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ISSN 2039-0394 (Print Edition)
ISSN 2039-0408 (Online Edition)

with the support of

world biodiversity association

SEPTEMBER 2012, 3(3): 157-260

FOR NATURALISTIC RESEARCH
AND ENVIRONMENTAL STUDIES

Julodis andreae Marseul, 1865 - Turkey

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1.*Julodis armeniaca* Marseul, 1865, Turkey, Bingol Yayla, 20.VII.2007.
2. *J. aristidis*, Tunisia, Tozeur, 8.IV.2004.
3. *J. lucasi*, Tunisia, Gafsa, 11.IV.2006.
Cover: *J. andreae,* Turkey, Adiyaman - Nemrut Dagi, 12.VI.2007 (photos-collection M. Gigli).

The genus *Julodis* Eschscholtz, 1829 (Coleoptera Buprestidae). The genus *Julodis* is the type genus of the Subfamily Julodinae Lacordaire, 1857. Species in this group are quite unusual Buprestidae for their general appearance, but especially for the larval biology. They don't live in wood or other plant tissues like other larvae of this family, but they dig into the soil and feed on the roots of various plants. In contrast to larvae of other Buprestidae, they are covered by long bristles directed obliquely backwards, helping in the movement in the ground, just like in the larvae of Coleoptera Cetonidae. The genus *Julodis* is currently divided into two groups. One in South Africa (especially Cape Region) and Namibia, represented by 34 taxa (species and subspecies), and another group primarily Palaearctic, spread from Spain and North Africa to the steppes of Central Asia (up to the Chinese province of Gansu), with some species in the Oriental Region (Pakistan and India) and other taxa in Kenya (92 species and subspecies). Many species are extremely variable. The widespread polymorphism and the existence of many similar species has led to the description of many species, then falled into synonymy, and probably others will follow the same fate, especially in certain groups of Palaearctic species. The European Fauna includes six species and subspecies, in the Iberian Peninsula, South France, Italy (Lampedusa Is.) and Balkans. *Julodis onopordi* s.l. is one of the most polymorphic species. It, according to actual conception, is widespread in the range of Mediterranean climate in Spain and southern France, and in North Africa, from Morocco to Sinai, with many different populations, some of which are considered subspecies. It is the only species in the genus in the Italian Fauna (Lampedusa Is., only). Another very variable species, spread over extensive territories, is *Julodis andreae* s.l., who lives in all the lands between Turkey, Iran and Azerbaijan. On the contrary, there are species very localized, limited to areas with well-defined characteristics of the soil and climatic conditions. Among these, three species live in the vast steppe and pre-desert around the large salt lakes of Northern Africa: *Julodis aristidis* Lucas, 1860, *Julodis chrysesthes* Chevrolat, 1860, *Julodis kerimi* Fairmaire, 1875. They are differently distributed over the territory in consequence of the type of soil, the presence of salt, gypsum, and its granulometry, of great importance for the needs of the larvae. Adults mostly feed on leaves and young twigs of shrubs and small trees, but also on herbaceous plants.

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Biodiversity Journal, 2012, 3 (3): 159-164

New data of the freshwater fish genera *Laubuca* Bleeker, 1860 (Cypriniformes Cyprinidae) and *Phenacostethus* Myers, 1928 (Atheriniformes Phallostethidae) in Thailand

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ABSTRACT In the present paper are reported, for Thailand, additional records of the cyprinid fish *Laubuca siamensis* Fowler, 1939 and priapium fish *Phenacostethus smithi* Myers, 1928 respectively from Mekong Basin, Meklong Basin, Southeast Basin and from upstream of Bangpakong Basin and Yom Basin. Description and distribution data of the two freshwater fishes are also provided.

KEY WORDS *Laubuca*; *Phenacostethus*; Cyprinidae; Phallostethidae; Thailand.

Received 02.03.2012; accepted 26.06.2012; printed 30.09.2012

INTRODUCTION

Freshwater fishes of the genera *Laubuca* Bleeker, 1860 and *Phenacostethus* Myers, 1928 are poorly known in Thailand.

Currently, for *Laubuca* genus two valid species have been recorded in Thailand, namely *L. caeruleostigmata* and *L. laubuca* (Smith, 1931; 1945; Bănărescu, 1971).

The cyprinid fish *Laubuca siamensis* was descripted by Fowler (1939) and the distribution of this species is known only in waterfall at Trang Province, peninsular Thailand.

In Thailand, *Laubuca* species taxonomic status is still unclear, especially as far as concerns *L. laubuca*: in 1971, *L. siamensis* was reported as a junior synonym of *Chela laubuca* by Bănărescu (1971) and in 2009, the genus *Chela* Hamilton, 1822 in Thailand was considered a junior synonym of genus *Laubuca* based on a molecular study of phylogenetic interrelationships (Fang et al., 2009).

Phenacostethus are small priapium fishes, found in large rivers and river estuaries in Southeastern Asia.

The genus is separated into three valid species, namely *P. trewavasae* Parenti, 1986 from Sarawak, Malaysia, *P. posthon* Roberts, 1971 from peninsular Thailand (Satul Province, Pungah Province), Malaysia and Sumatra, Indonesia and *P. smithi* Myers 1928 widely distributed: Mekong Basin (Thailand and Cambodia); Chantaburi River, Southeast Basin; Lower Chaophaya Basin (Bangkok); Malay Peninsula, Sarawak, Borneo, Malaysia (Myers, 1928; Roberts, 1971; Parenti, 1986; Rainboth, 1996; Parenti & Lim, 2005).

In the present paper, the authors re-examine all specimens of the cyprinid fishes *Laubuca* stored in Inland Fisheries Resources Research and Development Institute, Department of Fisheries, Thailand [NIFI]. The authors found that the specimens named as *L. laubuca* from Thailand are clearly different from the original description of Hamilton

(1822) of *L. laubuca* by the combination of the following characters: lateral line scales, body depth, number of anal fin rays, pelvic fin length, pectoral fin length and absence of tubercles on lower jaw. The authors suggest the valid name of these specimens to be *L. siamensis* Fowler, 1939. In addition, the authors report additional records on the distribution of *L. siamensis* in Mekong Basin, Meklong Basin and Southeast Basin, Thailand.

As far as concerns the project at Yom River and upstream of Bangpakong Basin, Kabin Buri District, Prachin Buri Province and Nakhon Nayok Province, Central Thailand, we found many specimens of *P. smithi* in both areas, an additional record of this species in Thailand.

ACRONYMS. Standard length (SL); Head length (HL).

RESULTS

Order Cypriniformes Bleeker, 1859
Family Cyprinidae Cuvier, 1817

Laubuca siamensis Fowler,1939

Chela laubuca. Smith, 1945: Peninsular Thailand); Sontirat et al., 2006: Southeast Basin, Thailand.

EXAMINED MATERIAL. NIFI 0079, 2 specimens, Aun River, Sakon Nakhon Province, Northeast Thailand, I.1967, legit Sopa Trirat. NIFI 1227, 1 specimen, Klong Sang, Chav Raan Reservoir, Surat Thani Province, South Thailand, IV.1983, legit Karnasuta, J. NIFI 1968, 1 specimen, Tapi River Basin, Phrasaeng Suratthani Province, South Thailand, IV.1985, legit Sonkphan, L. (Fig. 1). NIFI 1969, 4 specimens, same data of NIFI 1968. NIFI 2527, 2 specimens, Tanow Sri River, Sune Pung Distric, Ratchaburi Province, West Thailand, XII.1993, legit Chavalit Vidthayanon (Fig. 2). NIFI 2966, 4 specimens, Klong Phraya W.S. Krabi Province, South Thailand, 1970, legit J.N.

DESCRIPTION. *L. siamensis* is distinguished from other species of *Laubuca* genus by the combination of the following characters: lateral line scales complete, with 31-33 + 2 scales; transverse line scales on body with 6-7/ 1 / 2-4½ scales; body depth is 28.6-33.0%SL. Pectoral fin is short not extending to the anus; anal fin with 3 unbranched rays and 18½ - 21½ branched rays; pelvic fin is long (93.8-136.6%HL) reaching beyond the anus; pectoral fin length is 34.6-39.4%SL; a clearly black blotch above the pectoral fin base; thin black longitudinal stripe along the body; caudal peduncle with clearly dark blotch; lower jaw smooth, no tubercles on skin.

Particularly, *L. siamensis* is compressed, body depth is 28.6-33.0%SL. Body width is 9.6-13.2%SL. Scales in lateral series are medium to large, lateral line scales complete, with 31-33 + 2 scales, transverse line scales on body with 6-7/ 1 / 2- 4½ scales and 16-18 predorsal scales. Head length is 21.2-26.8%SL. Eye is large, eye diameter is 25.5-37.9%HL (6.5-8.6%SL). Post orbital length is 39.0-43.8 %HL (8.6-10.1%SL), snout length is short, 25.9-31.9%HL (6.0-7.1 %SL) and interorbital width is 46.9-50.4 % HL (10.9-11.8 % SL), shorter than postorbital width (48.3-54.9 %HL or 12.0-12.5 %SL). Dorsal fin origin coincides with posterior anal fin origin, predorsal fin length is 62.8-71.8%SL, prepectoral fin length is 25.4-30.6 %SL, prepelvic fin length is 43.0-46.5 %SL and preanal fin length is 64.1-68.9 %SL.

Caudal peduncle depth is 9.2-10.9 %SL; pectoral fin is long but not reaching beyond the anus, the pectoral fin length is 34.6-39.4%SL showing 11-12 branched fin rays. Pelvic fin is long reaching beyond the anus, the pelvic fin length is 93.8-136.6%HL or 20.8-36.6%SL with 5 branched fin rays. Anal fin base is longer than dorsal fin base, the anal fin base length is 26.0-28.5%SL, dorsal fin with 3 unbranched rays and 8 branched rays and anal fin with 3 unbranched rays and 18½-21½ branched rays. The dorsal fin base length is 10.5-14.0 %SL.

DISTRIBUTION. This species is distributed in Mekong Basin, Meklong Basin, Southeast Basin and peninsular Thailand.

COMPARATIVE NOTES. Other examined material. *L. caeruleostigmata*: NIFI 0041, 1 specimen, Poung Klong Nong Moa, Ayuttaya Province, Central Thailand, XII.1966, legit Theachareon, p. NIFI 2602, 23 specimens, Pasak, Lopburi Province, Central Thailand, I.1994, legit Chukajom T. (Fig. 3).

L. siamensis was descripted by Fowler (1931) from waterfall at Trang Province, peninsular Thailand, and "*siamensis*" is referring to Siam, the old name of Thailand, the type locality of this species; this species has been considered unil now a junior

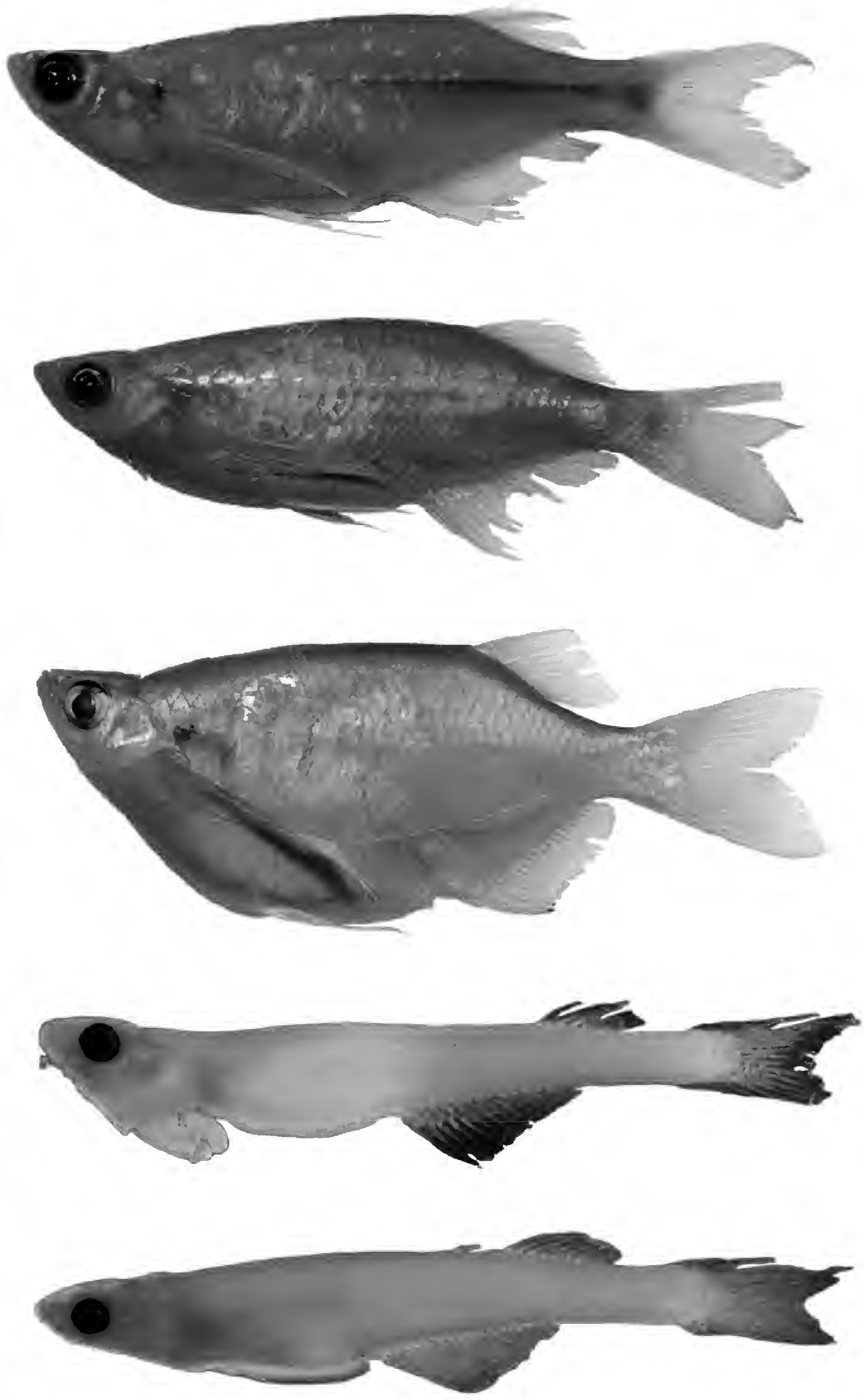

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Figure 1. *Laubuca siamensis*, 48 mm SL, Tapi River Basin, Phrasaeng, Suratthani Province, South Thailand. Figure 2. *L. siamensis*, 51 mm SL, NIFI 2527, Tanow Sri River, Ratchaburi Province, West Thailand. Figure 3. *L. caeruleostigmata*, 61 mm SL, NIFI 2602, Pasak River, Lopburi Province, Central Thailand. Figures 4, 5. *Phenacostethus smithi*, 15-17 mm SL, male (above) and female (below), NIFI 4545, Yom Basin, VIII.2011, Siriwan Suksri leg., Thailand.

synonym of *Chela laubuca* (Bănărescu, 1971) and genus *Chela* in Thailand has been considered a junior synonym of *Laubuca* (Fang et al., 2009).

The current status of *L. siamensis* is *L. laubuca*. *L. laubuca* was descripted by Hamilton (1822) from Northern parts of Bengal (NorthEast India; Bangladesh). From a comparison with documents and specimens from Thailand, we strongly believe that, in Thailand, the valid name of *L. laubuca* is *L. siamensis*.

L. siamensis from Thailand is distinguished from the original description of Hamilton (1822) and the report of Günther (1868), who re-examined the *C. laubuca* specimens of Dr. Bleeker's Collection from Bengal, by the combination of the following characters: lateral line scales are 31-33 + 2 (in *L. laubuca* they are 37), body depth is 3.00-3.50 times greater than SL (in *L. laubuca* is 2.75-2.80 times); transverse line scales is 6-7/ 1 / 2-4½ scales (in *L. laubuca* is 7½/1/4); pelvic fin shows 5 rays (in *L. laubuca* 7); pectoral fin is short not extending to the anus (in. *L. laubuca* it reaches the anus); pelvic fin is long extending beyond the anus (in *L. laubuca* is shorter, not reaching beyond the anus); first ray of pelvic fin appears like a filament (in *L. laubuca* it is undivided from the other branched rays); a thin black longitudinal stripe along the body and a clearly dark blotch on caudal peduncle (which are absent in *L. laubuca*).

L. siamensis is clearly different from *L. caeruleostigmata* of Thailand by many characters such as: body depth is 3.0-3.5 times greater than SL (in *L. caeruleostigmata* is 2.25 times), lateral line scales includes 31-33 scales (in *L. caeruleostigmata* 34-35). *L. siamensis* has one black blotch above the pectoral fin base, a thin black longitudinal stripe along the body and a clearly dark blotch on caudal peduncle (*L. caeruleostigmata* shows 4-5 dark vertical stripes above pectoral fin base on both sides of the body) (Smith, 1931; Smith, 1945).

Order Atheriniformes Rosen, 1966
Family Phallostethidae Regan, 1913

Phenacostethus smithi Myers 1928

EXAMINED MATERIAL. NIFI 4545, 8 specimens, Yom Basin, Pakpot Subdistrict, Moung District, Su-

Figure 6. Collection area, floodplain of Yom Basin in Sukhothai Province, Thailand.

khothai Province, Thailand, 25.VIII.2011, legit Siriwan Suksri (Figs. 4-5). NIFI 4548, 3 specimens, upstream of Bangpakong Basin, Kabin Buri District, Prachin Buri Province, Thailand, 5.II.2011, legit Sitthi Kulabtong.

DESCRIPTION. *P. smithi* has a slender and cylindrical body. Body depth is 16.5-19.9%SL. Head is short, head length is 18.2-19.1 %SL. Eye is big, eye diameter is 33.5-37.6 %HL. Post orbital length is 33.5-37.7%HL, snout short (23.5-24.3 %HL) and mouth is upward. First dorsal fin is very small, but second dorsal fin is large. Pre-second dorsal fin length is 70.6-70.7 %SL and the second dorsal shows 6-7 rays.

Dorsal fin origin is clearly posterior the anal fin origin. Pre-anal fin length is 52.3-52.9%SL and the anal fin comprises 14-17 rays. Second dorsal fin base is shorter (12.4-14.4%SL) than anal fin base (23.5-25.1%SL). The body is translucent, with tiny scales. Head with membranous dome. Males have a priapium (reproductive organ) below the head at the base of pectoral fin.

The priapium of *P. smithi* is ruffled and hence distinguished from that (i.e. smooth) of other *Phenacostethus* occurring in Thailand.

VARIABILITY. Priapium, the reproductive organ is found in males only.

BIOLOGY AND DISTRIBUTION. *Phenacostethus smithi* were found in several habitats of Yom Basin (Fig. 6); floodplain canal and mainstream, characterized by slow and turbid waters and muddy bottom. In each habitat, submerged or marginal plants, such as green algae, papyrus and grass, were found. Specimens from upstream of Bangpakong Basin were found in a small stream nearly the mountain, with shallow, turbid and slow waters.

This species is known from Yom Basin, Bangpakong Basin, Lower Chaophaya Basin and Southeast Basin in Thailand; Mekong Basin in Thailand and Cambodia; Malay Peninsula, Sarawak, Borneo in Malaysia.

ACKNOWLEDGEMENTS

A special thanks to reviewers for reviewing this manuscript. Authors wish to thank Dr. Rohan Pethiyagoda, Wildlife Heritage Trust in Sri Lanka and Dr. Sorin Stefanut, Institute of Biology Bucharest, Romanian Academy, Romania for providing the original description of many species of *Laubuca* genus. Finally we are grateful to all partners for their support.

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Biodiversity Journal, 2012, 3 (3): 165-172

Evaluation of the toxicity of metal pollutants on embryonic development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata Echinoidea)

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ABSTRACT Bioassays are frequently used to evaluate biological effects of pollutants on marine organisms. The objective of such tests is the detection of toxic effects on populations that are representative of a given ecosystem. Sea urchin is a model organism employed in the field of environmental toxicology due to its sensitivity towards various pollutants, particularly heavy metals. Preliminary bioassay tests on embryos and/or larvae of *Paracentrotus lividus* (Lamark, 1816) from Madagh (Oran, Algeria) were used to assess the potential toxicity and determine the LC_{50} of four metal pollutants, Cadmium, Copper, Lead and Zinc.

KEY WORDS Bioassays; LC_{50}; Madagh; Heavy metals; *Paracentrotus lividus*.

Received 22.05.2012; accepted 06.07.2012; printed 30.09.2012

INTRODUCTION

Inland aquatic and marine systems represent containers for virtually all contaminants, via direct and indirect contributions (Peijnenburg et al., 1997). Research on the action of heavy metals on the development of the sea urchin eggs represent an important contribution to the progress of knowledge in the field of embryonic determination.

Sea urchin is a preferred model in such a research, due to a number of reasons including external growth of embryos, rapid cell division rate and cell transparency, thus being commonly employed in the field of Environmental Toxicology (Guillou & Michel, 1993; Quiniou et al., 1997). Bioassays or bio-tests frequently use several techniques to measure, predict and control the effect of the release of toxic substances on marine organisms.

Present study examines the impact of four heavy metals, Cadmium, Copper, Lead and Zinc, on the embryonic development of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata Echinoidea).

MATERIALS AND METHODS

We investigated the site of Madagh bay (Oran, Algeria: 35°37'952" N; 000°104'243" W) (Fig. 1), a non-impacted area, closed at its ends by two small caps reducing the action of winds. Moreover, the proximity of the Habibas island, which is considered a marine protected area, could make this site a reference station for comparative studies regarding the monitoring of pollution impacts in the coastal marine ecosystem of western Algeria, a site rich in algae and *Posidonia* meadows (Benghali, 2006).

Collection of sea urchins was carried out during the period March-June 2010, when spawning is at its peak in this echinoid species. Spawning was induced by injection of 0.5 ml of 0.5 M KCl into the

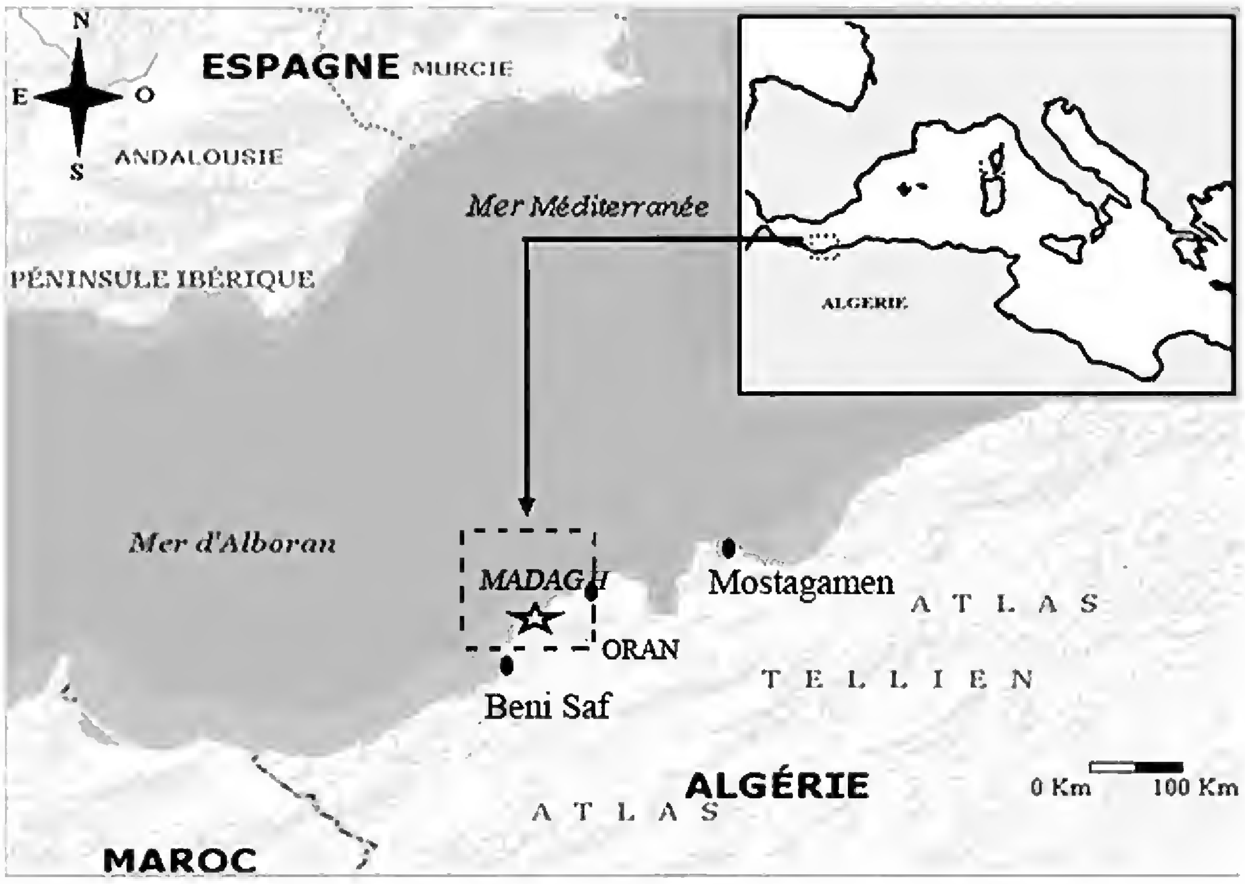

Figure 1. Sampling site: Madagh bay, Oran, Algeria.

coelomic cavity of the sea urchin (Harvey, 1940); the male sex products were recovered "dried" and stored in melting ice. Moreover, sperms of several males were pooled. Females were placed in a 250 ml Erlenmeyer flask containing filtered seawater (FSW) so that the genital pore was in contact with the surface of the water. After spawning, eggs were sieved with a 160 micron sieve and collected in a test tube. Volume was adjusted to 500 ml using FSW and homogenized. Subsequently, the first 100 ml of the solution (containing eggs) was removed and replaced by FSW. This operation was repeated a second time (Dinnel et al., 1988; Quiniou et al., 1999; Guillou et al., 2000).

Once suspensions of gametes were obtained, eggs and sperms were recovered separately in 2 ml of FSW. Fertilization was performed in beakers containing 1500 to 2000 oocytes to which 250 µl of sperm was added. After one hour of contact, we checked the fertilization success under a light microscope. Bioassays were carried out according to Coteur et al. (2003). A 15 well plate was used for each metal pollutant (Cd, Pb, Cu and Zn).

Four different increasing concentrations (10 µg/l; 50 µg/l; 100 µg/l; 200 µg/l) and a control solution (FSW), employed as blank, were used. Each well contained 10 ml of each solution, then 250-300 embryos were transferred to each well and incubated for 72 hours at 21 ± 1 °C.

At the end, larval development was stopped by adding neutral formalin (35%) and the percentage of anomalies was determined according to the criteria of Klöckner et al. (1985). Number of malformed eggs/larvae, expressed in percentage, was assessed under the optical microscope by scanning slides containing about 100 eggs each. The number of dead cells was adjusted by the formula: % mortalities corrected = (Po – Pt)/(100 – Pt), where Po is the percentage of mortalities observed and Pt is the percentage of mortalities in controls (Abbott, 1975).

Five replicates were performed for each concentration and each metal. The statistical treatment of experimental data was performed by the probit method (Bliss, 1935), which is useful for experiments with reduced number of animals and particularly suitable for research on marine invertebrates, as confirmed by Bendimerad (2000).

RESULTS

The mean percentages of embryonic abnormalities observed in each heavy metal solution ± standard deviation are shown in Table 1.

As shown in Table 1, concentrations of 10 µg/l and 50 µg/l determined minor negative effects on larval development. At 100 µg/l, the malformation percentage is about double or more (respect to 50

[µg /l] Metals	10	50	100	200
Cd	7.31 ± 1.84	18.26 ± 2.97	79.32 ± 15.38	88.00 ± 14.60
Pb	5.00 ± 1.68	12.20 ± 7.85	22.40 ± 7.97	40.33 ± 4.04
Cu	5.93 ± 1.76	28.93 ± 4.52	41.91 ± 4.13	52.66 ± 24.61
Zn	10.60 ± 2.70	17 ± 3.98	36.73 ± 10.32	40.5 ± 24.02

Table 1. Mean percentages of abnormal embryos ± standard deviation observed in the sea urchin *P. lividus* from Madagh bay (Oran, Algeria) treated with four heavy metal solutions at different concentrations.

µg/l) depending on the metal, in particular, it resulted 79.32% for Cd, 41.91% for Cu, 22.40% for Pb and finally 36.73% for Zn (Figs. 2-5).

Graphs show results we partly expected: the more the concentration increases, the more the percentage of malformations is important, although, surprisingly, percentage of malformations observed after treatment with Cadmium at 200 µg/l is higher (88%) (Fig. 2) than that detected with the other metals: 52.66% (Copper, Fig. 3), 40.33% (Lead, Fig. 4) and 40.50 % (Zinc, Fig. 5).

LC_{50}, calculated according to the method of Bliss (1935), resulted 61.65 µg/l for Cd, 158.48 µg/l for Cu, 389.04 µg/l for Zn and 446.68 µg/l for Pb.

DISCUSSION

Geffard (2001), using Pb solutions at 10 µg/l and 50 µg/l, obtained, as percentages of malformations, 14.8 ± 6.7% and 17.2 ± 3.9%, respectively; whereas, 100% of larvae with abnormalities were observed at 1200 µg/l. The LC_{50} was 482.0 ± 101.0 µg/l. The LC_{50} value we found for Pb is 446.68 µg/l. When comparing the two values, they appear to be very similar.

In a previous study, carried out on the same species and in the same biotope, Dermeche (2010) reported, for Cd and Pb solutions at 10 µg/l, percentages of malformations of 8.33 ± 0.47% and 10.66 ± 0.47%, respectively. At 200 µg/l the percentages passed to 82.33 ± 0.94% and 40.67 ± 0.94% with a LC_{50} of 69.18 µg/l for Cadmium and 436.51 µg/l for Lead. Once again, these values are close to the values discussed in the present paper (61.65 µg/l for Cd and 446.68 µg/l for Pb). Many authors use Zinc and Copper to determine the LC_{50} by using the sea urchin larvae. According to Hall & Golding (1998), Ghirardini et al. (2005) reported that a concentration of 30 µg/l of Copper shows a negligible effect, while it takes a concentration of 50 µg/l to observe the first malformed larvae. These results are consistent with those obtained by His et al. (1999) who observed developmental defects after treatment with a Copper solution at 60 µg/l.

Our study gives a percentage of 28.93 of malformations at a concentration of 50 µg/l, a result that remains consistent with results obtained by different authors. Bougis & Corre (1974) suggested that the effect of Copper varies depending on the quality of brood stock. This would explain different results obtained. It is likely that gametes of poor quality are more sensitive to a toxic agent.

Although echinoderms are capable of removing accumulated contaminants, the residence time in the body and the principal mode of elimination appear to depend on the metal (Warnau et al., 1997; Mannaerts, 2007). According to Basuyaux et al. (2009) and Pétinay et al. (2009), larvae can develop up to a Copper concentration of 90 µg/l but malformations start appearing at 50 µg/l. Copper leads to a significant reduction in growth at 30 µg/l with larvae showing spicules reaching 464 ± 7 microns while normal ones generally are up to 495 ± 9 µm. Bielmyer et al. (2005) noted that abnormalities in larval development and, above all, the stop at pluteus stage were manifested at concentrations ranging from 40 to 80 µg/l.

According to Fernandez & Beiras (2001), Cd causes 100% of arrest of development of the

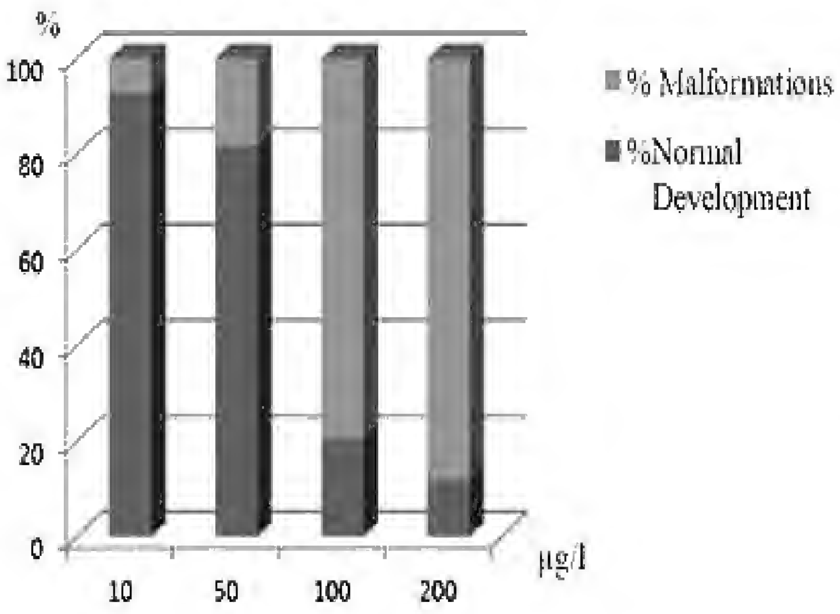

Figure 2. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after tretament with Cadmium solutions.

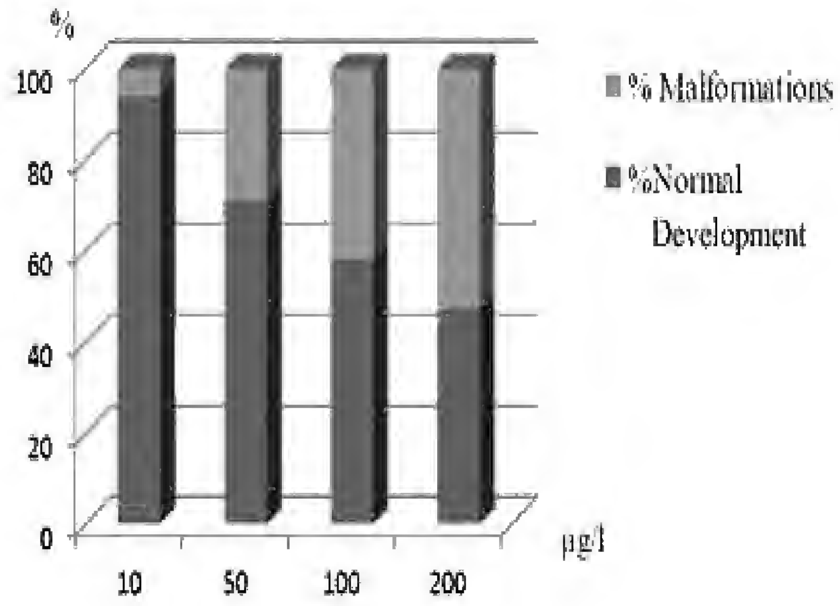

Figure 3. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after tretament with Copper solutions.

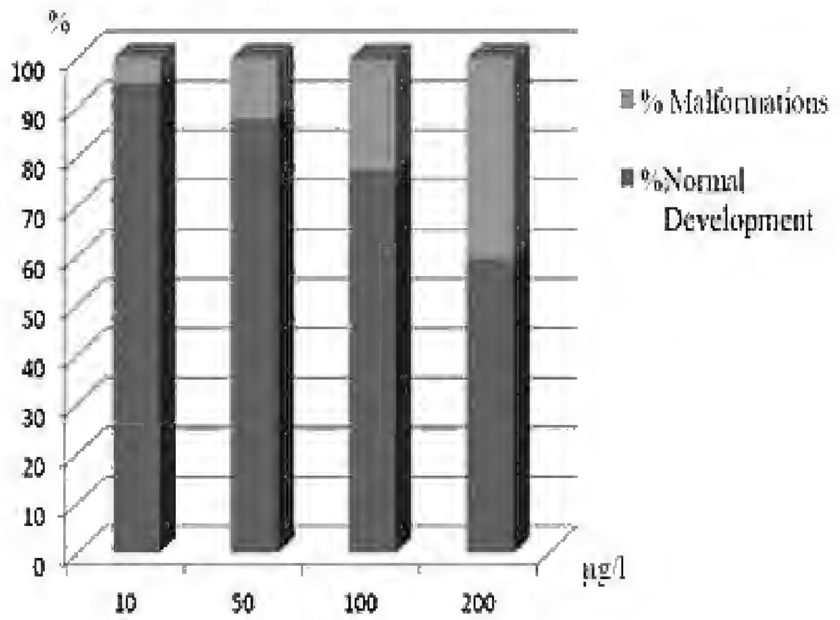

Figure 4. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after treatment with Lead solutions.

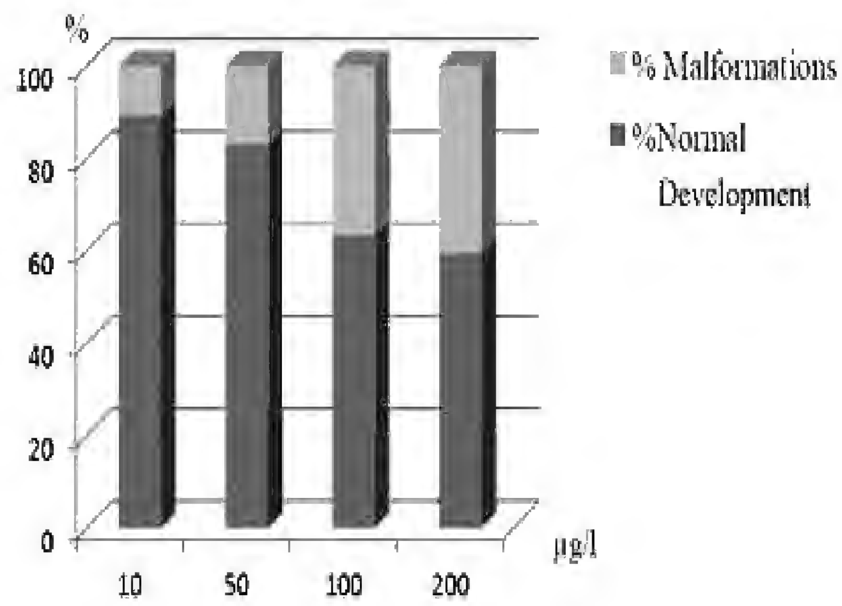

Figure 5. Percentage of deformed and normal larvae of *Paracentrosus lividus* observed after treatment with Zinc solutions.

pluteus at 16 µg/l and the stop at blastula and gastrula stage at concentrations from 32 to 64 µg/l, respectively. Several studies demonstrated sensitivity of sea urchin embryos to heavy metals solutions in the range of 0.01-0.1 mg/l for Hg and Cu, and 0.1-10 mg/l for Cd and Pb (Waterman, 1937; Kobayashi, 1981; Carr, 1996; Warnau et al., 1996).

In a Cu solution at 64 µg/l, embryonic development was arrested at gastrula stage, and at morula stage at 128 µg/l. The toxic effects of Zinc on the larval development of sea urchin were as follows: the highest concentration used, 480 µg/l, completely inhibited the embryonic development; at very low concentrations (7.2 µg/l) no inhibitory effects were observed at first cleavage or at pluteus formation; exogastrula and Apollo-like gastrula were observed at concentrations ranging from 14 to 58 µg/l. At higher concentrations (120 to 240 µg/l), embryonic development and the elevation of the fertilization membrane showed signs of delay and even malformations, as well as polyspermies, permanent blastula, or exogastrula (Kobayashi & Okamura, 2006).

Other metals known to cause exogastrulation in echinoids are: sodium selenite, cobalt chloride, zinc chloride or acetate, nickel, mercury chloride or acetate, the trivalent chromium and manganese chloride (Rulon, 1952; 1956; Timourian, 1968; Timourian & Watchmaker, 1970; Kobayashi, 1971; 1990; Murakami et al., 1976; Pagano et al., 1982;

Species	Cu	Cd	Pb	Zn	References
Paracentrotus lividus	<32 µg/l	> 11 µg/l	----	----	Pagano et al., 1986
	----	----	----	> 33µg/l	Ramachandra et al.,1997
	----	----	0.21 - 0.26 µg /l	----	Warnau & Pagano, 1994
	----	----	482.68 µg /l	----	Geffard, 2001
	158.48 µg/l	61.65 µg/l	446.68 µg/l	389.04 µg/l	present study
Strongylocentrorus purpuratus	6.3 µg/l	----	<9.7 µg/l	----	Dinnel, 1990
	----	0.5 µg/l	----	23 µg/l	Ramachandra et al.,1997
Strongylocentrorus intermedius	----	0.5 to 2.5 µg/l	----	----	Gnezdilova et al., 1985
Arbacia lixula	----	----	----	10-100 µg/l	Castagna et al., 1981

Table 2. Toxicity (LC_{50}) of heavy metal (Cd, Cu, Pb, Zn) solutions at different concentrations for different sea urchin species from the Mediterranean.

Mitsunaga & Yasumasu, 1984; Vaschenko et al., 1994); according to Lallier (1955) and Timourian (1968), skeletal malformations of pluteus were caused by Zinc whereas delay in skeletal development was always caused by Cadmium and Cobalt (Kobayashi, 1990; Mannaerts, 2007).

King & Riddle (2001) showed that exposure of *Sterechinus neumayeri* embryos to various concentrations of Copper caused significant damages to the development at different stages and, particularly, at the stage of blastula; moreover, significant abnormalities were observed at a concentration of 4-5 µg/l. High mortality of embryos was estimated at a concentration of 16 µg/l, and abnormalities were observed at a concentration of 32 µg/l; a Copper concentration of 11.4 µg/l caused 50% of developmental abnormalities after 6 to 8 days of exposure.

Radenac et al. (2001) reported, for Cu solutions at 50 µg/l, about 36.9% of malformations which approximates the results (28.93%) observed, for the same concentration, in this study; however, at 100 µg/l this rate reached 99.80% which exceeds our result (41.91%); 100% of larval malformations were obtained at 250 µg/l while our study showed 52.66% of malformations at 200 µg/l. For Pb solutions, at 250 µg/l, a high percentage (96.6%) of malformation was reported. On the contrary, in our study, at 200 µg/l, we obtained only 40.33% of malformations. These results seem to suggest a different sensitivity of different species to heavy pollutants; concerning Zn, our results are coherent with those of Radenac et al. (2001).

