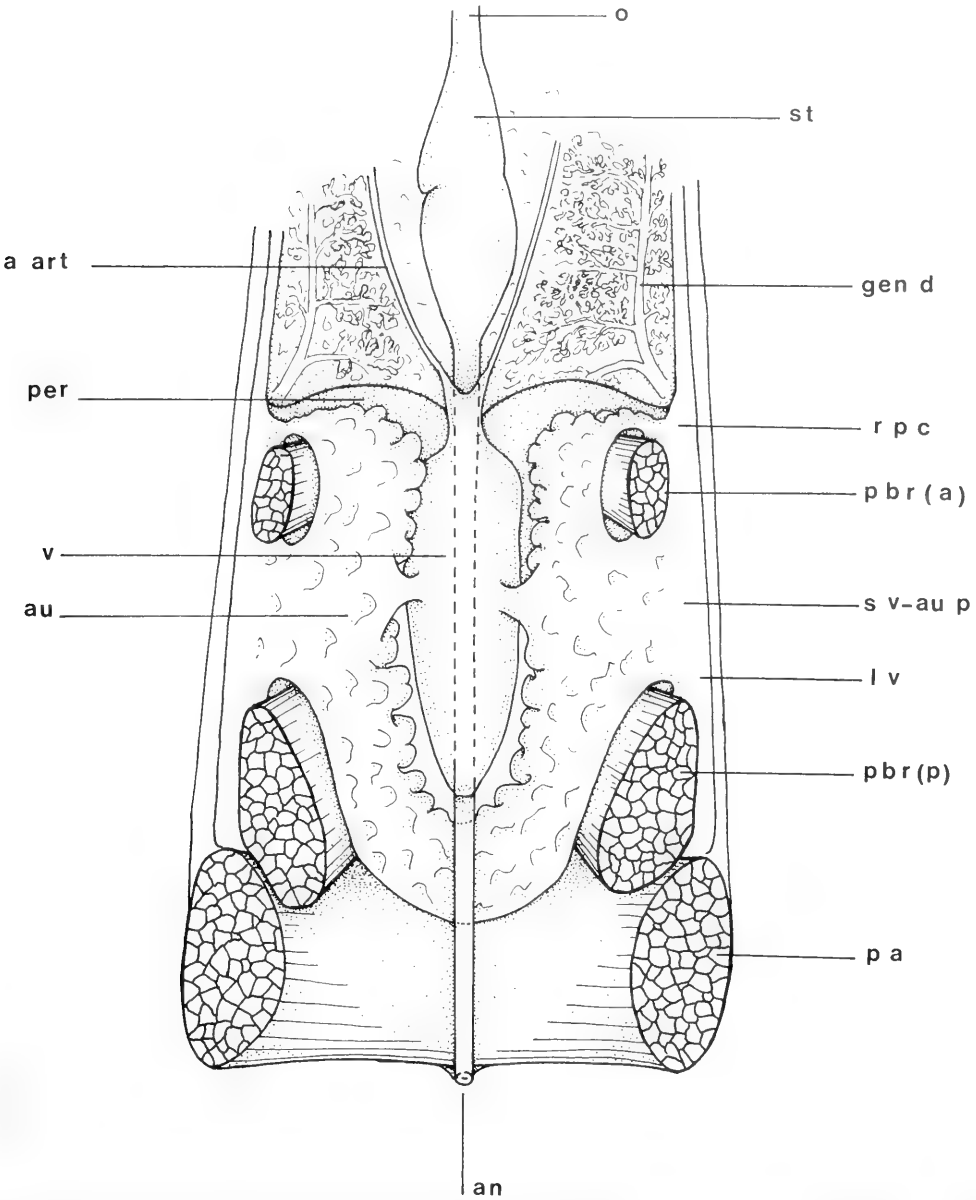

FIG. 12. Dorsal view of the heart showing the narrow anterior connection of the auricles with the longitudinal vein through the reno-pericardial channel, and the wide secondary connection between the anterior and posterior bundles of the posterior byssal retractor muscles.

of this mussel is the extent of ventral fusion of the mantle lobes. It is achieved by normal fusion of the inner folds anteriorly, but posteriorly it is achieved by extraordinary development of the valvular siphonal membrane. This structure is represented in some other mytilids by a thin, transverse, oblique partition from the antero-ventral edge of the branchial septum, partly occluding the incurrent aperture (see Yonge, 1955; *Botula* Wilson & Tate, 1984, fig. 3). In this case the postero-ventral part of the mantle cavity is enclosed by the valvular siphonal membrane except for the short central byssal-pedal gape. Neverthe-

FIG. 13. Photograph of living mussels *in situ*; R–V *Alvin* Dive 885, courtesy of Dr. Robert Hessler.

less the free edges of the inner folds posteroventrally are extensible and, judging from photographs of the animal in life (Fig. 13), must channel water along the ventral side of the body to that gape.

