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ANATOMY OF *SHINKAILEPAS MYOJINENSIS* SASAKI, OKUTANI & FUJIKURA, 2003 (GASTROPODA: NERITOPSINA)

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ABSTRACT

The anatomy of *Shinkailepas myojinensis* Sasaki, Okutani & Fujikura, 2003, was examined by gross dissection, scanning electron microscopy, and histological serial sections. The organization of the soft part conforms to general neritopsine pattern, especially in pallial complex, digestive system, reno-pericardial system, and nervous system. New character states previously unknown in neritopsine gastropods were revealed mainly in female and male reproductive systems, sense organs, and glands in pallial cavity. Comparison of our observations with published descriptions of various gastropods confirmed that the species of *Shinkailepas* are assigned to the superfamily Neritoidea among Neritopsina. The inclusion of *Shinkailepas* in the family Phenacolepadidae as in previous studies is also supported, although the number of their uniquely shared character is rather limited. Infrafamilial taxa of phenacolepadids so far anatomically studied are clearly divisible into deep-sea (*Shinkailepas* and *Olgasolaris*) and shallow-water (*Phenacolepas* and *Cinnalepeta*) groups in characters of the shell, operculum, head-foot external morphology, mantle margin, digestive tract, and female reproductive organ. At species level, members of *Shinkailepas* are diagnosed by morphology of the eye stalks, epipodial fold, and penis, as well as shell, radular and opercular characters.

Keywords: *Shinkailepas*, Phenacolepadidae, Neritopsina, comparative anatomy.

INTRODUCTION

In the recent systematics, Neritopsina includes six to nine families, namely, Neritidae, Neritiliidae, Phenacolepadidae, Hydrocenidae, Neritopsidae, including Titiscaniidae, and three helicinoidean families (Helicinidae, Ceresidae, and Proserpinidae, or three subfamilies of Helicinidae) (Thompson, 1980; Ponder, 1998; Sasaki, 1998; Kano & Kase, 2000, 2002). They comprise a robust clade phylogenetically (Ponder & Lindberg, 1997; Sasaki, 1998) and exhibit remarkable ecological diversification from deep-sea to terrestrial habitats (Kano et al., 2002; Sasaki & Ishikawa, 2002). Among them, three genera, *Shinkailepas*, *Olgasolaris*, and *Bathynerita,* have been known only from deep-sea chemosynthesis-based biological communities. The first two genera are currently assigned to the Phenacolepadidae (Beck, 1992; Warén & Bouchet, 2001; Sasaki et al., 2003), together with the shallow-water genera *Phenacolepas* and *Cinnalepeta*, and *Bathynerita* is regarded as a member of the Neritidae (Warén & Bouchet, 1993, 2001). They represent part of characteristic molluscan elements endemic to vent/seep environments.

In the genus *Shinkailepas* Okutani, Saito & Hashimoto, 1989, four species have been hitherto described: (1) *S. kaikatensis* Okutani, Saito & Hashimoto, 1989, from Kaikata Seamount, off Ogasawara Islands, Japan, 470 m deep, (2) *S. tufari* Beck, 1992, from Manus Back-Arc Basin, 2,450–2,505 m deep, (3) *S. briandi* Warén & Bouchet, 2001, from Mid-Atlantic Ridge, Menez Gowen to Logatchev site, 850–3,500 m deep, and (4) *S. myojinensis* Sasaki, Okutani & Fujikura, 2003, from Myojin Knoll, Ogasawara Ridge, Japan, 1,260–1,340 m deep. In addition, unidentified species were also reported from Mariana Back-Arc Basin (Hasegawa et al., 1997) and Okinawa Trough (Sasaki et al., 2003), suggesting the presence of more new species in the genus.

Although some anatomical descriptions have been published (e.g., Fretter, 1984; Sasaki, 1998; Kano & Kase, 2002), there is considerable uncertainty in anatomical organization of phenacolepadids and other possibly related

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neritoidean groups. Hence, further anatomical comparison is significant to understand relationships among *Shinkailepas*, *Olgasolaris*, shallow-water neritoideans, and the remaining less known neritopsines. All of known species of *Shinkailepas* have been described based chiefly on the shell, radula, operculum, and external morphology of the animal (Okutani et al., 1989; Beck, 1992; Warén & Bouchet, 2001; Sasaki et al., 2003), and only limited anatomical descriptions have been published for internal organs of the genus.