CONCLUSIONS

Considering different stages of development of *P. lividus*, embryos and larvae were found to be the most sensitive and best suited to study heavy metal toxicity. Moreover, they can be used when testing short (embryotoxicity) and long-term (larval growth) issues. Information reported in this study show that responses are indicative of embryo sensitivity. *P. lividus* fulfills the characteristics of a good indicator species as accumulative bio-indicator of the health of a given environment.

Cadmium, Copper, Lead and Zinc are considered among the most toxic and persistent pollutants, with a very long biological half-life (16 to 33 years) leading to accumulation in organs (Guthrie & Perry, 1980). It is therefore an urgent need for further research and adequate scientific-environmental strategies to encourage studies of this type and use the results for the management of harmful polluting sources.

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Biodiversity Journal, 2012, 3 (3): 173-178

Metoncholaimus sp. (Nematoda Oncholaimidae) pseudoparasite of *Mullus surmuletus* (Linnaeus, 1758) (Perciniformes Mullidae) in the western Algerian Sea

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ABSTRACT This study was carried out between October 2009 and July 2010 to determine nematode parasites of the red stripped mullet, *Mullus surmuletus* (Linnaeus, 1758), in the gulf of Oran (western Algeria), located at 35°43' N - 0°37' W. A total of 100 fishes caught from the local fishermen by gill-net were investigated. Our investigation revealed the presence of three nematodes (one female and two males) located in the intestine of two infected fishes, these nematodes were alive and not attached to the mucosa of the fish host. The examination of the nematodes recovered showed that they belong to the genus *Metoncholaimus* Filipjew 1918 (Oncholaimidae Oncholaiminae). These nematodes are free living mostly in the coastal marine sediment; *Mullus surmuletus* might acquire them accidentally while either feeding on them or along with other food items taken from the bottom.

KEY WORDS *Mullus surmuletus*; Nematode; Pseudoparasites; Oran; Western Algeria.

Received 04.05.2012; accepted 26.07.2012; printed 30.09.2012

INTRODUCTION

The striped red mullet *Mullus surmuletus* (Linnaeus, 1758) (Perciniformes Mullidae), a benthic perciform fish with a widely known distribution, is very common in the Algerian coasts and is a commercially important species (Figs. 1-3).

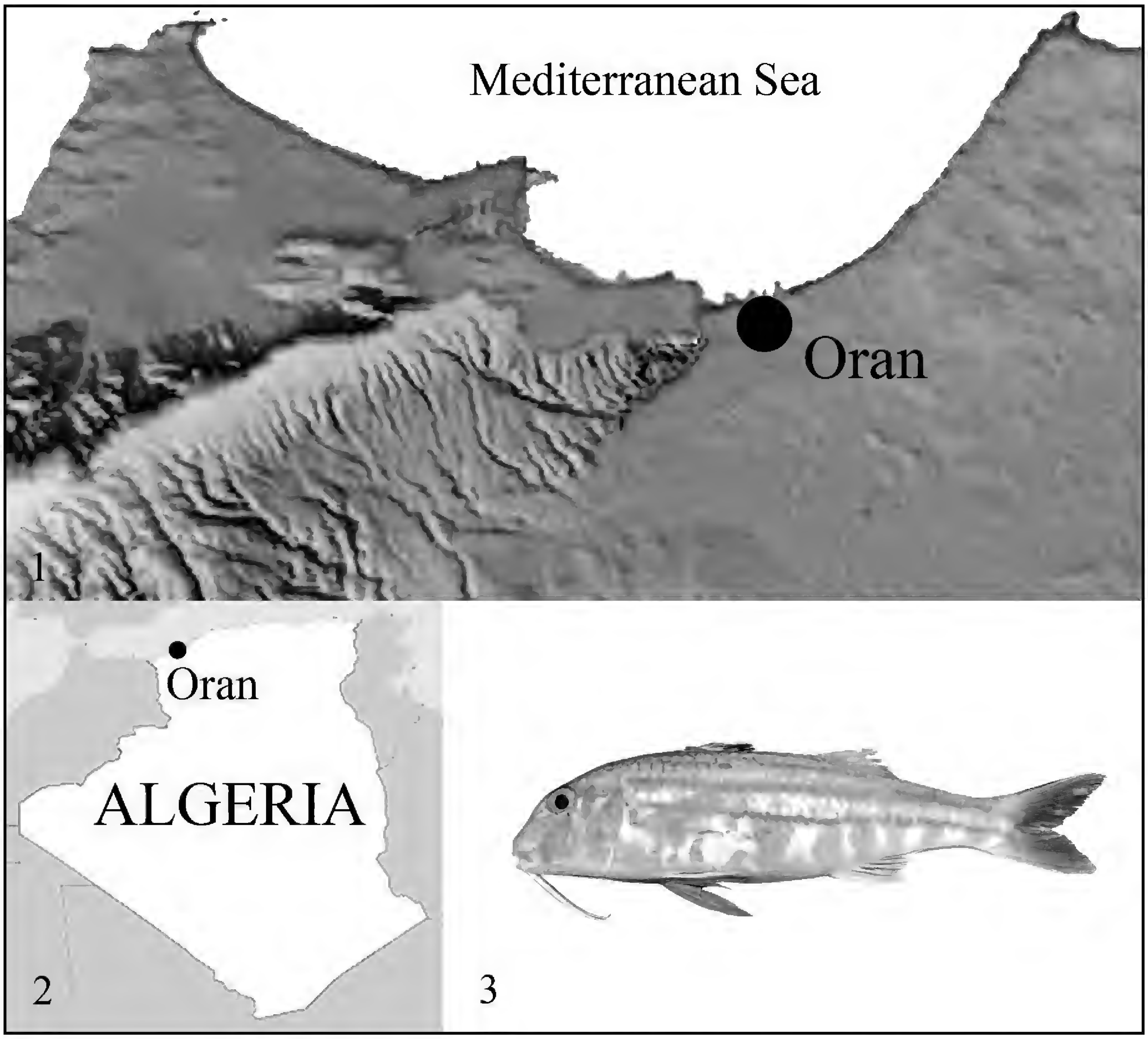
On the other hand, its nematode parasites in the western Mediterranean sea are poorly known although having been studied since more than one century by numerous authors who considered the system Helminthes-*Mullus* as the richest one and the most diversified of the Mediterranean sea (Bayoumy et al., 2008; Ferrer et al., 2005; Neifar et al., 2007; Ternengo et al., 2009). In this regard, we began a helminthological study during which we faced the problem posed by pseudoparasitism by known species of free-living nematodes.

MATERIALS AND METHODS

Fish were collected by means of a trammel net at a mean depth of 15 m, according to the traditional local small-scale fishery techniques. Individual body weight and size (total length), sex, and maturity stage were recorded. The range of fish size (total length) was 10.5-22 cm.

The whole gastro-intestinal tract was removed immediately after capture and all portions (stomach, pyloric caeca and intestine) were opened by a longitudinal incision. Removal of contents was obtained by successive washes with a wash bottle in a Petri dish, the food material collected was examined under a dissecting microscope Zeiss Stemi 2000.

Only helminthes infesting this tract were examined. The parasites were hand sorted and placed ini-



Figures 1, 2. Sampling site: Madagh bay, Oran, Algeria. Figure 3. *Mullus surmuletus* (Linnaeus, 1758).

tially in 2% NaCl saline solution and then stored in 75% ethanol. The nematodes specimens were stained in Acetic-Carmin, dehydrated and mounted in Canada balsam.

The collected nematodes were cleared in glycerin for examination. Drawings were made with the aid of a Camera Lucida connected to a Wild bright field microscope.

For the identification of nematodes, drawings were compared with those of specialists in parasitic nematodes of fishes (Anderson, 1992; Moravec, 1998). Identification of nematode pseudoparasites did require the consultation of specialist works on free-living nematodes (i.e. Hope & Murphy, 1972; Tarjan, 1980; Platt & Warwick, 1983).

RESULTS

Systematic position

Phylum Nematoda Rudolphi, 1808
Class Adenophorea Linstow,1905
Order Enoplida Filipjev, 1929
Family Oncholaimidae Filipjev, 1916
Genus *Metoncholaimus* Filipjew, 1918

Metoncholaimus sp.

Description (Figs. 4-7). Body elongated, somewhat tapering to both cephalic and caudal regions.

Cuticle. Smooth without transverse striations, thick, particularly in the cervical and the caudal regions.

Anterior extremity. The head bears a crown of tubular and non-segmented sensilla; mouth opening is spacious, roughly hexagonal, with narrow membranous margin provided with small papillae; lips are very distinct and developed; buccal capsule large, with well sclerotized walls, sub-terminal next to a simple muscular esophagus very long and somewhat expanded at its posterior part. Nerve ring encircling esophagus anteriorly, excretory pore not located. Outer sensory organs represented by a crown of well developed tubular and non-segmented bristles, never seen beyond the nerve ring.

Caudal region: the tail of males and females is conical, tapered and curved, short bristled mainly located in both preanal regions in both sexes.

Genital equipment, males: testes initiating at a short distance below the end of the muscular esophagus; strong spicules, regularly pairs are arched without individualized capitulum and the distal end is flared into two points. Solid gubernaculum, consisting of two distinct parts, central bulging and curved ventral apophysis. Numerous short bristles (8 to 10) are present in the cloacal opening region.

Genital equipment, females: only one female was found, it was non-gravid with monodelphic uterus. Ovary anteriorly starting below esophagus end. Genital opening situated in posterior end of the body.

DISCUSSION

The examination of stomach contents of *Mullus surmuletus* revealed the presence of several preys difficult to identify because of their advanced state of digestion. Generally, they are mainly composed of fragments of polychaetes, small fish or crustaceans (N'Da, 1992).

The stomach content analysis of *Mullus surmuletus* revealed a wide food range that can explain the observed parasite richness (Klimpel et al., 2008). Only parasites resist to digestion which led us to believe that the three *Metoncholaimus* specimens found alive and intact were nematode parasites of the fish. More precisely, these nematodes are pseudoparasites, i.e. *Mullus surmuletus* may accidentally ingest them from marine sediments, the diet of the latter being composed largely of benthic prey (Quéro & Vayne, 1997) that the mullet harvests from the sediment through its burrowing and tactile barbells (Bougis, 1949).

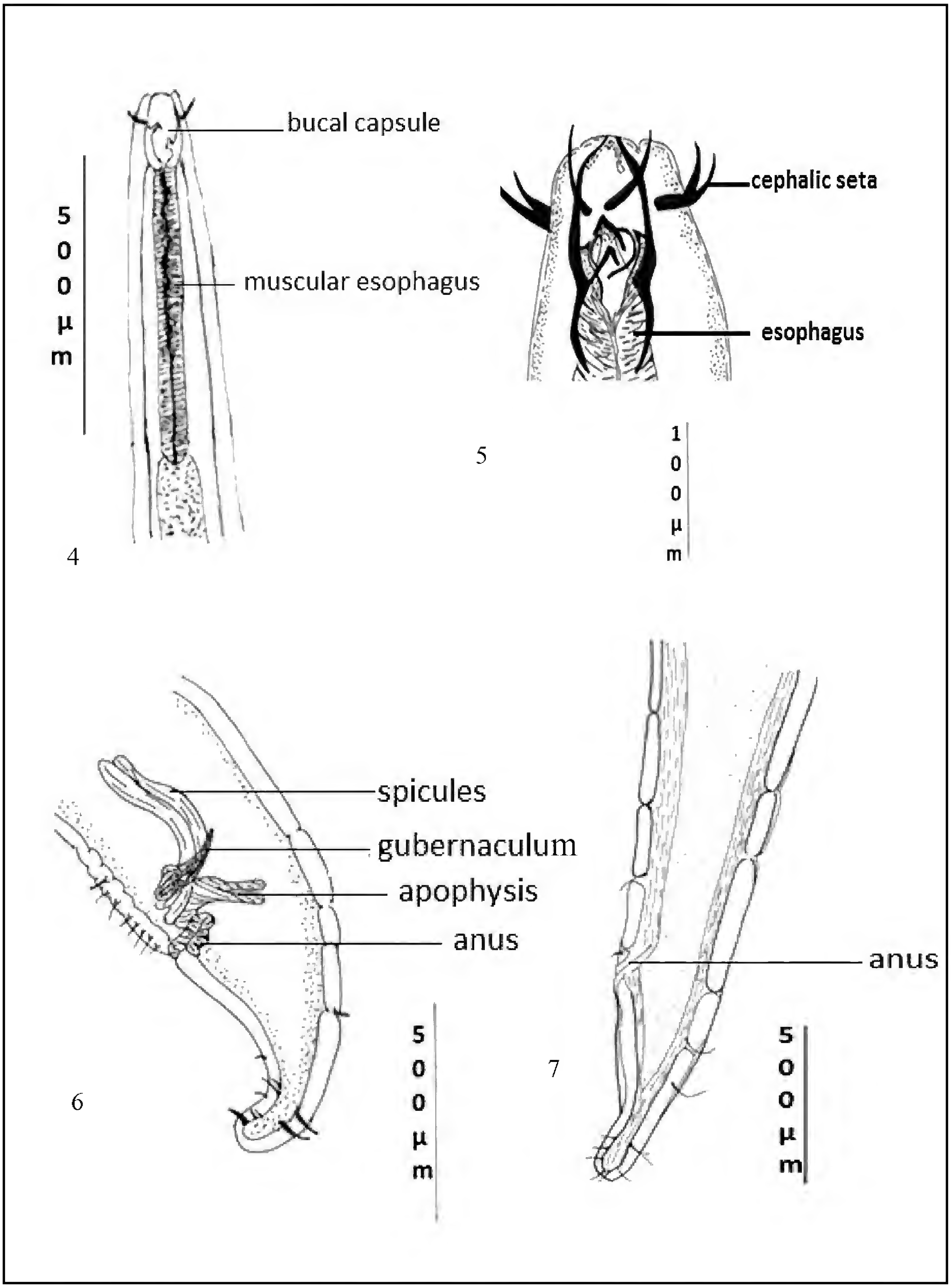
Pseudoparasitism is a fairly rare phenomenon, but was nevertheless described; indeed, pseudoparasites were found alive and in perfect condition in the fish *Haemulon sciurus* (Shaw, 1803) in Brazil. On a total of 50 fish examined, 13 contained nematode pseudoparasites at the rate of 2 to 50 specimens per fish.

These nematodes were identified as *Metoncholaimus amplus* Filipjev, 1918, Oncholaimidae (Moravec et al., 1990) that is a species of free living nematodes described for the first time by Hopper (1967). There are small differences between *Metoncholaimus amplus* specimens which may be considered to be within the limits of intraspecific variability.

This species was recorded in the coast of Florida, Bermuda Islands and also from the Suez canal (Riemann & Rachor, 1972). Comparison of our designs with those of Hopper (1967) did not allow us to assign our specimens to this species, however, we can assign it to the genus *Metoncholaimus* whose identification keys according to Keppner & Tarjan (1989) are:

- Cuticle smooth without transverse striations.
- Non-segmented cephalic setae located in one to three crowns before the nerve ring.
- Simply muscular and cylindrical esophagus without vesicular cells.
- Well-developed buccal capsule.
- Tapered shank short and curved.
- Spicules paired, fronted a gubernaculum.
- Presence or no of ventral apophysis.
- Short caudal setae concentrated primarily on the preanal region of male.

From a comparison of *Metoncholaimus amplus* Hopper, 1967 with our specimens, a few differences came to our attention: (i) *M. amplus* has two crowns of cephalic setae, *Metoncholaimus* sp. just one; (ii) as far as concerns shape and size of the copulatory apparatus, *M. amplus* shows thin and slender spicules and lacks of a ventral apophysis, our specimens - on the contrary- had stronger spicules and a ventral apophysis; (iii) in *M. amplus* the nerve ring of esophagus is situated at the mid-length of esophagus while in *Metoncholaimus* sp. is located more anteriorly.



Figures 4-7. *Metoncholaimus* sp. pseudoparasite of *Mullus surmuletus*. Fig. 4: anterior end; Fig. 5: detail of the anterior end; Fig. 6: posterior end of male; Fig. 7: posterior end of female.

It should be noted that *M. amplus* approximates our specimens particularly by the appearance of the head region, the esophageal structure, the shape of the buccal capsule, the measurements of the body and the implementation of the bristles in the caudal region.

The genus *Metoncholaimus* has twelve species and, in the Mediterranean Sea, is mainly represented by the species *Metoncholaimus pristiurus* Zur Strassen, 1894 (Gerlach & Riemann, 1973), with an abundance of 68.54% of the total population of free-living nematodes (Hedfi et al., 2010).

CONCLUSIONS

It is clear that *Metoncholaimus* sp. is not a real parasite of *Mullus surmuletus*. Indeed, setae on its cephalic and caudal regions indicate a free-living mode of life. Nevertheless, the mode of survival of pseudoparasites into the digestive tract and their resistance to digestive enzymes remain unknown although they are known to be biomonitoring tools in coastal ecosystems due to their extreme sensibility to any environmental stress (Mahmoudi et al., 2002).

Pseudoparasitism by known species of free living nematodes is rare, however it is interesting to report it in order to attract the attention of some ichtyoparasitologists who would easily confuse free living nematodes with parasitic nematodes of fishes because, despite nematodes are the most frequent and the most important parasites of fishes constituting a significant part of the parasite fauna of these hosts in freshwater, brackish-water or even marine environments throughout the world, there is a world-wide shortage of specialists capable of identifying unknown helminthological materials (Moravec, 2007).

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Biodiversity Journal, 2012, 3 (3): 179-188

Biodiversity and conservation of Wildlife at the Wafra area in Kuwait

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ABSTRACT In response to the increasing needs to conserve wildlife and to enhance biodiversity, the Joint Operations-Wafra (JO-Wafra) protected their natural environment by fencing and dedicating areas for conservation of biological diversity. The main objective of this study is to conduct a wildlife baseline assessment in the oilfields of JO-Wafra and to identify potential habitats of endangered or threatened species that could occur on site. The wildlife survey covered the winter and early spring seasons. Although short and insufficient to provide a detailed assessment, the field data collected indicated significant differences in the number of individuals and wildlife fauna species within the fenced and unfenced oilfields. It also showed that the fenced JO-Wafra has rich and diverse wildlife fauna species, an indication of ecological health. In addition to JO-Wafra oilfield, it is recommended to protect the South Umm Guddair (SUG) oilfields from livestock grazing and wildlife hunters. The protected area could, therefore, increase wildlife habitats and might harbor some endangered wildlife species. It is also recommended to connect the two oilfields with native shrubs and trees planted along the road, to serve as "green corridor", shelter and additional source of food for the animals of both oilfields.

KEY WORDS Endangered wildlife; Oil fields; Wildlife fauna; Wildlife habitat; Wildlife monitoring.

Received 07.06.2012; accepted 02.08.2012; printed 30.09.2012

INTRODUCTION

Biological diversity refers to the variety of life forms including the genes they contain, and the ecosystems they form.

There are three different levels of biodiversity: genetic diversity which refers to the variety of genetic material contained in all the individuals, species diversity which refers to the variety of living species and ecosystem diversity which refers to the variety of habitats and ecological processes. In other words, it is reflected by the variety of all forms of life on earth, which provides the building blocks for human existence.

The total number of species (defined as a population of organisms which are able to interbreed freely under natural conditions) is estimated to range from 5 million to 100 million globally; though less than 1.7 million have actually been described (BUDEST, 1993; FEPA, 2003; Maffi, 2005; Sarkar, 2006).

Biodiversity conservation is of a major importance internationally because humans derive their food, medicines and industrial products from biological diversity of the wild and its domesticated components. It also gives future generations the opportunity to enjoy nature. In addition, biodiversity is important for the recycling of essential elements, such as carbon, oxygen, and nitrogen.

It is also responsible for mitigating pollution, protecting watersheds and combating soil erosion; accordingly, experiencing and increasing our knowledge about biodiversity transforms our values and beliefs (McGregor, 1994; FEPA, 2003; Borokini et al., 2010).

The state of Kuwait covers an area of about 17600 Km^2 at the north-eastern corner of the Arabian peninsula, between 28° 30' N and 30° 05' N in latitude and between 46° 33' E and 48° 35' E longitude. Kuwait's environment is characterized by scarcity of rainfall (100 mm/yr) and extreme temperature variations throughout the year.

The summer temperature is very hot (over 40-50°C), while the winter is cool to mild with a mean temperature of 12.7°C. The water resources are very limited in Kuwait. There is no fresh surface water supply and very limited renewable groundwater. The adverse climate conditions of the state of Kuwait affected its biological diversity and environmental ecosystems (Omar et al., 2001). The desert of Kuwait has long been known as an important source of food, livestock grazing and wildlife hunting. Desert plants were used for fuel and medication purposes.

Urbanization, rapid increase in population, overgrazing, recreation usage, environmental factors, and destruction due to Iraqi invasion in 1990 are main contributing factors to the ecological degradation of the country (McGregor, 1994; Omar, 2000; Selby, 2005). Biodiversity conservation has become one of the challenging priorities for many countries, including the state of Kuwait, to combat species extinction. The State of Kuwait ratified the International Convention on Biodiversity and the National Strategy for Biodiversity Conservation was adopted in order to conserve and enhance biological diversity in the country. Numerous wildlife research studies were conducted in the State of Kuwait. From all these studies, information on flora and fauna of Kuwait has been collected.

A list of desert animals has been prepared. Also, threatened desert animals and those to be extinct were identified such as Houbara Bustard (*Chlamydotis undulata macquennii*), Desert Monitor (*Varanus griseus*), Ostrich (*Struthio camelus*) and Arabian Oryx (*Oryx leucoryx*) (KISR., 1999; Delima et al., 2005; Zaman et al., 2005).

In December 1922, a partitioned neutral zone (PNZ) was established by agreement between the Kingdom of Saudi Arabia and the State of Kuwait, in order to allow tribesmen from both countries to use this favorite grazing ground (Chichester, 2000).

In 1938-1940, oil was discovered from the Al-Burgan area, near PNZ. In 1948, a 60 year concession was granted by the Kuwait government to the Aminoil, a small group of oil companies to explore and exploit the Kuwait side of the PNZ. Similarly in 1949, the Getty Oil Company (formerly Pacific Western Oil Corporation) gained grants from the Kingdom of Saudi Arabia (KSA) to explore the PNZ (KSA side).

The Joint Operations (JO) was born in 1960 when the two oil companies formed a joint committee to oversee and supervise their operations with the resultant productions divided equally to both parties: the Kuwait Oil Company (KOC), which operates the Kuwaiti concession and the Texaco Incorporated, Saudi Arabian Texaco (SAT) that operates the Saudi Arabian concession.

The 3,600 Km^2 partitioned neutral zone oilfields were not spared during the Iraqi invasion of Kuwait in 1990. The oil wells were destroyed and burned contributing to the environmental catastrophe man had ever known. In 1999-2002, the perimeter fence around the JO-Wafra main oilfield was constructed to prevent livestock grazing and wildlife hunters from the area.

This resulted to the gradual rehabilitation of the flora and fauna in the oilfield. Literature on the wildlife fauna of the Wafra area is very limited. Example is the "Insect Fauna of Kuwait" by Al-Houty (1989), when some insect samples were collected in the Wafra area.

An environmental impact review prepared by Chichester (2000) described the fauna of the upland deserts and sabkha of the Partitioned Neutral Zone (PNZ) as "Over 220 species of birds have been observed in the PNZ in recent years… fauna of the upland deserts and sabkha includes common insects; ants and beetles; lizards and snakes; such as Sand Boa, Rat Snake, Blue-throated Agamid, Desert Monitor, and Dhub. Small, nocturnal mammals include Jerboa, Jirds, Desert Fox, and Long-eared Hedgehog".

The current study involves fauna baseline assessment within the JO-Wafra territories with the following main objectives: assess the wildlife fauna in JO-Wafra main area; and compare the quality of habitat inside and outside the JO-Wafra main area. The work has been implemented between Kuwait Institute for Scientific Research (KISR) and Kuwait Gulf Oil Company (KGOC).

MATERIALS AND METHODS

The wildlife fauna study commenced in December 2005 and terminated in March 2006. This report covers only the study period (i.e. from January to March, 2006), wherein 15 field data collection exercises were performed with a total of 345 field data collected from 11 selected wildlife (fauna) study sites, within the fenced JO-Wafra main oilfield and the unfenced SUG (South Umm Guddair) oilfield. Selections of wildlife study sites at the JO-Wafra oilfield were performed during reconnaissance surveys. The criteria used in selecting the possible wildlife study sites were: (1) type of habitat that include soil and vegetation cover; and (2) location within the oilfield, disturbed or undisturbed (see Table 1).

Several wildlife survey methodologies were implemented to study the wildlife biodiversity, na-

Types of habitat	Wildlife study sites number
1.Habitat with good vegetation cover	2, 3, 4, 8, 9 and 10
2.Habitat with poor vegeta tion cover	1 and 5
3.Windblown sand covered habitat	6 and 7
4.Overgrazed and unprotec ted habitat	11

Table 1. Different Wildlife study sites according to types of habitat.

mely: 1) Line Transects (LT): of 5 km to record animals within a specific habitat type. 2) Pitfall Trapping (PFT): to catch ground crawling animals such as reptiles and invertebrates. 3) Baited Mammal Trap Line: is usually a one kilometer long trap line. The large mammal trap (MTL) is placed between two small mammal traps (MTS) at a distance of 250 meters. 4) Mark-Release-Recapture (MRR): to estimate the population dynamics of an area. MRR models were developed for field studies in which the count statistics are numbers of marked and unmarked animals caught (Nichols, 1992; Grenwood, 2000).

RESULTS

From December 2005 to March 2006, 17 trips were made to the JO-Wafra main (fenced) and the unfenced South Umm Guddair (SUG) oilfields. A total of 15 field data collecting exercises were performed. This included line transects, baited mammal trappings and pitfall trappings, conducted over at least three consecutive days (Table 2; Figs. 1-2).

Line Transects (LT): a total of 49 LT exercises were performed, covering the winter and early spring periods of the country. It was noticeable that during the last days of line transect exercises, more birds were observed and even the shy Red fox (*Vulpes vulpes*) was recorded. This may be due to the rise in temperature and the pleasant spring weather in the air. More than 34 species of wildlife fauna were recorded from the line transects performed.

These included 27 species of birds, one species of mammal, one species of reptile and more than six species of invertebrates (butterflies, dragonflies, flies and ground hoppers). Table 3 lists the species recorded during line transect exercises at the JO-Wafra oilfields. The list is not conclusive, as it was taken during winter and early spring seasons.

No. of Field Trips	Type of Trips	Location of Trips
2	Field orientations, reconnaissance, site selections and installation of field study equipment e.g., mammal traps and pitfall traps with drift fences	JO-Wafra main and SUG oilfield (south Um Guddair)
15	Field data collection resulting to the following field data collected: 49 from line transects (LT) 148 from two (x2) small mammal traps at each study site 74 from one (x1) large mammal trapping at each study site 74 from pitfall traps in association with drift fences (1x5) at each study site	11 wildlife (fauna) study sites within the fenced JO-Wafra and the unfenced SUG oilfields

Table 2. Trips made by Wildlife Survey Team to JO-Wafra Oilfields.

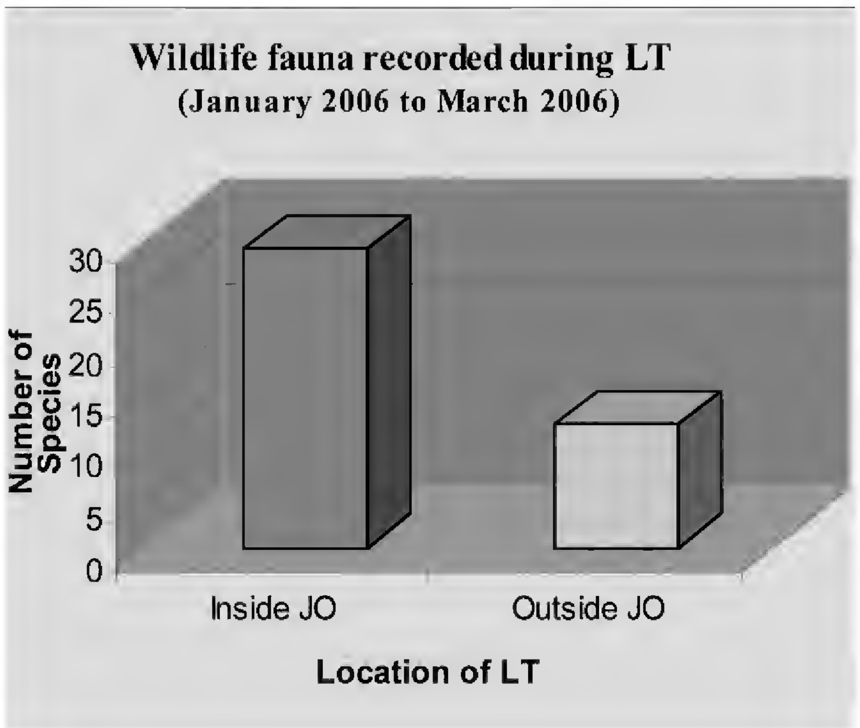

Figure 1. Wildlife species recorded during line transect inside and outside JO-Wafra oilfields

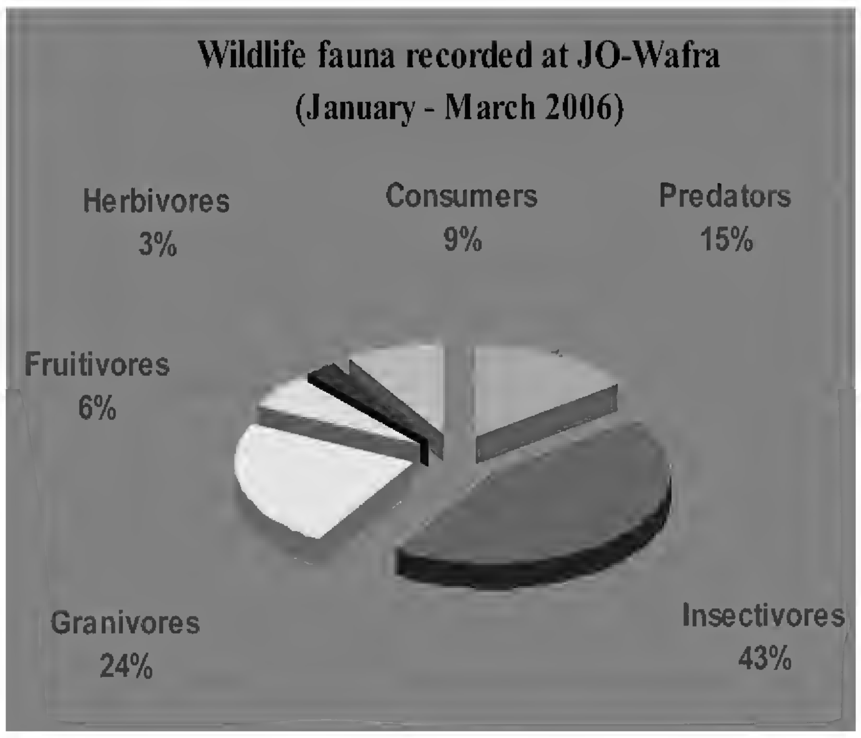

Figure 2. Different trophic levels in the wildlife fauna recorded at JO-Wafra oilfields from January to March 2006.

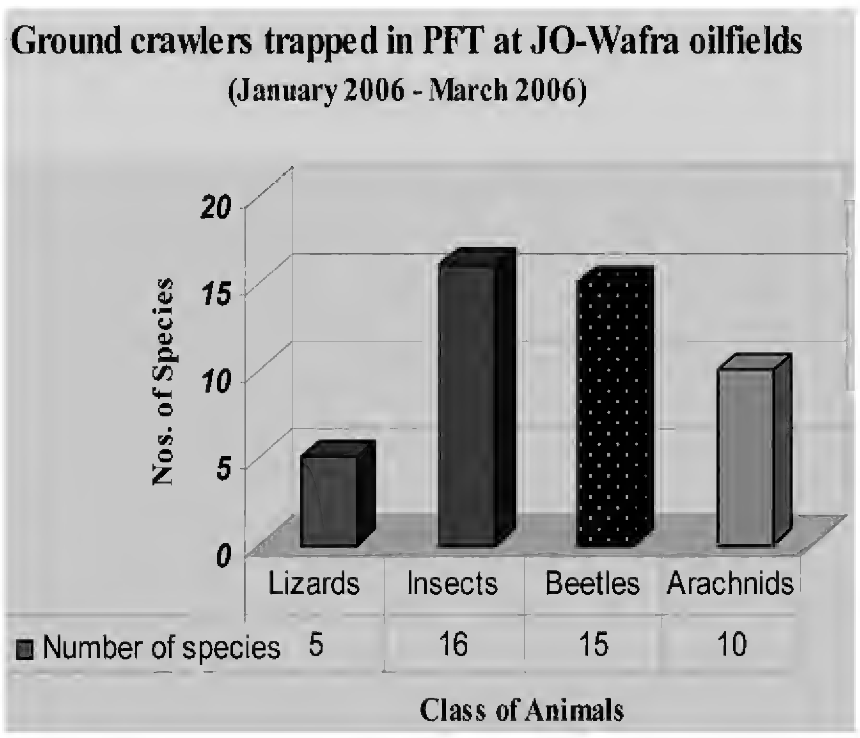

Figure 3. Classes of animal species trapped in PFT at JO-Wafra oilfields.

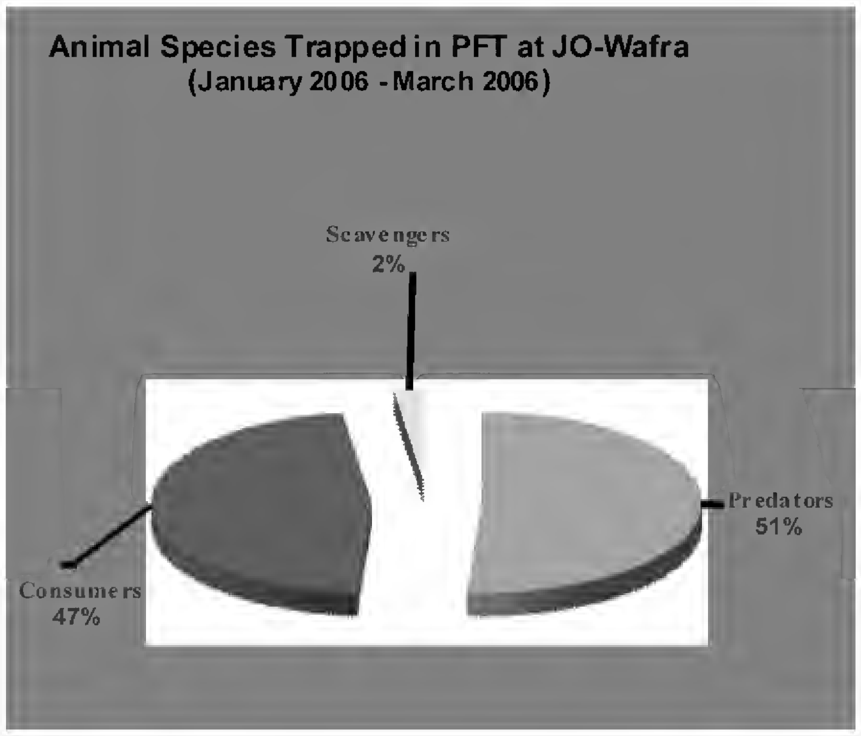

Figure 4. Animals recorded from PFT at JO-Wafra are shown according to their trophic levels.

More animal species are expected to be recorded if the survey covers the four seasons, especially the two migration periods of the country. Except for the residents, such as the Black-crowned finch lark, Crested lark, House sparrow and the Feral pigeon, the birds might only be over wintering in the country e.g., Tawny pipit, Short-eared owl, Blue rock thrush, Pied wheatear, Woodchat shrike, Great grey shrike, and Hoopoe lark among others.

The list is only 7.7% from the total number of bird species recorded in the country. The Black-crowned finch larks (*Eremopterix nigriceps*) were recorded breeding at study area, while both the Crested larks (*Galerida cristata*) and the Isabelline wheatears (*Oenanthe isabellina*) were also observed displaying courting behaviors.

Pitfall trappings: there were 64 pitfall trapping exercises performed at the fenced main JO-Wafra and the unfenced SUG oilfields. More than 46 animal species were recorded during these exercises, including 10 species of arachnids; lizards (5 species); beetles (15 species); and 16 species of insects (Figs. 3-4 and Table 4). Baited mammal trappings: there were seven (x7) mammal trapping exercises performed at the JO-Wafra study sites, for the duration of the study period.

		Study Site				
SN	**Animal Species**	**G**	**SLT**	**NLT**	**G**	**SUG**
1	Black-crowned Finch Lark, *Eremopterix nigriceps* (Gould, 1839)	x	x	x		
2	Barn swallow, *Hirundo rustica* (Linnaeus, 1758)				x	x
3	Blue Rock-Thrush, *Monticola solitaries* (Linnaeus, 1758)	x	x			
4	Lepidoptera sp. 1	x	x	x		
5	Chiffchaff, *Phylloscopus collybita* (Vieillot, 1817)			x		
6	Cream-coloured courser, *Cursorius cursor* (Latham, 1787)	x				
7	Crested lark, *Galerida cristata* (Linnaeus, 1758)	x	x	x	x	x
8	Arabian babbler, *Turdoides squamiceps* (Cretzschmar, 1827)	x	x	x		
9	Desert wheatear, *Oenanthe deserti* (Temminck, 1825)	x	x	x		
10	Dhub, *Uromastyx microlepis* (Blanford, 1874)	x	x	x		
11	European roller, *Coracias garrulous* (Linnaeus, 1758)	x	x	x		
12	Feral pigeon, *Columba livia* (J.F. Gmelin, 1789)	x	x	x	x	x
13	Great grey shrike, *Lanius excubitor* (Linnaeus, 1758)	x	x	x	x	x
14	Ground hopper, *Tetrix undulata* (Sowerby, 1806)	x				
15	Hoopoe, *Upupa epops* (Linnaeus, 1758)	x				
16	Greater Hoopoe lark, *Alaemon alaudipes* (Desfontaines, 1789)	x	x	x		
17	House sparrow, *Passer domesticus* (Linnaeus, 1758)	x	x	x		
18	Isabelline wheatear, *Oenanthe isabellina* (Temminck, 1829)	x	x	x	x	x
19	Kestrel, *Falco tinnunculus* (C.L. Brehm, 1855)	x	x	x		
20	Northern wheatear, *Oenanthe oenanthe* (Linnaeus, 1758)	x	x	x		x
21	Olivaceous warbler, *Iduna pallida* (Hemprich et Ehrenberg, 1833)	x	x	x		
22	Pallid harrier, *Circus macrourus* (S.G. Gmelin, 1770)	x	x	x		
23	Pied wheatear, *Oenanthe pleschanka* (Lepechin, 1770)	x	x	x	x	x
24	Red-backed shrike, *Lanius collurio* (Linnaeus, 1758)	x	x	x	x	x
25	Red fox, *Amphicoma vulpes vulpes* (Fabricius, 1792)	x				
26	Sand martin, *Riparia riparia* (Linnaeus, 1758)					x
27	Short-eared owl, *Asio flammeus* (Pontoppidan, 1763)				x	x
28	Short-toed eagle, *Circaetus gallicus* (Gmelin, 1788)	x	x	x		
29	Short-toed lark, *Calandrella brachydactylal longipennis* (Eversmann, 1848)	x	x	x		
30	Stable flies, *Stomoxys calcitrans* (Linnaeus, 1758)	x	x	x	x	x
31	Sundevall jird, *Meriones crassus* (Sundevall, 1842)			x		
32	Swift, *Apus barbatus* (Sclater, 1866)		x			x
33	Tawny pipit, *Anthus campestris* (Linnaeus, 1758)	x	x	x		
34	Turtle dove, *Steptopelia turtur* (Linnaeus, 1758)	x				

Table 3. Animal species recorded during line transect at the JO-Wafra Oilfield. Legend: G=General line transect. SLT=South LT. NLT=North LT. SUG= Unfenced JO-Wafra oilfield LT.