The gills of *Bathymodiolus,* also, are anomalous among mytilids for which gill structure is known. Although they have the typical 'W' shape, the gills are unusually thick and lack food grooves at the ventral edges of the demibranchs. Perhaps the deep grooves at the free edges of the ascending lamellae are functional food grooves but even in that case the conclusion is inescapable that this mussel does not filter suspended particles in the usual mytilid way.

Further evidence for a different feeding mechanism is found in the alimentary tract of *Bathymodiolus.* The short, direct gut lacking a recurrent loop and the internal structure of the stomach are quite unlike those of any other mytilid so far described. In *Mytilus* (Graham, 1949; Owen, 1955), *Modiolus* (Nelson, 1918; Reid,1965; Morton, 1977), *Leiosolenus* (Purchon, 1957, *Adula* (Fankboner, 1971) and *Musculista* (Morton, 1974) the stomach is a tumid, two-chambered organ with a deep antero-ventral sorting caecum and a prominent left pouch and dorsal hood posteriorly. But in *Bathymodiolus* the stomach is elongate, there is a small lateral pouch on the left side of the anterior chamber but no deep sorting caecum, and a left pouch of the posterior chamber is lacking. In those genera the major typhlosole originates in the caecum but in *Bathymodiolus* it originates on the ventral floor of the anterior chamber and runs straight down the mid-line to the entrance of the intestine. In other genera the intestinal groove originates in the posterior left pouch and curves around the posterior chamber into the sorting caecum of the anterior chamber on the left side of the major typhlosole. In *Bathymodiolus* it originates in the posterior digestive gland duct on the left side and runs straight forward along the left side of the major typhlosole, curves around its anterior end and returns down its right side to the entrance of the intestine. The transverse plications on the floor of the stomach between the two arms of the intestinal groove are not matched by any similar structures in other mytilids. Finally, in the other mytilid genera specified above the ducts of the digestive gland are numerous and asymmetrically grouped, while in *Bathymodiolus* there are three distinct pairs which enter the stomach laterally.

Although the presence of a crystalline style, gastric shield and plications on the stomach floor confirm that the stomach of *Bathymodiolus* is a particle-sorting chamber, it has a much more simple structure and organization than the stomachs of other mytilids which are extremely complex. If simplicity indicates primitiveness then it can be concluded that *Bathymodiolus* has a primitive gut. The disposition of the paired and separate digestive ducts also may be regarded as primitive (Purchon, 1957).

From these observations it is evident that the water circulation system within the mantle cavity, the capture and carriage of particles on the gills, and the sorting processes within the gut of *Bathymodiolus thermophilus* are atypical of the Mytilidae and that feeding in this species must differ from the usual.

High concentrations of chemoautotrophic, sulphur-oxidizing bacteria occur in the sulphur-rich water in the vicinity of the hydrothermal vents (Corliss *et al.,* 1979; Galápagos Biology Expedition Participants, 1979). These bacteria could be food for the filter-feeding animals living there (Jannasch & Wirsen, 1979; Rau & Hedges, 1979; Karl *et al.,* 1980; Williams *et al.,* 1981). Suspensory feeding on such high concentrations of suspended bacteria might indeed involve mechanisms different from the usual ones.

There is also evidence that *Bathymodiolus* may obtain some or all of its nutrients through symbiosis with sulphur-oxidizing bacteria in the gills. Cavanaugh *et al.* (1981) discovered prokaryotic cells in the trophosome of the vestimentiferan tubeworm *Riftia pachyptila* Jones. Felbeck (1981) demonstrated the presence of sulphur-oxidizing and Calvin-Benson cycle enzymes in that organism, suggesting that sulphur-oxidizing bacteria exist in a symbiotic relationship with it. Subsequently Felbeck *et al.* (1981) have shown that the vent clams *Calyptogena pacifica* Dall and *C. magnifica* Boss & Turner (1980), and the mussel described here, also show evidence of sulphur-oxidizing enzyme activity in the gill tissues. They suggested that these bivalves inhabiting the sulphide-rich environments of the hydrothermal vents "are not only able to tolerate these toxic habitats, but in addition are capable of exploiting the energy of sulphide to drive net CO_2 fixation and, thereby, reduce their dependence on ingestion of

photosynthetically fixed carbon." The simple structure of the gills, labial palps and alimentary tract in *Bathymodiolus* is quite consistent with this possibility. Nevertheless, the anatomical evidence indicates some degree of ciliary feeding on suspended particles, probably bacteria.