In this study, we attempted to provide detailed account of anatomical organization of *Shinkailepas myojinensis* by gross dissection, scanning electron microscopy, and histological serial sections. The results of observations are compared with existing knowledge of organ systems of other nertiopsines, and their significance is discussed in terms of comparative anatomy and systematics.

MATERIALS AND METHODS

Materials examined in this study were selected from part of paratype series of *S. myojinensis* and those preserved at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). Details of sampling data are shown in the original description. Samples fixed in formalin were used for anatomical observations. After removed from the shell, soft parts of five specimens were dissected under a binocular microscope. Pieces of dissected soft parts were dried with a freeze-drier (Hitachi ES2030) and observed with SEM (Hitachi S2400). Whole animals of two females and three males were thin-sectioned at the thickness of 6 µm after embedding in paraffin. They were stained by a standard method of Mayer's Hematoxylin and Eosin staining. Whole series of sections (UMUT RM28647–28651) and SEM stub (UMUT RM28652) are deposited in the Department of Histological Geology and Paleontology, the University Museum, the University of Tokyo (UMUT). The terminology used in the descriptions chiefly followed that of Sasaki (1998) and Kano & Kase (2002).

RESULTS

Shell and Operculum

See Sasaki et al. (2003).

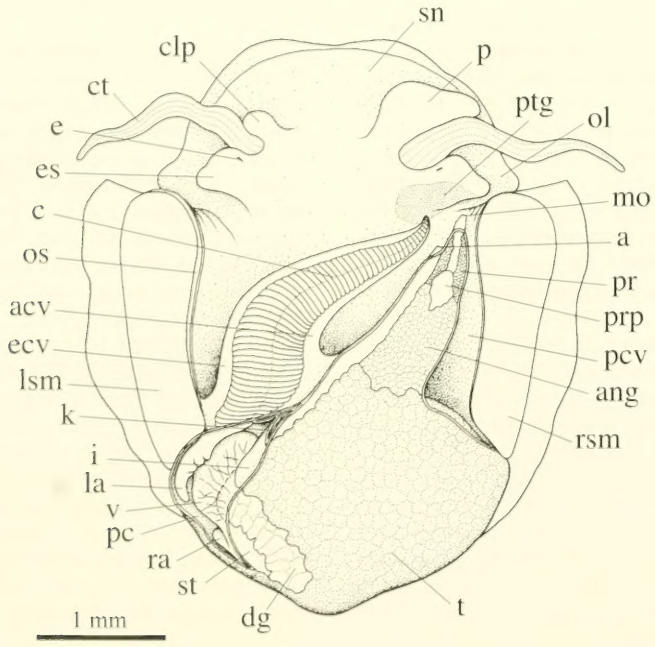

FIG. 1. Dorsal view of animal, male, with mantle removed.

External Anatomy

The animal is dorsoventrally flattened and symmetrical in outline. The visceral mass is not spirally coiled (Fig. 1). The dorsal surface of the animal is covered with a thin mantle. The entire surface of the mantle carries dense filamentous mantle processes (mp: Fig. 3A), which are multicellular and contain fiber-like structure internally (Fig. 4A). These processes are not branched and some of them penetrate through whole thickness of the shell at positions corresponding to the shell pores (cf. Sasaki et al., 2003: fig. 12C). The dorsal side of mantle margin also bears similar processes (Fig. 3B).

The mantle margin (mm: Figs. 2, 4C) is prominently thickened, divided into the outer and inner folds by the periostracal groove (pg: Figs. 3B, 4C). Its inside contains muscle fibers and blood sinus. The portion of the mantle covering the pallial cavity is provided with extensive blood sinus with numerous hemocytes inside (pls: Fig. 4F) and probably functions as a respiratory surface.