		Study Site										
SN	**Animal species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	Arabian darkling beetle, *Pemelia arabica* (Kaszab 1982)	x	x	x	x	x	x	x	x	x	x	x
2	Brilliant ground weevil, *Bembidion* sp.		x					x			x	
3	Camel spider, *Galeodis arabs* (Koch, 1842)	x	x	x	x	x	x	x	x	x	x	x
4	Capsid bug, *Eurydema ornatum* (Linnaeus, 1758)							x				
5	Centipede, *Craterostigma* sp.	x			x							
6	Churchyard beetle, *Blaps kollari* (Seidlitz G von, 1896)								x			x
7	Desert runner, *Cataglyphis niger* (Andre, 1981)	x	x	x	x	x	x		x			
8	Domino beetle, *Anthia duodecimguttata* (Bonelli, 1813)	x	x	x	x	x	x	x	x	x	x	x
9	Elevated stalker, *Adesmia stoeckleini* (Koch, 1940)	x	x	x	x	x	x	x	x	x	x	x
10	Giant black ant, *Camponotus xerxes* (Forel, 1904)	x	x	x	x	x	x	x	x	x	x	
11	Golden-tipped ant, *Camponotus sericeus* (Forel, 1904)	x	x	x	x	x	x	x	x	x	x	
12	Ground mantis, *Eremiaphila braueri* (Krauss, 1902)											x
13	Hairy capsid bug, *Tropinota squalida* (Scopoli, 1763)											x
14	Joker bee, *Parachistus pulchellus* (Greathead, 1980)					x				x		
15	Jumping spider, Salticidae			x			x		x			
16	Lesser scarab, *Mnematium* sp.		x	x	x				x			
17	Lesser yellow scorpion, *Uroplectes alstoni* (Purcell, 1901)				x							
18	Long-legged spider, Pholcidae	x	x	x	x	x	x	x	x	x	x	x
19	Mesopotamian beetle, *Sepidium mesopotamicum* (R.,1904)				x	x		x	x			
20	Mosquito, *Anopheles pharoensis* (Theobald, 1901)											x
21	Opossum beetle, *Mesostena puncticollis* (Solier, 1835)	x	x	x	x	x	x	x	x	x	x	x
22	Orb-weaver spider, Araneidae	x	x	x	x	x	x	x	x	x	x	
23	Pill bug, *Armadillidium vulgare* (Latreille, 1804)		x	x			x				x	
24	Pinstripped ground weevil, *Ammocleonus aschabadensis* (Ft.,1884)	x	x		x							
25	Pitted beetle, *Adesmia cancellata* (Klug, 1830)	x	x	x	x	x	x	x	x	x	x	
26	Meloe "Queen", *Meloe omanicus* (Kaszab, 1983)		x	x							x	
27	Rack beetle, *Tentyrina palmeri* (Crotch, 1872)	x	x	x	x	x	x	x	x	x	x	x
28	Rock gecko, *Bunopus tuberculatus* (Blanford, 1874)			x		x	x	x	x	x	x	
29	Sand gecko, *Stenodactylus doriae* (Blanford, 1874)										x	
30	Saber-toothed beetle, *Scarites guineensis* (Dejean, 1831)				x							
31	Scorpion (Black), *Androctonus crassicauda* (Olivier, 1807)							x				x
32	Seville row beetle, *Paraplatyope arabica* (Koch, 1965)	x										
33	Short-nosed lizard, *Mesalina brevirostris* (Blanford, 1874)			x	x	x	x	x	x	x	x	
34	Silverfish, *Thermobia domestica* (Packard, 1837)	x	x				x	x	x			
35	Small black ant, *Monomorium gracillimum* (Smith, 1861)	x	x	x	x	x	x	x	x	x	x	x

SN	Animal species	Study Site										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
36	Small red ant, *Monomorium pharaonis* (Linnaeus, 1758)	x	x	x	x	x	x	x	x	x	x	x
37	Scutte lizard, *Acanthodactylus scutellatus* (Audouin, 1827)			x	x	x	x	x	x	x	x	
38	Schmidt lizard, *Acanthodactylus schmidti* (Haas, 1957)				x	x			x			
39	Tiger moth, *Utetheisa pulchella* (Linnaeus, 1758)		x									
40	Ugly trox, *Scleron sulcatum* (Kulzer, 1956)							x				
41	Variable stalker, *Adesmia cothurnata* (Forskal, 1775)	x	x	x	x	x	x	x	x	x	x	x
42	Winged ant, *Podalonia* sp.							x				
43	Wolf spider, Lycosidae							x		x		
44	unidentified moth		x	x					x			
45	unidentified larva	x		x	x							
46	Lepidoptera sp. 2				x				x			

Table 4. List of animal species trapped at different PFT in JO-Wafra Oilfields.

The twenty two (x22) MTS trapped 15 rodents from one species (*Meriones crassus*). Whereas the collapsible Tomahawk traps (MTL) trapped one species (feral dog). There were seven rodent recaptures and three of the feral dog, which is 'trap happy' because it was recaptured every trapping exercises. Table 5 shows the animal species trapped and caught during mammal trapping exercises at the JO-Wafra oilfields.

Mark-release-recapture (MRR): the field data collection exercises were performed during the winter, when the temperature ranged from 3^0C to 8^0C and during early spring, when the temperatures started to rise (14^0C to 18^0C). Therefore, the field data collected is not representative of the entire population of each study site but indicative only for the duration of the study period.

There were 32 trapping exercises performed at the JO-Wafra oilfields. This includes eight exercises each for the PFT; baited MTS1; MTS2; and MTL. These trapping exercises caught a total of 74 wildlife fauna (including recaptures) from different study sites at JO-Wafra. Five (5) species of lizards were caught in the PFT, namely: *Mesalina brevirostris* (27 individuals); *Acanthodactylus scutellatus* (x8); *A. schmidtii* (x2); and 22 geckos (*Stenodactylus slevini* and *Bunopus tuberculatus*).

Nine recaptures were recorded for the lizards during pitfall trappings; the first three species of the above-mentioned lizards were recorded to be active in winter, while the two species of geckos were trapped only when the ambient temperature at the study sites were higher at around 20^0C.

The fringed-toed lizards i.e. *A. scutellatus* and *A. schmidtii* seemed to be concentrated only at the low lying soot covered habitat, with good vegetation cover. The two species of geckos: *S. slevini* and *B. tuberculatus* were trapped in the PFT only at the start of spring, when the temperatures at the study sites were higher. These species were recorded during the last two exercises. Two (2) species of mammals (*Meriones crassus* and *Canis domesticus*) were trapped during baited mammal trapping exercises.

There were 19 Sundevall jirds (*Meriones crassus*) captured by the small mammal traps from eight study sites within the JO-Wafra main oilfield. Seven re-captures were recorded.

DISCUSSION AND CONCLUSIONS

The current study was conducted to assess the wildlife fauna in JO-Wafra main area; and to compare the quality of habitat inside and outside the JO-Wafra main area. The work has been implemented between Kuwait Institute for Scientific Research (KISR) and Kuwait Gulf Oil Company (KGOC).

Seventeen field visits to the JO-Wafra oilfields were undertaken by the wildlife team, to perform 15 field data collection exercises that resulted in 444 field data collected. The oil fires that occurred during the Iraqi invasion of Kuwait were the main cause of diversity loss in the country. It had significant effect on species and ecosystem, this effect shifted species distribution and caused reductions in population size that could be due to reduction in survival and fecundate rates.

This negative effect is well documented in other studies that investigated similar items (Da Fonseca et al., 2005; Parmesan, 2006; Fischlin et al., 2007). Conserving wildlife and biodiversity is increasingly recognized as an essential element of life. Its importance involves production of plants and animals for food, providing recreational resources, flood and pest control, providing chemicals for treatments. Accordingly, biodiversity conservation is strongly related to finance, economy and poverty degree in a society.

Petts & Platt (1990) demonstrated that most of the benefits derived from wildlife and biodiversity conservation are potentially quantifiable and can significantly add to the economy of a society. Many worldwide studies in the literature relate biodiversity to social poverty (Adams et al., 2004; Treves et al., 2005; Fisher & Christopher, 2007). Consequently, wildlife and biodiversity conservation became a priority in the world.

The approach of protecting natural resources and increasing extent of protected areas has been described and used in other worldwide studies to conserve wildlife and biodiversity (McNeely & Schutyser, 2003; UNEP, 2006). In addition to resource protection, the current study also applied the technique of monitoring wildlife populations. It involves collecting, analyzing, and interpreting ecosystem information. Such techniques develop wildlife and natural resource management approaches. Monitoring wildlife and ecosystems provide information that managers and companies such as Kuwait Oil Company can use to adjust or modify their commercial activities so that they minimize negative effect on natural resources. These techniques have been used worldwide to conserve wildlife and biodiversity (Adger et al., 2003; Fischlin et al., 2007).

The field data collected showed that the fenced JO-Wafra main oilfield has rich and diverse wildlife fauna species, which suggests that ecological health in this area is significantly better than the unfenced and unprotected SUG oilfields.

Although short and insufficient to provide a detailed assessment of the studied areas, the field data collected showed that the fenced JO-Wafra main area is rich and diverse in wildlife fauna, indicating significant ecological health compared to the unfenced and overgrazed South Umm Guddair oilfield, which is located approximately 29 kilometers northwest of the main oilfield. The field data collected also indicated that additional surveys and monitoring activities for the wildlife at the JO oilfields are necessary and conducted to cover the different climatic seasons and migration periods of the country.

The field data collected showed that there are at least four types of wildlife habitats at the JO-Wafra oilfields: high and low lying habitat with good vegetation cover; high and low lying habitat with poor vegetation cover; windblown sand covered habitat; and over-grazed and unprotected habitat. The first three types of habitats are located inside the fenced oilfield, while the latter is at the unfenced South Umm Guddair (SUG) oilfield. Because of the perimeter fence constructed in 2000, the JO-Wafra main oilfield enjoyed protection from livestock grazing and wildlife hunters.

This has brought to the gradual rehabilitation of the flora and fauna within the perimeter fence. There were more than 78 wildlife fauna species recorded at the JO-Wafra main oilfield. This includes the 19 species (24.36%) of wildlife fauna recorded at the unfenced SUG oilfield. The timing of the field data collection might have impact on the numbers of individuals and fauna species recorded from both study areas because desert animals tend to hibernate during winter and only come out during spring. It is suggested and recommended that the SUG oilfield be fenced and protected from livestock overgrazing and wildlife hunters. The two oilfields then could be connected with native shrubs and trees planted along the road.

The fenced SUG and the additional plants will augment and increase the possible areas for resting, feeding and even breeding of some threatened and endangered migrating fauna that pass through the country during their migration movements (examples are the Houbara bustard, *Chla-*

mydotis undulata macquennii and the Imperial eagle, *Aquila heliaca*). The Houbara bustard because of the size of its habitat requirement may become the "umbrella" species in the wildlife conservation program. In other words, the presence of Houbara bustard bird in any habitat reflects richness in biodiversity in that habitat (Gregory, 2005). Accordingly, it is important that wildlife conservation programs are oriented specifically at particular species of most concern such as Houbara bustard. Such programs should be implemented based on regional, national and international scope (Young, 1997; Mawdsley et al., 2009).

The weather during the field data collection exercises (occurrences of rains and low temperature) might have impact on the animals' availability. Ectothermic (cold blooded) animals tend to hibernate during cold weather (winter) and come out only from hibernation when the temperature is favorable (spring). This could be true because the Sand gecko (*Stenodactylus slevini*) and the Rock gecko (*Bunopus tuberculatus*) were trapped only during the PFT trapping exercises in March, when the temperature in the oilfields had risen to above 20^{0}C, whereas most of the beetles, ants and the fringe-toed lizards, such as *Acanthodactylus scutellatus*; *A. schmidtii*; and *Mesalina brevirostris* were trapped when the temperatures in the field ranged from 3^{0} to 8^{0}C.

The large percentage of predators (51%) of animals recorded from the PFT indicated good supply of prey or food resources i.e. consumers 47% and scavengers 2%. The low numbers of captures in the baited mammal trappings were expected because of the timing of the field exercises, winter. Desert animals tend to hibernate during winter and come out during spring and summer (examples are hedgehog, gerbil, jerboa, etc.). More animal species are expected to be recorded if the survey was to cover the four seasons, especially the two distinct migration periods of Kuwait.

Expanding the survey period and applying more conservation strategies and programs is recommended in future work. No conservation program or strategy is optimal, some strategies have to be oriented to a specific target. Development of a set of strategies or approaches that complement each other is sometimes important to create useful conservation tools and to fulfill requirements needed for an appropriate wildlife conservation approach.

ACKNOWLEDGEMENTS

The authors would like to thank Joint Operation management for facilitating the research assessment in Al Wafra fenced area. Thanks are also extended to KISR staff for their effort in executing field work.

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Biodiversity Journal, 2012, 3 (3): 189-190

On the presence of the Caddisflie *Stenopsyche siamensis* Martynov, 1931 from Central Thailand (Trichoptera Stenopsychidae)

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ABSTRACT In the present paper, caddisflie larvae and pupae of *Stenopsyche siamensis* Martynov, 1931 (Trichoptera Stenopsychidae) are recorded from upstream of Maewong Basin, Central Thailand.

KEY WORDS Caddisflies; *Stenopsyche siamensis*; Stenopsychidae; Trichoptera.

Received 08.07.2012; accepted 26.07.2012; printed 30.09.2012

INTRODUCTION

Caddisflies are aquatic insects of the order Trichoptera Kirby, 1813. This order of aquatic insects is a very large one comprising more than 10,000 species in the world (Sangpradub & BoonSoong, 2006).

In tropical Asia, Caddisflies include about 28 families (Dudgeon, 1999) and, more particularly, 491 species were recorded in Thailand (Malicky & Chantaramongkol, 1999). In natural freshwater ecosystems Caddisflies are important and fundamental components.

Caddisflies of the family Stenopsychidae Martynov, 1924 are different from other families of Trichoptera by the combination of the following morphological and ecological characters: a) head longer than 2 times as long as wide; b) mesopleuron not extended anteriorly; c) dorsum of abdominal segment 9 without sclerotized plate; d) larvae of the family building nests between large stones (Sangpradub & BoonSoong, 2006).

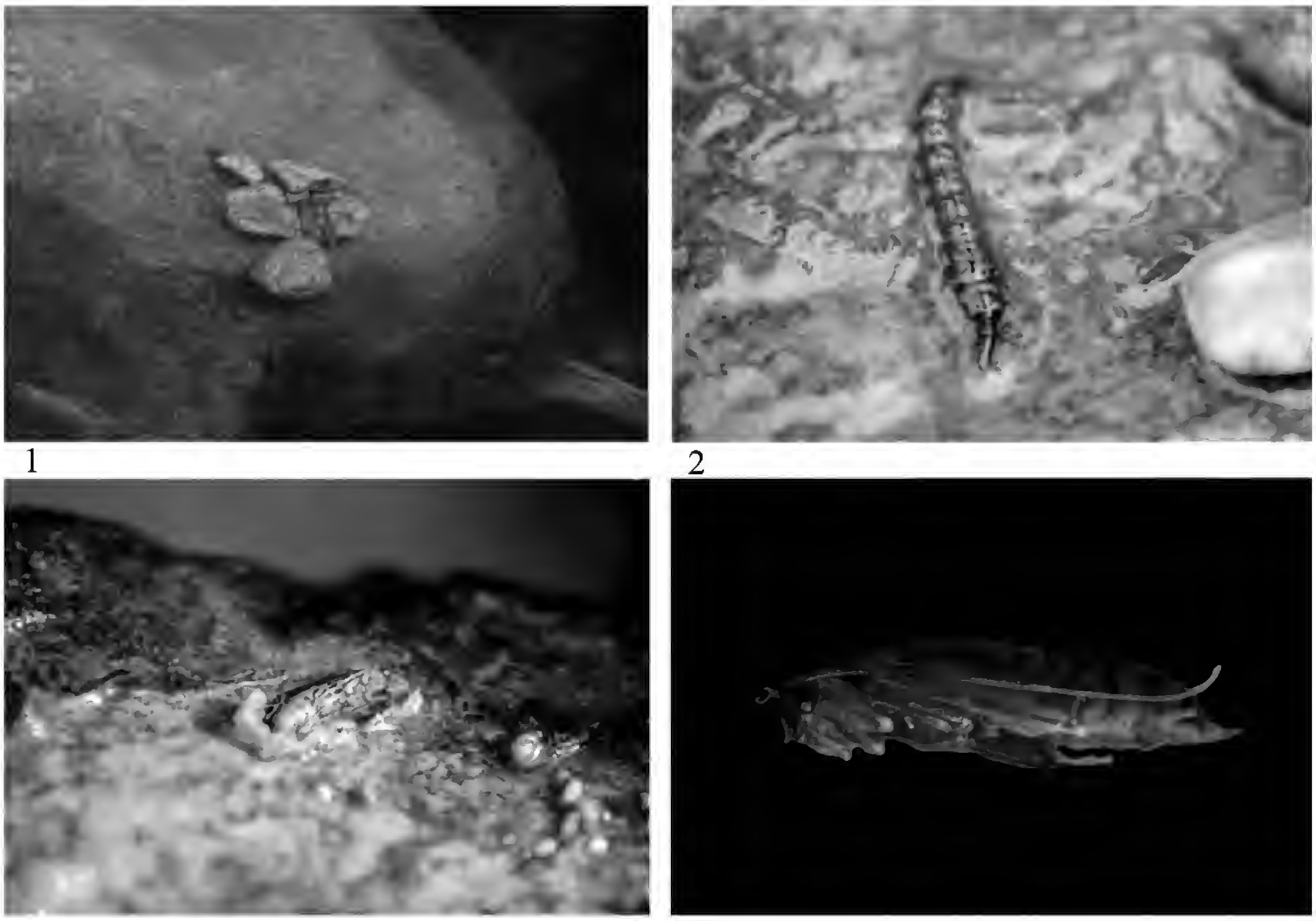
In Thailand, Stenopsychidae comprise one genus, *Stenopsyche* McLachlan, 1868, and six species (Malicky & Chantaramongkol, 1999) including *Stenopsyche siamensis* Martynov, 1931; life cycle and feeding habits of *S. siamensis* in Thailand were studied by Laudee & Chantaramongkol (2003).

Stenopsyche siamensis Martynov, 1931

This species is widely distributed in Thailand and Malaysia (Laudee & Chantaramongkol, 2003). In a survey project of aquatic ecology at upstream of Maewong Basin, Pangsira Thong District, Kamphaeng Phet Province, Central Thailand, carried out in May 2012, we found many specimens of *S. siamensis* at larvae and pupae stage.

This report is important for faunistic and ecological aspects, considering that all the Trichoptera are good ecological indicators. Particularly, larvae and pupae of *S. siamensis* (Laudee & Chantaramongkol, 2003), were found in upstream mountainous areas or islets of Maewong Basin, where water is of high-quality, the stream is transparent and running fast, on average about less than 1 m deep and the stream ground is made of rough sand and large stones.

These larvae make protective cases by fibers between large stones and/or under stones, stay in-



1 2 3 4

Figures 1-4. Residence constructions under stones (Fig. 1), larva (Fig. 2) and pupa (Fig. 3, 4) of *Stenopsyche siamensis* from Maewong Basin, Thailand.

side the construction and live in the stream since they get to pupa stage when, the small sticky fiber, reinforced by rough sand from the stream ground, is wrapped around the body of the insect (Figs. 1-4).

Present paper reports on an additional record of the species from Central Thailand confirming indirectly the good water quality of upstream of Maewong Basin.

ACKNOWLEDGEMENTS

The authors are grateful to reviewers for reviewing this manuscript. A special thank to Mr. Thanapol Saranark and Mr. Wathunyu Kalumpuk, for supporting the field survey.

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Biodiversity Journal, 2012, 3 (3): 191-200

A contribution to the knowledge of the terrestrial Mammalian fauna of Comino and its satellite islets (Maltese Archipelago)

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ABSTRACT The present work aims to contribute to existing knowledge on Mammalia species occurring on Comino and its satellite islets and to provide additional records collected between the years 1998-2012. At the present state of knowledge, on the islands of the Maltese Archipelago there are 19 different species of terrestrial mammals (Erinaceomorpha: *Atelerix algirus*. Soricomorpha: *Suncus etruscus*, *Crocidura sicula*. Chiroptera: *Rhinolophus ferrum-equinum*, *R. hipposideros*, *Miniopterus schreibersii*, *Myotis punicus*, *Eptesicus serotinus*, *Nyctalus noctula*, *Pipistrellus pygmaeus*, *P. kulii*, *Plecotus austriacus*, *Tadarida teniotis*. Lagomorpha: *Orictolagus cuniculus*. Rodentia: *Apodemus sylvaticus*, *Rattus rattus*, *R. norvegicus*, *Mus musculus*. Carnivora: *Mustela nivalis*), more than half of which are bats. Out of 13 species listed here, 8 species are represented as new to the islands while 3 species are confirmed and 2 species are listed as unlikely occurring.

KEY WORDS Mammalia; Comino; Satellite islets; Maltese Islands.

Received 08.07.2012; accepted 18.08.2012; printed 30.09.2012

INTRODUCTION

Very little work is directed to Maltese Mammalia, and even less is known about their distribution, especially with respect to the smaller islands. Some scattered notes provide some literature of past records.

The first data on the mammalian fauna of the Maltese Archipelago were reported by Gulia (1890). Busuttil & Borg (1925) were the first to attempt to list the mammalian fauna present on Comino Island.

Unfortunately their list only constitutes of Maltese vernacular names and this often causes difficulty in determining the precise species as local names change through time and may refer to a number of closely related species.

Lanfranco (1969) repeats these records in his work; Savona-Ventura (1982) focuses on giving new data on the mammals living on Comino and Cominotto. Baldacchino & Schembri (2002) along with the life history of mammalia and herpetofauna of the Maltese Islands, give also some localities where the latter species were recorded.

Recently Aloise et al. (2011) represented the possibility that *Crocidura sicula* (Miller, 1900) is present also on Comino. This latter record is confirmed in the present work.

What follows is the current mammalian fauna known to occur or have occurred on the latter islands. In addition to bibliographic data available, data were collected through direct observations, by collection of dead specimens and through discussion with local people.

All non-flying mammals of the Maltese Archipelago, however, are thought to have been introduced, at different times, by Men.

MATERIALS AND METHODS

Study area

The Maltese Archipelago consists of three main islands, which are Malta, Gozo (Ghawdex) and Comino (Kemuna) and a number of minor islands, islets and rocks. Comino, the third largest island of the Archipelago, is surrounded by following satellite islands: Cominotto, Old Battery`s Rock, Lantern Point Rock, Comino Cliff Face Rock/ Pigeon Rock, Small Blue Lagoon Rock, Large Blue Lagoon Rock, Ghemieri Rocks (Fig. 1; Table 1).

Comino (Kemuna)

Comino is the third largest island of the Maltese Islands, with an area of 2.7 km^2. Comino is also considered to be the smallest of the three main islands, but it can be referred as the mainland with respect to its satellite islets. However, due to its mono-geological component of Upper Coralline Limestone above sea-level, it has much less habitats than Gozo and Malta. Moreover with its small size, the range in topography is also limited, with a genaral tilt from South to North, identical inclination to the one of Marfa Ridge.

Cliffs are only dominant on the eastern and southwestern perimeters of the island, while only two considerably long valleys exist there. Sand-dunes are minimal and localised. With respect to vegetation communites, Thyme garigue and *Anthyllis-Teucrium* and *Euphorbia melitensis* phryganas are most common, with dense *Hypericum aegyptium* garigues along the coast, followed by *Pistacia lentiscus* pseudomaquis further inland and few *Pinus halepensis* woodland patches.

Other phryganas are rare on Comino, but a considerable large population of *Senecio bicolor* exists on Comino, in some areas it is dense enough to contribute to its own phrygana. Elsewhere in Maltese Islands, this species seems common in Malta and Gozo, and rare on the islets, but always scatte-

Name of the islet/rock	Code (fig.1)	Rank by area	Height (m)	Width (m) W-E orientation	Length (m) N-S orientation	Distance from the mainland (m)	Topographical inclination	Surface Geology	Soils
Cominotto	O	3	>20	500	~200	125	South to North	Upper Coralline	Terra Rossa
Large Blue Lagoon Rock	M	6	20	170	57	50	South to North	Upper Coralline	Terra Rossa
Small Blue Lagoon Rock	N	11	18	27	70	110	West to East	Upper Coralline	Terra Rossa
Pigeon Rock	L	15	20	20	45	20	East to West	Upper Coralline	Inglin complex
Battery Rock	J	22	6-7	18	9	4	East to West	Upper Coralline	Terra Rossa
Lantern Rock	K	23	8	7	17	30	/	Upper Coralline	/
Ghemieri Rocks (3 rocks)	X	19	6, 1, 2	14,14,10	19, 20, 38	10,82, 30	/	Upper Coralline	/

Table 1. Characteristics of the Satellite islets of Comino (Maltese Archipelago).

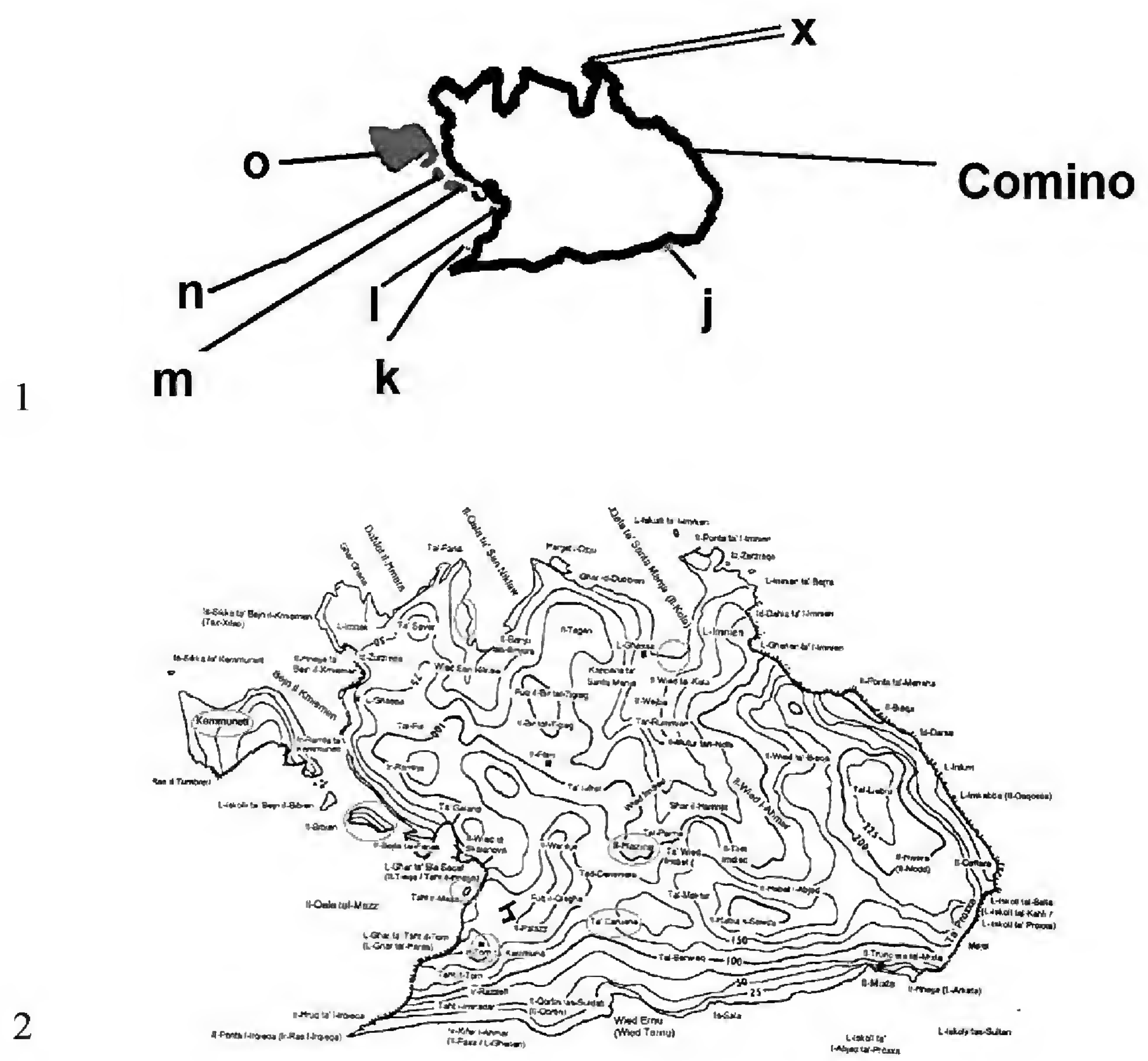

Figure 1. Satellite islets of Comino (Maltese Archipelago): Old Battery`s Rock (J), Lantern Point Rock (K), Comino Cliff Face Rock/ Pigeon Rock (L), Small Blue Lagoon Rock (M), Large Blue Lagoon Rock (N), Ghemieri Rocks (X), Cominotto (O).

red (not in dense clumps). Sub endemic rare plants have been noticed occuring sporadically on the island, such as the Pelagian-Maltese endemics *Linaria pseudolaxiflora* Lojacono and *Daucus lopadusanus* Tineo, while the other is *Senecio pygmaeus* D.C, a Sicilian-Maltese endemic.

Cominotto (Kemunett)

By far, Cominotto is the largest islet near Comino, with 9.9 ha; it is the second largest uninhabited island of Malta. The island has an S-shaped topographical orientation.

Cominotto also has a similar altitude of the other nearby islets on the south east of the island. Cliffs dominate the south coast, while a peninsula, larger than Small Blue Lagoon Rock, is connected on the south east of Cominotto.

Cominotto has three slopes, east to west from its highest point to its sandy beach and nearby coast, south to north and west to east from the highest point too.

Thymbra capitata, *Convolvulus oleifolius*, *Teucrium fruticans*, *Brachypodium retusum* and *Anthyllis hermanniae* are dominant species on the eastern side of the island, especially facing Comino. *Euphorbia melitensis*, and *Pistacia lentiscus* are dominant on the highest point of the island. *Darniella melitensis* is dominant on the south cliffs.

Euphorbia melitensis is also dominant on the northwest of the island. In winter, several annual species dominate the island, such as *Convolvulus althaeoides*, *Anthyllis vulneraria*, *Linum strictum*, *Linum trigynum*, and *Galactites tomentosa* in the disturbed patches of the island. *Phagnalon graecum* subsp. *ginzbergeri* is only present in the peninsula of the island (Sciberras & Sciberras, 2010).

Large Blue Lagoon Rock (Il-Hagra Ta' Bejn il-Kmiemen il-Kbira)

Large Blue Lagoon Rock is the second largest islet of Comino. The islet has one slope, which is slightly steeper than that of Small Blue Lagoon Rock. The south perimeter is dominated by cliffs, except for a sizeable sea cave on its eastern side, which goes right through the islet. Its topographic landscape shows that it used to form part of the collapsed western valley of Comino.

The upper half is inhabited by vegetation. *Hypericium aegypticum* is the dominant species all over the islet, while *Daucus carota* is dominant on the west side of the islet. Some patches are dominated by *Convolvulus oleifolius* (Sciberras & Sciberras, 2010).

Small Blue Lagoon Rock (Il-Hagra Ta'Bejn il-Kmiemen iz-Zghira)

Situated between Large Blue Lagoon and Cominotto, the Small Blue Lagoon Rock is the third largest islet of Comino. Most vegetation also occurs on its upper half. *Arthrocnemum macrostachyum* and *Daucus carota* are the dominant species all over the islet.

Lygeum spartum entirely covers a small patch of soil. *Lavatera arborea* (*Malva dendromorpha*), with only four specimens, barely survives near the *Lygeum spartum* and is only present on this islet in Comino`s Archipelago (Sciberras & Sciberras, 2010).

Comino Cliff Face Rock/Pigeon Rock/Ta' Taht il-Mazz Rock(Il-Gebla ta' Taht il-Mazz)

Ta' Taht il-Mazz rock is the fourth largest islet near Comino. The islet is very steep, with vertical sheer cliffs on its east side facing the western cliffs of Comino, while its western is slightly less steep, but still sheer.

The majority of species of plants occur on its west side and upper half of it, while only one species inhabits its east side. *Inula crithmoides* is a dominant species on the eastern face of the islet, while *Darniella melitensis* is dominant on the western cliff face of the islet. *Daucus carota* and *Limonium melitensis* are also dominant, but on a lesser extent. *Anthyllis hermanniae* and *Pistacia lentiscus* are rare on the islet.

No soil exists on the rock, vegetation is growing on the debris (the accumulating debris) and in rough weather the lower area is inundated by wave action (Sciberras & Sciberras, 2010).

Old Battery's Rock (Gebla ta` taht il –Batterija)

This small rock is situated on the southeast of Comino under the old battery it is the second smallest rock of Comino. Remnant soil exists on the islet which is inundated by water wave action during rough weather. Till 2010 the flora of the islet consisted of 22 individuals of *Inula crithmoides* (Sciberras & Sciberras, 2010).

Lantern Point Rock (Gebla Tal-Ponta Rqiqa)

Lantern Rock it is slightly smaller than Old Battery's Rock, it supports very little vegetation, only two species, *Limonium melitense* and *Inula crithmoides*. It is a large boulder of no more than 7 m high, with another small boulder lying on top of it (Sciberras & Sciberras, 2010).

RESULTS

Erinaceomorpha Gregory, 1910
Erinaceidae G. Fischer, 1814

Atelerix algirus (Lereboullet, 1842)
Algerian Hedgehog

Taxonomy of Hedgehog of Maltese Islands was uncertain for a long time. The Hedgehog was first recorded for the Malese Islands by Gulia (1858), which, however, erroneously considers this population belonging to *Erinaceus europaeus*. After the taxonomic revision of the Maltese specimens (Lanfranco, 1969), currently all the populations occurring in the Maltese Archipelago belong to the species *Atelerix algirus*.

Busuttil & Borg (1925) mention that a species of hedgehog was imported to Comino from the neighbouring islands of Malta or Gozo probably by the ornithologist A. Schembri, in the 19th Century, to control infestations of *Blattaria* sp. Lanfranco (1969) and Savona-Ventura (1982) seem not to respond to the presence of this species to the island while Baldacchino & Schembri (2002) state that this species is not present.

Two of the authors (A. Sciberras and J. Sciberras) have recorded this species 8 times over a time span of 14 years and in one occasion (23.XI.2005) a nest was found containing 5 young. Specimens were encountered mostly in the centre of Comino Island, particularly in a location known as Ta` Caruana. Several naturalists informed the authors however that during the 1990`s several specimens saved from Maltese roads, especially from Mġarr to Miżieb area, were released on the island for safety reasons.

Both white and dark forms were encountered on the island. The status of the population present in Comino is unknown.The Algerian Hedgehog is present in Comino, Malta and Gozo, but is absent from all the other smaller islands.

Soricomorpha Gregory, 1910
Soricidae G. Fischer, 1814

Crocidura sicula (Miller, 1900)
Sicilian shrew

In the Maltese Archipelago is confirmed the presence of two species of Soricidae: *Suncus etruscus* (Savi, 1822) and *Crocidura sicula*. On the basis of current knowledge *S. etruscus* occurs only in Malta, and *C. sicula*, classified as *C. suaveolens*, is recorded only from the island of Gozo (Schembri & Schembri, 1979). Only recently has advanced the possibility that this species is present on Comino (Aloise et al., 2011).

Two specimens were obtained from the analysis of *Asio otus* (Linnaeus, 1758) pellets found from beneath the nest of the latter. Although most likely the latter specimens were caught from Comino, there is also the possibility that the specimens were caught from Gozo by the predator and then expelled on the nesting site. Similar pellets were collected by A. Sciberras containing *Rattus* sp. remains and were retrieved where *Asio flammeus* (Pontoppidan, 1763) was occasionally sighted; presumably these remains belong to the latter species. On 18.III.2012 a dead specimen of *Crocidura sicula* was found drowned in a bucket presumably full of rain water close to the Northern Coast, confirming the presence of a population of this species on the island.

On 19.X.2011 a lower jaw bone of *Crocidura* sp. was found on Cominotto (A. Sciberras, J. Sciberras and L. Pisani, unpublished data) but the presence of a population of *Crocidura* Wagler, 1832 on this islet is not confirmed. The Sicilian shrew is absent from all the other smaller islands.

Chiroptera Blumenbach, 1774
Rhinolophidae Gray, 1825

Rhinolophus hipposideros minimus (Heuglin, 1861) Lesser Horseshoe Bat

This species was already more commonly known in Gozo and less in Malta, which is reported by the early work on the Maltese Islands (see Borg et al., 1997 and references therein).

On 12.III.2004 a dead specimen was found in a location known as Il-Ħazina on Comino Island by one of the authors (A. Sciberras). This species t is a new record for this island.

Vespertilionidae Gray, 1821

Myotis punicus Felten, Spitzenberger & Storch, 1977 Mediterranean Mouse-eared Bat

Taxonomy and distribution of the Mouse-eared bat of the Maltese Islands have been debated for a long time, because of its morphometric peculiarities. In the past reported as *M. oxignatus* (Lanza, 1959; Van den Brink, 1967; Lanfranco, 1969), was then reported to *M. blyti punicus* and studied in detail (Felten et al., 1977; Savona-Ventura, 1984a, 1984b; Borg, 1998; Borg & Cachia-Zammit, 1988, 1994; Borg et al., 1990; 1997; Borg, 1998). *M. punicus* actually up to now is found on Malta and Gozo.

On 12.III.2004 and 22.XI.2005 a specimen of this species was found in a subterranean area close to the Comino tower. This is the first record for the island. On the Maltese Archipelago, this species was formerly common but has suffered immense decrease in the 1980`s and according to Baldacchino & Schembri (2002) the current population consists of only around 250-300 specimens.

Pipistrellus kuhli (Kuhl, 1817)
Kuhl's Pipistrelle

This species was presumed rare in the Maltese Archipelago before 1969 (Gulia, 1890; Lanfranco, 1969) but according to a 1990 study (Borg et al., 1990, 1997) the latter was found to be more frequent and it had a distribution all over the three main islands: Malta, Gozo and Comino.

Pipistrellus pygmaeus (Schreber, 1774)
Soprano Pipistrelle

Busuttil & Borg (1925) record the presence of two species of bats, noting that one preferred roosting in cracks while the other in caves. Lanfranco (1969) repeats this record. Savona-Ventura (1982) observed several specimens in flight and entering crevices at Santa Marija bay on VIII.1977. None were captured, and so the records are only from direct observations. It was suggested to be similar to *Pipistrellus pipistrellus* (Schreber, 1774). Nowadays it is being considered that *Pipistrellus pygmaeus* (Leach, 1825) is the likely species to exist in the Maltese Archipelago (Baldacchino & Schembri, 2002).

Several specimens were noted in flight by the three of the authors (A. and J. Sciberras, M. Sammut) and from field observations they look to be a *Pipistrellus* sp., but none were ever recovered for taxonomic identification. Several specimens were also observed flying over Cominotto and landing in crevices of Comino Cliff Face Rock.

Plecotus austriacus (Fischer, 1829)
Grey Long-eared Bat

Present on Malta, Gozo and Comino, the species was reported already by Gulia (1890, 1914), Lanza (1959) and Van den Brink (1967) as *P. auritus* (Linnaeus, 1758). Baldacchino & Schembri (2002) state that this species had a distribution all over the three main islands. Although the authors never observed this species on Comino a dead specimen was found on Cominotto Beach by one of the authors (A. Sciberras) on 6.VII.2001. This is the first record for Cominotto island.

Tadarida teniotis (Rafinesque, 1814)
European free-tailed bat

A skull of this species was retrieved from *Larus sp.* pellet from Large Blue Lagoon Rock (13.6.2011 A. and J. Sciberras leg.). Although the shape and size of the island suggest that, probably, the animal was preyed on a different site than where the remains were found, this is an interesting record because besides being new to the islet, this species is extremely rare and was reported from the Maltese Islands twice before the discovery of this skull.

The first time was in 1993 in a locality known as Cittadella on the island of Gozo where 2 specimens were observed hunting insects under street lights. The same specimens were noted till mid - May in the same locality. Another specimen was recorded flying close to the cliffs of Ħad - Dingli in Malta in November of 1996 (Baldacchino & Schembri, 2002). To add to the interest on the same islet where the skull was located, on 8.VIII.2010 the same authors observed gulls, identified as *Larus michahellis* (Naumann, 1884) by M. Sammut, hunting bat specimens that approached the vicinity of a colony of 7 gulls that were resting on the islet.

This was just before sunset. It could be that this species does occasionally predate on bats as it was noted to predate on other terrestrial species such as *Podarcis filfolensis* (Bedriaga, 1876). It could also be that the bat was caught away from this site even offshore and it was regurgitated locally.

Carnivora Bowdich, 1821
Mustelidae Fischer, 1817

Mustela nivalis (Linnaeus, 1766) Weasel

Baldacchino & Schembri (2002) state that this species locally is restricted in the Maltese Islands to the island of Malta. It is very scarce on the main island but it is widespread and observations of this species range from the North to the South of the main island of Malta.

Whilst most observations of this species are of single individuals, a den with cubs was observed in Balluta area (Wardija Limits) (A. Sciberras, 5.IV.2002, unpublished data) and an adult with five cubs was observed at Mtahleb (M. Sammut & C. Cachia Zammit, 13.XI.2010, unpublished data), although illegal, most of the specimens (from Qammieħ and Aħrax headlands in Mellieħa) have been killed for taxidermal purposes. On 23.IV.2012, one of the authors (M. Sammut) saw an adult specimen of this species at a location known as Il-Hazina (Comino island) moving swiftly from behind a rubble patch.

Following that it climbed from behind a rubble wall and popped up on the rubble wall where it was seen very well and could be identified without any doubt. It disappeared again as swiftly as it had appeared. It was seen in the afternoon and though the author remained in the area for over an hour it was not seen again.

An indication of the presence of species on the island is the finding of 3 dead specimens of *Rattus norvegicus* and a young specimen of *Oryctolagus cuniculus* near Comino`s pig farm (6.V.2004, A. Sciberras, unpublished data). These carcasses showed signs that they had been killed and dragged from the neck, and less than 50 cm away a den similar to the one observed at Balluta was discovered.

No cubs or adults were observed despite the constant monitoring. Interestingly, people who have lived all their lives and constantly patrol the island of Comino have never seen the species on the island (S. Vella, pers. comm.). It is also worth noting that *Mustela putorius furo* (Linnaeus, 1758) was once observed on Comino in the 1970`s (M. Psaila, pers. comm.).

In the past feral specimens have escaped from rabbit hunters which visited the island (S. Vella, pers. comm.). One of the authors (A. Sciberras) also has reports on *Mustela* sp. being present in Gozo but after checking the site (an area known as Munxar) 3 feral specimens of *M. putorius furo* were noted roaming free. One of the authors (J. Sciberras) received reports by locals of *Mustela* sp. at San Blas Bay.

After observing the site and description of the locals it was confirmed that a few specimens of *M. putorius furo* were present at the site. Lanfranco (1969) also records the latter feral species in Malta and it is suggested that these must be escapees from *O. cuniculus* hunting parties.

Rodentia Bowdich, 1821
Muridae Illiger, 1815

Rattus norvegicus (Berkenhout, 1769)
Brown rat

The Brown Rat is considered common, but can be very common and, under certain conditions, can become particularly problematic.

Its presence has been detected on all the major islands, Cominotto and many other smaller islands. The presence of rats on the Comino and Cominotto Islands is referred by Busuttil & Borg (1925), while Lanfranco (1969) mentions this species and *Rattus rattus*, Savona-Ventura (1982) observed a specimen of the species on Cominotto in IX.1975. Baldacchino & Schembri (2002) also record the presence of the species on Comino.

Presently including the period of observations stipulated above, this species is numerous on the islands and was recorded on the following islets (A. Sciberras, unpublished data): Manoel Island, Qawra Point or Ta' Fra Ben islet, Selmunett Island, Large Blue Lagoon Rock, Ħalfa Rock, and Taċ-Ċawl Rock. On Fungus rock and Selmunett Island, it is known of its devastating impact on the Insular biodiversity (Baldacchino & Schembri, 2002; Sciberras, 2007; Sciberras & Schembri, 2008).