Nutrition based on symbiotic sulphur-oxidizing bacteria is now described for several bivalves that live in sulphide-rich environments (Reid & Bernard, 1980; Cavanaugh, 1983; Felbeck, personal communication). In discussing such symbioses in marine invertebrates, Reid & Bernard (1980) commented on the need for a burrow or tube in pogonophorans "to contain and confine the related organisms and prevent the dissipation of useful solutes." The extreme degree of ventral mantle fusion in *Bathymodiolus* may also serve this function.

In several of the preserved specimens, small particles of bright yellow particulate matter, presumed to be elemental sulphur, were observed trapped in mucus on the gills. Jones (1981) made a similar observation in *Riftia.* One might expect that an animal living in such a toxic environment would have a specially efficient excretory system. It is surprising, therefore, to find that the kidney in *Bathymodiolus* is so small. The vascular system, on the other hand, is unusually well developed. The clue to interpretation of these unusual structures may lie in the physiology of nutrition based on suspended or symbiotic sulphur-oxidizing bacteria, or in the mussels' physiological tolerance to the sulphur-rich environment.

The phylogenetic affinities of *Bathymodiolus* remain problematical. The similarity of the modioliform shell to that of *Modiolus* is clearly a case of parallelism for the anatomical characters are very different. The extent of mantle fusion, the simple gut, the lack of ventral food grooves on the gills, the small kidney and the large auricles with second connections to the longitudinal veins, are all characters which have not been previously described in the Mytilidae. The physiological implications of these features indicate a different life-style and evolutionary origins to the Mytilinae, Modiolinae, Lithophaginae, Crenellinae or Musculininae. We confidently introduce the new subfamily Bathymodiolinae for this new genus and species.

There are several small, modioliform mytilids in the Pacific region. Knudsen (1970) described *Modiolus abyssicola* (Fig. 14B) from 3670–3270 m in the Gulf of Panama (5°49′N, 78°52′W). This small mussel (max. length recorded 17.2 mm) has an arcuate modioliform shell similar to that of a medium to large-sized *Bathymodiolus thermophilus* although juvenile specimens of the latter are not arcuate (Fig. 14A). Because of this similarity and the proximity of the localities, early consideration was given to the possibility that the large mussels from the hydrothermal vents might be adults and the Panama ones juveniles of one species. Independently we have examined the anatomy of preserved specimens in the type-series of *M. abyssicola,* through the courtesy of Dr. Knudsen. The posterior retractors of this species are divided but there is no siphonal development, the branchial septum is short and simple, there is a large incurrent aperture (gape) from the ventral side of the branchial septum to the anterior adductor, and the intestine is short but looped as in *Modiolus.* Gonads, gonoducts and a prominent gonad aperture were observed in the larger specimens, dispelling any suggestion that these are juveniles. For these reasons, we conclude that *M. abyssicola* may be a true *Modiolus* or at least a modiolinid, and the possibility of a relationship with the species described here can be rejected.

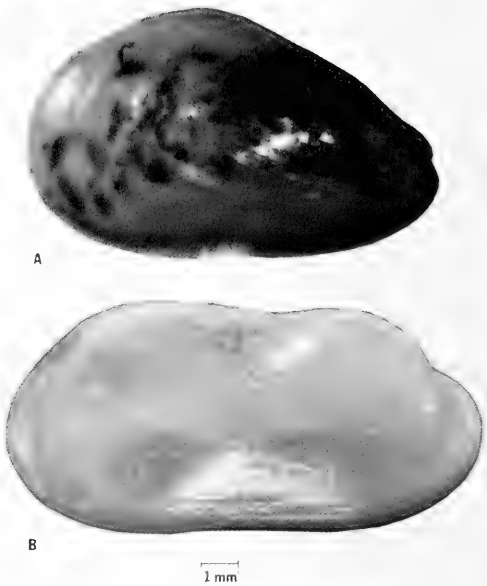

FIG. 14. A. Small specimen of *Bathymodiolus thermophilus.* B. Holotype of *Modiolus aybssicola* Knudsen.