Major part of the ventral side of the animal is occupied by a circular, flattened pedal sole (ps: Fig. 2). Its anterior margin is marked by the anterior pedal groove (apg: Figs. 2, 4B) with the opening of the anterior pedal gland (apd: Fig. 4B). The ventral side of the head is extended as the oral lappet (ol: Figs. 2, 3C). The circumference of the mouth is thickened, papillate, and wrinkled (Fig. 3C). The lateral foot is smooth without any protrusive structure. The epipodial fold is extended between posterior dorsal rim of the foot and the mantle margin and provided with triangular tentacles (Figs. 2, 3D). The epipodial tentacles lack micropapillae, sense organ and ciliated structure. Their number ranges from 11 to 19, varying among specimens examined. The operculum is firmly attached to the dorsal surface of the foot musculature below the visceral mass.

The head consists of the snout, cephalic tentacles, cephalic lappets, and eye stalks (Fig. 1). The snout is stout, short and not tapered. The cephalic tentacles are paired in equal form and size, symmetrically positioned on each side of the head, not covered with sensory papillae, and striated by internal longitudinal muscle. The cephalic lappets are symmetrical, equal in size in female, but the right one is greatly enlarged in the male as the penis (p: Fig. 1). The eye stalks are posterior to the cephalic tentacles, flattened, and morphologically identical between both sexes. Only the posterior half of the right eye stalk is covered with a patch of tall glandular cells, which are here termed the "post-tentacular gland" (ptg: Figs. 1, 4F). Black eye spots are visible externally in anterior middle part of the eye stalks (Fig. 1).

The shell muscle is divided into right and left portions (lsm, rsm: Fig. 1), leaving separated scars on the internal surface of the shell. Each muscle is not subdivided into bundles, nor penetrated by blood vessels. The head retractor muscles are merged with shell muscles and do not produce independent attachment areas. The mantle is devoid of particular retractor muscle inserted on the shell, but instead attached to the shell by penetration of pallial processes.

Pallial Cavity

The pallial cavity is deep and attains the level of the posterior end of both shell muscle attachments. It contains the ctenidium, osphradium (os: Fig. 4E), anus, the kidney opening (ko: Fig. 10C), and genital opening(s).

The ctenidium is single, extended from the posterior left to the anterior right (Fig. 1). The ctenidial lamellae are bipectinate in alternating

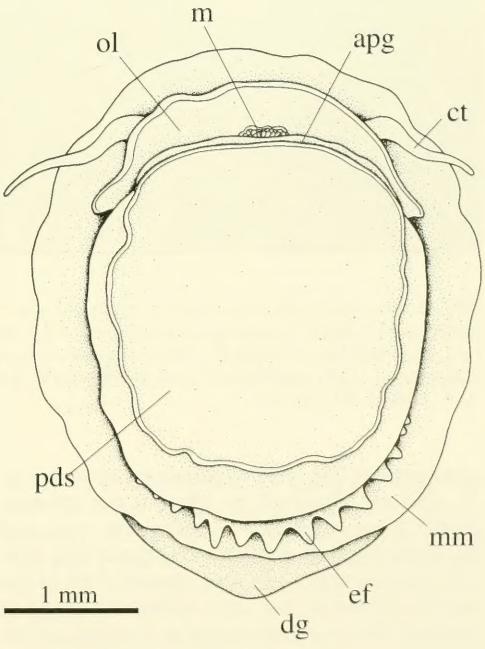

FIG. 2. Ventral view of animal.