Rattus rattus (Linnaeus, 1758) Black Rat

The Black Rat is reported as present and common on Malta, Gozo, Comino and some of the satellite islets. Busuttil & Borg (1925) record the presence of rats on the Comino and Cominotto Island while Lanfranco (1969); Savona-Ventura (1982) states in III.1978 he found a dead specimen of this species.

Baldacchino & Schembri (2002) and Aloise et al. (2011) also record the presence of this species on Comino including Malta and Gozo and state that it is common. The species is also known for Fungus rock (Borg & Sultana, 2003).

From experience with data also collected from a local Pest control company, this species is numerous where it is present but it does have a restricted distribution. Most records of specimens come from Valletta and neighbouring harbour cities. In all the years of observations and data collection, this species was never encountered on Comino or its satellite islets.

Mus musculus (Linnaeus, 1758)
House mouse

Widespread and very common, the House Mouse is abundand throughout. Present on all major islands, although not proven, its presence can be regarded as likely also on the islands of smaller size, because of its unique ecological characteristics.

Busuttil & Borg (1925) reported the presence of a mouse on Comino and so did Savona-Ventura (1982) when he noted a specimen at Santa Marija Bay in 1977. Since it was not caught, it could not be certain whether it was this species or *Apodemus sylvaticus* (Linnaeus, 1758). Several specimens were caught annually on the north Coast of Comino as a part of pest control treatment. From 34 studied samples all specimens resulted in being *M. musculus*.

Lagomorpha Brandt, 1855
Leporidae Fischer, 1817

Oryctolagus cuniculus (Linnaeus, 1758)
Wild Rabbit

Busuttil & Borg (1925) reported the presence of a rabbit on Comino and this was repeated by Lanfranco (1969). This species was very common at their time and it is presumed that this species was introduced around 1890`s on the latter Island. Prisoners and soldiers during 1914-1918 did short work on the rabbit population on Comino.

In 1969 Comino was declared a protected area from hunters and the rabbit population must have benefited, however Savona-Ventura (1982) assumed the survival of the animal through its scattered dropping and not so much on the sightings. He also recorded the species on Cominotto by finding droppings of the latter in IX.1975. In the 1980`s, Myxomatosis was introduced and the Maltese population was virtually wiped out as happened with the Comino population.

A local resident introduced this species from Gozo again in several occasions as he did with other species (Sciberras, 2009). Today the Comino population is the largest in density when compared to the size with other Maltese Islands.

The populations recorded in past literature on Cominotto and Selmunett are extinct (Sciberras unpublished data). It is interesting to note that in the Maltese Archipelago, two colour morphs occur: the brown form and the yellow form. The Maltese population consists of almost entirely the brown form with occasional yellow and hybrids with domestic rabbits. Domestic rabbits are generally set free because of some kind of illness such as VHD (Viral Hemorrhagic Disease), Myxomatosis and the most commonly found Ear Cancer. If these survive they sometimes interbreed with the wild stock (Sciberras, 2006).

The Gozo population has both forms in equal numbers, whilst the Comino population constitutes entirely of the yellow form and only on very rare occasions, slightly darker specimens are noted.

CONCLUSIONS

At the present state of knowledge, on the islands of the Maltese Archipelago are 19 different species of terrestrial mammals (Erinaceomorpha: *Atelerix algirus*; Soricomorpha: *Suncus etruscus*, *Crocidura sicula*; *Rhinolophus ferrum-equinum*, *R. hipposideros*, *Miniopterus schreibersii*, *Myotis punicus*, *Eptesicus serotinus*, *Nyctalus noctula*, *Pipistrellus pygmaeus*, *P. kulii*, *Plecotus austriacus*, *Tadarida teniotis*; Lagomorpha: *Orictolagus cuniculus*; Rodentia: *Apodemus sylvaticus*, *Rattus rattus*, *R. norvegicus*, *Mus musculus*; Carnivora: *Mustela nivalis*), more than half of which are bats.

As regards the island of Comino and its satellite islands, most of these species are present (68,4%), not being up to now verified the presence of *S. etruscus* and *A. sylvaticus*, among the non-flying, and *R. ferrum-equinum*, *M. schreibersii*, *E. serotinus* and *N. noctula* among the bats.

With the species listed above, Busuttil & Borg (1925) reported that a certain Captain Stivala released on the island of Comino a pair of *Gazella sp.* These bred successfully but were eradicated by prisoners during the First World War.

A population of *Felis silvestris catus* (Schreber, 1777) introduced in the 1980`s was exceeding over 20 specimens around Comino hotel (Northern Coast) in the late 1990`s and it was eradicated by environmentalists for the protection of the native wildlife of Comino. This update on the mammalian fauna was a result of observations, made indirectly while the authors were conducting other studies or surveys mostly entomological, herpetological or ornithological.

Upon further investigation, especially on species most critical (eg. *Crocidura sicula* and Chiroptera), would be necessary to verify the status of the population, to guarantee their conservation in the Archipelago.

ACKNOWLEDGEMENTS

A. and J. Sciberras are in debt to Esther Sciberras, Romario Sciberras and Luca Pisani for their constant assistance in the field. Special thanks go to Professor Patrick J. Schembri, Alfred. E. Baldacchino and Pietro Lo Cascio for providing useful contacts and some literature. Thanks also go to Mario Gauci for his hospitality during Gozo visits. Mark Psaila and Salvu Vella are acknowledged for sharing their observations.

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Biodiversity Journal, 2012, 3 (3): 201-228

New and little known land snails from Sicily (Mollusca Gastropoda)

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ABSTRACT In the present paper are reported new and little known land snails from Sicily (Mollusca Gastropoda). Particularly, *Platyla similis* (Reinhardt, 1880) (Aciculidae) and *Rumina saharica* Pallary, 1901 (Subulinidae) are first recorded in the island; new taxonomic data, useful for a better systematic classification, are provided on two little-known taxa, *Lampedusa lopadusae nodulosa* Monterosato, 1892 (Clausiliidae) and *Cernuella (Cernuella) tineana* (Benoit, 1862) (Hygromiidae); and finally, a new species of slug, *Tandonia marinellii* n. sp. (Milacidae), currently known from North-Western Sicily, is described.

KEY WORDS Mollusca; land snails; Sicily; taxonomy; new species.

Received 10.07.2012; accepted 26.08.2012; printed 30.09.2012

INTRODUCTION

During 19th century several taxa of terrestrial molluscs of Sicily were established, many of which are still little known and of uncertain taxonomic status, nevertheless some genera or families of molluscs, including slugs, have been neglected for a long time. The study of terrestrial molluscs of Sicily undertaken by present authors in the last decade, despite the complexity of the subject, continues with this further contribution in which some new faunal reports are provided, little known taxa are examined and, in addition, a new species of slug is described.

ACRONYMS. APP = anterior portion of palatal plica; BC = bursa copulatrix; BCD = diverticulum of bursa copulatrix; CL = columellar lamella; DE = distal epiphallus; DG = digitiform glands; DSC = dart sac complex; DBC = duct of the bursa copulatrix; DCP = distal caviti of the penis; DGS = dart gun; DG = digitiform glands; DSC = dart sac complex; DSO = dart sac opening; E = epiphallus; F = flagellum; FO = free ovidutto; FR = frenula; G = penial papilla; GA = genital atrium; ISO = inner stylophore opening; L = lunella; OSO = outer stylophore opening; P = penis; PCP = proximal caviti of the penis; PD = penial diverticulum; PE = proximal epiphallus; PL = parietal lamella; PLL = parallel lamella; PP = principal plica; PPP = posterior portion of (upper) palatal plica; PR = penial retractor muscle; SCL = subcolumellar lamella; SL = spiral lamella; SP = sutural plica/plicae; V= vagina; VAG = vaginal accessory gland; VC = vaginal chambre; VD = vas deferens; VP = vaginal pleats.

The materials used for this study are deposited in the following Museums and private collections: M. Bodon collection, Italy, Genova (CB); D.P. Cilia collection, Santa Venera, Malta (CC); S. Giglio collection, Cefalù, Italy (CG); Laboratory of Cytogenetics and Molecular Biology, University of Urbino, Italy (LCMBU); F. Liberto collection, Cefalù, Italy (CL); Museo Civico di Storia naturale di Comiso, Italy (MCSNC); Museo Civico di Storia Naturale di Genova "G. Doria", Italy (MSNG); G. Nardi collection, Nuvolera, Italy (CN), A. Reitano collection, Tremestieri Etneo, Italy (CR); I. Sparacio collection, Palermo, Italy (CS); The Steinhardt National Collections of Natural History, Zoological department, Tel-Aviv University, Israel (TAU).

MATERIALS AND METHODS

All specimens were collected by sight on the soil and under the rocks or by sieving litter and soil.

Observations on ecology of these organisms and their feeding behavior were made directly in the field. Dry shells have been studied as regards size, colour, morphology, sculpture, aperture, plicae and lamellae, lunella and clausilium. Photographs were taken with a digital camera. In order to study and illustrate genital organs, the specimens were drowned in water and fixed in 75% ethanol.

Reproductive apparatus was extracted by means of scalpel, scissors and needles. Illustrations of genitalia were sketched using a camera lucida. Height and maximum diameter of the shell along with some parts of genitalia were measured (in millimeters) by a digital gauge. Voucher specimens were stored in collections indicated below. Toponyms (place-names) are reported following the Portale Cartografico Nazionale (PCN, http://www.pcn.minambiente.it /PCN/), Map IGM 1:25000. Each locality and/or collection site is named in the original language (italian). The material used for the molecular analysis was collected on the field during February 2012. All the specimens were studied and observed at the steromicroscope (Leica MZ 7.5).

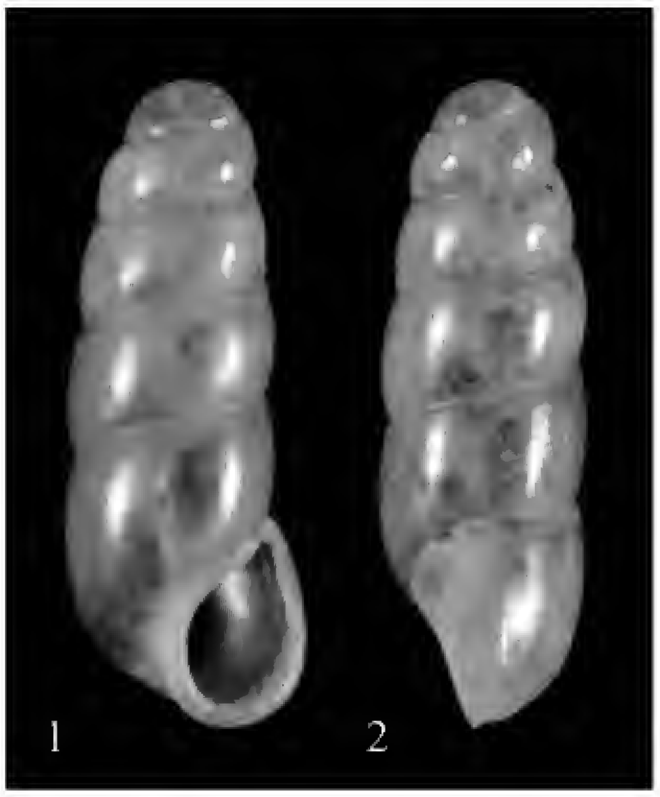
Genetic study described in the present study was based on a comparative analysis of COI partial sequences which are frequently used as markers in the investigation of evolutionary processes at the specific level. Briefly, the study was conducted by DNA isolation, PCR amplification, sequencing, alignment of the sequences and phylogenetic reconstructions using the Maximum Likelihood algorithm. For a detailed description see below.

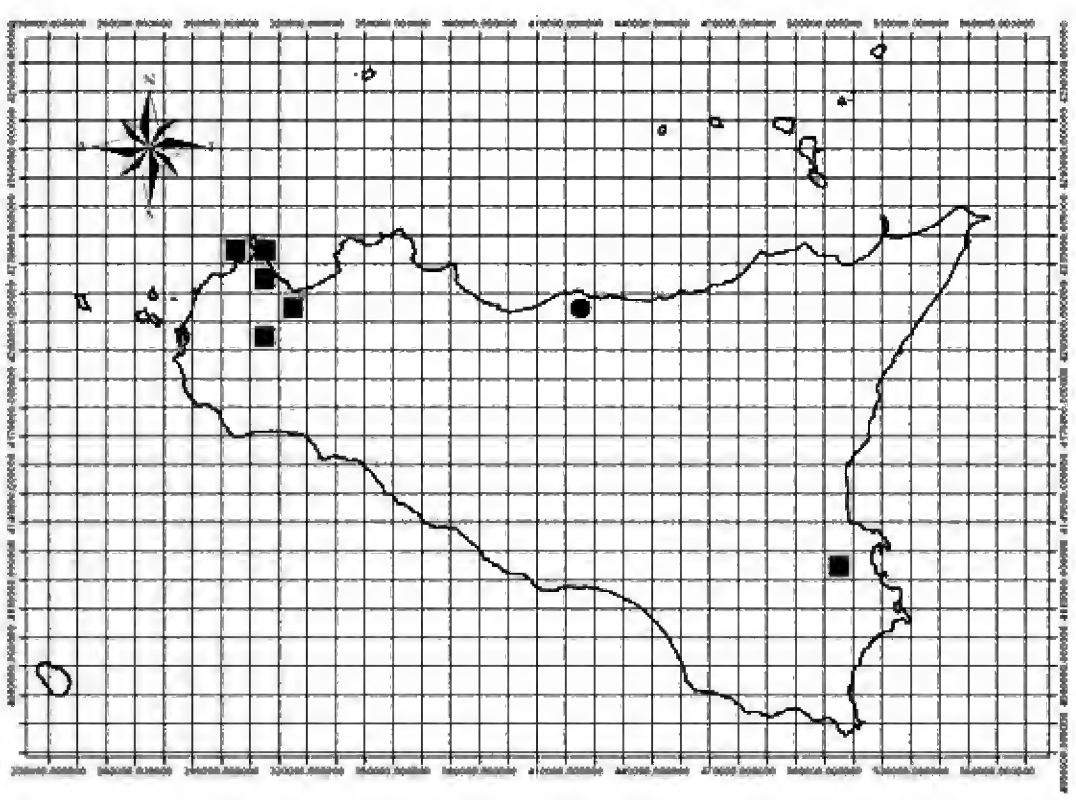
RESULTS

Class Gastropoda Cuvier, 1795
Ordo Architaenioglossa Haller 1890
Family Aciculidae J.E.Gray, 1850

Platyla similis (Reinhardt, 1880)

Examined material. Italy, Sicily, Cefalù, Rocca di San Nicola, 37°59'07"N 14°02'42"E, 600 m, 01.VI.2008, 6 specimens (CG); Cefalù, Cozzo Carcarello, 37°59'29" N, 14°03'05"E; 320 m, 15.VI.2008, 8 specimens (CL); idem, 23.VIII.2009, 22 specimens (CL); idem, 23.IV.2012, 12 specimens (CL).





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Figures 1,2. Shell of *Platyla similis* from Cefalù, Cozzo Calcarello, h: 2.32 - D: 0.89. Figure 3. Geographic distribution of *Platyla similis* (circles) and *P. subdiaphana* (squares) in Sicily (personal data).

DESCRIPTION. Morphologically, this sicilian population of *P. similis* has typical characters of the species: shell conical (Figs. 1-2), slender, colorless, height 2.16-2.58 mm, width 0.80-0.98 mm, with 4-5 whorls slightly convex, last portion of last whorl slightly ascending in relation to the penultimate, aperture slightly oblique, sinulus little depth, external peristomal rib consists of a weak thickening, not clearly defined anteriorly and posteriorly.

BIOLOGY AND DISTRIBUTION. *P. similis* lives in woodland litter, on the soil, in the cracks and at the base of the calcareous rocks. *P. similis* has South-Oriental European distribution including Bulgaria, Croatia, Serbia, Greece, Romania, Kosovo, and Central and Southern Italy (Boeters et al., 1989; Bodon et al., 1995; Bodon & Cianfanelli, 2008; Bank, 2012).

REMARKS. We report the presence of *P. similis* for the first time in Sicily, from Nothern Madonie mountains, on the calcareous rocks named "Rocca di San Nicola" and "Cozzo Calcarello". Shells were collected by sieving litter and soil, sampled in cracks of the calcareous rock, at the base of rocky walls or under boulders in the woods of oaks.

In Sicily it was known until now only the endemic species: *P. subdiaphana* (Bivona, 1839) (Boeters et al., 1989; Bodon et al., 1995; Bodon & Cianfanelli, 2008; Bank 2012) (Fig. 3). Boeters et al. (1989) distinguish all species of *Platyla* Moquin-Tandon, 1856 into three groups on the basis of the presence or absence and conformation of the external peristomal rib (see also Bodon & Cianfanelli, 2008).

P. similis is inserted into the second group characterized by an external peristomal rib not robust and not clearly delimited posteriorly. *P. subdiaphana* belongs to the third group characterized by a robust external peristomal rib bounded by a sharp line or by a large groove. *P. subdiaphana*, also, is distinguished from *P. similis* for bigger size (height 3.5-4.45 mm).

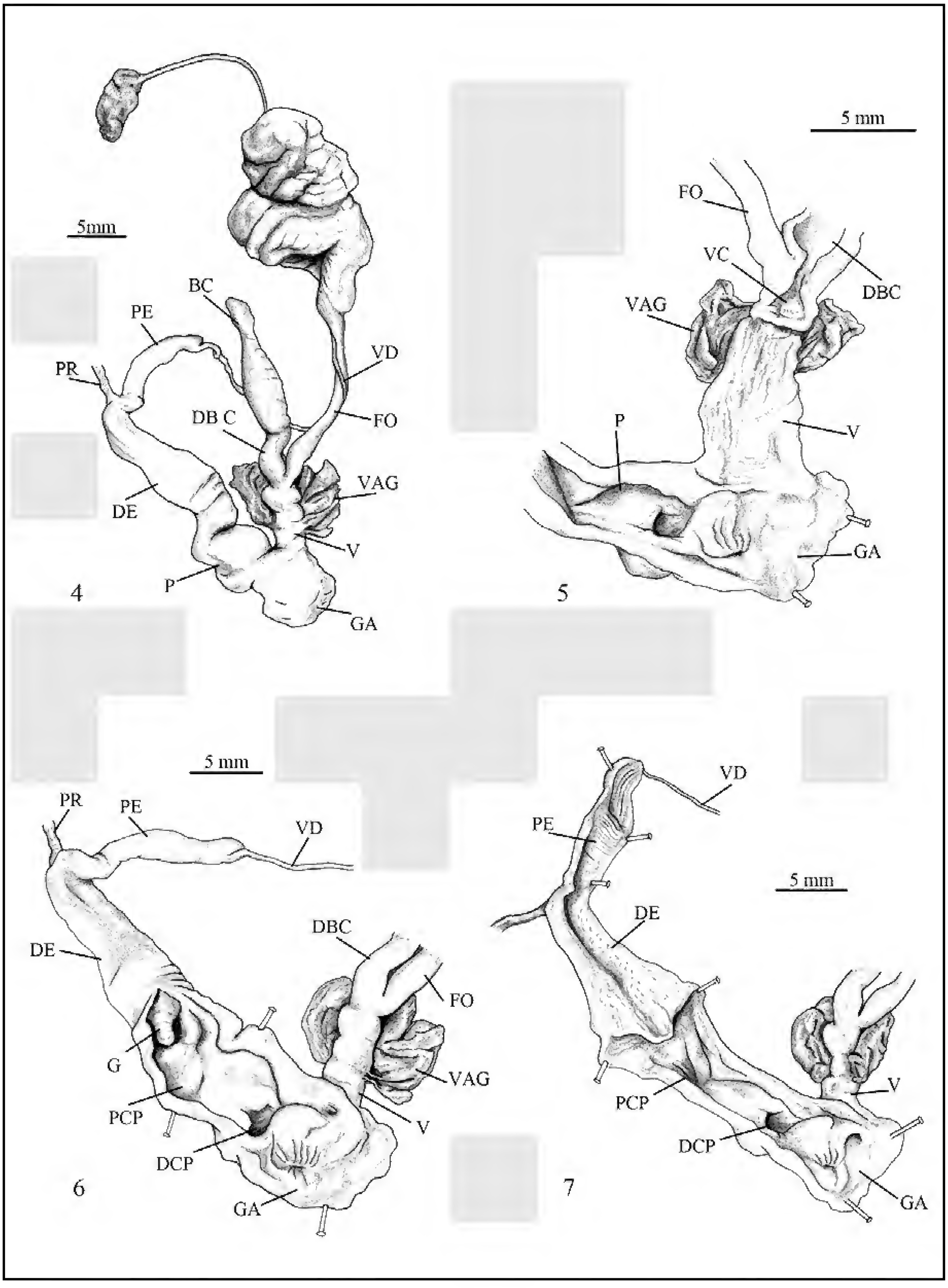
Ordo Stylommatophora A. Schmidt, 1855
Family Milacidae Germain, 1930

Tandonia marinellii n. sp.

EXAMINED MATERIAL. Holotypus: Italy, Sicily, Custonaci, Monte Sparagio, Pizzo Giacolamaro, 38°03'18"N 12°44'35"E, 665 m, 08.I.2012 (MSNG 56989). Paratypi: Italy, Sicily, Custonaci, Monte Cofano, 38°06'11"N 12°40'39"E, 255 m, 14.IV.1991, 2 specimens (CS); San Vito lo Capo, Macari, Pizzo Castelluzzo, 38°07'28"N 12°44'41"E, 364 m, II.2007, 3 specimens (CR); Erice, 38°02'24"N 12°35'34"E, 500 m, 03.X.2011, 1 specimen (CN); Calatafimi, Cozzo Gessi, 37°54'44"N 12°50'41"E, 264 m, 20.XI.2011, 3 specimens (CL); idem, 2 specimens (CG); Calatafimi, Monte Bernarco, 37°54'56"N 12°49'45"E, 370 m, 20.XI.2011, 6 specimens (CL); Castellammare del Golfo, Fraginesi, 38°01'06"N 12°50'08"E, 180 m, 4.XII.2011, 1 specimen (CS); Custonaci, Monte Sparagio, Pizzo Giacolamaro, 38°03'18"N 12°44'35"E, 665 m, 8.I.2012, 1 specimen (CL); idem, 1 specimen (TAU 76575); idem, 1 specimen (MCSNC 4411); Custonaci, Monte Sparagio, Pizzo Giacolamaro, 38°03'17"N 12°44'57"E, 716 m, 4.II.2012, 8 specimens (CL); Trapani, contrada Chinèa, near the Lago Rubino, 37°53'49"N 12°44'05"E, 260 m, 18.II.2012, 2 specimens (CS); Custonaci, Muciara, 38°03'27"N 12°43'64"E, 542 m, 4.III.2012, 11 specimens (CS); idem, 2 specimens (MSNG 56990; MSNG 56991).

DESCRIPTION OF HOLOTYPUS. Slug medium-sized, length 55 mm after preservation (the specimen is contracted). Clypeus about 1/3 of body length, superficially granulated, with horseshoe-shaped groove, and a hollow near keel; evident carina running from clypeus to posterior apex of body. Body and mantle brownish-yellow with blackish pigment forming irregular reticulation and dots which disappear toward the sole, keel orange. Foot sole tripartite, creamy-coloured, mucus thick, viscous, white-yellowish. Shell (limacella) nail-like, oval, well calcified, white, with apex posterior and situated on major axis, at the highest point, convex above, slightly concave ventrally (Figs. 18-20); length: 7.5 mm; diameter: 4.9 mm.

Genitalia (Figs. 4-7, 11). Vas deferens thin, ending laterally at proximal epiphallus tip. Epiphallus very long (20 mm), divided by a slight constriction in a narrow cylindrical proximal portion with thin walls (proximal epiphallus) and in an ample conic distal portion with thick walls (distal epiphallus). Internally, the proximal epiphallus is crossed by around 15 anular crests more evident in the central zone, and 5-6 longitudinal folds in the slight broadening apical knob, while the distal epiphallus has a very narrow duct; penial retractor muscle ending



Figures 4-7. Genitalia of *T. marinellii* n. sp., holotypus: whole genitalia (Fig. 4), internal structure of vagina (Fig. 5), internal structure of penis (Fig. 6), internal structure of penis and epiphallus (Fig. 7).

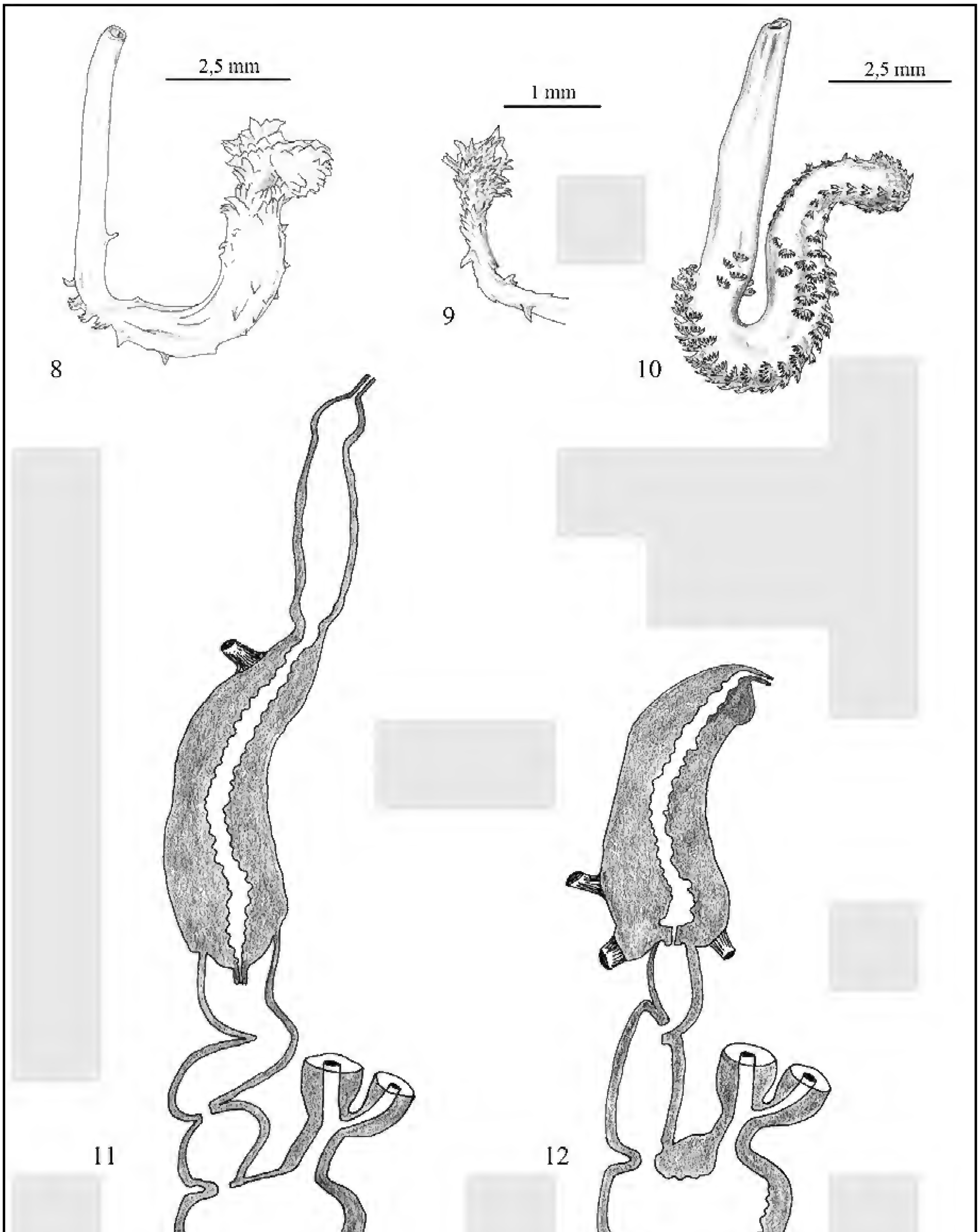


Figure 8. Spermatophore of the holotypus of *T. marinellii* n. sp. Figure 9. Anterior portion of spermatophore of *T. marinellii* from Calatafimi. Figure 10. Spermatophore of *T. sowerbyi* from Novara di Sicilia, Rocca Novara, Sicily. Figures 11, 12. Scheme of genitalia of *T. marinellii* n.sp., (Fig. 11) and of *T. sowerbyi* by Wiktor (1987), modified (Fig. 12).

laterally on penial complex where slight constriction separates distal epiphallus from proximal epiphallus. Border between epiphallus and penis externally evident, inside the epiphallus protrudes into the penis with a semispheric extension, bearing at its apex a small cylindrical papilla. Penis irregular, cylindrical, 11 mm long, approximately ½ of length of epiphallus, with thin walls, inside with some striations and divided by a constriction in two cavities: a narrower, oblong proximal cavity (8 mm) and a short, wide distal cavity (2.2 mm). A thin sheath envelopes the penial complex, keeping proximal epiphallus bent on the distal epiphallus and the penis contracted; wide and short genital atrium, with fine folds around the genital opening.

Vagina long (9.8 mm), inside there are some discontinuous longitudinal folds. Vaginal accessory gland enters by means of thin canaliculi at about midway along vagina. An annular pad separates the real vagina from a small chamber (vaginal chamber) with thick, smooth wall, where the duct of bursa copulatrix and the free oviduct end. Duct of bursa copulatrix short and slender. Bursa copulatrix elongate with a narrow apical prolungation. Long and slender free oviduct, slightly wider near vagina.

Spermatophore (Fig. 8) worm-like, glossy, golden. Anterior apex lacking (broken), the remaining anterior portion is regularly tubular and bare, only on the distal part there are some short spines (with 4-5 apexes), the posterior portion is covered with some short spines with bifurcate point (two apexes) or simple (one apex); posterior apex of the spermatophora, bigger, completely covered of bifurcate or simple spines. The spermatofore was found in the channel of the bursa copulatrix with the posterior apex contained into "vaginal chamber".

Variability. Body colour variable (Figs. 13-16) from uniform orange with some spots hardly visible to orange-brown speckled with darker patches; keel clear, orange or cream; clypeus with blackish horseshoe-shaped groove and sometimes with a dark central line; genitalia: proximal epiphallus and distal epiphallus generally have the same length, but in some specimens the proximal portion is slightly shorter; the epiphallus protrudes into the penis with a semispheric or conic extension, penis length varying from 6 mm to 10 mm; vagina length varies from 2 mm to 9 mm. Spermatophore variable in size (Fig. 9) and color from red to yellow-gold; these were found in the channel of bursa copulatrix with the big apex contained into "vaginal chamber".

Etimology. The new species is dedicated to Aldo Marinelli (Roma), as sign of appreciation for creating the forum "Natura Mediterraneo" (available at: http://www.naturamediterraneo.com/forum/).

Biology and Distribution. Species rather common in natural environments with forests, mediterranean maquis or garrigue, nocturnal, during the day specimens shelter under rocks and logs; sexual maturity occurs in winter. *T. marinellii* n. sp., at present, is known only from North-Western Sicily (Fig. 21).

Comparative notes. *T. sowerbyi* (Férussac, 1823) was the only known species in Sicily (Wagner, 1931, Giusti, 1973; Manganelli et al., 1995; Bank 2012) (Fig. 21). In this region it seems to be native as widely widespread, especially in natural environments, and having been reported since 1800 (Lessona & Pollonera, 1882 sub *Amalia carinata* and *A. carinata* var. *oretea*; Minà Palumbo, 1883 sub *Amalia carinata*; Pollonera, 1891 sub *Amalia carinata*). The populations of eastern Sicily examined by us show the typical morphological characters of *T. sowerbyi* (Wiktor, 1987, Giusti et al., 1995) (Figs. 10, 12). The epiphallus in *T. sowerbyi* is of medium length and cone-shaped, while in *T. marinellii* n. sp. the epiphallus is very long and equipped with an evident proximal portion completely absent in *T. sowerbyi*; the penis retractor muscle in *T. sowerbyi* ends at about 2/3 of the length of the epiphallus, while in *T. marinellii* n. sp. ends at half the length of the epiphallus exactly where the distal portion ends and the proximal one begins; a pair of short supplementary muscles inserted at the distal end of epiphallus observed in *T. sowerbyi*, lack in the new species.

The penis in *T. sowerbyi* is divided by a constriction in a short distal portion followed by a longer proximal chamber, while in *T. marinellii* a proximal long portion is followed by a short distal one, wide and sac-shaped; penial papilla in *T. sowerbyi* is squished, of vestigial type, while in *T. marinellii* is of cylindrical shape; the fold-like thickening (reduced "stimulator") present in *T. sowerbyi*, lacks in *T. marinellii*; the spermatophore in *T. sowerbyi* has a posterior portion covered with very branched spines (Fig. 10), whereas in *T. marinellii* is covered by scattered spines not branched or

Figures 13-16. *T. maninellii* n.sp., Custonaci, Monte Sparagio, Giacolamaro, 08.I.2012: variability of the body colour. In a specimen (Fig. 16) is visible the white-yellowish mucus.

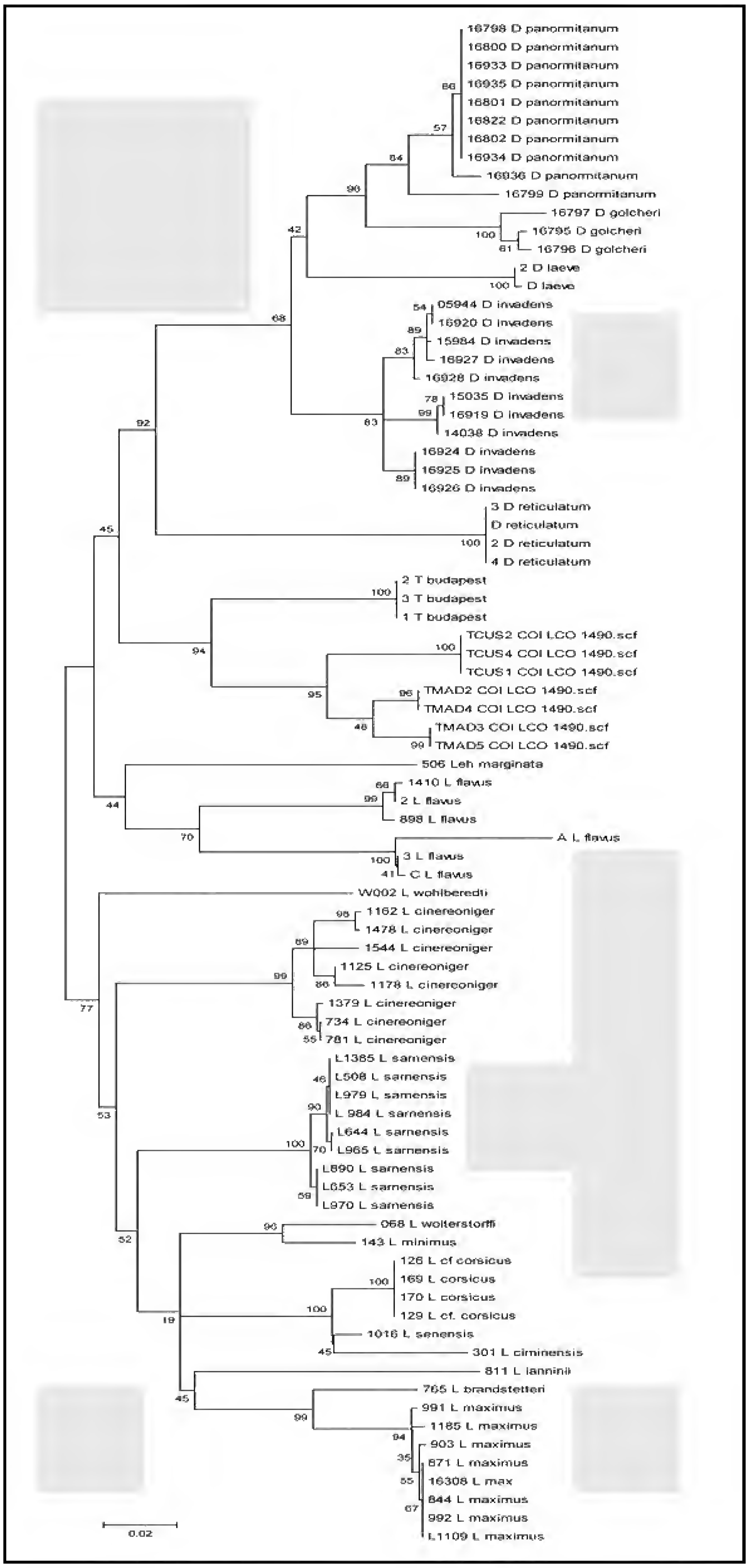


Figure 17. Maximum Likelihood consensus tree (rooted with respect to the genus Limax) inferred from a dataset of 83 (seven sequences obtained in the present paper and 76 retrieved from GenBank database) mitochondrial COI gene partial sequences. Numbers above branches represent bootstrap values.

at most bifurcate with wider base, and the posterior apex is bigger.

Examined material of *Tandonia sowerbyi*. Italy, Emilia Romagna, Castiglione dei Pepoli, Roncobilaccio, 44°06'59"N 11°13'42"E, 593 m, 5.XI.2011, 2 specimens (CS). Italy, Sicily: Castelbuono, Cozzo Luminario, Piano Sempria, 37°54'18"N 14°03'59"E, 1192 m, X.1990, 4 specimens (CS); Palermo, Parco della Favorita, Vallone del Porco, 38°10'07"N 13°20'39"E, 243 m, 13.XI.1990, 4 specimens (CS); Monreale, Bosco Ficuzza, Diga Scanzano, 37°55'14"N 13°22'25"E, 536 m, 1.XII.1990, 6 specimens (CS); Palermo, Fiume Oreto, Ponte delle Grazie, 38°04'4"N 13°19'25"E, 95 m, 19.XI.1990, 5 specimens (CS); idem, 3.XII.1990, 2 specimens (CS); Isnello, Contrada Montaspro, 37°54'42"N 13°59'30"E, 857 m, 5.III.1991, 1 specimen (CS); Collesano, Contrada Croce, 37°55'23"N 13°55'20"E, 511 m, 5.III.1991, 6 specimens (CS); Cammarata, Monte Cammarata, Cozzo Panepinto, 37°38'16"N 13°36'34"E, 984 m, III.1992, 4 specimens (CS); idem, 2 specimens (CL); Petralia Sottana, Fiume Imera Meridionale, 37°48'26"N 14°05'01"E, 808 m, 2.XI.1992, 3 specimens (CS); San fratello, Monte Soro, Pizzo Muto, 37°56'16"N 14°38'16"E, 1410 m, X.2001, 1 specimen (CS); idem, 1 specimen (CL); Melilli, Riserva Naturale Integrale Grotta Palombara, 2008 (CR); Monreale, Ponte Arcera, 37°55'42"N 13°23'01"E, 470 m, 14.XI.2008, 2 specimens, (CL); Melilli, Riserva Naturale Integrale Grotta Palombara, 2008 (CR); Vizzini, Contrada Rubala, near the F. Vizzini, 37°08'28"N 14°44'15"E, 376 m, 11.I.2009, 5 specimens (CL); Prizzi, Fontana Grande, 37°42'53"N 13°25'43"E, 800 m, 15.XI.2009, 4 specimens (CL); Torrenova, Rocca Scovoni, Piano Scodoni, 38°05'38"N 14°41'26"E, 25 m, 06.XII.2009, 1 specimen (CL); Castelbuono, S. Guglielmo, near the creek San Calogero, 37°55'04"N 14°04'22"E, 670 m, I.2010, 3 specimens (CL); Novara di Sicilia, Rocca Novara, 37°59'35"N 15°08'25"E, 1000 m, 07.XI.2010, 4 specimens (CL); Itala, Piano Fattaredda, 38°02'48"N 15°25'09"E, 612 m, 12.XII.2010, 2 specimens (CL); Isnello, Vallone Montaspro, 37°54'18"N 13°58'55"E, 783 m, 26.II.2012, 2 specimens (CL).

Tandonia rustica (Millet, 1843) has an European central and southern distribution and is found in the northern regions of Italy and along the Apennines up to the central regions. This species is characterized by a very long epiphallus externally similar to that of *T. marinellii*. However, *T. rustica* is characterized by its whitish or creamy coloration, somewhat violetish, with numerous, small black dots; the penial complex (epiphallus+penis) is smaller (around 10 mm) compared to *T. marinellii* (20-31 mm), and has a different structure: penis proportionally shorter and epiphallus showing internally long longitudinal rows of papillae, penial papilla proportionally larger and much more ornate; the place where musculus retractor inserts is not constricted; atrium is narrow and tube-shaped while in *T. marinellii* is short and very broad.

Examined material of *Tandonia rustica*. Italy, Emilia Romagna, Castiglione dei Pepoli, Roncobilaccio, 5.XI.2011, 1 specimen (CS); Italy, Lombardia, Brescia, Valvestino, Armo, 45°46'N 10°35'E, 666 m, 22.X.2000, 1 specimen (CN); idem, Anfo, S. Petronilla, 45°46'N 10°29'E, 524 m, 4.V.2008, 1 specimen (CN); idem, Ghedi, 45°24'N 10°16'E, 85 m, 20.IX.1996, 1 specimen (CN), idem, Marone, Vello, 45°45'N 10°05'E, 200 m, 01.V.2007, 1 specimen (CN).