Modiolus projectus Verco, 1908, from 200

fathoms off South Australia is another small species (holotype length 10.9 mm) of similar form. It is characterized by a conspicuous "projecting lamina" below the ligament. The generic affinities of this species remain undetermined but this unusual character makes a relationship with *Bathymodiolus* improbable.

Adipicola Dautzenberg, 1927, is a genus containing several small deep water modioliform mytilids. The prosogyrate umbos are situated well back from the anterior end and there is an anterior (lunular) keel as well as a posterior one. A sub-ligamental ridge is lacking, and the ligamental plate, though more or less vertical posteriorly, curves under the margin near the umbos. In *A. simpsoni* (Marshall, 1900) and *A. argenteus* (Jeffreys, 1876) there are pseudo-taxodont teeth or denticles on the dorsal margin beneath and behind the ligament; these are lacking in the type-species *A. pelagica* (Woodward, 1854). The anatomy of these species is unknown to us although the posterior retractor muscle scars are not divided in *A. simpsoni* at least. The Pacific genus *Terua* Dall, Bartsch & Rehder, 1938, appears to be very close to *Adipicola*. The type-species *T. pacifica* Dall, Bartsch & Rehder, 1938, and *T. japonica* (Habe, 1971) also have hinge denticles. Dried specimens of the latter species in the USNM (204525) show a long ventral gape, no excurrent siphon, and undivided posterior retractor muscles. This complex of small modioliform mytilids is in need of revision. In the meantime, even in the absence of much information about their anatomy, we are confident that they are not closely related to the new species from the Galapagos hydrothermal vents. Nevertheless, an affinity for *Bathymodiolus* might eventually be established in this direction.

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NOTE

The following appeared while this paper was in press (see also p. 255, footnote 3):

FIALA-MEDIONI, A., 1984, Mise en évidence par microscopie électronique à transmission de l'abondance de bactéries symbiotiques dans la branchie de Mollusques bivalves de sources hydrothermales profondes. *Comptes Rendus de l'Académie des Sciences* [Paris], III, 298: 487–492, 2 pl.

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ED.

MALACOLOGIA, 1985, 26(1–2): 273–275

LETTER TO THE EDITORS

DERIVATIONS OF ARENOPHILIC MANTLE GLANDS IN THE ANOMALODESMATA

Club-shaped, multicellular glands lining at least a portion of the mantle edge, have been found in various members of the bivalve subclass Anomalodesmata. These organs, first described from the verticordiids by Allen & Turner (1974) and named by these authors "radial mantle glands," have since been found in members of the Lyonsiidae (Prezant, 1979a, 1981a), Periplomatidae (Morton, 1981), Parilimyidae (Morton, 1982), and Clavagellidae (Morton, 1984a,b). At least in the Lyonsiidae, these glands secrete an adhesive mucin (bi-layered, weakly acidic mucopolysaccharide and glycoprotein) atop the periostracum that functions in the attachment of extraneous material (often sand) to the outside of the shell (Prezant, 1979a,b, 1981a,b). Based on this function, Prezant (1981a) termed these organs "arenophilic radial mantle glands" or "arenophilic glands." This external coating may serve several functions including: protection of relatively thin shelled specimens; dissuasion of boring predators (especialy naticids); camouflage; weighting and increased frictional resistance of relatively light, smooth shells in animals with weak byssi (e.g. *Lyonsia*) for increased stability in potentially shifting substrata (Prezant, 1979a,b, 1981a,b,c).

In Morton's many studies on the Anomalodesmata, he has described a variety of members with "radial mantle glands" as occurring in the middle mantle fold (1981, 1982, 1984a,b). Morton (1984a), for instance, states that for *Clavagella australis* (Sowerby), ". . . the tips of the siphonal crown possesses specialized subepithelial, basiphilic, glands, termed by Prezant (1979) for the Lyonsiidae, "radial mantle glands". . . Such glands are developed in the middle folds, not the outer folds as suggested by Prezant. . . ."