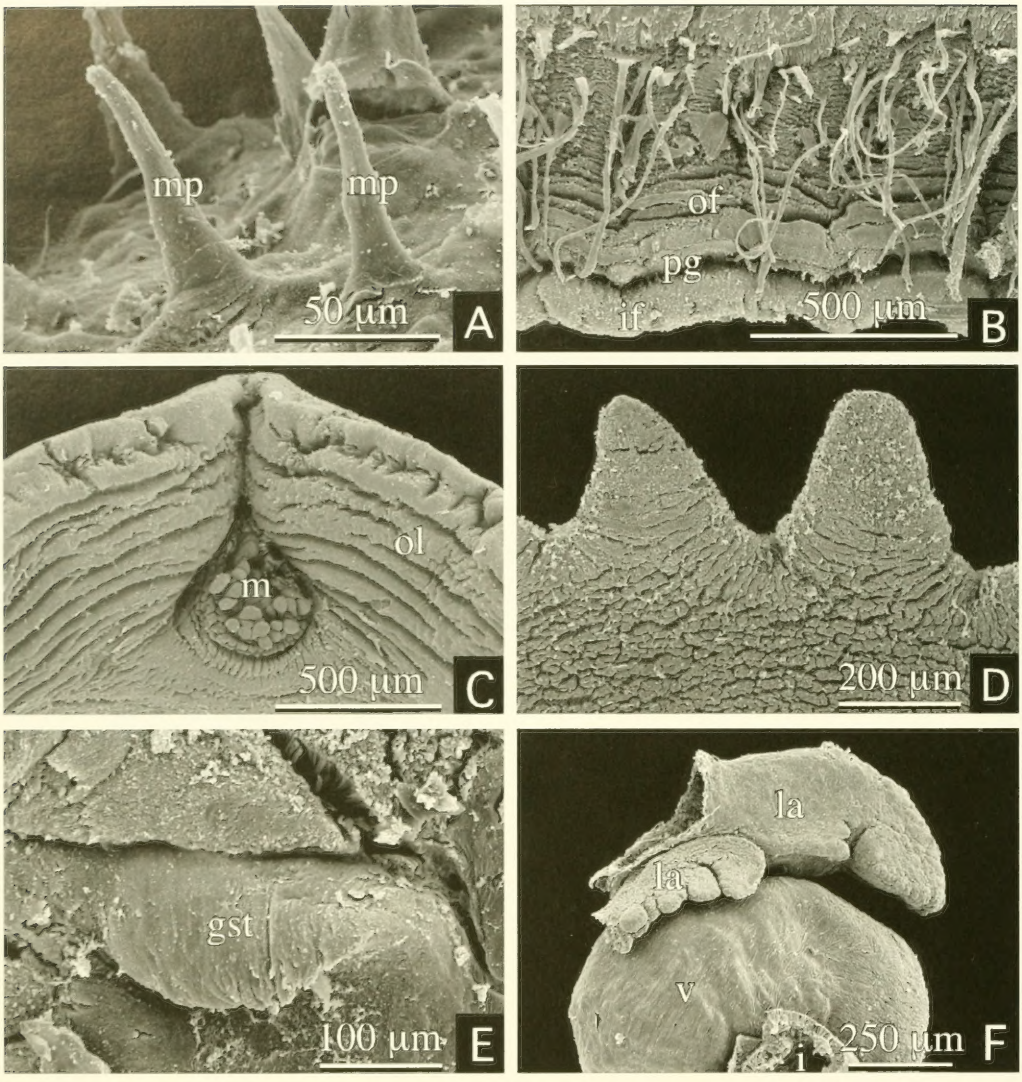

FIG. 3. Scanning electron micrographs of external and internal organs. A. Mantle processes (mp) on surface of mantle covering visceral part. B. Dorsal view of mantle margin. C. Ventral view of mouth (m) and oral lappet (ol). D. Ventral view of epipodial fold. E. Tooth of gastric shield (gst) inside of stomach. F. Left auricle (la) and ventircle (v) penetrated by intestine (i), removed from pericardium. A–F. UMUT RM28652.

arrangement (Fig. 4D), ridged along midline (Fig. 1), and not attached by afferent nor efferent ctenidial membranes. The skeletal rods and buriscles (sensory pockets) are absent (Fig. 4D).

A hypobranchial gland is absent. Part of the mantle opposing the post-tentacular gland is covered with a tall glandular epithelium, which is termed the "anterior pallial gland" (apl: Fig. 4F). This gland is also extended to cover the distal tip of the pallial gonoduct. Dorsoventrally paired, two glands, the post-tentacular gland on ventral side and the anterior pallial gland on dorsal side, are developed in the same position and size in both sexes. The epithelium of equivalent part on the left side is not specialized as gland.