MOLECULAR ANALYSIS. Seven *Tandonia* specimens, three from Custonaci (TP) and four from Madonie mountains (PA), labelled as TCUS and TMAD respectively, were analyzed. Samples were stored in 75% Ethanol at -20 °C in test tubes. For each individual, a piece of about 40-50 mg was used for total DNA extraction. Pieces of each specimen were deposited as vouchers at University of Urbino, Lab. of cytogenetics and molecular biology. COI amplicons (654 bp) were obtained by LCO1490/HCO2198 universal primers (5'-GGTCAACAAATCATAAAGATATTGG-3'/5'-TAAACTTCAGGGTGACCAAAAAATCA-3') as in Folmer et al. (1994) with a PCR cycle of 95 °C for 5 min; 95 °C for 1 min, 42 °C for 1 min, 72 °C for 1 min (37 cycles); 72 °C for 10 min. Sequencing of the purified PCR products was carried out using automated DNA sequencers at Eurofins MWG Operon (Germany). Finally, sequence chromatograms of each amplified fragment were browsed visually. Sequences generated in this study were analysed with additional seventy-six Limacidae COI sequences retrieved from GenBank (IDs: AF239733-34, AM259702-06, AM259712-14, EF128217, FJ481179, FJ481181, FJ606455-71, FJ606481, FJ606483, FJ606485, FJ606487, FJ606489, FJ606491, FJ606493, FJ606495,

FJ606497, FJ606499, GQ145509, GQ145523, GQ145525, GQ145527, GQ145538-39, GQ145553, GQ145572-75, JN248291-99, JN248300-15; see also Reise et al., 2011). Sequences were visualized with BioEdit Sequence Alignment Editor 7 (Hall, 1999), aligned with the ClustalW option included in this software and double checked by eye. Standard measures of nucleotide polymorphism and phylogenetic analyses were conducted in MEGA 5.0.3 (Tamura et al., 2011). The best-fit evolution model of nucleotide substitution resulted GTR+G (General Time Reversible+Gamma). The evolutionary history was inferred by using the Maximum Likelihood method; the bootstrap consensus tree was inferred from 500 replicates; a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.4467). Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. Divergence among TCUS and TMAD groups (Dxy), assessed as p distance, was 5.9%.

Although genetic differences (p distance) are only indicative when assigning a group to a given taxonomic rank, the distance we assessed (5.9%) between COI sequences obtained from specimens collected in Madonie mountains and Custonaci not only is in line with values considered discriminatory at the specific level in Mollusca (i.e. Herbert et al., 2003; Pfenninger et al., 2006), but it is even greater than estimated distances separating entities accepted as distinct species (*L. corsicus*/*L. senensis*, 2.3%; *L. ciminensis*/*L. senensis*, 4.2%; *L. minimus*/*L. wolterstorffi*, 4.4%).

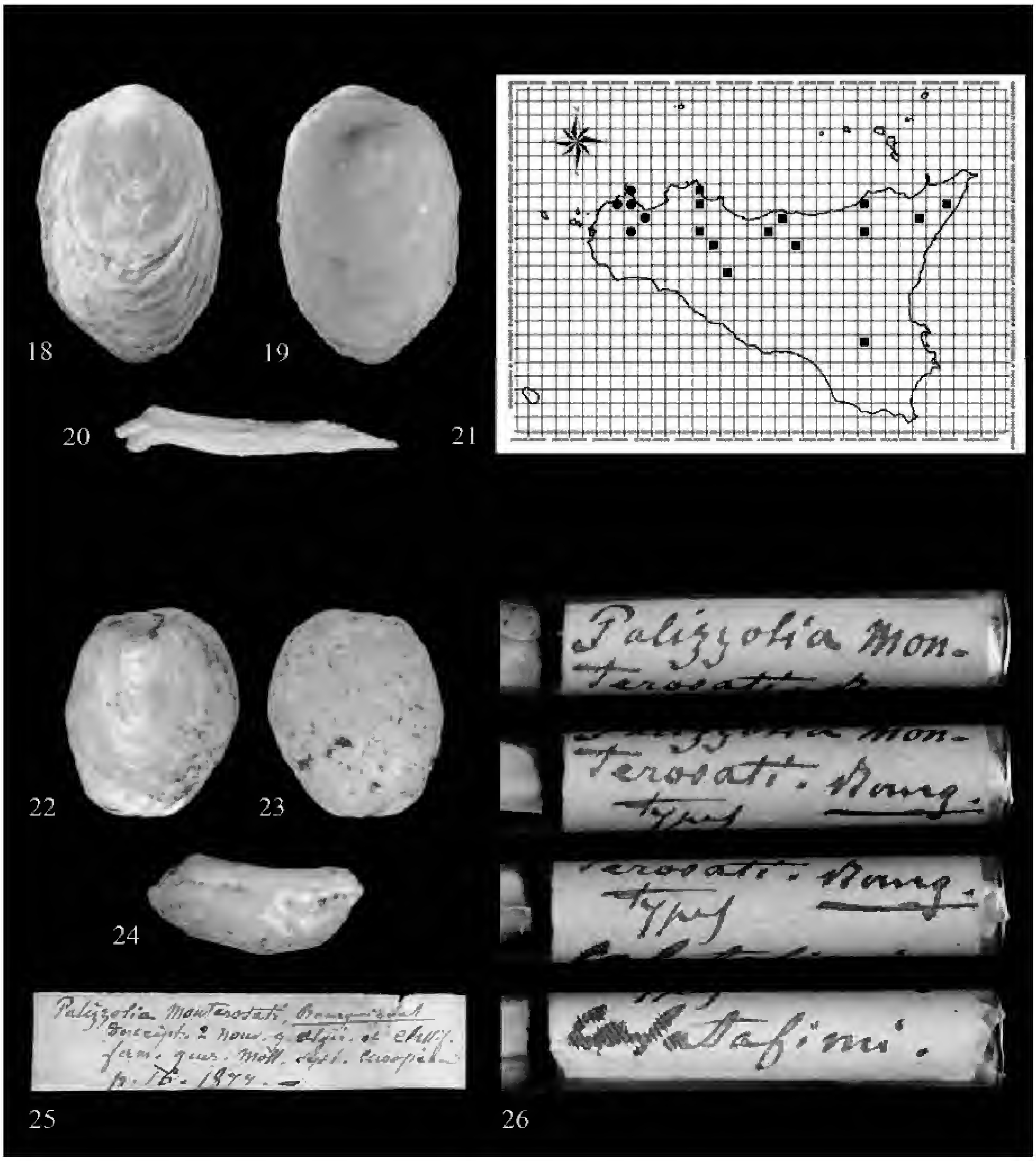
In conclusion, phylogenetic tree (Fig. 17) and genetic distance between TCUS and TMAD groups firmly support the hypothesis that specimens from Custonaci and Madonie mountains belong to two distinct *Tandonia* species.

Remarks. The genus *Tandonia* Lessona & Pollonera, 1882 has European-Mediterranean distribution extended to the Black Sea coasts (Wiktor, 1987, Giusti et al., 1995). In Italy it's verified the presence of six species (Bank, 2012): *T. nigra* (C. Pfeiffer, 1849), *T. budapestensis* (Hazay, 1880), *T. robici* (Simroth, 1884), *T. rustica*, *T. simrothi* (Hesse, 1923), *T. sowerbyi*. Among them, *T. sowerbyi* and *T. rustica* are morphologically the more similar to *T. marinellii* n. sp. that, on the other hand, sharply differs from these taxa for all the characters described above. Noteworthy, in bibliography about Sicilian and surrounding geographical areas a few taxa of uncertain taxonomic value are reported. These taxa are examined below. *Amalia marginata* var. *oretea* Lessona & Pollonera, 1882 is a taxon described for Sicily (locus typicus: "Palermo presso il fiume Oreto") only on external morphological characters: "Typica, clipeo tantum zonula nigra longitudinali mediana instructo" (Lessona & Pollonera, 1882). The body coloration in *Tandonia* (=*Amalia* Moquin-Tandon, 1855) is quite variable and topotypic specimens studied by us can be traced back, due to the shape of genitalia, to *T. sowerbyi* (Figs. 10-12). *A. marginata* var. *oretea* is then confirmed as a synonym of *T. sowerbyi* (Giusti, 1973; Bank, 2012). It would have been critical examination of typical material, but the specimens described by Lessona & Pollonera (1882) are no longer available in their collection housed at the Museo Regionale di Scienze Naturali di Torino (E. Gavetti in litteris).

Bourguignat (1877) established a new genus and a new species, *Palizzolia monterosati*, on a single Milacidae shell from Calatafimi with the following description: "Limacelle ovalaire, épaisse, très-bombée (comme sphérique) en dessous, caractérisée en dessus: 1° Par une surface plane, sur laquelle on distingue un cucléus médian, circonscrit par une profonde dépression; 2° par une forte échancrure à sa partie médiane supérieure"

Lessona & Pollonera (1882) recognise in *Palizzolia* diagnostic characters of the shell of Milacidae (oval, medial nucleus) and put *Palizzolia* in homonymy with the genus *Milax* Gray, 1855 (sub *Amalia*). This choise was followed by Cockerell (1991), Kennard & Woodward (1926), Wiktor (1987) although with a question mark, Alzona (1971) and Barker (1999). However, the shell of Milacidae offers no morphological characters useful for a reliable classification at both genus and species level and therefore it is possible that *Palizzolia* could be an older synonym of *Tandonia*.

The taxon *monterosati* was usually treated as doubtful species (Lessona & Pollonera, 1882; Minà Palumbo, 1883; Cockerell & Collinge, 1893; Wiktor, 1987; Cockerell, 1991;) because the rear hollow, the considerable thickening of the lower part of the shell and the groove around the apex are abnormalities detectable in different species of both *Milax* and *Tandonia* genera; only Kennard & Woodward (1926) pose the taxon *monterosati* in synonymy with *Milax gagates* (Draparnaud, 1801).



Figures 18-20. Shell (limacella) of *T. marinellii* n.sp., holotypus, h: 7.65 mm - D: 5.05 mm. Figure 21. Geographic distribution of *Tandonia marinellii* n. sp (circles) and *T. sowerbyi* (squares) in Sicily (personal data). Figures 22-24. Typus of *Palizzolia monterosati* (MHNG BGT 2385), h: 4.5 mm - D: 3.8 mm, thickness 2.2 mm. Figure 25. Original label of *P. monterosati* (MHNG BGT 2385). Figure 26. Four sequential visions of the ampoule rotated progressively so that to allow the overall vision of the label of *P. monterosati* (MHNG BGT 2385).

Giusti et al. (1995) note that *Tandonia* shells are generally thicker and oval, however examination of the *Palizzolia monterosati* type (MHNG BGT 2385) (Figs. 22-26) does not provide indications for univocal taxonomic attribution. At Calatafimi, locus typicus of *P. monterosati*, we surveyed *M. nigricans* (Philippi, 1851) and *T. marinellii* n. sp., but the presence of *M. gagates* and *T. sowerbyi* cannot be definitely ruled out. Hence, it appears that the taxon/binomial *Palizzolia monterosati* Bourguignat 1877 is a nomen dubium, attributable with certainty at neither genus nor species level. In these cases, on the basis of article 75.5 of ICZN, the Commission may be asked, in order to settle all taxonomic doubts, to set up a neotype which, in our opinion, should be *Milax gagates* since *Palizzolia*, as prevalent use, has been considered a synonym of the genus *Milax*, and *monterosati* synonym of *Milax gagates*.

For North Africa, particularly Tunisia and North Eastern Algeria, no species of the genus *Tandonia* was ever reported (Cockerell, 1891; Wiktor, 1987; Abbes et al., 2010). *Milax gasulli* Altena, 1974 and *Amalia ater* Collinge, 1895 are well known morphologically (genitalia) and considered as valid species of the genus *Milax*. Wiktor (1987) based on specimens of Algeria (without additional indications) puts *Limax scaptobius* Bourguignat, 1861 in synonymy with *Milax gagates* and both *Amalia cabiliana* (Pollonera, 1891) and *A. gagates* (var. or subsp.) *mediterrana* Cockerell, 1891 in synonymy with *Milax nigricans*.

Amalia maculata Collinge, 1895, described for the surroundings of Algiers, was considered by Wiktor (1987) synonymy of *T. sowerbyi*, but the original description does not allow per se a certain assignment to the genus *Tandonia*. The taxon *maculata* Collinge 1895, however, is pre-occupied by *Amalia maculata* Koch & Heynemann, 1874 =*Lytopelte maculate* (Koch & Heynemann, 1874) of the family Agriolimacidae (see Wiktor, 1987) and, for this reason, Hesse (1926) published, in its place, the new taxon *Milax collingei*. *Limax eremiophilus* Bourguignat 1861 (locus typicus Algiers, Algeria) was described only based on color and remains a taxon of uncertain allocation at both genus and species level. *T. sowerbyi* is reported for the regions of Southern Italy, attested with certainty up to Basilicata (Ferreri et al., 2005).

For Calabria two little-known taxa were described by Paulucci (1879), unreported even by Alzona (1971): *Amalia mongianensis* (locus typicus: Monte Pecoraro, Mongiana, Catanzaro) and *A. fulva* (locus typicus: "Monte Sant'Elia, Palmi"). The specimens from Calabria we could examine are to be considered as *T. sowerbyi*, although there are some morphological features that require further study.

Family Subulinidae Thiele, 1931

Rumina saharica Pallary, 1901

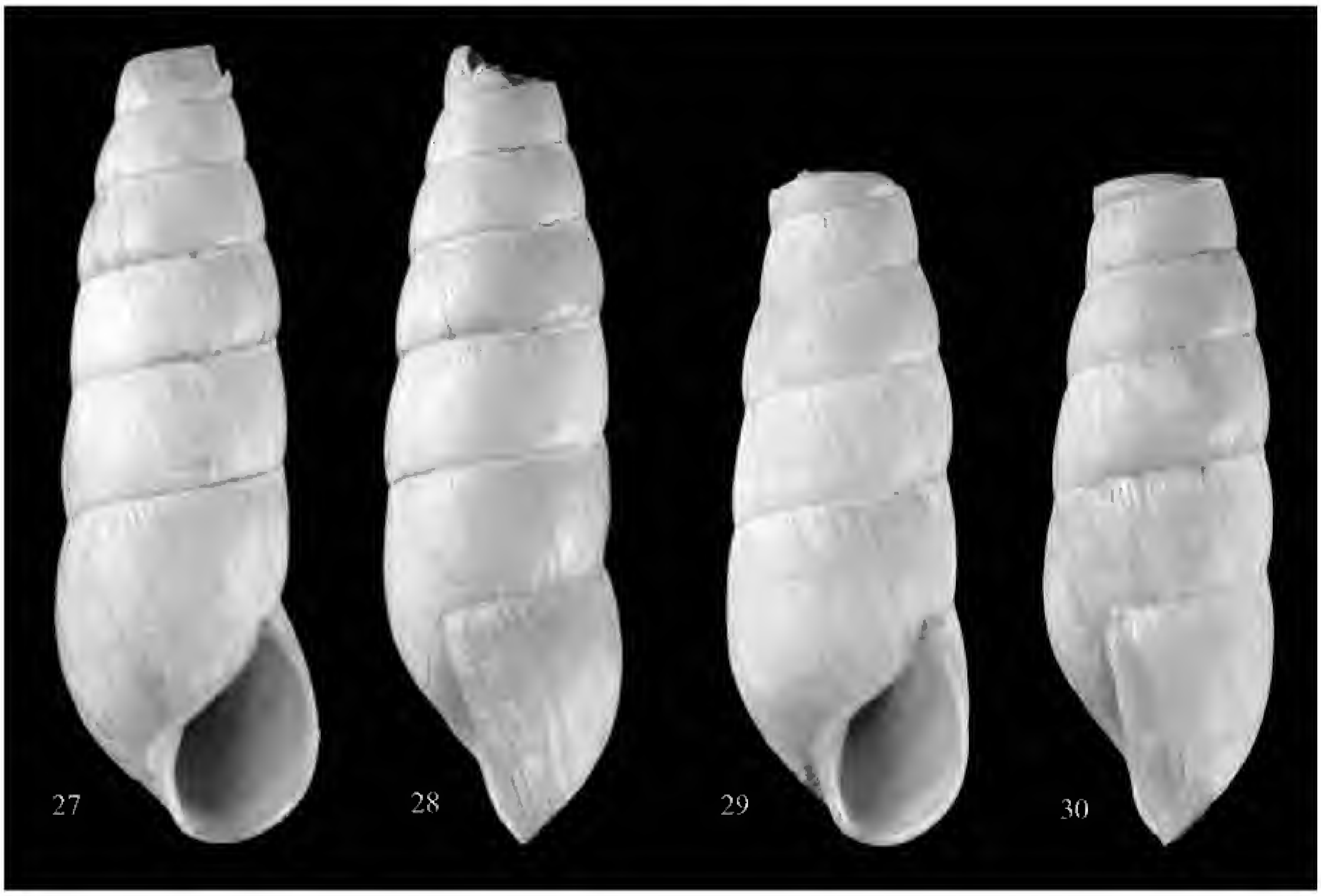
EXAMINED MATERIAL. *Rumina saharica*. Italy, Sicily, Egadi Islands, Marettimo, admist Case Romane and Buccerie 200-250 m, 37°58'N, 12°03'E, 30.V.2010, 6 specimens, 1 shell (CL); idem, 18 shells (CS); idem VIII.2012, 2 specimens, 10 shells (CC).

DESCRIPTION. Shell dextral (Figs. 27-30), whitish, truncated, height 30.5 mm, maximum diameter 10 mm, slender, sub-cylindrical, with slightly convex sides, the last whorl is wider than the penultimate whorl. Animal white. Genitalia (Figs. 31-32) characterized by vagina internally with longitudinal pleats and penis internally with some sparsely distributed papillae towards the proximal end.

BIOLOGY AND DISTRIBUTION. *R. saharica* is a thermophilic and xeroresistant species. The genus *Rumina* Risso, 1826 has Mediterranean distribution extending to Macaronesia, but it was dispersed by man in some extra-Mediterranean countries (United States, Mexico, Cuba, Bermudas, China, Japan). Currently, *R. saharica* seems to prevail in the north African-East European area (Carr, 2002; Prèvot et al, 2007).

REMARKS. Prèvot et al. (2007) with molecular analyses demonstrated the presence in the Mediterranean area of two groups of species: *R. decollata* and *R. saharica*. They also showed the presence in *R. decollata* of two clades genetically distinct but morphologically similar. In addition, Mienis (2002) re-evaluates the validity of *R. paviae* (Lowe 1861) from Morocco, Algeria, and Tunisia. In Sicily, actually, is known only *R. decollata* (Manganelli et al., 1995; Bank, 2012) (Figs. 23-24).

Carr (2002) signals, in the collection of Natural History Museum of London, the presence of three shells similar to *R. saharica* collected in Sicily, however he points out that without data on the genitalia the classification of *Rumina* species is not certain. The population of *Rumina* from Marettimo Island (Western Sicily) which we examined shows the typical morphological characters of *R. saharica* (sensu Carr, 2002) with the exception of the duct of bursa copulatrix which is slightly longer.



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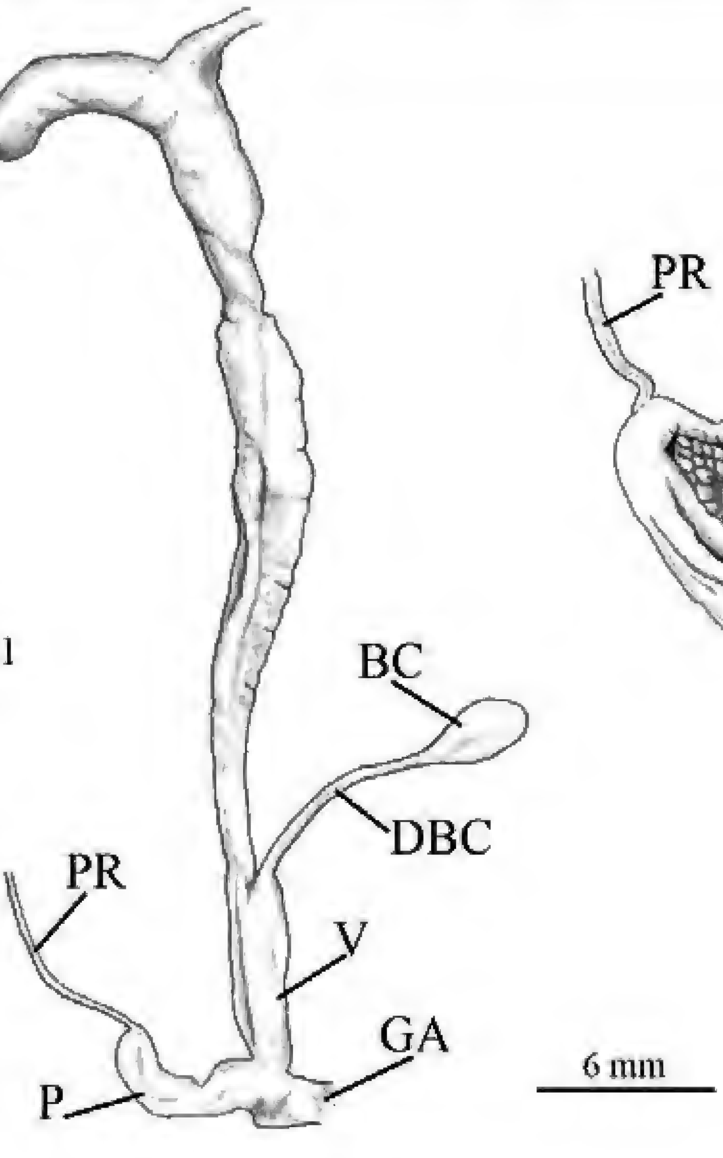

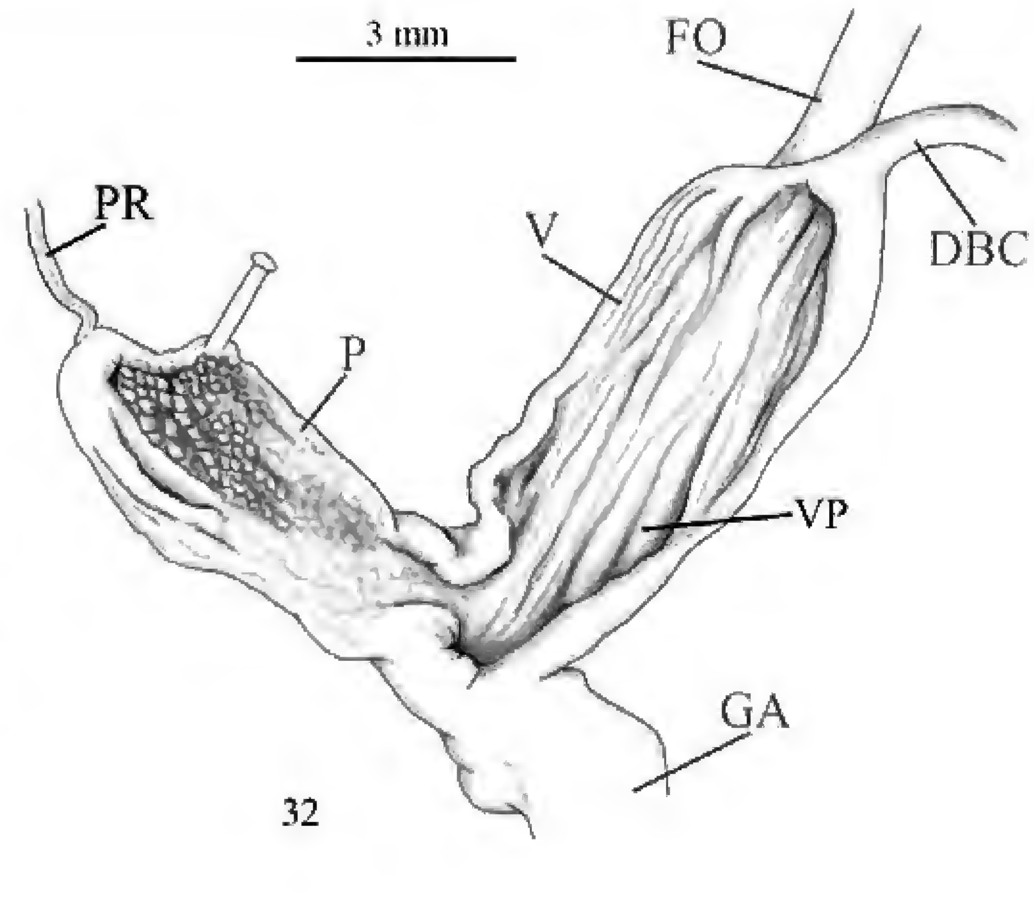

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Figures 27, 28. Shell of *Rumina saharica*, Marettimo, h: 31.9 mm - D: 10.2 mm. Figures 29, 30. Idem, h: 26.4 mm - D: 9.8 mm. Figure 31. Genitalia of *R. saharica,* Marettimo. Figure 32. Idem, internal structure of penis and vagina.

Family Clausiliidae Mörch, 1864

Lampedusa lopadusae nodulosa Monterosato, 1892

Clausilia (Lopadusaria) nodulosa - Monterosato, 1892: 29
Clausilia (Lopedusaria) nodulosa - Kobelt, 1893: 303
Clausilia (Lopedusaria) nodulosa - Kobelt, 1897: 292
Clausilia lopadusae var. *nodulosa* - Westerlund, 1901: 105
Clausilia lampedusae var. - Giglioli, 1912: 217
Lampedusa lopadusae nodulosa - Alzona in Zavattari, 1961: 427
Delima (Lmpedusa) lopedusae - Alzona, 1971: 92
Lampedusa lopadusae, (synonym) *nodulosa* - Holyoak, 1986: 217
Lampedusa lopadusae, (synonym) *nodulosa* - Beckmann, 1992: 22
Lampedusa lopadusae, (synonym) *nodulosa* - Cianfanelli, 2002: 61, T. 9, f. 29
Lampedusa lopadusae, (synonym) *nodulosa* - Bank, 2012

EXAMINED MATERIAL. Italy, Sicily, Pelagian Islands, Lampione, 31.VIII.2009, 12 specimens, legit T. La Mantia (CS); idem, 09.IX.2009, 23 specimens, legit A. Corso (CL); idem, 23.VII.2010, 5 specimens, legit T. La Mantia and S. Pasta (CS).

DESCRIPTION. Shell sinistral (Figs. 33-36), medium-sized (height 13-18.7 mm; maximun diameter 3.6-4.4 mm), fusiform, apex obtuse, elongated and inflated at half of its height, rather thick and robust, yellowish-brown in colour when fresh; external surface with oblique, thin and close ribs, 54-81 ribs on penultimate whorl. Spire with 9-10 convex whorls slowly and regularly growing, last whorl distinctly narrower than penultimate whorl and tapering downwards, rather gibbous near umbilicus. Sutures deep, subcrenulated; umbilicus slit-like, internally closed; aperture about ¼ of shell height (height 3.5-4.4 mm; maximun diameter 2.9-3.7 mm), irregularly ovalar or sub-squared, peristome continuous, reflected, little thickenek. Aperture with 5 lamellae on parietum and columellar side and 3 or 4 plicae and lunella on palatum. On parietum (Figs. 41-42), starting from suture, there are: parallel lamella in the form of small relief, spiral lamella at centre of parietum, columellar lamella, a little subcolumellar lamella, and a tooth like parietal lamella (upper lamella); only columellar lamella and parietal lamella are visible trough the opening (in apertural view). On the palatum (Fig. 43) there is a lateral lunella and, starting from suture: a long, well developed sutural plica; a second sutural plica variable in length: as long as the first one, shorter, or sometimes absent; principal plica thin and raised; palatal plica showing a rear portion merged with the upper part of lunella, a central indistinct part and an anterior part in the form of relief just visible. Clausilium elongated, plough-like (Fig. 44).

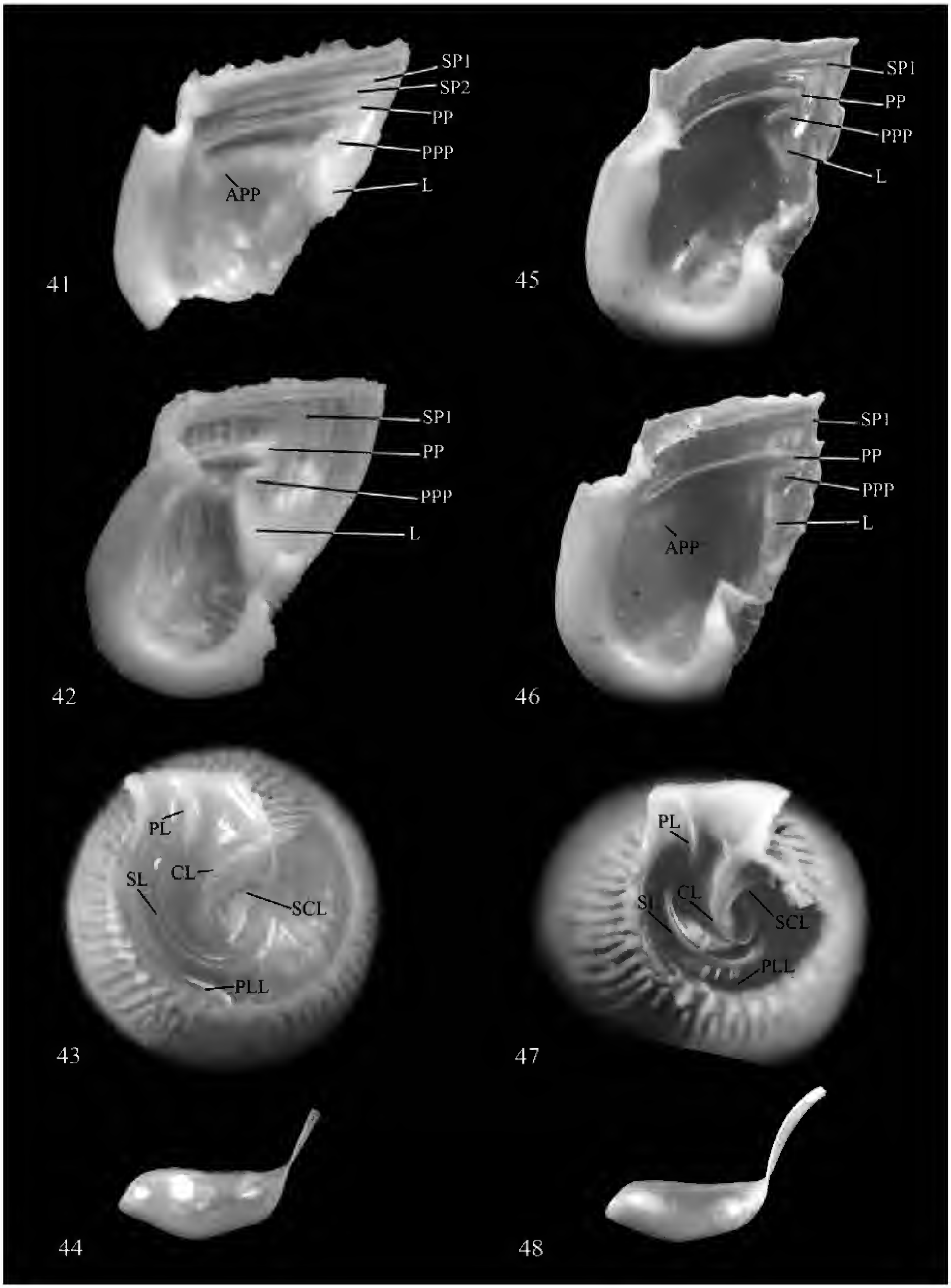
Body. Animal oval-elongate, narrow, posteriorly pointed, white-yellowish; upper tentacles short, cylindro-conical, apically widened, with small black eyes; lower tentacle very short (Fig. 51; see also Cianfanelli, 2002 fig. 29).

Genitalia. Anatomical organization of the genitalia (Fig. 49) is similar to *L. lopadusae* (Soòs, 1933; Pintér & Varga, 1984; Holyoak, 1986; Giusti et al., 1995) with penial complex consisting of flagellum, epiphallus, penis and penial diverticulum; flagellum short and slender; epiphallus divided by insertion of penial retractor muscle into proximal (1.5-1.6 mm) and distal (1.2-1.9 mm) portions; long, hook-like penial diverticulum (1.5-1.88 mm) arising on border between epiphallus and penis; penis long (2.6-3.6 mm); on the inner wall there are 5 longitudinal crests which are parallel in the distal portion and rather indistinct towards the penial diverticulum. Vagina long; short, wide copulatory duct branched in a short and slender duct of bursa copulatrix with small oval bursa copulatrix, and a short diverticulum of bursa copulatrix; short free oviduct.

BIOLOGY AND DISTRIBUTION. At the base of vegetation, at the soil, under stones (T. La Mantia in verbis). *L. lopaduse nodulosa* is endemic of the little isle of Lampione (Fig. 52), Pelagian Islands, between Sicily and Tunisia.

REMARKS. Monterosato (1892) described "Clausilia (Lopadusaria) nodulosa" from the island of Lampione, with the following words: "Conchiglia solida, striata quasi obliquamente (nella C. Lopadusae le coste sono perpendicolari ed esattamente lamellate); apertura a bordi ben rivoltati, porcellaniosi; colorazione bianchiccia; anfratti cochleaeformi, apice più ottuso. Dimensione quasi la stessa." ["Solid shell, ribbed almost sideways (in *C. lopadusae* the ribs are perpendicular and exactly lamellated); opening with edges well turned, porcelain-like; whitish colour; cochlea-shaped whorls, apex more obtuse. Almost the same size"].

Figures 33-36. Shell of *L. lopadusae nodulosa*, Lampione, h: 16.94 mm - D: 4.16 mm. Figures 37-40. Shell of *L. lopadusae lopadusae*, Lampedusa, h: 17.11 mm - D: 4 mm.



Figures 41-44. *L. lopadusae nodulosa*, Lampione: palatum of two specimens (Figs. 41-42), parietum (Fig. 43) and clausilium (Fig. 44). Figures 45-48. *L. lopadusae lopadusae*, Lampedusa, palatum of two specimens (Figs. 45-46), parietum (Fig. 47) and clausilium (Fig. 48).

Subsequently, this taxon is reported by Kobelt (1893; 1897) while Westerlund (1901) and Alzona (1961) consider it respectively as variety and subspecies of *L. lopadusae*. Alzona (1971), Holyoak (1986) and Beckmann (1992) put *L. nodulosa* in synonymy with *L. lopadusae*. No news of this taxon is reported by Manganelli et al. (1995) and Cossignani & Cossignani (1995). Cianfanelli (2002), despite considering it a synonym of *L. lopadusae*, reports that the population of the island of Lampione "… presenta dei caratteri piuttosto distinti sia nella conchiglia che nell'animale" ["... shows pretty distinct characters both in the shell and in animal"]. Nordsieck (2007) did not mention it in his catalog on the Clausiliidae of the world, Bank (2012) still considers it a synonym of *L. lopadusae*. *L. nodulosa* differs from *L. lopadusae* (Figs. 37-40. Figs. 45-48. Fig.51) for shell less robust, darker in color i.e. yellowish-brown (yellowish-grey in *L. lopadusae*), with deeper sutures, and whorls more convex so that the shell profile, in frontal view, appears less linear than *L. lopadusae*; peristome is less develo-

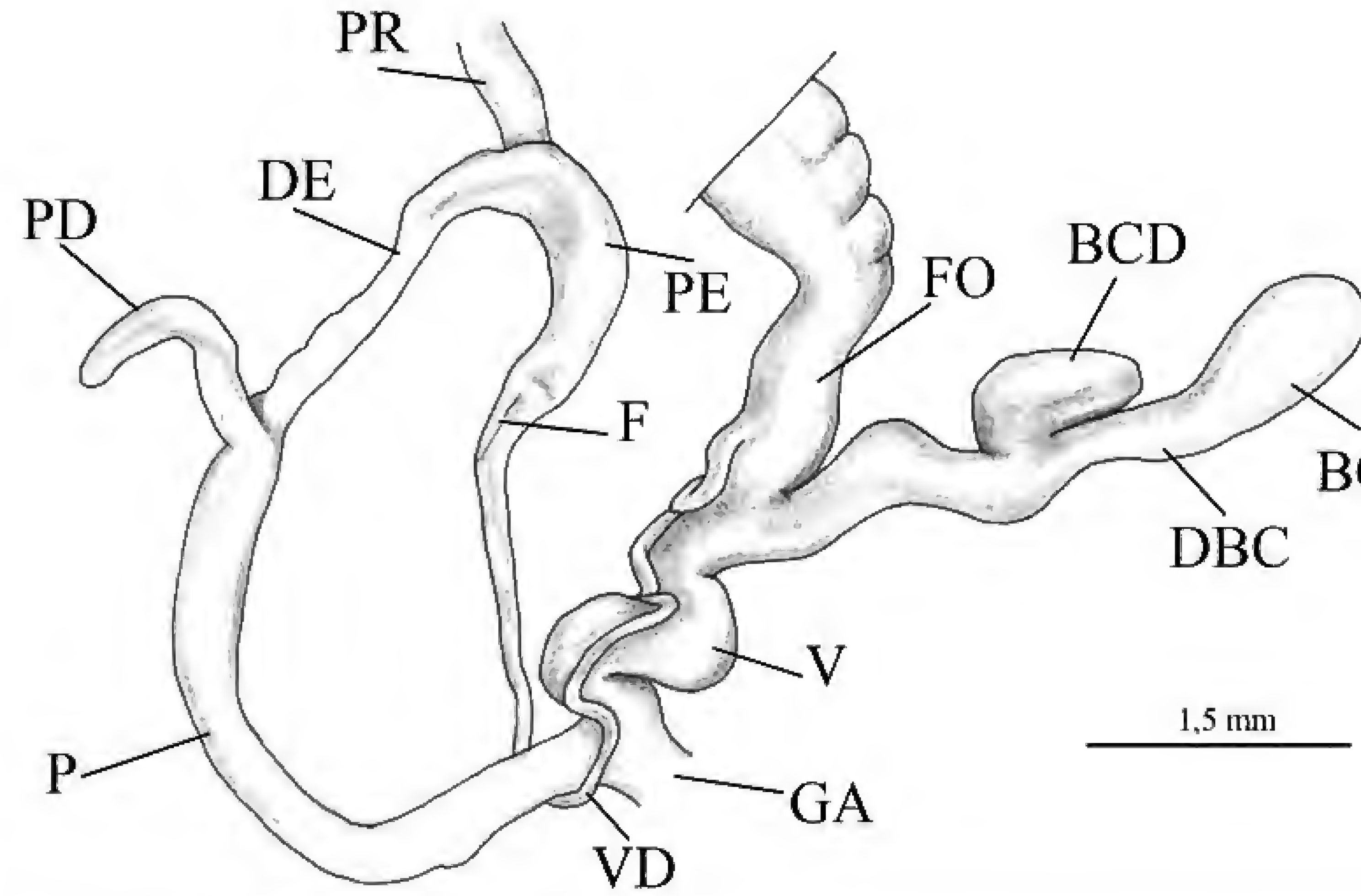


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Figure 49. Genitalia of *L. lopadusae nodulosa*. Figure 50, 51. Body colour in *L. lopadusae lopadusae* (Fig. 50) and *L. lopadusae nodulosa* (Fig. 51). Figure 52. Lampione island (photo P. Lo Cascio).

ped and calcified; ribs are more oblique, more numerous and less robust; in the internal structure of the shell, *L. nodulosa* mostly shows a second sutural plica (rarely present in *L. lopadusae*). Genitalia of *L. nodulosa* differ from *L. lopadusae* for penial diverticulum slightly longer; the animal is lighter in color. As reported in the original description of Monterosato (1892), highlighted by Cianfanelli (2002) and confirmed by our observations, *L. nodulosa* presents some morphological differentiations with respect to *L. lopadusae* and therefore we believe it is worthy of taxonomic reconsideration, at least at the sub-specific level, also in view of its peculiar geographical isolation. Indeed, the island of Lampione, where *L. nodulosa* lives, reaches its maximum altitude at 36 m above sea level and is approximately 17.5 Km far from Lampedusa, from which is separated by a stretch of sea -80 m deep. Despite its very little size, this islet harbours a very rich pool of plant and animal species, particularly some local endemics of high biogeographic interest (Lo Cascio & Pasta, in press.)