I found that the glands in members of the lyonsiid *Entodesma* open distal to the periostracum-secreting cells (i.e. ventral to the periostracal groove on the inner surface of the outer mantle fold) (Prezant, 1981a). The glands in members of the genus *Lyonsia* are found proximal to the periostracum-secreting cells and thus open directly into the periostracal groove. In both genera a secretory sheath encircles the gland. In *Lyonsia* this sheath is derived from the outer epithelium of the middle mantle fold, and in *Entodesma* from the inner epithelium of the outer fold. In both genera, however, the central gland (composing the bulk of the organ) is derived from the outer mantle fold epithelium.

Adhesive mucins from the arenophilic glands of both *Lyonsia* and *Entodesma* serve similar functions and are both released upon the periostracum. In the latter the glands are more abundant in thinner shelled juveniles where they may still be active in their ascribed functions (adult members of *Entodesma* may be thick-shelled crevice dwellers with strong byssi) (for a discussion of this see Prezant, 1981a). In specimens of *Entodesma* the secretion is deposited upon the periostracum presumably by the action of some proteolytic enzyme (probably incorporated within the glycoprotein layer) that allows the adhesive to periodically penetrate through the overlying periostracum (secretion deposited as radial tufts in *Entodesma* as opposed to continuous radial lines in *Lyonsia*). The third genus within the Lyonsiidae, *Mytilimeria,* lacks arenophilic glands but has a heavy concentration of marginal mucocytes dispersed along the mantle edge. *M. nuttalli* Conrad is typically an endosymbiont of compound ascidians and has consequently lost arenophilic glands with attainment of this protective and secure habitat.

The arenophilic glands of lyonsiids are derived as invaginations of the outer mantle fold epithelium (with a surrounding middle fold sheath). If, as suggested by Morton (1981, 1982, 1984a,b), the glands proper were of middle fold origin, it would necessitate an ontogenetic migration of these organs through the periostracal groove in *Entodesma.* This is almost certainly not the case.

Based on the limited evidence available, a derivation of these glands from the middle mantle fold in anomalodesmatans outside the Lyonsiidae might be hypothesized. The evidence for this, however, is not strong as the only detailed work thus far in print on these organs is that of Prezant (1979a, 1981a) for the lyonsiids. Also, as Morton states (1981), it is very likely that these are "specialized glands evolved in some comon ancestor of

the Anomalodesmata and retained in a diverse series of descendants." It is indeed plausible that these organs can serve as evoutionary markers but we must first expand our knowledge of them and also clear the literature of discrepancies.

In a report on tube construction in *Brechites,* Morton (1984a) reports that "Similar glands occur in members of the Verticordiidae, Lyonsiidae, Pholadomyidae, Periplomatidae, Poromyidae and Parilimyidae (Allen & Turner, 1974; Prezant, 1979[a]; Morton, 1980, 1981a,b, 1982a). . . ." There is, however, no indication in any of these cited references that either poromyids or pholadomyids possess arenophilic mantle glands. In fact Morton (1982; table 1) lists both the Pholadomyidae and Poromyacea as lacking radial mantle glands. In his 1980 publication on *Pholadomya candida* Sowerby, Morton notes that "Radial mantle glands . . . do not occur in *P. candida."*

Among the Anomalodesmata additionally reported not to possess arenophilic mantle glands are the Pandoridae (Prezant, 1981a), Thraciidae (Prezant, 1981a), Laternulidae (Prezant, 1981a), Cleidothaeridae (Morton, 1984) and Cuspidariidae (Morton, 1982). The Myochamidae have yet to be adequately examined for these organs. While arenophilic glands have been described by Morton (1981) from the periplomatid *Periploma (Offadesma) angasi* Crosse & Fischer, no such glands have been found in serially sectioned mantle of adult *P. fragile* (Totten) nor *Cochlodesma praetenue* (Pulteney) (Prezant, 1981a).

Arenophilic mantle glands have been found by Morton (1982) in *Parilimya fragilis* (Grieg) and apparently also function in adhesion of sand grains to the periostracum. Here, Morton states that "Clearly the glands have an uneven distribution in Anomalodesmata. This may be a case, however, so often encountered in the Anomalodesmata, of a primitive feature, possibly evolved during the Palaeozoic period of radiation in shallow water deposits, subsequently retained in a wide but disjointed assemblage of descendants." I concur that the glands are of an "uneven" distribution among members of the subclass. I also suggest that they may be of "uneven" structure and derivation in recent Anomalodesmata. It is unlikely that such specialized glands arose separately in any major group of anomalodesmatans. The Middle Ordovician stock of this subclass likely possessed an "anlage" of arenophilic glands in the form of widely dispersed and nonconsolidated mucocytes. The secretions from these simple glands perhaps helped support the walls of the bivalves' burrows. The glands diversified early in their evolution into the structured organs we see today. Extant members of the subclass that possess arenophilic glands may reflect a bifurcation from this early stock with the structure developing, phylogenetically, either inside or outside the periostracal groove.