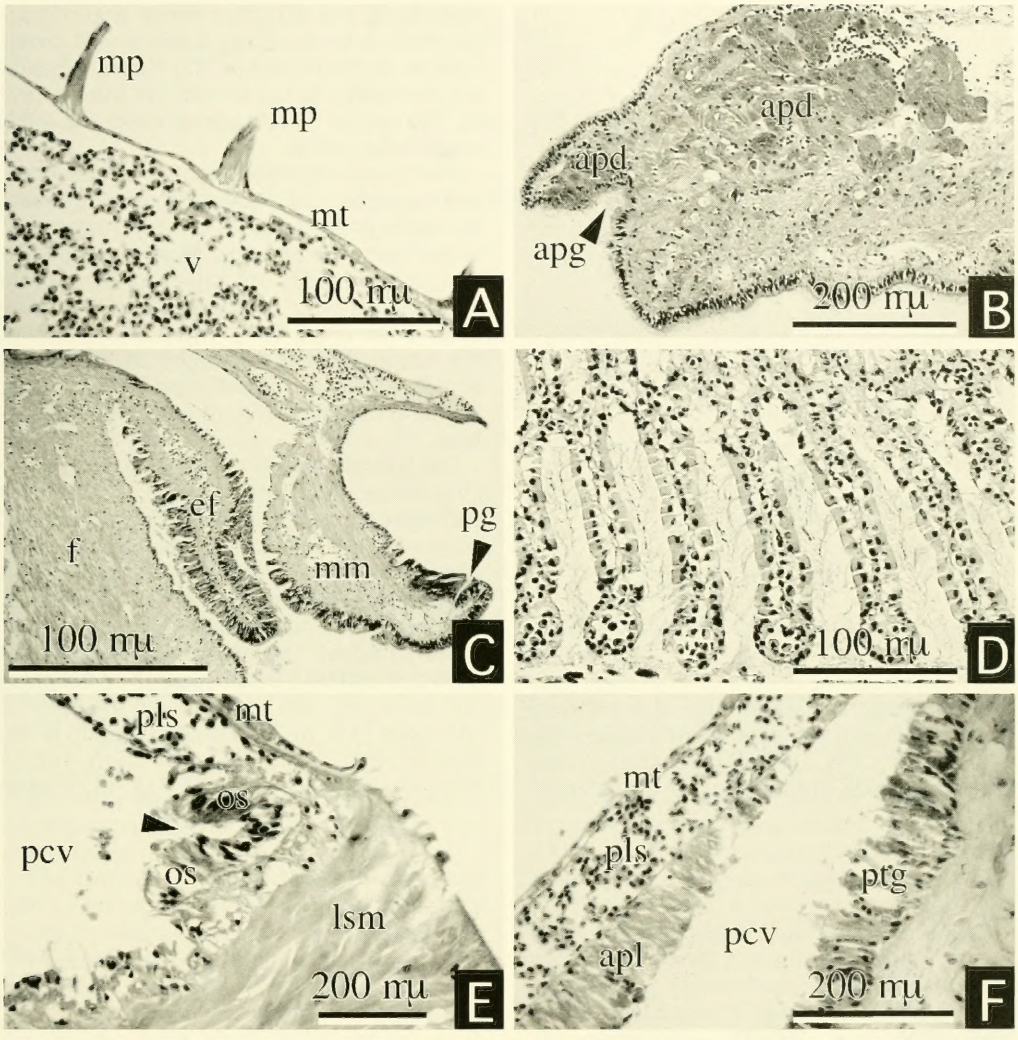

FIG. 4. Histology of external and pallial organs. A. Vertical section of mantle processes (mp) arising from mantle (mt) above ventricle (v). B. Longitudinal section of anterior tip of foot, showing anterior pedal gland (apd) and anterior pedal groove (apg). C. Longitudinal section of posterior part of foot (f) and mantle margin (mm). Epipodial fold (ef) arises from part of foot. D. Longitudinal section of ctenidial lamellae. E. Cross section of osphradium (os) along left shell muscle (lsm). Arrowhead indicates longitudinal groove on osphradium. F. Cross section of two opposing glands in pallial cavity near right eye stalk. A, E–F. UMUT RM28648. B–D. UMUT RM28651.