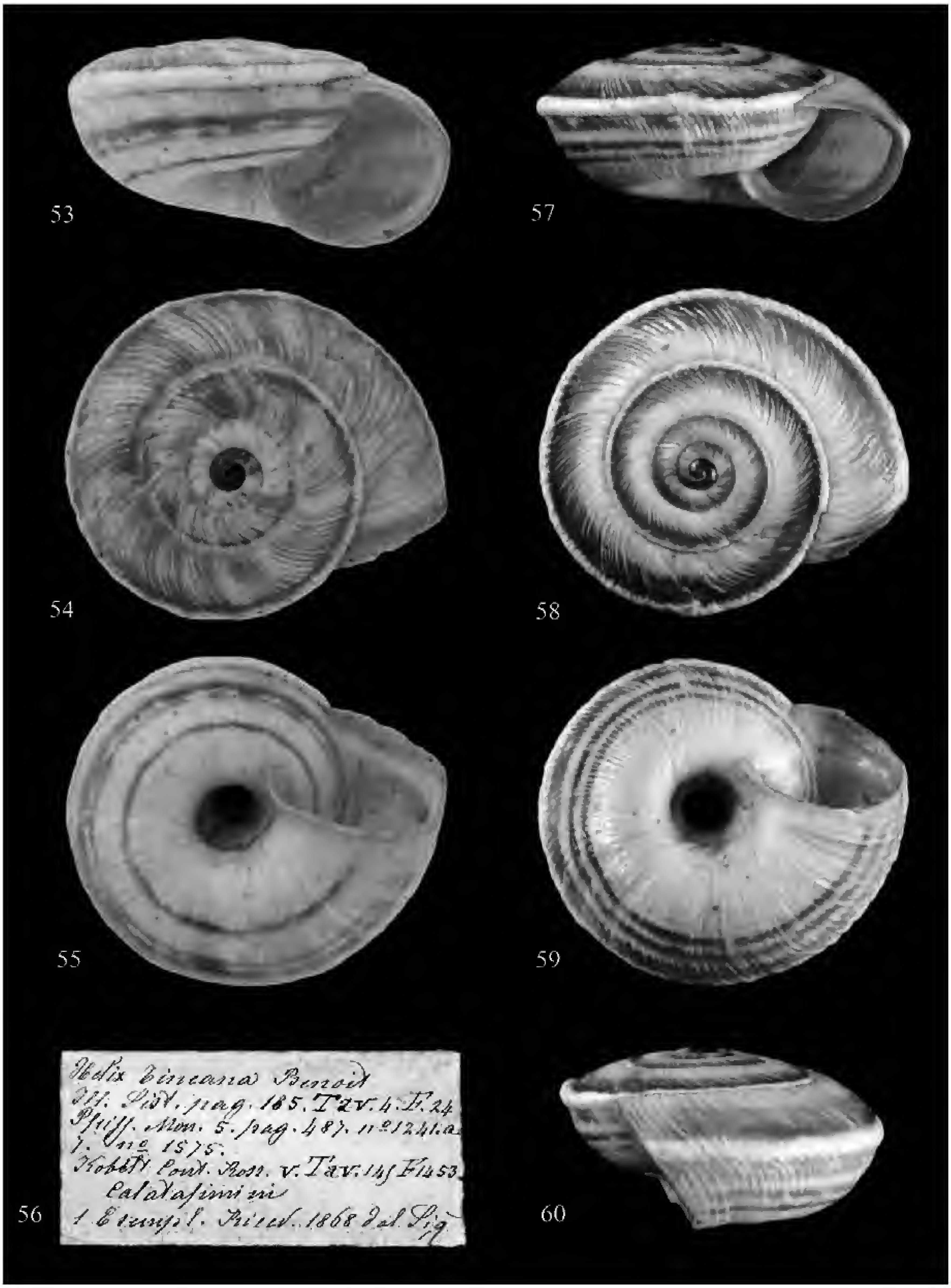
Family Hygromiidae Tryon, 1866

Cernuella (Cernuella) tineana (Benoit 1862)

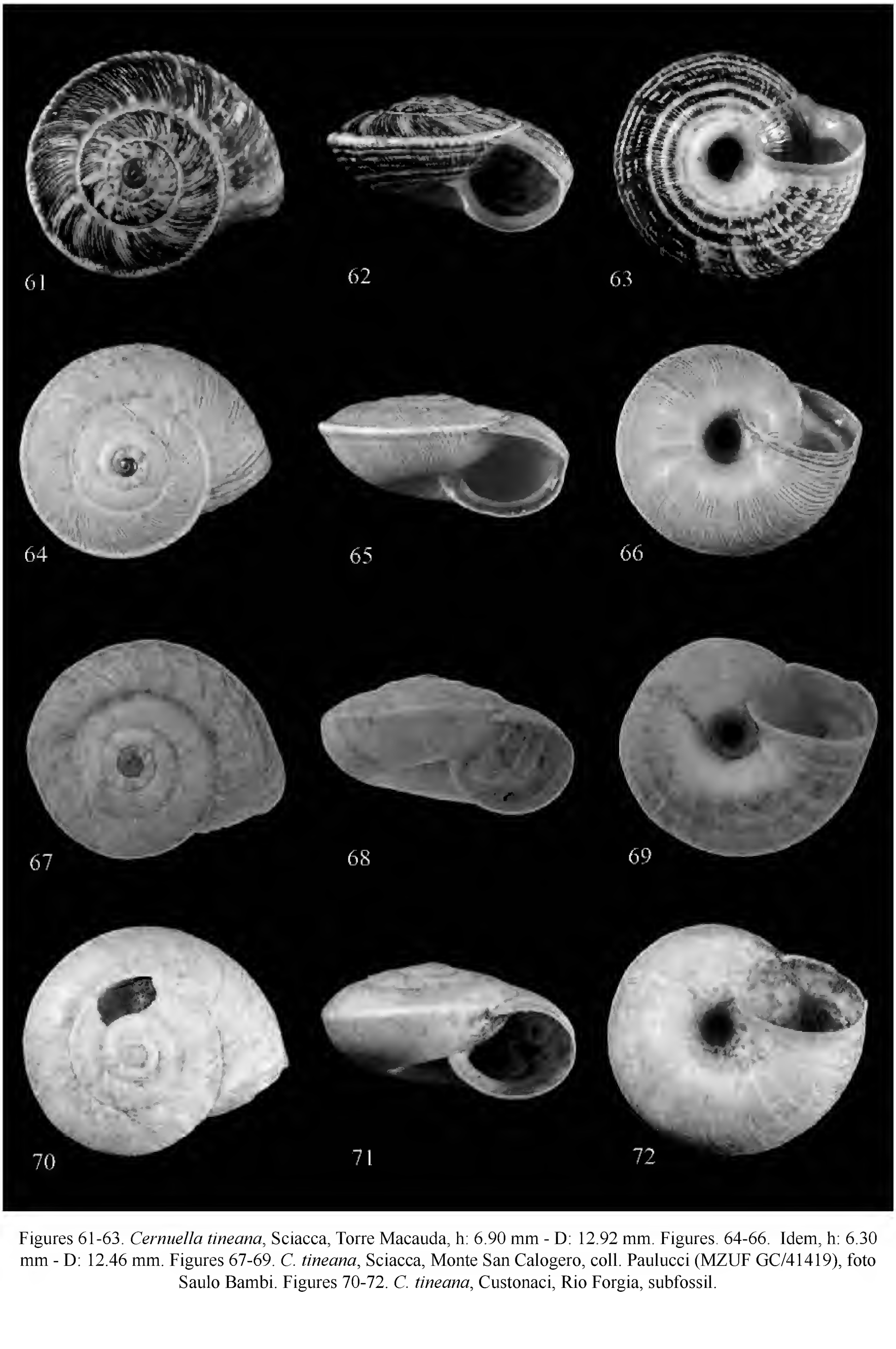
Helix tineana - Benoit, 1862: 185-187, t. 4, fig. 24 (Calatafimi)
Helix tineana - Pfeiffer, 1868: 487 (Sicilia, Calatafimi)
Helix tineana - Benoit, 1875: 14 (Calatafimini)
Helix Xerophila tineana - Kobelt, 1875: 18 (Calatafimi)
Helix (Xerophila) Jacosta tineana -Westerlund, 1876: 104 (Sicilia)
Helix (Xerophila) Jacosta tineana var. *kobeltiana* - Westerlund, 1876: 104
Helix tineana - Kobelt in Rossmassler, 1877: 103-104, fig. 1452 (Sicilia, Calatafimi)
Xerophila (Jacosta) tineana - Kobelt, 1881: 47 (Sicilien)
Xerophila (Jacosta) tineana kobeltiana - Kobelt, 1881: 47 (Sicilien)
Helix tineana - Benoit, 1882: 37 (Calatafimini)
Helix (Helicella) Jacosta tineana - Tryon, 1887: 253 pl. 62 fig. 92-94 (Sicily)
Helix (Xerophila) Jacosta tineana - Westerlund, 1889: 318 (Sicilien, Calatafimini)
Helix (Xerophila) Jacosta var. *mista* - Westerlund, 1889: 318-319 (Sicilien)
Helix (Xerophila) Jacosta tineana var. k*obeltiana* - Westerlund, 1889: 319 (Sicilien)
Helix (Xerophila) Jacosta tineana var. *mista* -Westerlund, 1890: 61 (Sicilien)
Helix (Xerophila) Jacosta tineana var. *kobeltiana* - Westerlund, 1890: 61 (Sicilien)
Helicella Jacosta tineana - Pilsbry, 1894: 260
Helicella Jacosta tineana var. *mista* - Pylsbry, 1894: 260
Helicella Jacosta tineana var. *kobeltiana* - Pylsbry, 1894: 260
Helicella (Xerotropis) tineana - Alzona, 1971: 174
Helicella (Xerotropis) tineana mixta - Alzona, 1971: 174
Helicella (Xerotropis) tineana kobeltiana - Alzona, 1971: 174

EXAMINED MATERIAL. Italy, Sicily, Monte Cofano, Gorgo Cofano, 38°06'07"N 12°40'31"E, 228 m, 14.X.1984, numerous specimens (CS); Italy, Sicily, Sciacca, Torre Macauda, 37°28'58"N 13°10'59"E, 59 m, 1.VII/31.VII.1986, numerous specimens (CS); Italy, Sicily, Monte Cofano, Gorgo Cofano, 38°06'07"N 12°40'31"E, 228 m, 14.IV.1991, numerous specimens (CS); Italy, Sicily, Ribera, Contrada Castello, 37°30'18"N 13°15'04"E, 153 m, IX.2005, 3 specimens, 10 shells (CR); idem, 37°30'23"N 13°14'12"E, 144 m, IX.2005, 2 specimens, 12 shells (CR); Italy, Sicily, Cava a Nord di Ribera, 30.XII.2007, 3 specimens, M. and E. Bodon (CB); Italy, Sicily, Sciacca, Torre Macauda, 37°28'58"N 13°10'59"E, 59 m, 24.V.2008, numerous specimens (CS); idem 22.II.2009, 23 shells (CL); Italy, Sicily, Sciacca, Torre Macauda, 37°28'58" N 13°10'59" E, 60 m, 22.XI.2009, 1 specimen, 8 shells (CL); Italy, Sicily, Custonaci, Rio Forgia, 38°03'42"N 12°39'32"E, 56 m, 6.II.2011, 3 subfossil shells (Figs. 70-72); Italy, Sicily, Monte Cofano, Gorgo Cofano, 38°06'07"N 12°40'31"E, 228 m, 20.XI.2011, numerous specimens (CS); Italy, Sicily, Monte Cofano, Gorgo Cofano, 38°06'07"N 12°40'31"E, 228 m, 4.III.2012, numerous specimens (CS); Italy, Sicily, Custonaci, Baglio Cofano, 38°06'11"N 12°40'40"E; 250 m, 05.VIII.2012, 29 shells (CL).

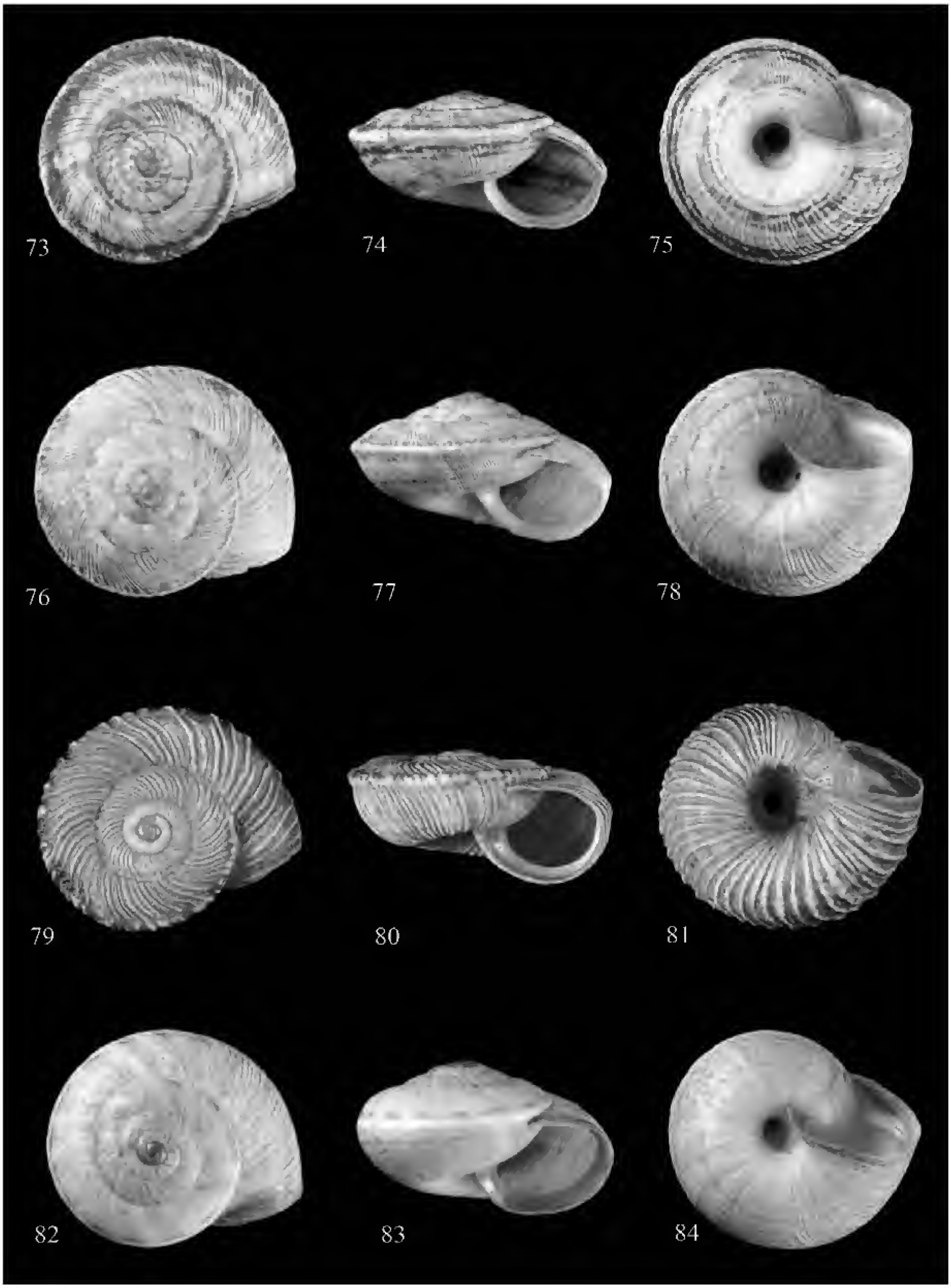
DESCRIPTION. Shell dextral (Figs. 53-55, 57-75), medium-sized (height: 7.2 mm, maximum diameter 17 mm), depressed, robust, whitish or greyish-yellow in colour with brown band and dark apex; external surface finely and regularly ribbed, opaque,



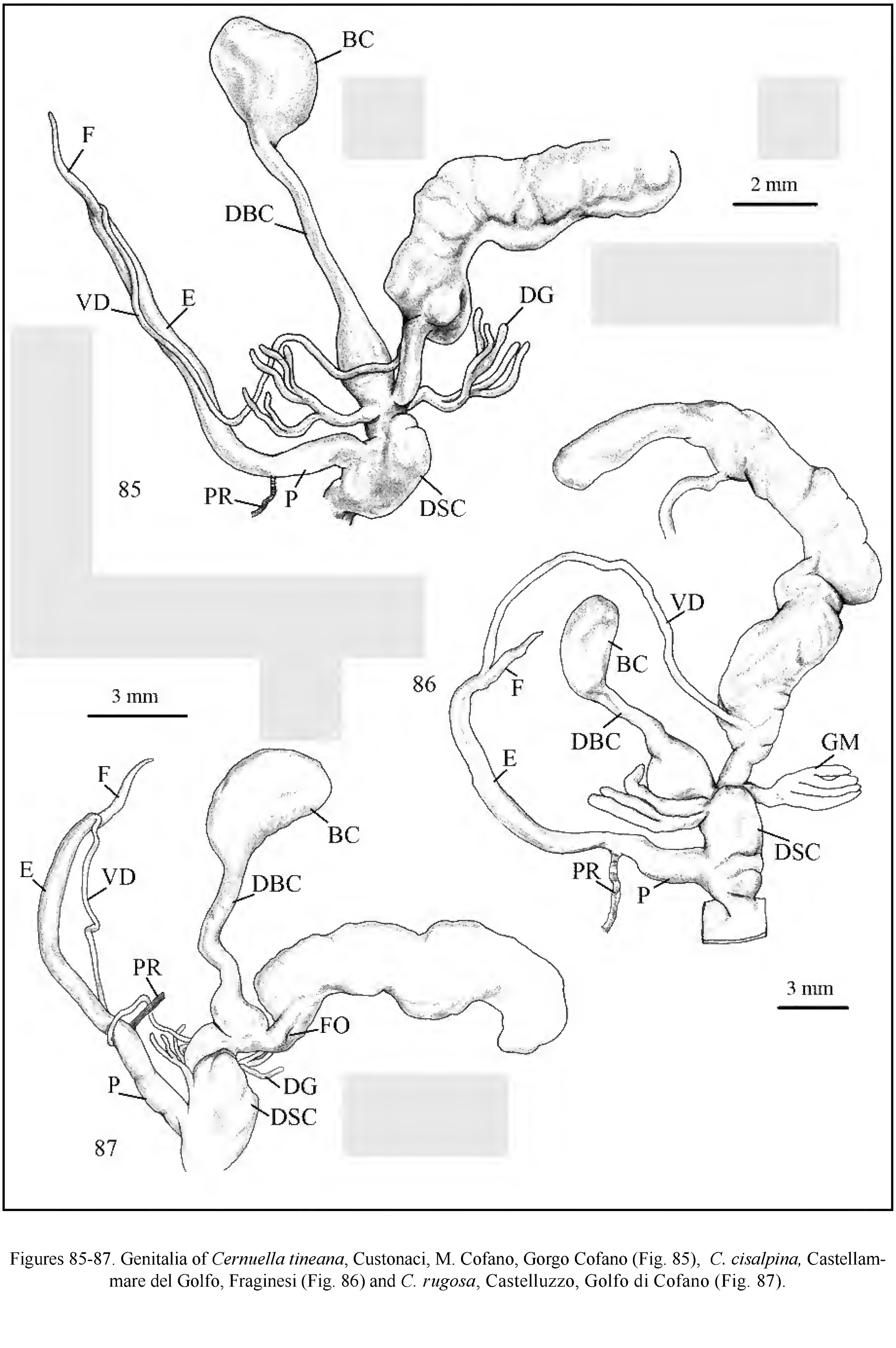
Figures 53-56. *"Helix" tineana*, Calatafimi, Paulucci collection (MZUF GC/10825) (Figs. 53-55) and original label (Fig. 56), photos Saulo Bambi. Figures 57-60. *C. tineana*, Custonaci, Monte Cofano, Baglio Cofano, h: 5.55 mm - D: mm 11.10 mm.



Figures 61-63. *Cernuella tineana*, Sciacca, Torre Macauda, h: 6.90 mm - D: 12.92 mm. Figures. 64-66. Idem, h: 6.30 mm - D: 12.46 mm. Figures 67-69. *C. tineana*, Sciacca, Monte San Calogero, coll. Paulucci (MZUF GC/41419), foto Saulo Bambi. Figures 70-72. *C. tineana*, Custonaci, Rio Forgia, subfossil.



Figures 73-75. *Cernuella tineana*, Sciacca, Torre Macauda, h: 7.07 mm - D: 12.42 mm. Figures. 76-78. *C. amanda*, San Vito lo Capo, Salinelle, h: 12.18 mm - D: 7.12 mm. Figures 79-81. *C. rugosa*, Castelluzzo, Calette degli Agliarelli, h: 6.30 mm - D: 11.95 mm. Figures 82-84. *C. cisalpina,* Castellammare del Golfo, Fraginesi, h: 6.90 mm x D: 10.80 mm.



Figures 85-87. Genitalia of *Cernuella tineana*, Custonaci, M. Cofano, Gorgo Cofano (Fig. 85), *C. cisalpina,* Castellammare del Golfo, Fraginesi (Fig. 86) and *C. rugosa*, Castelluzzo, Golfo di Cofano (Fig. 87).

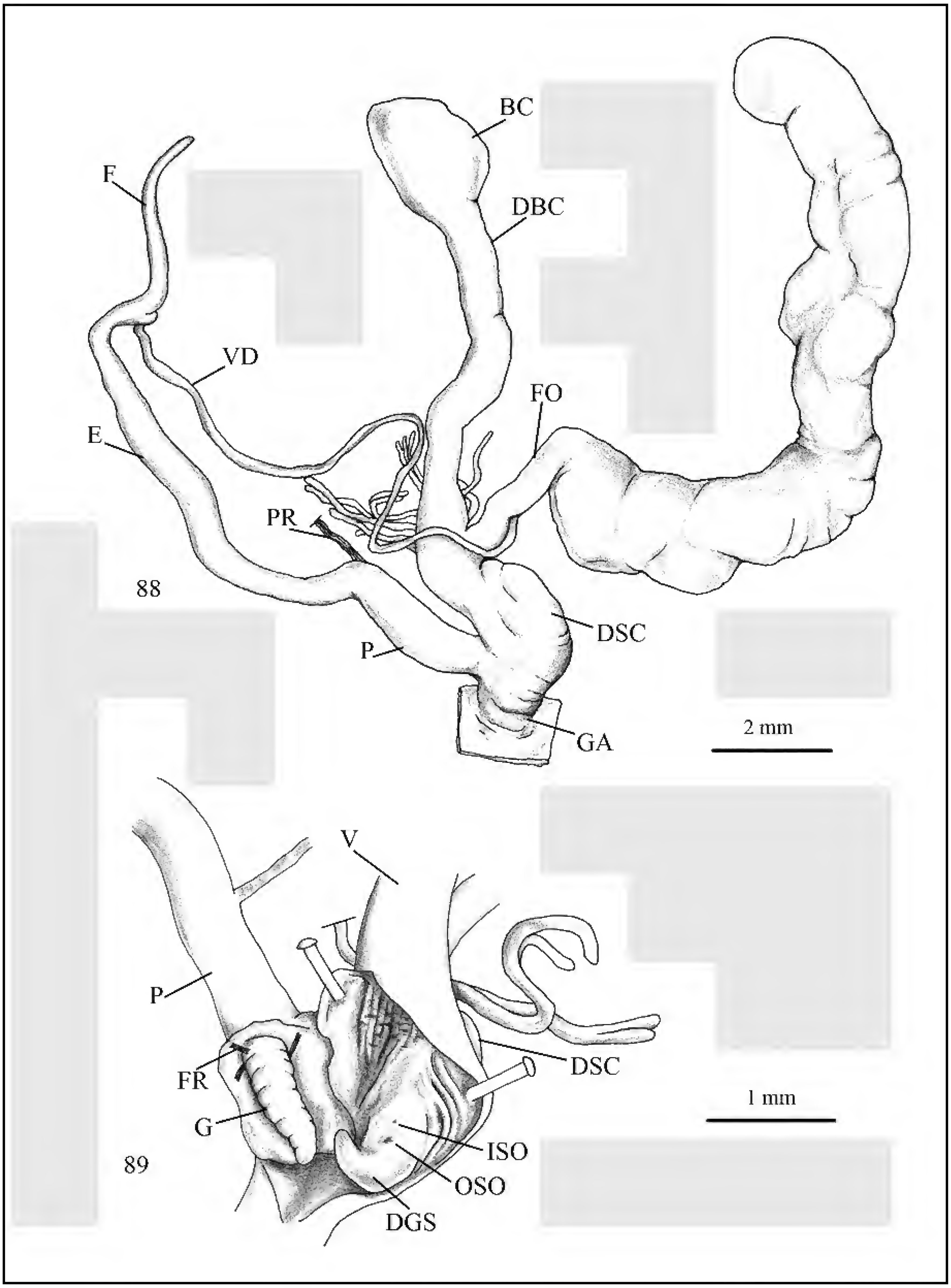


Figure 88. Genitalia of *Cernuella tineana*, Sciacca, Torre Macauda. Figure 89. Idem, internal structure of penis, dart sac and vagina.

spire more or less flat, with 5 regularly growing whorls, slightly convex; marked sutures; last whorl very convex below and keeled at its periphery; umbilicus deep and wide, about 1/3 of maximum shell diameter; aperture oval and slightly angled, peristome simple, interrupted, with internal rib.

Body. Animal whitish; dorsal region provided, more or less extensively, of dark spots.

Genitalia. Short free oviduct, duct of bursa copulatrix of medium length, with large base, ending in a sac-like bursa copulatrix; vagina short (1-1.6 mm), 2 tufts of digitiform glands with 4-5 slender lobes and 8-12 apexes, disposed on opposite sides of proximal vagina. Dart-sac complex consisting of a pair of stylophores located on one side of vagina; large outer stylophore containing dart. Penial complex composed of flagellum, epiphallus and penis; flagellum long (2-2.4 mm), ending where vas deferens enters penial complex; epiphallus long 3-5 times the length of the penis (4.5-5.5 mm), ending where penial retractor muscle contacts penial complex wall; penis short (1.5-2.2 mm); penial papilla cylindrical, elongate, with apical opening, and base connected to penial walls by three small muscles (frenula).

Biology and Distribution. *C. tineana* is found on the ground, usually on grass often under stones. It is endemic to Sicily, distributed with point populations in coastal and low-hill territories ranging from Custonaci (Monte Cofano) in the province of Trapani to Ribera in the province of Agrigento (Fig. 90).

Comparative notes. Anatomical character of *"Helix" tineana* suggest to ascribe this species to the genus *Cernuella* Schluter, 1838 sensu stricto: penial papilla with three basal frenula, two groups of digitiform glands on opposite sides of the vagina, proximal vagina short or absent, proximal portion of the duct of bursa copulatrix wide (Manganelli & Giusti, 1987; Manganelli et al., 1996a, b, 2001). Currently, five species of *Cernuella* s. str. are recognized in Sicily (Bank, 2012): *C. aradasi* (Pirajno, 1842), *C. metabola* (Westerlund, 1889), *C. cisalpina* (Rossmässler, 1837), *C. virgata* (Da Costa, 1778), *C. rugosa* (Lamark, 1822).

C. aradasi is a dune-species with limited distribution to the dunes near the lighthouse in Messina (North-east Sicily) and neighbouring sandy soils. It's distinguished from *C. tineana* for shell smaller, smooth, globose and without keel; genitalia characterised by relatively large penis and by epiphallus twice as long as penis.

C. metabola is an endemic species from Lampedusa island; it is distinguished from *C. tineana* by the shell with the globose shell with discontinuous, thick ribs and narrow umbilicus. A preliminary study on the genitalia of this species seems to highlight significant differences from the other *Cernuella* species (unpublished data).

C. cisalpina is a polymorphic species, with a Mediterranean distribution, for which several taxa of still difficult taxonomic interpretation were established. Shell of small-medium size, "small sized *Cernuella*" sensu Manganelli & Giusti (1987), subglobose, with thin ribs, sometimes well raised, last whorl usually rounded or angled at its periphery (keel-like) (Figs. 82-84). Some populations of *C. cisalpina* present a shell similar to that of *C. tineana*, but in addition to the morphological characteristics of *C. tineana* pointed out above, they are always distinguishable by their genitalia with epiphallus 2-3 times longer than penis, flagellum and penis proportionally shorter and digitiform glands lower, i.e. between the vagina and the inner dart sac (Fig. 86).

C. virgata is a polymorphic species showing a European-wide distribution. In Sicily it is common at low and medium altitudes where specimens can be found on grass and shrubs. *C. virgata* is distinguished from *C. tineana* for the shell which is smooth or with faint wrinkles, without keel and larger, "large sized *Cernuella*" sensu Manganelli & Giusti (1987); genitalia resembling those of *C. cisalpina* but with epiphallus longer and more numerous digitiform glands.

C. rugosa, endemic of Western Sicily known only for two locations (Figs. 79-81, 90), is an extremely vulnerable species deserving of protection. From the morphological point of view *C. rugosa* is distinguished from *C. tineana* for the shell with raised, irregularly spaced ribs and a cordlike, crenulated keel at its periphery, and for the penial complex (Figure 87; Manganelli at al., 1996b, Fig. 16) with penis longer, epiphallus and flagellum shorter.

C. tineana is morphologically well distinguishable from other *Cernuella* species sensu stricto. Differential diagnosis problems may arise with the shell of *Cernuella (Xeroamanda) amanda* Rossmässler, 1838 (see also Benoit, 1862-1857) (Figs. 76-78).

In the latter species the shell is as convex inferiorly as in the upper part, the keel is less obtuse than *C. tineana*, opening more angled and the umbilicus markedly funnel-shaped. However, an examination of genitalia can easily allow to distinguish the two species that belong to distinct subgenera (Manganelli et al., 1996).

REMARKS. *Helix tineana* was described by Benoit (1862) for the surroundings of Calatafimi, "Pizzo di grasso " and dedicated to the then Director of the Orto Botanico of Palermo, Vincenzo Tineo. Benoit (1857) provides, in addition to the detailed description of the shell, also a comparative analysis of *"Helix" rugosa* Lamark, 1822 and *"Helix" amanda* Rossmässler, 1838, and draws the three species in table IV, figs. 24, 25, 29. In his later works, Benoit (1875, 1882) reported this species citing only the locus typicus. Other authors cited this species: Pfeiffer (1868), Kobelt (1875), Kobelt in Rossmassler (1877), Tryon (1887). Westerlund (1876) reports it indicating the locality "Sicilia" and describes the variety *kobeltiana* on the basis of specimens received by Kobelt under the name " H. tinei Ben.". Subsequently, Westerlund (1889) re-describes *H. tineana* from " Sicilien bei Calatafimi " and adds to the variety *kobeltiana* the new var. *mista* with "Sicilien" as locus typicus.

Alzona (1971) ascribes *"tineana"* to the genus *Helicella* Férussac, 1821 subgenus *Xerotropis* Monterosato, 1892 and considers the two varieties described by Westerlund (1889) as valid subspecies. Neither Cossignani & Cossignani (1995) and Manganelli et al. (1995) nor Bank (2012) report *"Helix" tineana* for, respectively, the Italian fauna and the European fauna.

Despite repeated searches, we have not found this species in the locus typicus, Calatafimi. However, in Paulucci collection we saw a shell determined as "Helix tineana" (MZUF GC/10825), collected in Calatafimi by a sicilian naturalist De Stefani, in 1868 (Figs. 53-56). This topopypic sample corresponds with Benoit's original description and even with the specimens we have sampled and studied on Monte Cofano. The more southerly po-

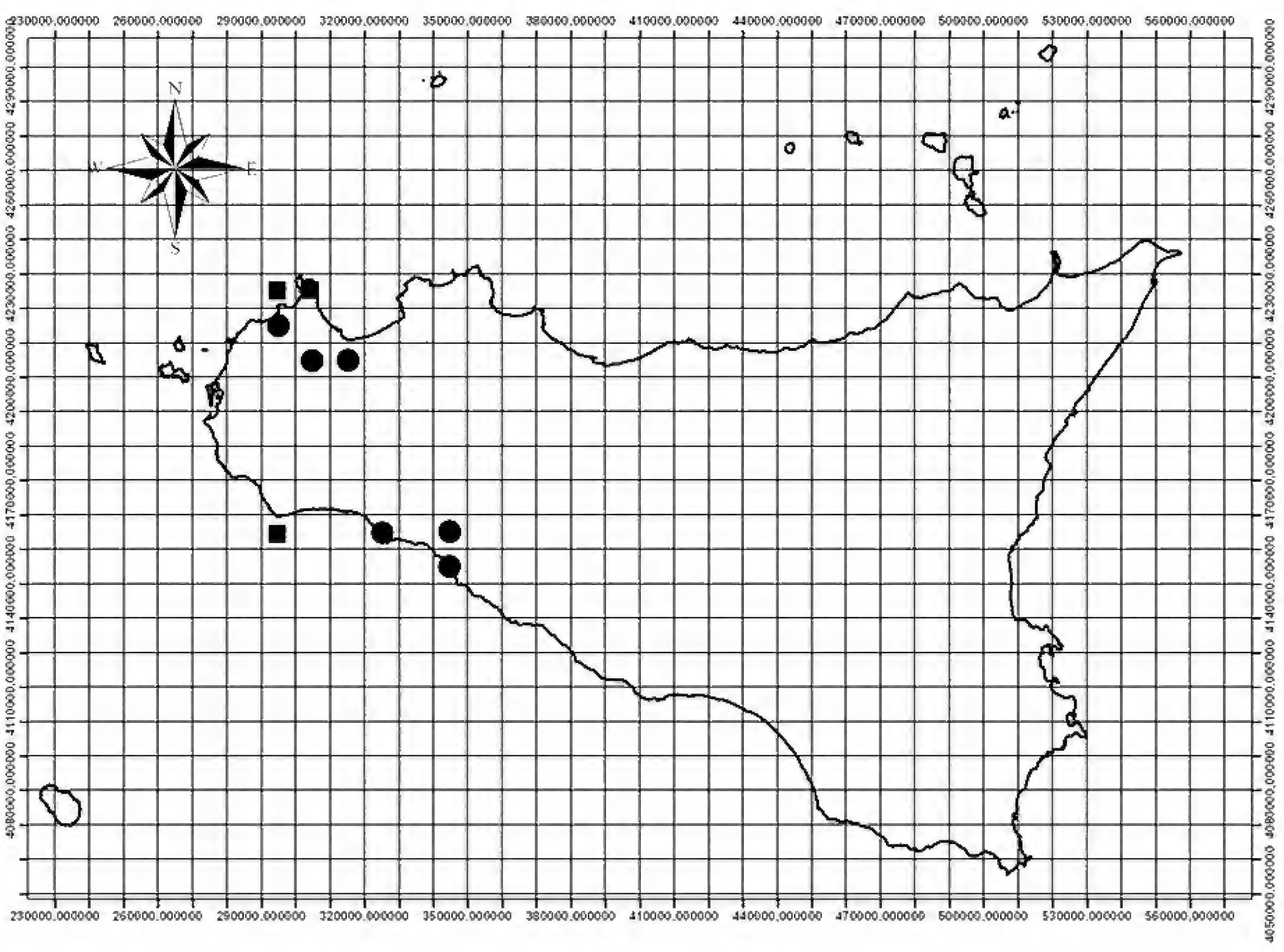


Figure 90. Geographic distribution of *C. tineana* (circles) and *C. rugosa* (squares) in Sicily.

pulations (Sciacca, Ribera) have slightly larger dimensions. To these populations we attribute, by comparison, also one sample from Paulucci collection sub *H. caficiniana* (MZUF GC/41419) picked up at Sciacca, Monte San Calogero (South-Western Sicily) (Figs. 67-69).

"Helix" caficii, described by Westerlund (1876) with locus typicus Sciacca, corresponds, in our view, with the populations of *C. tineana* of Sciacca. If the examination of the type in the Westerlund collection will confirm this assumption *"Helix" caficii* may be a synonym of *C. tineana*.

ACKNOWLEDGEMENTS

We wish to thank Luigi Barraco (Valderice, Trapani, Italy), Marco Bodon (Italy, Genova), Saulo Bambi and Simone Cianfanelli (Museo di Storia Naturale dell'Università di Firenze sezione di zoologia de "La Specola"), David P. Cilia (Santa Venera, Malta), Andrea Corso (Siracusa, Italy), Giulio Cuccodoro and Yves Finet (Muséum d'Histoire naturelle, Genève, Switzerland), Elena Gavetti (Museo Regionale di Scienze Naturali di Torino, Italy), Alessandro Hallgass (Roma, Italy), Tommaso la Mantia (Palermo, Italy), Pietro Lo Cascio (Lipari, Messina, Italy), Giuseppe Maraventano (Lampedusa, Agrigento, Italy), Gianbattista Nardi (Nuvolera, Brescia, Italy), Roberto Poggi (Museo Civico di Storia Naturale di Genova "G. Doria", Italy), Agatino Reitano (Tremestieri Etneo, Catania, Italy), András Varga (Andras Varga, Mátra Múzeum Gyöngyös, Hungary), Francisco Welter-Schultes (Zoologisches Institut, Berliner, Germany).

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Biodiversity Journal, 2012, 3 (3): 229-236

A stability assessment on seasonal variation of seaweed beds in the Trat peninsula of Thailand

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ABSTRACT Species diversity, biomass and distribution pattern of seaweed beds in the Trat peninsula, east coast of Thailand, were investigated in relation to environmental conditions from January to December 2011. The macroalgal samples and environmental factors were collected monthly; covering cool-dry (January-February, November-December), hot-dry (March-April) and rainy (May-October) seasons at four sampling stations; Ao Cho, Ao Lane, Laem Tien and Laem Sok. A total of 26 taxa of marine benthic algae were recorded, of which 16 species of red marine algae were the most diverse group. It was found that *Catenella nipae*, *Gracilaria salicornia*, *Gelidium pusillum*, *Hydropuntia changii*, *Hypnea hamulosa*, *Kyrtutrix maculans*, *Laurencia decumbents*, *Lyngbya majuscula*, *Peyssonnelia rubra* and *Ulva clathrata* were the most abundant throughout the sampling period. The highest number of marine flora species was obtained in March (25 species), whereas the lowest in June (12 species). Algal biomass had a maximum value in April (59.50 g/m^2 dry weight) and minimum value in July (20.14 g/m^2 dry weight).

KEY WORDS Seasonal variation; benthic algae; seaweed beds; Conservation; Trat peninsula.

Received 12.07.2012; accepted 03.09.2012; printed 30.09.2012

INTRODUCTION

The seaweed or marine macrophytic algae, which are a large and diverse group of eukaryotic photosynthetic organisms occurring in marine environment, are one of the major marine fishery resources in Thailand (Edwards et al., 1982). Seaweed beds are a common habitat in coastal inshore communities consisting of large benthic vegetation and distributed widely along coastline of Thailand (Lewmanomont, 1998; Prathep, 2005).

They are highly valuable ecologically and economically and perform a variety of functions within marine coastal ecosystems (Lobban & Harrison, 1994; Stachowicz et al., 2008). Most seaweed beds are served as a vitally important food sources for fish and aquatic invertebrates and provide breeding area for several species of marine animals (Zhang et al., 2008). Additionally, seaweeds are used around the world as food and fertilizers and for the extraction of valuable commercial products (Sambamurty, 2006, Graham et al., 2009).

Trat is one of the provinces located in the east of Thailand, and encloses the upper Gulf of Thailand adjacent to the border between Thailand and Cambodia. Along the coastline of Trat peninsula, there are many different types of coastal ecosystems including estuaries, mangrove areas, sandy shores and mudflats. In addition to coastal environment, there is a considerable amount of nutrient supply variation in the response of wave exposure gradient.

Because of the properties of coastal area features and environmental diversity, Trat peninsula has remarkably diverse marine fishery resource, especially macroalgal flora; wild populations of macroalgae are widely distributed in the intertidal and subtidal zones of Trat peninsula (Pirompug, 1976) and used as human food and for agar extraction (Edwards & Tam, 1984; Chirapart et al., 1992).

Currently, the abundance and diversity of Thai seaweed has been vulnerable to decline because of over-harvesting of natural populations and the ecological deterioration of several inshore and estuary ecosystems (Lewmanomont, 1998).

In Trat costal areas and adjacent waters, the rapid extension and development of fisheries activities by local fishermen and conversion of mangrove areas into shrimp farms and urban areas threaten aquatic organisms (Menasveta, 1997) and this situation has a potentially negative impact on the coastal ecosystem (Doydee, 2005). Accordingly, the change in ecosystem and environmental conditions, mangrove deterioration and costal land-use activities would result in a decrease of macroalgal biodiversity and biomass in Trat peninsula.

The need to promote a scenario of seaweed resource management is therefore important for sustainable conservation and restoration of coastal ecosystem. However, information and knowledge regarding to macroalgal assemblages and their ecology is very limited in this coastal region. In order to provide useful data for a possible preliminary management strategy for conservation of seaweed resources in Trat peninsula we determined seasonal variation in the species diversity, biomass and distribution pattern of macroalgae with reference to some environmental variables for better understanding of the recent situation in algal communities.

MATERIALS AND METHODS

Study area

The study site is located at the coastal area of Trat province, east of Thailand. Four coastal areas of intertidal habitat, which are different in shape and environmental condition, were chosen as research station: Ao Cho (site 1), Ao Lane (site 2) Laem Tien (site 3) and Laem Sok (site 4).

Among the stations, Ao Cho and Ao Lane are semi-exposed areas. Ao Cho is characterized by the formation of sandy beaches alternated with rocky shores and partly surrounded by mangroves, while natural habitat of Ao Lane is composed mainly by mudflat and this area is moderately occupied by indigenous fisheries community.

Laem Tien is a non-exposed area with muddy sand bottom fringed by mangroves, and some parts of this area are heavily exploited for aquaculture and shrimp farms. Additionally, Laem Sok, a fully exposed area with rocky shore habitat, is partially converted for commercial and industrial activities.

Sample collection and laboratory analysis

Species diversity, biomass and distribution

Field sampling was carried out once a month at four stations for a year (from January to December 2011). Benthic marine macroalgae were sampled thoroughly by wading or snorkeling. Complete thalli of live specimens were uprooted by hand or with paint-scraper, placed in plastic bags, labeled by location and date of collection, and transported to laboratory.

Algal samples were rinsed to remove sediment and debris, photographed, preserved as herbarium vouchers, or, on some occasions, preserved in 4% formalin-seawater solution, and deposited at the Algal Bioresources Research Center, Faculty of Fisheries, Kasetsart University. The species identification was based on gross morphology and internal features following Lewmanomont & Ogawa (1995); Huisman (2000) and Litter & Litter (2000; 2003). In order to determine algal biomass and distribution pattern, a quadrat method along a vertical transect line set across the intertidal zone perpendicularly to the coastal line was performed monthly throughout the study period.

Once a month 25 replicates of sampling quadrat (50×50 cm) from research stations were collected for determination of algal biomass. Algal samples from each quadrat were carefully cleaned with freshwater to remove sand, silt, epiphytes and other debris before weighting. Dry weight of algal biomass was obtained by drying the samples in the oven at 105°C for 48 hours (Wong & Phang, 2004).

Environmental parameters

Environmental parameters, including physical and chemical factors, were recorded seasonally at the moment of each sampling. For physical variables, seawater temperature, salinity, pH, turbidity and dissolved oxygen (DO) were measured in the field. Seawater temperature and DO were determined using a salinity compensated dissolved oxygen meter (YSI Model 57).

Salinity was determined by hand refractometer; pH was measured using a pH meter (YSI Model 60) and water transparency was estimated by a turbidimeter (LaMotte Model 2020).Total rainfall of Trat peninsula was obtained from the Meteorological Department of Thailand. For chemical variables, nutrient concentration in the seawater was evaluated.

Water quality was sampled from each study site and fixed in ice chests to examine alkalinity, hardness, ammonia, nitrate, nitrite and phosphate, using the methods of Sasaki & Sawada (1980) and Strickland & Parsons (1972).

Statistical analysis

Data for statistical analysis were tested initially for normality and homogeneity (Zar, 1984). Oneway analysis of variance (ANOVA) was employed to search for any significant difference among month, site and biomass data of each species.

Statistical significance was set at $p<0.05$ and the stability of the estimate reflected by 95% confident intervals. All tests and analyses were performed with SPSS version 12.0 (SPSS, Inc., Chicago, IL).

RESULTS

Species diversity

A total of 26 taxa were identified including 3 species of Cyanobacteria, 3 species of Chlorophyta, 4 species of Phaeophyta and 16 species of Rhodophyta (Tables 1, 2).

The number of species varied during the study period and ranged from 25 (March 2011) to 12 (June 2011). *Catenella nipae*, *Gracilaria salicornia*, *Gelidium pusillum*, *Hydropuntia changii*, *Hypnea hamulosa*, *Kyrtutrix maculans*, *Laurencia decumbents*, *Lyngbya majuscula*, *Peyssonnelia rubra* and *Ulva clathrata* were found throughout the sampling period.