The location of the glands in specimens of *Entodesma* precludes the possibility that these organs are derived from the middle mantle fold in the Lyonsiidae. Fig. 17 in Prezant's (1981a) article on arenophilic glands clearly shows such a gland opening inside (*i.e.* ventral to) the periostracal groove. Fig. 8 of the same paper shows the secretion from an arenophilic gland penetrating the periostracum. The lack of adequate longitudinal sections in other reports demonstrating the occurrence of similar glands prohibits the exact determination of specific locations and mantle origins. Cross-sectional representations, as exhibited in most reports (*i.e.* Morton, 1981, 1982, 1984a,b), strongly suggest the similar origin within the Anomalodesmata of arenophilic glands but do little to support the contention that the glands are middle mantle fold derivatives. Allen & Turner (1974) found that the radial mantle glands of the verticordiid *Policordia densicostata* Locard open "on to the edge of the sensory lobe of the mantle." The latter authors also report "radial mantle glands within the sensory lobe" of *Verticordia triangularis* Locard. Morton (1981), however, found the "description by Allen & Turner (1974) of similar glands in the mantle of members of the Verticordiidae . . . insufficiently detailed to facilitate comparison . . ." with the arenophilic glands of *Lyonsia* (as described by Prezant, 1979a). In addition, Morton (1981) found "No trace of retractor muscles for withdrawing the gland . . ." in *Offadesma angasi. . .* "as occur in *Lyonsia* (Prezant, 1979)" and suggests, that their inability to protrude may be ". . . because of their length. . . ." Though no total length measurements are offered in the latter paper (*i.e.* Morton, 1981), diagrammatic representation show glands in *O. angasi* that are likely equal to or shorter than protrusible glands along the siphonal mantle edge of some lyonsiids. While the glands of *O. angasi* may not be protrusible, it is not a reflection of length. This difference as well as other differ-

ences already mentioned may offer valuable clues to the taxonomy and phylogeny of the Anomalodesmata.

Because of our limited knowledge of arenophilic glands, it is presently difficult to account for intertaxon differences in mantle gland derivations in what is most likely a well established mantle feature. The loss of these glands in the lyonsiid *Mytilimeria,* the location of the glands deep in the periostracal groove of *Lyonsia,* and the establishment of the glands distal to the groove in *Entodesma* with the apparent concurrent development of some proteolytic enzyme involved in periostracal penetration, all point to the evolutionary plasticity of this organ in one family alone. The plasticity within the subclass is certainly greater.

Since arenophilic glands are present in several superfamilies of anomalodesmatans (*i.e.* Pandoracea, Verticordiacea, Clavagellacea, Thraciacea), it is likely that the glands, *sensu stricto,* evolved early, perhaps even in primitive pholadomyacean stock of the Paleozoic. Present day pholadomyids may lack these organs but this does not preclude the possibility of gland loss from earlier stock. The presence of similar mantle organs in numerous anomalodesmatan families argues strongly for plesiomorphy. Since mantle glands of members of the lyonsiid genus *Entodesma* are scarce in large adult specimens, it is still possible that similar organs will be found in other anomalodesmatan families once complete serial sections are taken of mantle edges from complete growth series. Unfortunately, Morton (1980) had only a single specimen of *Pholadomya candida* available for histological analyses and was thus unable to serial section the entire mantle edge. A large portion of the mantle edge of this primitive bivalve was left unexamined. Whether or not arenophilic glands are present in *P. candida* (and it is unlikely that they are present) does not discount the possibility of similar glands in stock pholadomyaceans or at least a densely packed series of mucocytes lining the mantle edge. This might well have been the incipient evolutionary stage of present day arenophilic glands.

I suggest we acknowledge the possibility that the possession of arenophilic glands *per se* is symplesiomorphic, but presently variable in form and ontogenetic origin. These subtle differences may offer strong clues to the phylogenetic progression of the Anomalodesmata. The solutions to the presumed discrepancies await ontogenetic evidence of the ultimate origin of arenophilic radial mantle glands in each group in which they occur.

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An asterisk (*) denotes a new taxon

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