On the other hand, *Brachytrichia quoyi*, *Chaetomorpha crassa*, *Cladophora sp.*, *Dictyota dichotoma*, *Hydroclathrus clathratus*, *Padina australis*, *P. sanctae-crucis*, *Acanthophora spicifera*, *Bostrycia tenella*, *Centroceras clavulatum*, *Ceramium flaccidum*, *Erythrotrichia sp.*, *Gelidiopsis intricatum*, *Gracilariopsis irregularis*, *Neosiphonia savatieri* and *Palisada papillosa* had only single occurrences in time.

Biomass and distribution pattern of marine benthic algae in each season and site

Total marine macroalgal biomass gradually increased from January to March, reached to the peak in April (hot-dry season) with 59.50 g/m^2 dry weight and dramatically decreased in July (rainy season) with 20.14 g/m^2 dry weight, and then steadily increased from August to December, reaching to the peak again in November (cool-dry season) with 55.46 g/m^2 dry weight. The seasonality of macroalgae biomass at the site was less uniform. During both dry and wet seasons, *Gracilaria salicornia* and *Hydropuntia changii* attained the maximum biomass mean value at 29.51 g/m^2 dry weight and 14.82 g/m^2 dry weight, respectively.

Acanthophora spicifera, *Hydroclathrus clathratus* and *Padina sanctae-crucis* had greatest biomass during the cool-dry season, and less biomass during the wet season. In contrast, *Gracilariopsis irregularis*, *Hypnea hamulosa*, and *Padina australis* exhibited higher biomass during the hot-dry season than in the wet season.

Most seaweed biomass exhibited greatest abundance in Ao Cho e.g. *Gracilaria salicornia*, *Gracilariopsis irreguralis*, *Hypnea hamulosa*, *Hydropuntia changii*, *Palisada papillosa* and *Padina sanctae-crucis*. Some species, e.g. *Acanthophora spicifera*, *Hydroclathrus clathratus*, *Hypnea hamulosa*, *Hydropuntia changii*, *Palisada papillosa* and *Padina australis*, were common in Ao Lane.

The brown algae, *Padina australis* and *P. sanctae-crucis*, however, were common in Laem Tien. Some species, e.g., *Gracilaria salicornia*, *Hypnea hamulosa*, and *Palisada papillosa* were common in Laem Sok. In general, there were no patterns found in biomass of algae among different sites.

Ten species were found throughout the entire study period. These included two species of cyanobacteria, *Kyrtutrix maculans*, *Lyngbya majuscula*, one species of green algae, *Ulva clathrata*, seven species of red algae, *Catenella nipae*, *Gracilaria salicornia*, *Gelidium pusillum*, *Hydropuntia changii*, *Hypnea hamulosa*, *Laurencia decumbents* and *Peyssonnelia rubra*.

	Ao Cho												Ao Lane											
TAXA	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
CYANOBACTERIA																								
Brachytrichia quoyi (Agardh) Bornet et Flahault	+	+	+																					
Kyrtutrix maculans (Gomont) Umezaki	+	+	+					+	+	+	+	+	+	+	+									+
Lyngbya majuscula (Dillwyn) Harvey	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+			+
CHLOROPHYTA																								
Chaetomorpha crassa (C.Agardh) Kützing	+	+	+	+	+						+	+	+	+	+									
Cladophora sp. Kützing	+	+	+	+	+	+				+	+	+	+	+	+									
Ulva clathrata (Roth) C.Agardh	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHAEOPHYTA																								
Dictyota dichotoma (Hudson) Lamouroux		+	+										+	+	+									
Hydroclathrus clathratus (C.Agardh) M.A.Howe	+	+	+									+				+	+							
Padina australis Hauck													+	+										
Padina sanctae-crucis Børgesen	+	+	+					+	+	+	+	+												
RHODOPHYTA																								
Acanthophora spicifera (M. Vahl) Børgesen													+	+	+	+							+	+
Bostrycia tenella (J.V. Lamouroux) C.Agardh																								
Catenella nipae Zanardini	+	+	+	+	+	+	+	+	+	+	+	+												
Centroceras clavulatum (C.Agardh) Montagne		+	+																					
Ceramium flaccidum (Harvey ex Kützing) Ardissone	+	+									+	+												
Erythrotrichia sp. Areschoug	+	+	+	+	+						+	+	+	+	+	+								
Gelidiopsis intricatum (C.Agardh) Vickers	+	+	+	+	+	+	+	+	+	+														
Gelidium pusillum (Stackhouse) Le Jolis	+	+	+	+	+	+	+	+	+	+	+	+												
Gracilaria salicornia (C.Agardh) E.Y.Dawson	+	+	+	+	+	+	+	+	+	+	+	+												
Gracilariopsis irregularis Abbott													+	+	+	+	+							
Hydropuntia changii (Xia et Abbott)Wynne	+	+	+	+	+	+	+	+					+	+	+	+	+	+	+	+	+	+	+	+
Hypnea hamulosa (Esper) J.V. Lamouroux	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						+	+
Laurencia decumbents Kützing	+	+	+	+	+	+	+	+	+	+	+	+												+
Neosiphonia savatieri (Hariot) M.S.Kim	+	+	+	+	+						+	+	+	+	+	+	+	+					+	+
Palisada papillosa (C.Agardh) K.W.Nam	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+						
Peyssonnelia rubra (Greville) J.Agardh	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+

Table 1. Seasonality of macroalgae at Trat Peninsula January (=1)/December (=12) 2011: Ao Cho and Ao Lane.

TAXA	Laem Tien												Laem Sok											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
CYANOBACTERIA																								
Brachytrichia quoyi (Agardh) Bornet et Flahault																								
Kyrtutrix maculans (Gomont) Ume zaki	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lyngbya majuscula (Dillwyn) Harvey	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+
CHLOROPHYTA																								
Chaetomorpha crassa (C.Agardh) Kützing	+	+	+						+			+		+										+
Cladophora sp. Kützing	+	+	+	+	+	+	+					+						+	+					
Ulva clathrata (Roth) C.Agardh	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHAEOPHYTA																								
Dictyota dichotoma (Hudson) Lamouroux																								
Hydroclathrus clathratus (C.Agardh) M.A.Howe																								
Padina australis Hauck	+	+	+	+																				
Padina sanctae-crucis Børgesen	+	+	+	+																				
RHODOPHYTA																								
Acanthophora spicifera (M. Vahl) Børgesen																								
Bostrycia tenella (J.V. Lamouroux) C.Agardh																				+	+	+	+	+
Catenella nipae Zanardini																								
Centroceras clavulatum (C.Agardh) Montagne																								
Ceramium flaccidum (Harvey ex Kützing) Ardissone																								
Erythrotrichia sp. Areschoug	+	+	+	+									+	+	+	+							+	+
Gelidiopsis intricatum (C.Agardh) Vickers	+	+	+	+	+	+							+	+	+	+	+							
Gelidium pusillum (Stackhouse) Le Jolis	+	+	+	+	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+
Gracilaria salicornia (C.Agardh) E.Y.Dawson													+	+	+	+	+	+	+	+	+	+	+	+
Gracilariopsis irregularis Abbott																								
Hydropuntia changii (Xia et Abbott)Wynne																								
Hypnea hamulosa (Esper) J.V. Lamouroux	+	+	+	+									+	+	+	+	+	+	+				+	+
Laurencia decumbents Kützing																								
Neosiphonia savatieri (Hariot) M.S.Kim	+	+	+											+	+									
Palisada papillosa (C.Agardh) K.W.Nam													+	+	+	+	+	+						
Peyssonnelia rubra (Greville) J.Agardh	+	+	+	+	+	+	+						+	+	+	+	+	+	+	+	+	+	+	+

Table 2. Seasonality of macroalgae at Trat Peninsula January (=1)/December (=12) 2011: Laem Tien and Laem Sok.

Physical and chemical factors study

There were insignificant variations in water temperature ($p>0.05$) among sites but significant variations in water temperature ($p<0.05$) among months. The water temperature was 27.5-33.5°C during the dry season and 25.5-31.5°C during the rainy season. The range was rather wide and was likely to influence the species diversity, biomass and distribution pattern of seaweed beds. In addition, there were insignificant differences in salinity ($p>0.05$) among sites but significant differences in salinity ($p<0.05$) among months. The salinity during the dry season was 32-37 ‰ and 15-27 ‰ during the rainy season.

Such a rather wide range was likely to influence the marine macroalgae. There were insignificant differences in turbidity ($p>0.05$) among sites but significant variations in turbidity ($p<0.05$) among months. The turbidity was 4.70-346.67 NTU during the dry season and 10.33-983.33 NTU during the rainy season. These differences could influence the species diversity, biomass and distribution pattern of marine algae

ANOVA revealed that there were insignificant variations in PO_4^{3-} ($p>0.05$) and TIN (total inorganic nitrogen: $NH_4^+ + NO_3^- + NO_2^-$) ($p>0.05$) among sites but significant variations in PO_4^{3-} ($p<0.05$) and TIN ($p<0.05$) among months. The phosphate during the dry season was 0.0004-0.0391 mg/l and 0.0030-0.0670 mg/l during the rainy season. And the TIN was 0.0134-0.2642 mg/l during the dry season and 0.0343-0.2800 mg/l during the rainy season. These ranges were rather wide, and were likely to influence the marine macroalgae.

DISCUSSION

Species diversity study

A total of 25 genera and 26 species of marine benthic algae were recorded, of which 16 species of red marine algae (a characteristically abundant and diverse group in the tropics) were the most assorted. Red algae occupy a wide range of irradiance environments, including high-latitude and high-intertidal habitats subjected to long periods of full sunlight (Graham et al., 2009).

Twenty-four percent higher species richness of marine algae was found at our study site compared to the study of Laehyeb (2011), in which only 21 species were reported throughout Trat peninsula; only three field collections were made in that previous study as compared to the four field collections in this study. Thus, the number of visits for field collection as well as the collection efforts could be important for appraising species diversity more accurately. In addition, the differences we observed suggested that there was temporal variation in species diversity of marine algae.

Many marine macroalgae have posed ecological problems in some marine ecosystems, which is probably due to environmental changes linked to decreasing of mangrove forest, sewage discharges, shrimp culture, tourism and factories activities (Doydee, 2005; Thongroy et al., 2007; Laehyeb, 2011). These activities have had serious impact on coastal environments.

Biomass and distribution pattern study

Among all four study areas of Trat peninsula, marine macroalgal biomass and diversity were the highest at Ao Cho during the dry season. This area is semi-exposed, sandy bottom with rocks and partly surrounded by mangroves. This same phenomenon was observed in macroalgae at Sirinart Marine National Park by Prathep (2005) in Thailand. Water motion is one of the most important variables influencing marine macroalgae, because it regulates turbidity, light penetration and nutrient availability (Nishihara & Terada, 2010; Kang et al., 2011).

At Ao Cho, marine macroalgae were exposed to intermediate levels of water motion, which allows the exchange of gases and uptake of nutrients (Lobban & Harrison, 1994; Kang et al., 2011). Ao Lane is a semi-exposed area as same as Ao Cho but is characterized by large mudflats and harbours a lot of fishery communities. This place is very sensitive to environmental changes due to human activity stressing the natural environment, such as sewage discharges of fishery communities, which can cause the decrease of the species diversity and of seaweed abundance. Laem Tien is a wave-sheltered area covered by mangroves with a large muddy sand bottom. Some parts of this place are used for aquaculture.

This site showed the lowest biomass and species diversity. Taking into account that the area is rather sheltered from wave action, marine macroalgae may be exposed to some physiological stress due to limited circulation of nutrients and gas exchange (Prathep & Tantiprapas, 2006; Thongroy et al., 2007).

In addition, this site is subjected to environmental changes because of water pollution from the shrimp farms. Laem Sok is a wave-exposed area with a large rocky shore habitat and many restaurants nearby the harbor. This place shows a high level of water motion, which is likely to affect species diversity and abundance of macroalgae (Prathep, 2005; Kang et al., 2011). In addition, in this area mangrove forests decreased in the last few years (Doydee, 2005; Laehyeb, 2011).

Generally speaking, mangroves are really important since these plants indirectly participate to habitat complexity and diversity of fauna and flora, particularly marine macroalgae (Ashton et al., 2003; Ellison, 2008, Doydee & Buot, 2011).

In fact by trapping nutrients and sediments from river runoffs from the uplands and transporting them to coastal waters (Anongponyoskun & Doydee, 2006; Ellison, 2008; Doydee, 2009) they contribute to improve shoreline stability and water quality. Therefore, the decrease of mangrove forests is certainly another reason affecting negative changes in species diversity and abundance of seaweed.

ACKNOWLEDGEMENTS

We thank the Japan International Research Center for Agricultural Sciences for partial financial support. Mr. Narongrit Muangmai for the invaluable editorial advice and Mr. Jiraweath Petsut for assistance with the field work.

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Biodiversity Journal, 2012, 3 (3): 237-242

Freshwater and brackish "oasis" fauna in the deep Black Sea

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ABSTRACT Present paper reports on the possible existence of recent freshwater fauna in the Black Sea. Based on information available from malacology, ecology, paleontology, stratigraphy, hydrogeology and observations in situ, the presence of freshwater biota on the shelf and continental slope is discussed, including the existence of aerobic life forms in the Black sea deep-water cavity.

KEY WORDS Aerobic; Biodiversity; Ecosystem; freshwater; submarine springs.

Received 03.08.2012; accepted 03.09.2012; printed 30.09.2012

INTRODUCTION

The Black Sea is a very specific marine basin with anaerobic water mass spreading from the maximal bottom depth (more than -2200 m) to about -200 m. This water thickness is valuated as about 85% of the whole Black Sea water volume (Zaitsev, 2006) and suggested as absolutely unfit for eukaryotic life (Vinogradov, 1997).

The Black Sea water salinity varies from about 17-18‰ on the inner shelf till 23‰ in the deepest basins' parts (Sorokin, 1982) which excludes any possibilities for freshwater biota inhabitance. Anyway, the possibility of existence of aerobic life "oases" related to fresh groundwater springs in sulfured hydrogen zone was hypothesized, taking into account a few unusual faunistic findings, signs "of island" speciation (Zaika, 2008).

Additionally, on the Black Sea continental shelf bottom surface were repeatedly found out the shells of several gastropod mollusk species typical of freshwaters or brackish complexes (Il'ina, 1966; Golikov & Starobogatov, 1972; Chukhchin, 1984). Most of these species - met nowhere else and described as extinct - supposedly re-deposited from ancient (Neoeuxinian) sediment layers formed in much more freshwater environmental conditions (Golikov & Starobogatov, 1972). However, among the above-mentioned gastropods, only one (*Theodoxus pallasi* Lindholm, 1924) is known for certain from Neoeuxinian sediment deposits.

During the R/V «Maria S. Merian» (Leibniz Institute for Baltic Sea Research, Germany) expedition, which was conducted in the Black Sea within the framework of the European project HYPOX in 2010, a live gastropod mollusk was sampled at the depth of 250 m (Sergeeva et al., 2011). If one believes that its presence in anoxic zone may be not casual, then it is necessary to admit the existence of oxygen input into the sulfured hydrogen water layer by sources which serve as freshwater springs.

The finding of meiobenthic crustaceans in the deep Black Sea (around -2000 m) and at a depth of 174 m (Korovchinsky & Sergeeva, 2008) may be considered as one of the biological and ecological proofs of the existence of aerobic fauna in anaerobic zone. In 1986, during a test diving on the submarine inhabited vehicle of the USSR Navy at a depth of 600-640 m in the Yalta canyon at bottom, one "oasis" of aerobic life was found out (Prof. Gevorkyan V.Kh. personal report); this information was under secret for a long time and therefore never published. The most credible reason for the existence of such an "oasis" is the occurrence of a zone of stable and powerful submarine unloading of oxygen-rich groundwaters.

Oceanological and geological researches confirmed the presence of submarine discharge of fresh water zones on the Black Sea continental shelf and slope (Trotsyuk et al., 1988; Shnyukov & Ziborov, 2004). Actually, these zones can create proper conditions for the existence of freshwater or brackish biota, both in aerobic and in anaerobic water masses of the Black Sea.

The aim of the present research, based on geological, biological and ecological data, is to investigate the existence of specific biocenoses in the Black Sea, related to the zones of the submarine unloading of freshwaters. A discovery and description of such a biocenosis would extend knowledge about the Black Sea biodiversity and give new ideas about possible ways of the “oasis” fauna evolution.

DISCUSSION

Geological and oceanological evidence

Submarine freshwater springs are known from many regions. Very recently, a Max Plank Institute science troop found rich bacterial life connected with freshwater springs even in the Dead Sea (Ionescu et al., 2012). Black Sea is also suitable for searching submarine sources of freshwater. On geological terms it is possible to distinguish three basic types of submarine sources of groundwater unloading: artesian, karstic and waters of subriverbed flow (Shnyukov & Ziborov, 2004).

Data on the dynamics of the artesian pools’ groundwater opened toward the Black Sea, testify movements of different hydrogeological floors. Actually these pools embrace all coastline of the Black Sea (Shnyukov & Ziborov, 2004). Submarine springs issued from the limestone massifs or other kinds of karsting rocks are widely spread in the Black Sea.

Researches, managing with submarine inhabited vehicles, showed the presence of numerous rocky outcrops from the bottom silty-mud sediments cover on a narrow and steep continental shelf and slope of south Crimea (Bondarev, 2008; 2009). Oceanological researches in 37th voyage R/V “Academician Vernadsky” showed submarine unloading of karsting-crack waters in head parts of many Crimean submarine canyons. The hydrochemical tests of near-bottom water allowed to set that in canyons salinity is lowered notably up to 12, 14, 15, 17‰ (with reference to base-line values of 21-22‰). Particularly, the desaltation of salt waters on the canyons bottom was found out on the continental slope of Turkey, north of Kefken Island and on the extreme north-west of the Turkish shelf (at 300-500 m below the sea level;13-17‰). Signs of desaltation were found also in the canyons of north-west Black Sea (Shnyukov & Ziborov, 2004).

Use of impermeable water samplers, vacuum degassing and chromatographic analysis allowed to set the presence of solved oxygen in the benthic layer of deep-water part of the Black Sea. In the area adherent to the estuarine part of Danube, oxygen in benthic water decreases from 4.2 ml/l in an off-shore zone practically to zero on the shelf edge. As recorded in eight near-bottom samples taken at different depths on the bottom and on the sides of a deep canyon up to 1340 m of depth, below the slope, up to the distal part of canyon fan, oxygen amount ranges from 0.3 to 1.6 ml/l. On the Anatolian side (the cone of dejection of Kyzil-Irmak river) water-solved oxygen was found out in two deep-water stations with bathymetric marks -2064 m and -2003 m, respectively. Measured oxygen concentrations were 0.5 and 0.7 ml•l. At depths of over 2000 m, near-bottom waters contained oxygen, as revealed by tests from the districts of western halistaza zone, interhalistaza zone and the east hollow of the Black Sea (Trotsyuk et al., 1988).

Major part of water samples containing oxygen was collected in submarine canyons or in the surroundings where the underground source of aerobic freshwaters binds to the submarine unloading. All these features do suggest the presence of specific freshwater aerobic biocenoses in the Black Sea is to be considered highly probable.

Biological and ecological evidence

Shells of gastropod molluscs of freshwater or brackish habitats were discovered on continental shelf and slope of the Black Sea (Golikov & Starobogatov, 1972), where water salinity is 19-21‰. Their list (Table 1) was recently filled up by one specimen of living gastropod from the near-Bosporus region, where background water salinity exceeds 22‰. Neritidae and Hydrobiidae mostly consist of marine species.

ditions, these shells should be absent. In addition, these shells are found in the middle and external part of shelf and are absent in littoral zone.

These circumstances and the sub-fossil state of seashells allowed to suppose they to origin from more ancient layers, maybe formed in a freshwater-

Taxa	Locality / inhabitance (after Kantor & Sysoev, 2006, others indicated)
Fam. Neritidae: *Theodoxus milachevitchi* Golikov et Starobogatov, 1966 *Theodoxus pallasi* Lindholm, 1924	Crimea offshore/ recent mud, depth 20-60 m Black Sea Neoeuxinian, Vityazean & Kalamitian layers, recent muds on the depth 18-158 m (Author's data)/Aral & Caspian Seas, very freshened littoral spots in the Azov Sea (Golikov & Starobogatov, 1972); rivers of Ural Mountains and Armenia
Fam. Hydrobiidae, Subfam. Pyrgulinae: *Caspia valkanovi* (Golikov et Starobogatov, 1966)	Crimea offshore, silt on the 20 m depth
Caspiohydrobia sp.	Near-Bosporus region, mud on the depth of 250m (Author's data)
Turricaspia crimeana (Golikov et Starobogatov, 1966)	Crimea, 15 m
Turricaspia iljinae (Golikov et Starobogatov, 1966)	Crimea offshore, mud on the 80-180 m depth
Turricaspia lirata marisnigri Starobogatov, in Alexeenko et Starobogatov, 1987	Crimea, *Modiolula phaseolina* (Philippi, 1844) contained mud.
Turricaspia nevesskae (Golikov et Starobogatov, 1966)	Crimea offshore, mud on the 80-180 m depth

Table 1. List of freshwater and brackish water gastropods found on the shelf and continental slope of the Black Sea.

However the genus *Theodoxus* Monfort, 1810 comprises species dwelling exceptionally in fresh or strongly refreshing (up to 5‰) waters of Eurasia. This characteristic allows to use them as bioindicators of freshwater environments (Goodwin, 2006). The Subfamilia Pyrgulinae Brusina, 1882 also includes genera and species from freshwaters (springs, rivers and lakes) of Europe and front Asia (Anistratenko, 1998).

A considerable part of these species inhabits brackish seas – Aral and Caspian. In the Azove-Black Sea basin the subfamily is represented by three genera whose species are usually encountered in the brackish zones of estuaries and rivers (Golikov & Starobogatov, 1972; Kantor & Sysoev, 2006). Hence, substantial differences in water salinity of sampling locations and in typical habitats of mollusk species led to hypothesize a possible introduced origin of shells from a freshwater environment. But, in that case, in the southern part of Crimea littoral, where there are special habitat con-

like environment (Golikov & Starobogatov, 1972). Such environmental conditions existed in a Neoeuxinian period of the Black Sea evolution (Il'ina, 1966); Neoeuxinian layers on the shelf of Crimea are covered by more young ground sedimentations, from 1 to 4 m thick (Shcherbakov et al., 1978).

Moving of shells through such a thick layer by means of natural processes is hardly plausible. But even if we assume such a possibility, then in Neoeuxinian deposits these species should be much more abundant than in recent sediments. However, species reported in Table 1 were found only in recent bottom deposits, with the exception of *Theodoxus pallasi*.

This species is the only one that is really characteristic for Neoeuxinian layers whereas in later deposits is quite rare. It is has been reported that shells of *T. pallasi* from recent bottom deposits have better saved surface and color pattern compared to those from Neoeuxinian sediments (Il'ina, 1966; Golikov & Starobogatov, 1972).

In our samples there is one *T. pallasi* subfossil specimen from a depth of 158 m from the recent bottom sediment of near-Bosporus region (Fig. 1). Maintenance of colouring of the shell testifies its relatively recent fossilization. Available data allow to suppose that the shells of the freshwater species complex discovered on Black Sea shelf belong to recent living species inhabiting within the limits of the freshwaters biotopes.

In 2010, during the Black Sea international expedition of R/V "Maria S. Merian" (Leibniz Institute for Baltic Sea Research, Germany) in a near-Bosporus region on a depth of 250 m, a live specimen, belonging to the genus *Caspiohydrobia* Starobogatov, 1970, was collected (Fig. 2). As at this depth in the Black Sea there are anoxic conditions, it was hypothesized that the animal had been rescued only by chance (Sergeeva et al., 2011). However if we admit the existence of specific aerobic freshwater fauna in the depths of the Black Sea, then the location of this mollusk corresponds to the biotope formed by a submarine unloading of freshwaters.

1

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Figure 1. Subfossil of *Theodoxus pallasi*, shell height: 5.8 mm, width: 7 mm, Bosporus region, depth 158 m, R/V "Maria S. Merian", 2010.
Figure 2. Live-collected *Caspiohydrobia* sp. (soft parts colored with "Bengal rose"), shell height: 3.4 mm, Bosporus region, depth 250 m, R/V "Maria S. Merian", 2010.

In 1986, during test dive of submarine inhabited vehicle of the USSR Navy in the Yalta canyon at a depth of 600-640 m, Dr. Gevorkyan (personal comm.) looked at one "oasis" of eukaryotic supposedly aerobic life. The biotope was characterized by unusually clear (for the Black Sea) water because of the absence, in the water column, of the characteristic particles of organic suspended debris (known as "marine snow"). On the outcropped rocks macrobenthos forms did remind hydroids. The most notable detail of the biota was the occurrence of fishes, exceeding 20 cm in size. The most likely hypothesis for the presence of such a biotope and a biocenosis is the possible existence of a powerful submarine spring of aerobic waters, stably existing since a long time.

Notably, generally speaking, strategy of reproduction [laying eggs attached to the substratum and non pelagic development, (see Chukhchin, 1984; Anistratenko, 1998,] and early ontogenesis peculiarities of these mollusks allow them to exist within the limits of localized biotopes showing parameters contrasting with those of surrounding environments. Another important ecological characteristic for allowing species to inhabit the deep Black Sea is the resistance to hypoxia. Hydrobiidae comprise mollusks adapted to the lack of oxygen (Chukhchin, 1984), and the specimen of *Caspiohydrobia* sp. discovered at a -250 m of depth belongs to this family. In 2002, during the international expedition on R/V Meteor (Germany) to two stations in the north-western part of the Black Sea at the depths of 1900 m and 2190 m, respectively, it was discovered a meiobenthic crustacean species unknown to science. The same species was found out in 2003 in an expedition on R/V "Yantar" (Russia) in north-eastern part of the Black Sea at a 171 m of depth. The specimen (Cladocera: Ctenopoda) allowed to describe a new species, *Pseudopenilia bathyalis* Sergeeva, 2004 and a new respective genus taxon. Consequently, also the Pseudopenilidae family Korovchinsky & Sergeeva, 2008 was described.

Hydrochemical analyses of near-bottom water in a deep-water place of sampling of *Pseudopenilia bathyalis* showed the presence of sulfured hydrogen in an amount of 4-12 ml/l at a salinity of 22-23‰.

However, finding out this crustacean species at a depth of 171 m in the hypoxia zone allows to suppose that we deal with an aerobic organism adapted to oxygen-deficit conditions.

Places of submarine water unloading are local phenomena in the Black Sea. Their spatial characteristics and stability in time could be substantially differentiated depending on sources regime. For example, water supplement varies seasonally and may lead to a temporary stop of unloading.

Characters of water springs related to the artesian layers are more stable. In addition, salt and solved gases composition may be different. Spatial structure of freshwater biotope can change as a result of dynamic influences of the surrounding water masses. The innate structure of such biotopes can be non-homogeneous, thus including several biota that differently react to presence/absence of oxygen and of refreshing/salting waters. Hence biotopes and biocenoses of submarine unloading zones may be extremely heterogeneous, various and very vulnerable ecosystems.

CONCLUSIONS

The existence of aerobic life in the deepest part of the Black Sea is traditionally contested and even denied (see Vinogradov, 1997). On the contrary, the present paper strongly supports the hypothesis reporting several evidence on the issue, including the occurrence of the endemic Pseudopenilidae family (Korovchinsky & Sergeeva, 2008) which suggests the evolutionally-long existence of specific fauna in the deep Black Sea. Probably, a freshwater relict fauna could have existed not only from Neoeuxinian time (27-10 thousand years ago) but also earlier.

In fact, the existence of submarine freshwaters springs is independent from the change of sea salinity levels and quantitative and quality composition of this fauna may have undergone transformation during time, depending also on the changes of sea salinity. Oceanological and hydrogeological researches (Shnyukov & Ziborov, 2004; Trotsyuk et al., 1988) reported on desalted waters in near-bottom layers from tide-mark to deep-water cavity bottom of the Black Sea. The general volume of the submarine discharge in the Black Sea is only approximately estimated. However, discharge volumes calculated for single areas show that this volume is ecologically significant. For example, only for the Crimean coast from Balaklava to Simheiz (less than 50 km), karst submarine springs were appraised as about 700 thousand m^3/day (Shnyukov & Ziborov, 2004). The same authors assessed the volume of subriver-bed flow as 1/3 of the volume of river flow. At the moment, water unloading in submarine canyons, although being demonstrated as a fact (Shnyukov & Ziborov, 2004), has not been calculated yet (not even preliminarily). The process of the submarine unloading can and must have ecological consequences.

This paper aims at not only discussing and contributing to a deeper knowledge of the refinement of the Black Sea biodiversity and of the recent state of the ecosystem, but also encourages the revision (by several colleagues) of some other aspects of the natural history of this ecosystem.

ACKNOWLEDGEMENTS

The Author is thankful to the Dr Gevorkyan V.Kh. (Institute of Geological Sciences NASU, Kyiv) for his personal communications and to Dr. Sergeeva N.G. (IBSS, NASU, Sevastopol) for having allowed the examination of macrobenthos samples collected in the expedition of R/V "Maria S. Merian"(Leibniz Institute for Baltic Sea Research, Germany) in 2010.

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Biodiversity Journal, 2012, 3 (3): 243-246

Description of two new species of *Carabus* Linnaeus, 1758 from China (Coleoptera Carabidae)

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ABSTRACT In the present paper two new species of *Carabus* Linnaeus, 1758, subgenus *Apotomopterus* Hope, 1838, are described and figured: *Carabus (Apotomopterus) francottei* n. sp. and *Carabus (Apotomopterus) eccoptopteroides* n. sp., comparative notes with the related taxa are provided.

KEY WORDS Coleoptera; Carabidae; *Carabus*; *Apotomopterus*, new species; China.

Received 28.08.2012; accepted 14.09.2012; printed 30.09.2012

INTRODUCTION

In terms of number of species the subgenus *Apotomopterus* Hope, 1838 is the largest subgenus of the genus *Carabus* L., 1758. The subgenus is widespread in Southeast China and adjacent Countries, in many places several species are sympatric (Deuve, 1997a, 1997b; Kleinfeld, 2009).

In the last decades thanks to the investigation of new or less known areas a large number of new species and subspecies was described (Kraatz, 1894; Boileau, 1896; Breuning, 1931, 1932-1936, 1950; Hauser, 1932; Deuve, 1991, 1995, 1997a, 1997b, 2001, 2002; Brezina, 2003; Deuve, 2004; Lassalle, 2006; Deuve & Li, 2009; Kleinfeld, 2009; Deuve, 2012a, 2012b).

The examination of some *Apotomopterus* specimens from North Guangdong and South Sichuan provinces in Southern China allowed to identify two new species described herein.

Carabus (Apotomopterus) francottei n. sp.

Examined material. Holotypus male (Fig. 1), China, North Guangdong province, Mts. Nanling Shan, VI.2009. The holotypus is deposited in the author's collection. Paratypus: 1 male, same data as holotypus. The paratypus is deposited in the author's collection.

Description of holotypus male. Length including mandibles: 33 mm, elytral width: 10.7 mm. Color black with very few metallic luster brownish-copper on dorsum and pronotum, mat.

Head moderately thickened. Frontal impressions deep and rugose, exceeding anterior margin of eyes; vertex slightly convex, surface of the vertex slightly punctured and rugulose; short neck. Surface faintly punctulate and rugulose. Mandibles moderately long, strong, and regularly curved. Palpi long, penultimate segment of labial palp multisetose (3-4). Eyes very convex and prominent. Antennae very long extending of 5 ½ antennomeres pronotal base and extending the second half of elytra. Pronotum very large and sinuate, transverse, about 1.34 times as broad as long, slightly convex; sides of pronotum narrow margined, slightly bent upwards; hind angles slightly protruding behind its base; surface of pronotum uniformly punctured; basal depressions large, roughly punctured.

Elytra elongate, sub-parallel sides, slightly emarginated at apex, moderately convex, maximum width behind middle; shoulders rather large and rounded; sculpture triploid homodyname, intervals uniformly convex, only the primary interrupted in the row in quite long links by small foveae, not punctured striae. Male aedeagus (Figs. 2, 3) small, regular curved; apical half slightly thickened, a little sinuate on the ventral side; apex a little narrowed spatulate.

VARIABILITY. No variability of paratypus. Body length 31 mm.

ETIMOLOGY. The new species is cordially dedicated to Dr. Auguste Francotte (Liege, Belgium) naturalist and specialist of Coleoptera Cerambycidae, my friend from many years.

COMPARATIVE NOTES. The new species is closely related with the sympatric *C. (Apotomopterus) sauteri nanlingensis* Deuve et Tian, 1999, but easy to be distinguished by the following characteristics (Deuve & Tian, 1999): larger and more sinuate pronotum; homodyname triploid sculpture of elytra with very regular intervals; larger and flat elytra; different color of elytra and pronotum; larger and curved median lobe of aedeagus.

Carabus (Apotomopterus) eccoptopteroides n. sp.

EXAMINED MATERIAL. Holotypus male (Fig. 4), China, South Sichuan province, Pu-Ge County, Lianxiang, Kakaliangzi, 1/11.VI.2012. The holotypus is deposited in the author's collection. Paratypi: 18 females, same data as holotypus. The paratypi are deposited in the author's collection.

DESCRIPTION OF HOLOTYPE MALE. Length including mandibles: 36 mm, elytral width: 11 mm. Color black with very faint cupper luster on dorsum and pronotum, mat. Legs, antennae and palpi black.

Head of normal shape, neck quite narrow, eyes small and slightly prominent. Flat vertex with a raised polish trilobate plate, the rest of the surface of the vertex rugulose. Mandibles short and stout. Palpi thin and very long, labial palp bi or three setose. Antennae long and thin, extending with 5 ½ antennomeres beyond pronotal base and extending the apical half of elytra. Pronotum rounded, slightly transverse, about 1.18 times as broad as long; disc of pronotum slightly convex; sides of pronotum very narrow margined, not bent upwards; hind angles very short, slightly protruding behind its base; surface of pronotum thin punctured; basal depressions small and not deep. Elytra very elongate, narrow, oval, moderately convex, maximum width just behind the middle; shoulders very narrow and rounded; sculpture triploid homodyname, intervals uniformly convex, with the primaries cut into quite long segments by small fovea; not punctured striae. Male aedeagus (Figs. 5, 6) very elongate, basal and median portion rectilinear and sub-cylindrical, apical portion strongly curved and very elongated; apex large and rounded.

VARIABILITY. Only females: the length of the body ranges from 36 mm to 43 mm. Pronotum more or less transverse: from 1.16 to 1.26 times as broad as long. Elytra very long, very narrow, rather convex, with very strong preapical emargination, the posterior angles are acuminate and very protruding, forming a sharp tooth. The apical half of elytra is marginated and bent upwards.

ETIMOLOGY. The given name wants to indicate the morphological vicinity of the present new species with *C. (Apotomopterus) eccoptopterus* Kraatz, 1894.

COMPARATIVE NOTES. *C. (Apotomopterus) eccoptopteroides* n. sp. is related with several *Apotomopterus* species: *C. (A.) aeneocupreus* Hauser, 1932; *C. (A.) benardi* Breuning, 1931; *C. (A.) eccoptopterus* Kraatz, 1894; *C. (A.) keithi* Deuve, 1995; *C. (A.) piriformis* Deuve, 1997 but easy separable by the following characters (Kraatz, 1894; Breuning, 1931; Hauser, 1932; Deuve, 1995, 1997b):

eccoptopterus: the new species is similar by the very elongate shape of body but differs by the strongly punctured pronotum, stronger preapical emargination of females elytra and by the shape of aedeagus strongly curved at the apical portion.

keithi: the new species differs by the larger size, strongly punctured pronotum, stronger preapical emargination of females elytra and by the shape of aedeagus strongly curved at the apical portion.

benardi: the new species is very different for the much more elongate body, the more convex elytra with very regular sculpture and the faintly punctured pronotum.

piriformis: the new species differs for the more elongate body, the more regular sculpture of elytra, the faintly punctured pronotum and the shape of aedeagus more strongly curved at the apical portion.

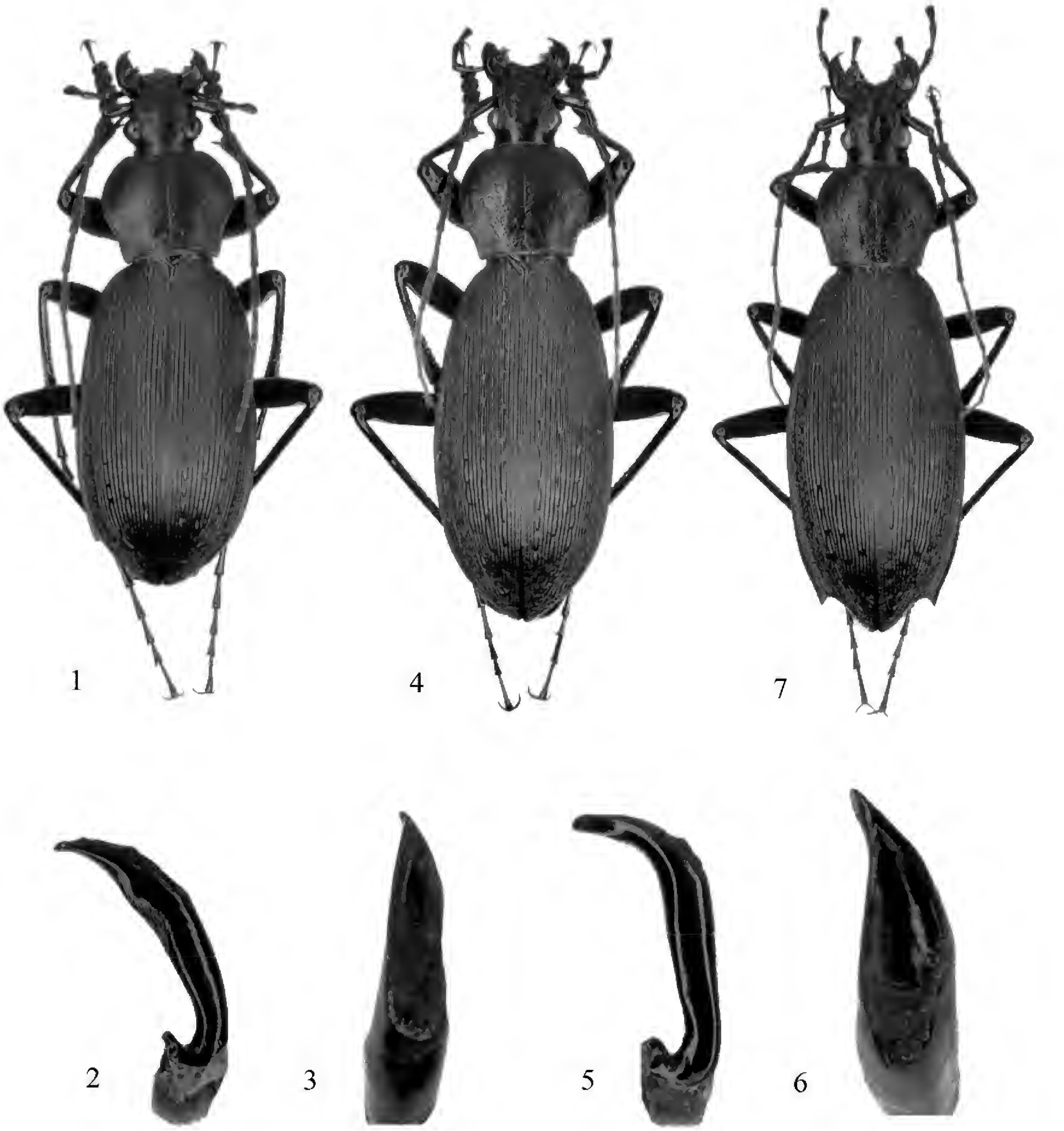

Figure. 1. *Carabus (Apotomopterus) francottei* n. sp. holotypus. Figures. 2, 3. idem, male edeagus lateral view (Fig. 2) and frontal view (Fig. 3). Figure. 4. *C. (A.) eccoptopteroides* n. sp. holotypus. Figures. 5, 6. idem, male edeagus lateral view (Fig. 5) and frontal view (Fig. 6). Figure. 7. *C. (A.) eccoptopteroides* n. sp. paratypus female.

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Biodiversity Journal, 2012, 3 (3): 247-250

Newly reported marine red alga, *Neosiphonia savatieri* (Hariot) M.S. Kim et I.K. Lee 1999 (Rhodophyta Rhodomelaceae) from Thailand

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ABSTRACT *Neosiphonia savatieri* (Hariot) Myung Sook Kim et In Kyu Lee, 1999 is reported for the first time from Thailand based on specimens collected from the Gulf of Thailand and Andaman sea. We herein describe the vegetative and reproductive morphology of the specimens. Important features for species identification include the thallus configuration, number of pericentral cells, cortication, branching pattern, origin of rhizoids, origin of branches, occurrence of trichoblasts and reproductive characteristics. Our results expand the known geographic distribution of this species and confirm its taxonomic features.

KEY WORDS Marine red alga; Morphology; *Neosiphonia savatieri*; Rhodomelaceae; Thailand.

Received 01.09.2012; accepted 15.09.2012; printed 30.09.2012

INTRODUCTION

The genus *Neosiphonia* Myung Sook Kim et In Kyu Lee, 1999 was segregated from *Polysiphonia* Greville, 1823 based on the generitype, *N. flavimarina* M.S. Kim et I.K. Lee, 1999 from Bangpo on the western coast of Korea (Kim & Lee, 1999). Currently, there are 30 assigned species (Guiry & Guiry, 2012) of which 15 species have been recorded for South East Asia region (Hô, 1969; Silva et al., 1987; Abbott et al., 2002; Kim et al., 2008).

N. savatieri (Hariot) M.S. Kim et I.K. Lee, 1999 was originally described based on collected material from Kanagawa Prefecture, Japan and subsequently it has been reported from Philippine, Korea, Malaysia, Norfolk Island, Hawaiian Island and Samoan Archipelago (Silva et al., 1987; Abbott, 1999; Millar, 1999; Masuda et al., 2001; Kim, 2005; Skelton & South, 2007; Kim et al., 2008).

In Thailand, only generic level of the genus *Neosiphonia* has been currently reported (Coppejans et al., 2010). During the collections under the project of the biodiversity inventory and information management in biodiversity hotspots, the tufting red alga, *Neosiphonia* sp. was collected in both Andaman Sea and the Gulf of Thailand.

Eventually we identified those specimens as *N. savatieri* and confirmed the taxonomic features based on morphological and anatomical characteristics of vegetative and reproductive plants.

MATERIALS AND METHODS

Specimens examined were hand-collected during October 2010 at sand dune area around the estuary of Pak Bara, Satun province, Andaman Sea (99°43’2”E; 6°51’27”N).

Additional material examined was from Ao Len, Trat Province, Gulf of Thailand (102°32'57"E; 12°4'13"N). Algal samples for morphological investigation were fixed and stored in 5% formalin/seawater or pressed onto herbarium sheets. Voucher specimens were deposited in the herbarium of Laboratory of Applied Research for Aquatic Plant and Plankton, Biodiversity and Aquatic Environmental Research Unit of Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

Specimens were stained with 1% aniline blue, acidified with 1N HCl and mounted in a 40% Karo®corn syrup on glass microscope slides. Digital images were photographed by microscope digital camera Olympus DP20 (Olympus, Tokyo, Japan) and eventually edited using Photoshop Elements 6 (Adobe, San Jose, CA, USA). Species identification was based on the literatures of *N. savatieri* from Japan, Korea and Malaysia (Hariot, 1891; Masuda et al., 2001; Kim, 2005; Kim et al., 2008).

RESULTS AND DISCUSSION

Based on diligent observations on morphological features of gametophytic and tetrasporangial thalli, we conclude that this is the first record of *N. savatieri* (Figs. 1-13) from Thailand.

Our Thai materials are in agreement with descriptions of previous studies of *N. savatieri* from other localities. The description below is based on the Thai materials.

Neosiphonia savatieri (Hariot) Myung Sook Kim et In Kyu Lee, 1999

Basionym: *Polysiphonia savatieri* Hariot, 1891: 226-227.

Habitat: Plants from Andaman Sea grew epiphytically on *Gracilaria minuta* Lewmanomont, 1994 and *G. salicornia* (Agardh) Dawson, 1954, which inhabited the lower intertidal on the sand dune nearby the river mouth. The specimens from the Gulf of Thailand were found on the thallus of *Hydropuntia changii* (Xia et Abbott) Wynne, 1989 at 1 m depth (Fig. 1).

The specimens of *N. savatieri* are erect, grow individually, and reach up to 5-10 mm high. Axes with 4 pericentral cells ecorticate, 90-180 µm in diameter (Figs. 2-3).

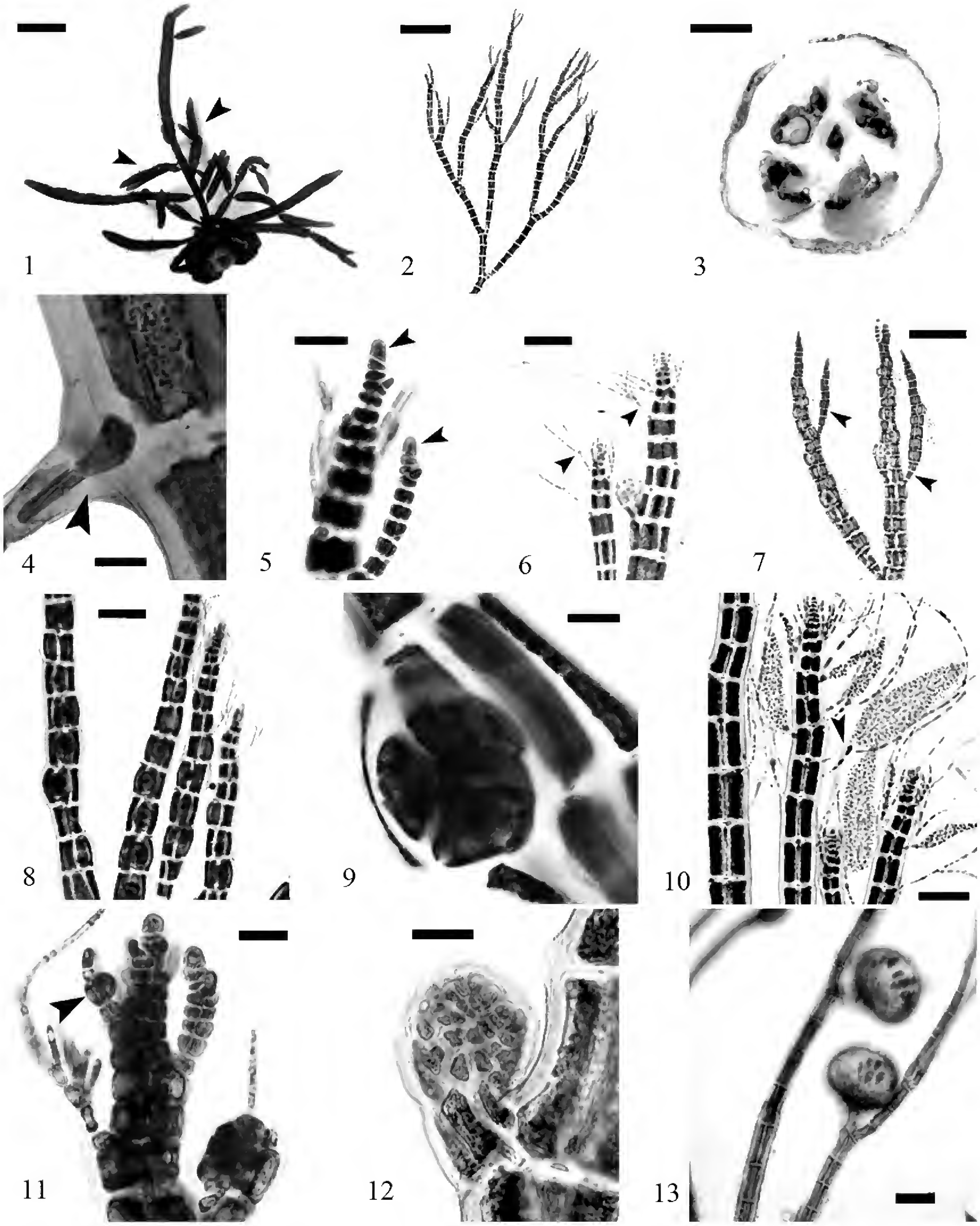
Specimens pseudodichotomously branched, showing a Y-shaped ramification with an angle of approximately 50 degrees (Fig. 2). Rhizoids aggregated in tufts in the lower segment of the axes, cut off from pericentral cells (Fig. 4), they penetrate into the tissue of *G. minuta*. Trichoblasts or branches are produced on successive segments (Fig. 5). Trichoblasts formed on every segment in a spiral arrangement and deciduous, leaving persistent scar cells (Fig. 6). Trichoblasts are abundant only at the apical part (Fig. 6). Lateral branches are exogenous, replacing trichoblasts (Fig. 5). Exogenous branches develop at various spots on the axis and grow from scar cells of the trichoblasts (Fig. 7).

Tetrasporangia arranged in slightly spiral series on the upper branches (Fig. 8). A single tetrasporangium is formed in each segment (Fig. 9). Mature tetrasporangia are prominent, 60-80 µm in diameter. Spores are tetrahedrally organized. Spermatangial branches are formed at the first dichotomy of fertile trichoblasts (Fig. 10). Mature spermatangial branches are lanceolate, 180-260 µm long and 60-80 µm wide, and lack sterile apical cells (Fig. 10).

An initial of carpogonial branch is formed on the second segment of the fertile trichoblast near the tip of lateral branches (Fig. 11). Procarp consists of a three-celled carpogonial branch and two sterile cells borne on the supporting cell. After fertilization, the gonimoblast gradually develops from the auxiliary cell (Fig. 12). Mature cystocarps are spherical with a slightly protruding ostiole, 180-220 µm in diameter (Fig. 13).

Based on the specimen collected from Kanagawa, Japan, Hariot (1891) originally assigned *N. savatieri* to the genus *Polysiphonia*, which is characterized by its four pericentral cells, unicellular rhizoids cut off by a cross wall from the proximal end of the pericentral cells and spermatangial branches arising as a primary branch of a trichoblast. Kim & Lee (1999) later assigned species with rhizoids cut off from pericentral cells, spiral arrangement of tetraspores, three-celled carpogonial branches and spermatangial branches on the primary dichotomy of trichoblast filament to a new genus, *Neosiphonia*.

The Thai specimens reported here agree well with the original description of *N. savatieri* and confirm the important identifying characteristics of this genus. Our Thai specimens were morphologically very similar to *N. savatieri* described from the



Figures 1-13 *Neosiphonia savatieri* from Thailand. Fig.1: plants epiphytic on *Hydropuntia changii* (arrows), Scale bar, 1 cm. Fig. 2: thalli pseudodichotomously branched with an angle of about 50 degree, Scale bar, 500 µm. Fig. 3: transverse section of the middle portion of a branch with 4 pericentral cells and a axial cell, Scale bar, 50 µm. Fig. 4: rhizoid (arrow) cut off from pericentral cells, Scale bar, 10 µm. Fig.5: apical portion of a branch showing oblique divisions of apical cells (arrows), Scale bar, 100 µm. Fig.6: trichoblasts (arrows) and scar cells of deciduous trichoblast arranged in spiral manner in the apical part of a cystocarpic plant, Scale bar, 100 µm. Fig.7: exogenous branches (arrows) of a tetrasporic plant, Scale bar, 200 µm. Fig.8: tetrasporic plants showing the spiral arrangement of tetrasporangia, Scale bar, 200 µm. Fig.9: mature tetrasporangium bearing one per segment, Scale bar, 20 µm. Fig.10: spermatangial branches arising on a branch of the fertile trichoblasts (arrow), Scale bar, 100 µm. Fig.11: procarp (arrow) developing on the second segment of a fertile trichoblasts at the tip of branches, Scale bar, 20 µm. Fig.12: post-fertilization stage, Scale bar, 50 µm. Fig.13: mature cystocarps, Scale bar, 70 µm.

Philippines, Hawaii, Malaysia, and Japan and Korea; all were relatively small epiphytic algae and have spermatangial branches formed on a branch of trichoblasts, spiraled tetrasporangia and an approximately 50 degree angle in the Y-shaped branching pattern (Silva et al., 1987; Abbott, 1999; Masuda et al., 2001; Kim, 2005; Kim et al., 2008).

Furthermore, Thai specimens of *N. savatieri* appear to be prevalent in river deltas surrounded by mangroves and grow specifically on gracilarioid algae. Our study shows that *N. savatieri* occur in both marine and brackish waters and that the distribution of this species extends to Thailand.

Additionally, Thai specimens of *N. savatieri* showed that young lateral branches are formed by replacing trichoblats, while Kim (2005) and Masuda et al. (2001) described branches that are not associated with trichoblasts in *N. savatieri* from Korea and Malaysia. According to Stuercke & Freshwater (2008), the origin of branches has been used as one of the important characteristics to separate species in *Polysiphonia* sensu lato.

It is important to take into account whether the relationship of branches and trichoblasts or other specific morphological characters are useful for species delineation of *N. savatieri*. Additional molecular and morphological analyses of *N. savatieri* will be needed in order to gain more insights into the species delimitation and differentiation among closely related species, especially in South East Asian region.

ACKNOWLEDGEMENTS

We thank those who helped us obtaining valuable specimens: Sunan Pattarajinda, Teerapong Duangdee and Wirayut Kuisorn. Sincere thanks are also due to Khanjanapaj Lewmanomont and John Bower for providing many useful suggestions and critical comments to the English. This research was partly funded by Office of Natural Resources and Environmental Policy and Training, Bangkok, Thailand.

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Biodiversity Journal, 2012, 3 (3): 251-258

Additional data on the genus *Muticaria* Lindholm, 1925 with description of a new species (Gastropoda Pulmonata Clausiliidae)

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ABSTRACT Morphological analysis and molecular genetic studies conducted on the genus *Muticaria* Lindholm, 1925 (Pulmonata Clausiliidae) in Sicily allowed to identify a new species which is described in the present paper.

KEY WORDS Clausiliidae; *Muticaria*; Sicily, new species.

Received 01.09.2012; accepted 18.09.2012; printed 30.09.2012

INTRODUCTION

The genus *Muticaria* Lindholm, 1925 has a distribution limited to South-East Sicily and Maltese Islands. Currently it includes three species: *Muticaria syracusana* (Philippi, 1836) and *M. neuteboomi* Beckmann, 1990 spread in southeastern Sicily and *M. macrostoma* endemic to the Maltese Islands, where it occurs with four subspecies: *M. macrostoma macrostoma* (Cantraine, 1835), *M. macrostoma scalaris* (L. Pfeiffer, 1850), *M. macrostoma oscitans* (Charpentier, 1852) and *M. macrostoma mamotica* (Gulia, 1861) (Beckmann, 1992; Giusti et al.,1995; Bank, 2012).

A preliminary molecular study on 16S rDNA partial sequences (Gregorini et al., 2008) carried out on Sicilian *Muticaria* revealed the existence of significant genetic differences between populations attributed either to *M. syracusana* or *M. neuteboomi*, including the topotypic ones.

Particularly, *M. neuteboomi* resulted the most widespread species with populations inhabiting inner areas of Iblean plateau (South Eastern Sicily), while *M. syracusana* resulted confined to a few coastal locality of Syracuse province.

A second and more detailed molecular study (Colomba et al., 2010) was conducted on topotypic specimens of *M. syracusana* and *M. neuteboomi* with a comparative analysis of mitochondrial 16S rDNA and cytochrome oxidase I (COI) gene partial sequences. This study, besides confirming preliminary data (Gregorini et al., 2008), strongly corroborated the validity of the two species.

As additional contribute to the research on the genus *Muticaria* in South Eastern Sicily and within the context of a wider and more detailed work, in the present paper the population of *Muticaria* from Spinagallo (Syracuse) is described as new species on the grounds of morphological and molecular data.

ACRONYMS. BC = bursa copulatrix; BCD = diverticulum of bursa copulatrix; CL = columellar lamella; DBC = duct of the bursa copulatrix; DE= distal epiphallus; FO = free oviduct; GA = genital atrium; L = lunella; P = penis; PD = diverticulum of penis; PE= proximal epiphallus; PL = parietal lamella; PLL = parallel lamella;

PP = principal plica; PR = penial retractor muscle; SL = spiral lamella; SP = sutural plica/plicae; V= vagina; VD = vas deferens.

The materials used for this study are deposited in the following Museums and private collections: A. Brancato collection, Syracuse, Italy (CB); S. Giglio collection, Cefalù, Italy (CG); Laboratory of Cytogenetics and Molecular Biology, University of Urbino, Italy (LCMBU); F. Liberto collection, Cefalù, Italy (CL); Museo Civico di Storia naturale di Comiso, Italy (MCSNC); Museo Civico di Storia Naturale di Genova "G. Doria", Italy (MSNG); Museo Naturalistico F. Minà Palumbo, Castelbuono, Italy (MNMP); A. Reitano collection, Tremestieri Etneo, Italy (CR); I. Sparacio collection, Palermo, Italy (CS).

Muticaria brancatoi n. sp.

Examined material. Holotypus: Italy, Sicily, Siracusa, Cugno Lungo, 37°00'25"N 15°10'47"E, 110 m, 02.IX.2012, legit A. Brancato (MSNG 57016). Paratypi: Italy, Sicily, Siracusa, Contrada Spinagallo, 37°00'12"N 15°10'50"E, 120 m, 12.III.2008, 5 specimens, 3 shells (CR); idem, 14 specimens, 30 shells (CR); Siracusa, V.ne Moscasanti, 37°00'58"N 15°09'53"E, 130 m, 28.XII.2010, 2 shells (CR); Siracusa, Cugno Lungo, 37°00'53"N 15°10'11"E, 135 m, 28.XII.2010, 2 specimens, 3 shells (CR) Siracusa, Cugno Lungo, 37°00'25"N 15°10'41"E, 110 m, 01.IV.2012, 16 shells (CL); Siracusa, Cugno Lungo, 37°00'27"N 15°10'48"E, 80 m, 01.IV.2012, 8 specimens, 86 shells (CL); idem, 2 specimens, 2 shells, legit F. Liberto (MCSNC 4412); idem, 6 shells (CG); Siracusa, Cugno Lungo, 37°00'25"N 15°10'47"E, 110 m, 02.IX.2012, 8 shells (CB); idem, 20 specimens, 32 shells (CS); idem, 2 specimens, legit I. Sparacio (MNMP).

Description of holotypus. Shell sinistral (Figs. 1, 2, 9), dimensions: height: 12.30 mm; maximum diameter: 4.20 mm, medium, cylindrical-fusiform, decollate, rather robust, light yellowish-grey in colour; external surface with minute, raised, close ribs, 69 ribs on penultimate whorl; last whorl with robust, evident and very spaced ribs; spire slowly and regularly growing, with 4 whorls; last whorl tapering downwards, with a very elevated and curved cervical keel; suture moderately deep; umbilicus slit-like, aperture about 1/3 of shell height, square, with 5 lamellae (on parietum and columellar side) and lunella and 4-5 plicae (on palatum); on parietum (Figs. 7, 8), starting from suture, there are: long, well developed, non-emerging parallel lamella; short spiral lamella, deviating from centre of parietum to adhere to parallel lamella, (upper) parietal lamella tooth-like; non-emerging columellar lamella; subcolumellar lamella internal; on palatum (Figs. 5, 6) there is an evident, lateral lunella and, starting from suture: two sutural plicae, the principal plica with a robust posterior portion, not fused to lunella apex, and a thin anterior portion, basal plica small, internally fused to base of lunella, very small sulcal lamella; clausilium triangular and slender (Figs. 3, 4), plough-like basal plate, apically pointed; peristome continuous, reflected, distinct from the wall of the last whorl.

Genitalia (Figs. 12-14). Genitalia are characterized by: short vagina, very short free oviduct, well developed ovispermiduct and a short copulatory duct ending in branched bursa copulatrix complex; one branch consisting of a short and wide diverticulum of the bursa copulatrix; other branch consisting of very short bursa copulatrix duct and oval and elongated bursa copulatrix. Penial complex consisting of flagellum, epiphallus, penial diverticulum and penis; epiphallus divided by point insertion of robust penial retractor muscle into proximal and distal portions, the latter very short; wide and pointed penial diverticulum arising on border between distal epiphallus and penis; penis short (2.5 mm). Internal walls of penis show a long, wide and elevated pleat and two thin and less evident pleats; left ommatophore long and well developed.

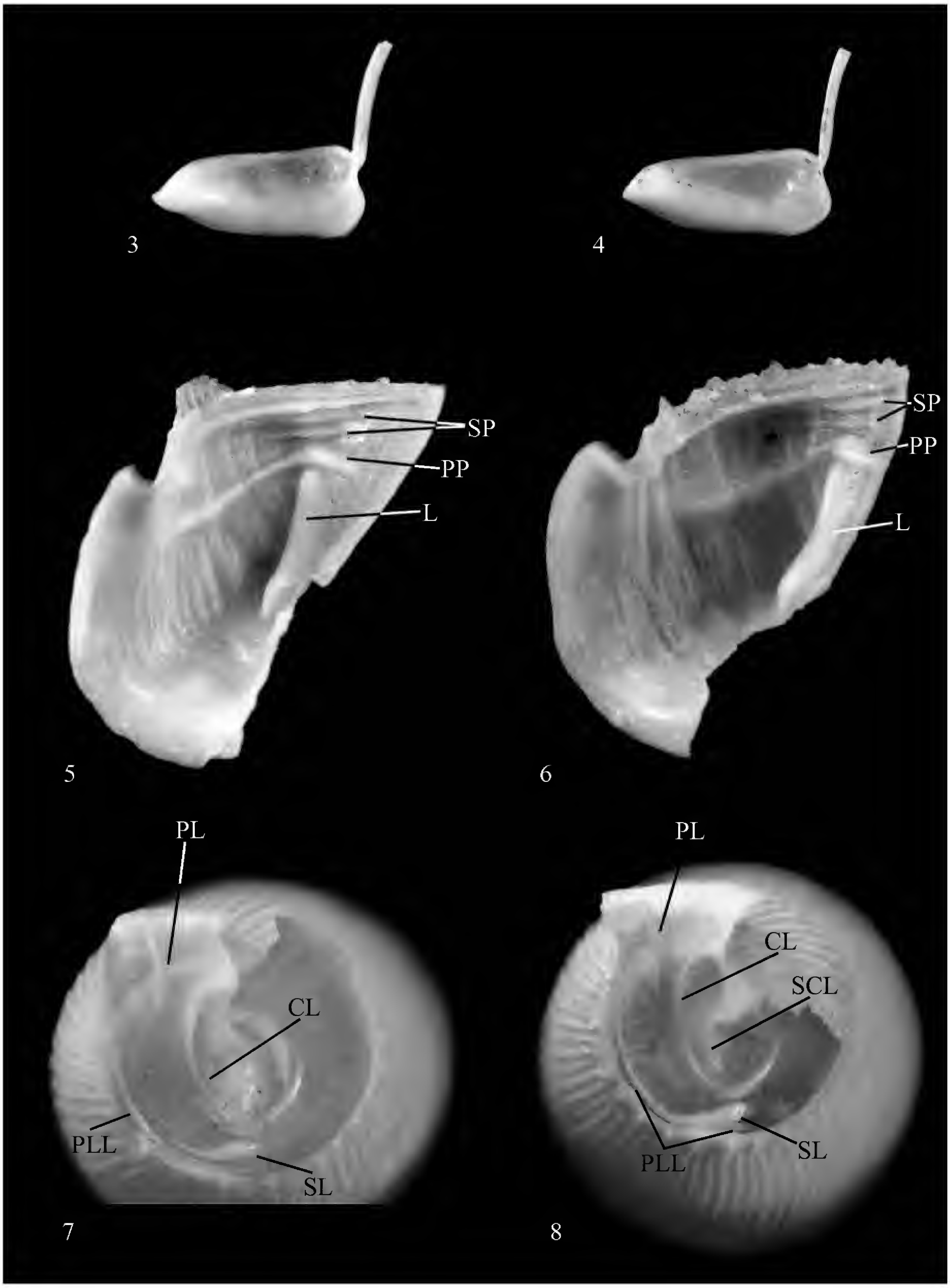
Variability. Dimensions in decollate specimens (4-5 whorls): height: 11.02-12.30 mm; maximum diameter: 4.16-4.55 mm. The number of ribs on the penultimate whorl of the shell ranges from 57 to 70 (on average, 67); in some specimens the principal plica is absent in its central portion.

Etimology. The new species is dedicated to Aldo Brancato (Syracuse, Sicily), dear friend and esteemed naturalist.

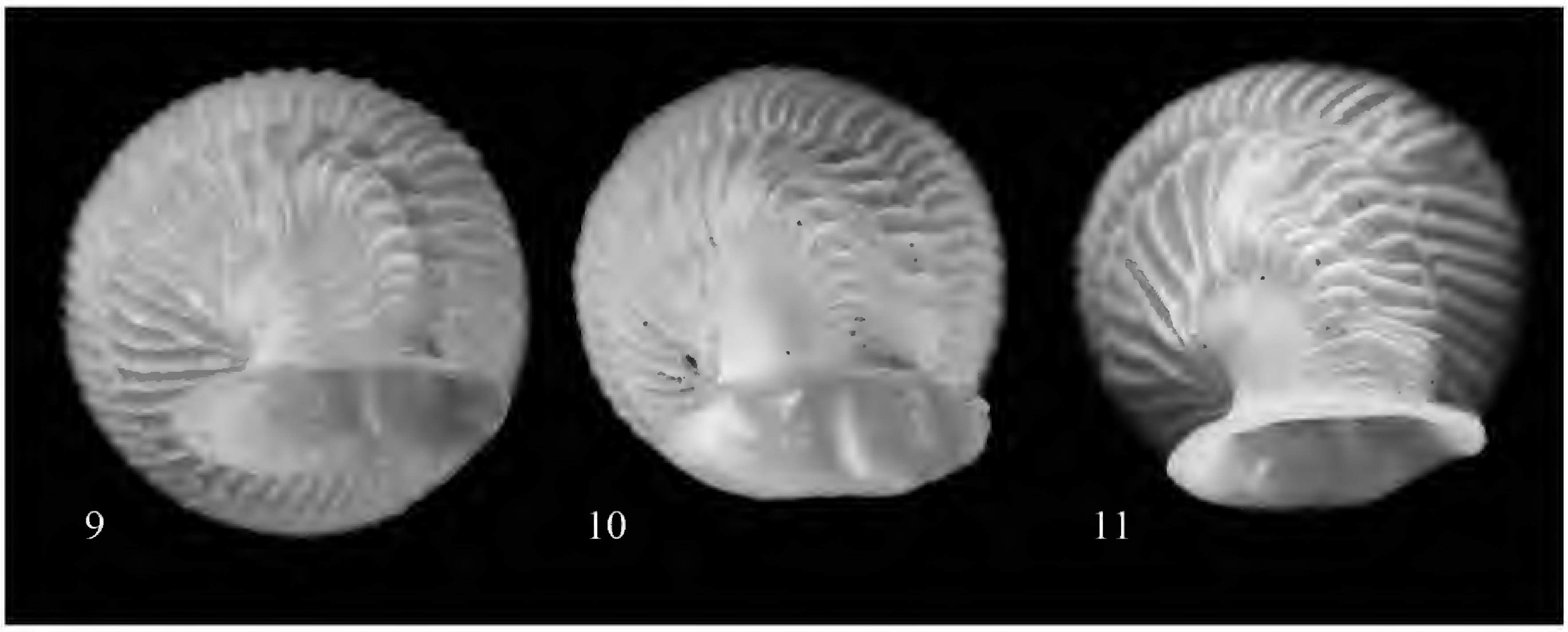
Biology and Distribution. This species lives on calcareous rock. It is found in cavities and under stone on stony soil. Endemic species to the South-Eastern Sicily, at the time known only for the locality of description.

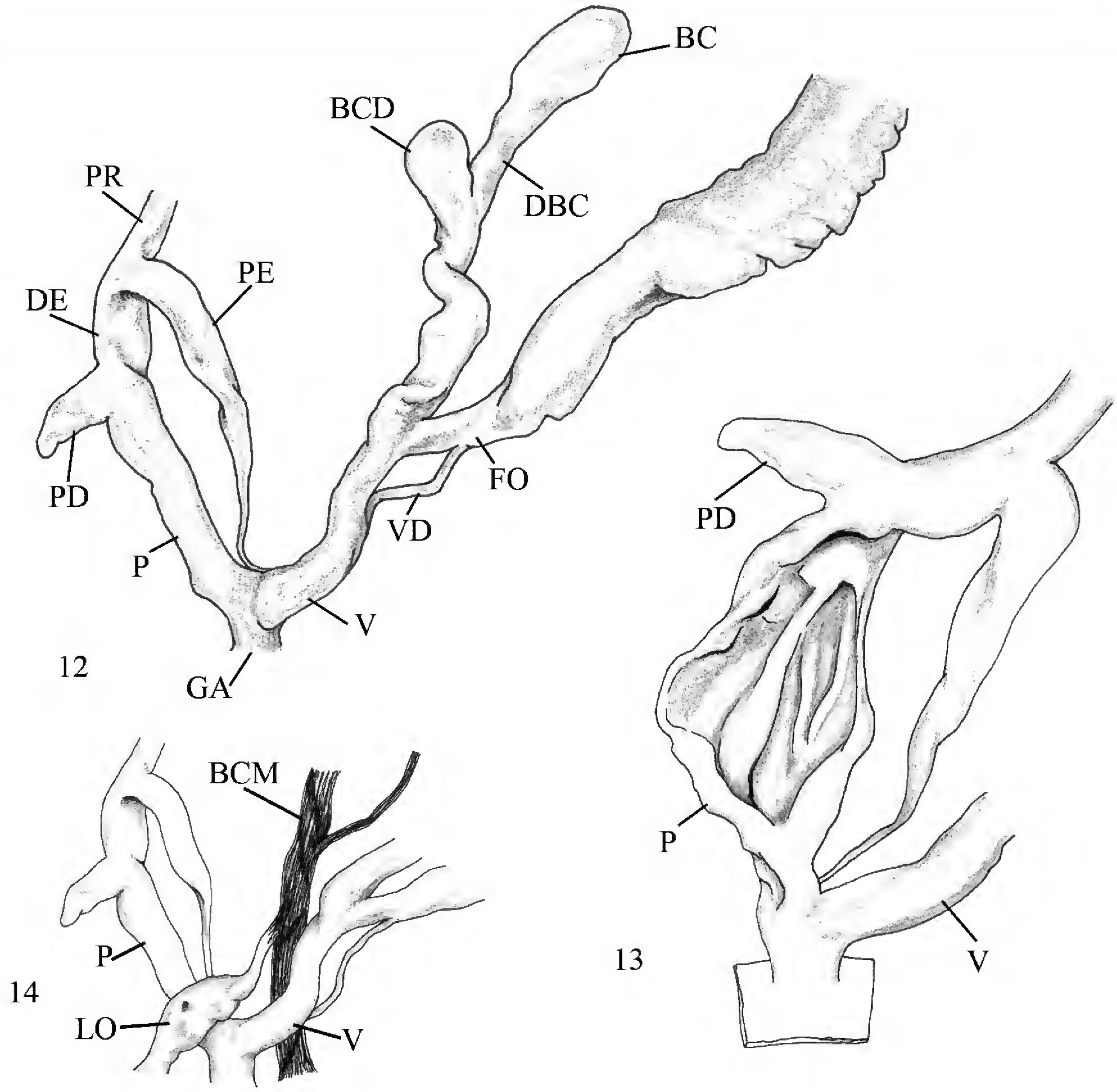
Comparative notes. *M. syracusana* shows slender and conical-fusiform shell with ribs on penulti-

Figure 1. Shell of *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo, h: 11.57 mm - D: 4.33 mm. Figure 2. idem, h: 12.27 mm - D: 4.29 mm.



Figures 3-8. *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo, clausilium of two specimens (Figs. 3, 4), palatum (Figs. 5, 6) and parietum (Figs. 7, 8).





Figures 9-11. Cervical keel in *Muticaria brancatoi* n. sp., Siracusa, Cugno Lungo (Fig. 9), *M. syracusana,* Siracusa, Teatro Romano (Fig. 10) and *M. neuteboomi,* Ragusa, Cava d'Ispica (Fig. 11). Figures 12-14. Genitalia of *M. brancatoi* n. sp., Siracusa, Cugno Lungo (Fig. 12) internal structure of penis (Fig. 13) and ommatophore (Fig. 14).

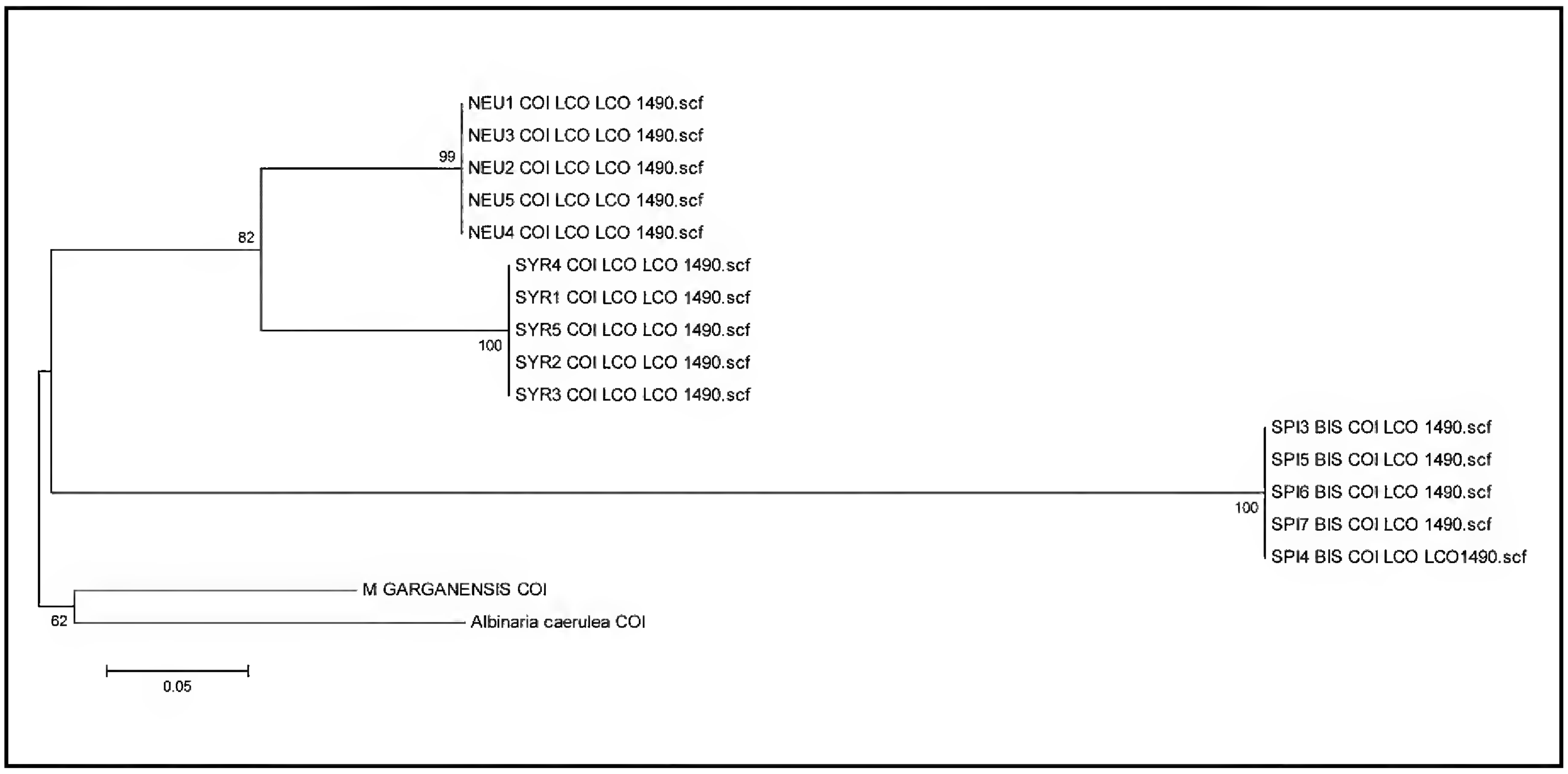


Figure 15. Maximum Likelihood consensus tree inferred from 500 replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values, i.e.the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches.

mate whorl more spaced and less numerous (27-54); on palatum, the principal plica is very short and fused to upper palatal plica. *M. neuteboomi* is characterized by fusiform shell, from slender to moderately ventricose, with more numerous ribs on penultimate whorl (56-97); on palatum, the principal plica is independent of upper palatal plica. All *Muticaria* from Maltese islands are characterized for a principal plica independent of the upper palatal plica.

Molecular Analysis. Five *Muticaria* specimens from C.da Spinagallo (Syracuse, SE Sicily), labelled as SPI, were analyzed. Samples were stored in 75% Ethanol at -20 °C in test tubes. For each individual, the entire animal was used for total DNA extraction (by Wizard Genomic DNA Purification Kit, Promega).

Para-voucher specimens, sensu Groenenberg et al. (2011) i.e. different specimens than the ones used for DNA analysis, but from the same sample or population, were stored by MSC (University of Urbino). COI amplicons (644 bp) were obtained by LCO1490/HCO2198 universal primers (5'-GGTCAACAAATCATAAAGATATTGG-3'/5'-TA-AACTTCAGGGTGACCAAAAAATCA-3') as in Folmer et al. (1994) with a PCR cycle of 95 °C for 5 min; 95 °C for 1 min, 42 °C for 1 min, 72 °C for 1 min (37 cycles); 72 °C for 10 min. Sequencing of the purified PCR products was carried out using automated DNA sequencers at Eurofins MWG Operon (Germany). Finally, sequence chromatograms of each amplified fragment were browsed visually. Sequences generated in this study were analysed with additional *Muticaria syracusana* (labelled as SYR) and *M. neuteboomi* (labelled as NEU) COI sequences, previously deposited by us in GenBank (IDs: HQ696869 and HQ696867, see also Colomba et al., 2010) *Medora garganensis* (ID: AY425595) and *Albinaria caerulea* (ID: NC_001761) COI sequences were employed as outgropus.

Sequences were visualized with BioEdit Sequence Alignment Editor 7 (Hall, 1999), aligned with the ClustalW option included in this software and double checked by eye. Standard measures of nucleotide polymorphism and phylogenetic analyses were conducted in MEGA 5.0.3 (Tamura et al., 2011). The best-fit evolution model of nucleotide substitution resulted T92+G (Tamura 3-parameter + Gamma). The evolutionary history was inferred by using the Maximum Likelihood method; the bootstrap consensus tree was inferred from 500 replicates; a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 2.1279). Codon positions included were 1st+2nd+3rd. All positions con-

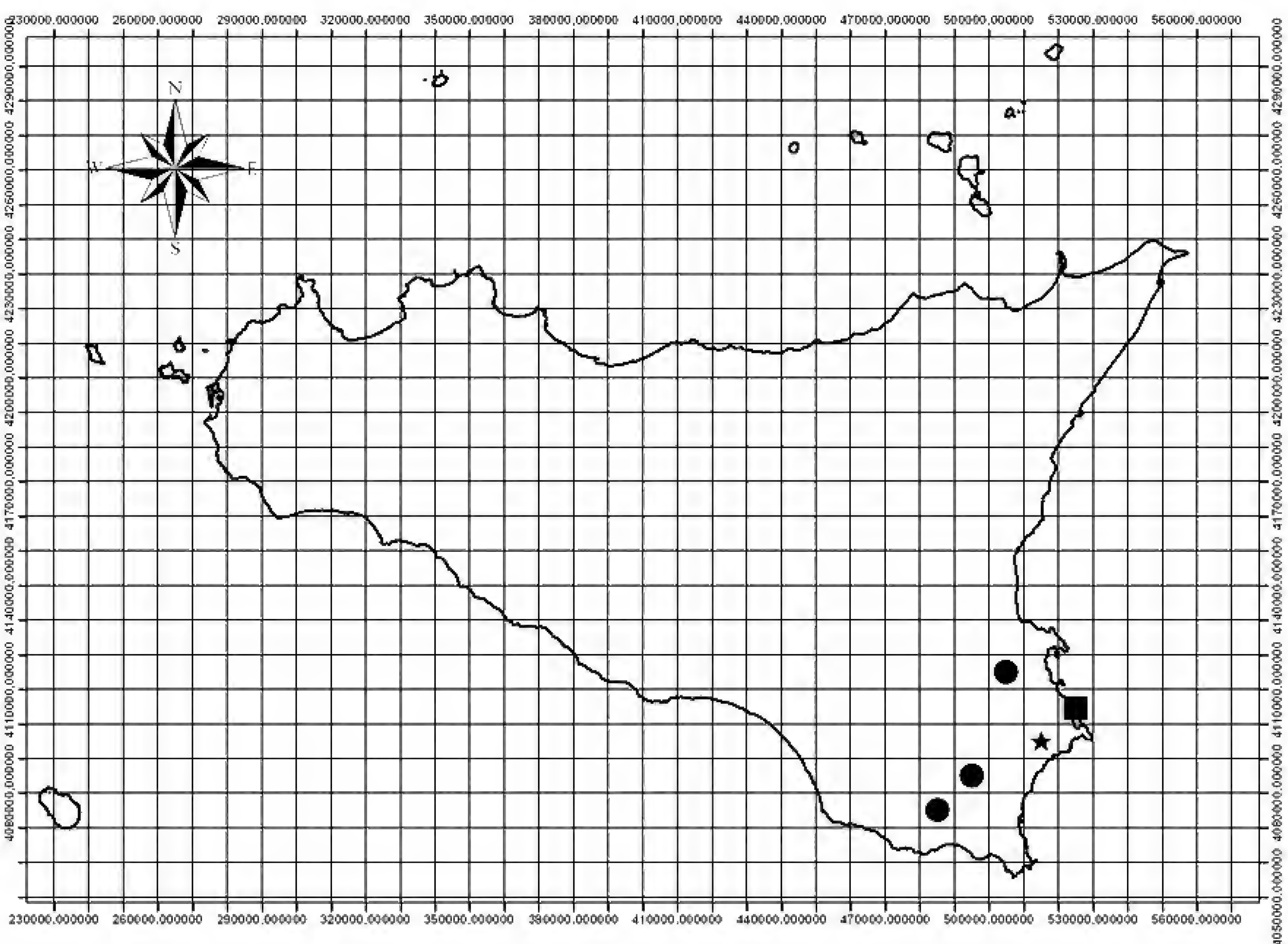


Figure 16. Geographic distribution of *Muticaria* species genetically analysed in SE Sicily: *Muticaria brancatoi* n. sp. (star), *M. syracusana* (square) and *M. neuteboomi* (dots).

taining gaps and missing data were eliminated. Divergences between SPI/SYR and SPI/NEU groups (Dxy), assessed as p distance, were 27.5% and 27%, respectively. Hence, phylogenetic tree (Fig. 15) and genetic distance between groups support the hypothesis that specimens from Spinagallo may be ascribed to a distinct *Muticaria* species.

REMARKS. *Muticaria brancatoi* n. sp. appears well differentiated morphologically from nearby and strictly related species currently known. Molecular data showed a good differentiation for Spinagallo populations already in preliminary studies conducted on 16S rDNA partial sequences (Gregorini et al., 2008), but with this survey, carried out by the analysis of cytochrome oxidase subunit I gene, p distance from the other species is considerably greater.

Based on available data no evolutionary and/or paleobiogeographic hypothesis is possible, nevertheless, this work highlights a remarkable complexity (Fig. 16) and differentiation within the genus *Muticaria* in Sicily (Gregorini et al., 2008; Colomba et al., 2010), much greater than supposed until now.

ACKNOWLEDGEMENTS

We wish to thank Andrea Corso (Syracuse, Italy)

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