Digestive System

The digestive system consists of the oral tube, sublingual pouch, buccal cavity, buccal mass, radula, esophagus, stomach, digestive glands, and intestine to anus (Fig. 5).

The mouth opens ventrally (Fig. 1). The oral tube is considerably short in front of the buccal mass and followed by the sublingual pouch ventrally and by the buccal cavity dorsally. The anterior inner wall of the buccal cavity is particularly thickened as the transverse buccal fold with a pair of distinctly cuticularized plates (cp: Fig. 10). The jaw with tooth-like elements is absent.

The sublingual pouch is well developed below the buccal mass (slp: Fig. 7). Its epithelium is thin and smooth. Paired sublingual

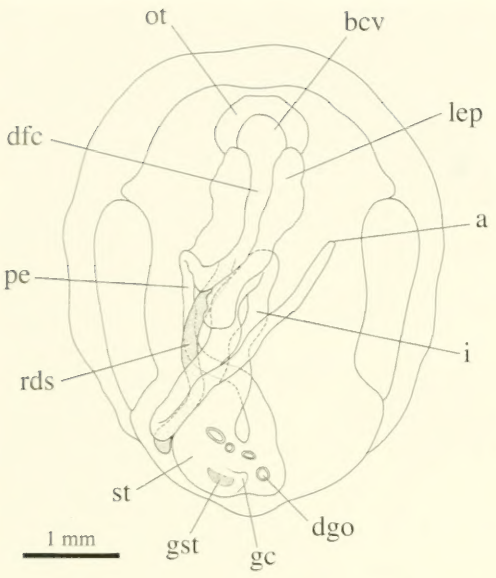

FIG. 5. Configuration of digestive tract in dorsal view.

glands project on each side of sublingual pouch and open into ventrolateral side of the oral tube (slg: Fig. 7). Their inner surfaces are roughened with irregular forms of glandular epithelium (Fig. 9B). The dorsal side of buccal cavity is thickened by a pair of well-developed dorsal folds (df: Fig. 9D). Salivary glands are not differentiated around the buccal cavity. The radular diverticulum is deep below the esophageal valve.

The buccal mass is elongated longitudinally and connected with body wall musculature with the lateral protractors (lp), inner and outer pairs of the ventral protractors (ivp, ovp), median and dorsal levators (ml, dl), posterior depressors (pd), anterior levators (al), and anterior tensors (at) (Figs. 6, 7). The posterior part of the odontophore is separated on both sides of radular sac and closely tied by postdorsal buccal tensor (pdt: Fig. 6) on the dorsal surface.

The buccal mass is internally supported by five odontophoral cartilages. The anterior cartilages are paired, elongated longitudinally, increasing in width backwards. The posterior cartilages are also paired, much smaller than the anterior pairs, firmly attached to the anterior pairs, and tapered posteriorly with pointed posterior ends. The median cartilage is unpaired, of almost the same length as the anterior cartilages, and lies between the anterior cartilages (Fig. 8). Its anterior one-fifth is demarcated by dorsal constriction and is smoothly swollen ventrally (Fig. 8). Ventral

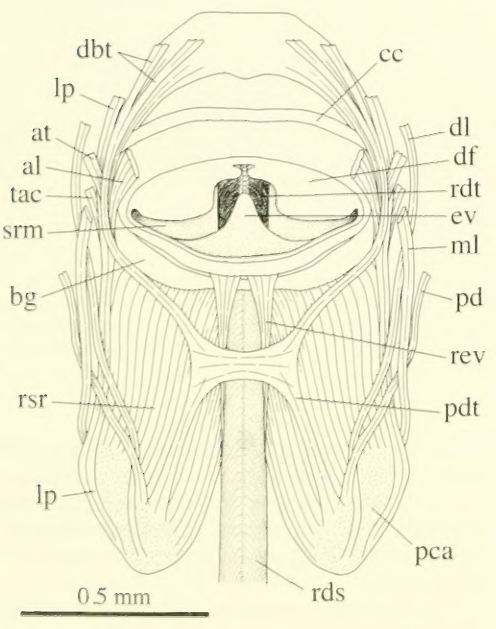

FIG. 6. Drosal view of buccal mass, with anterior digestive tract intact.

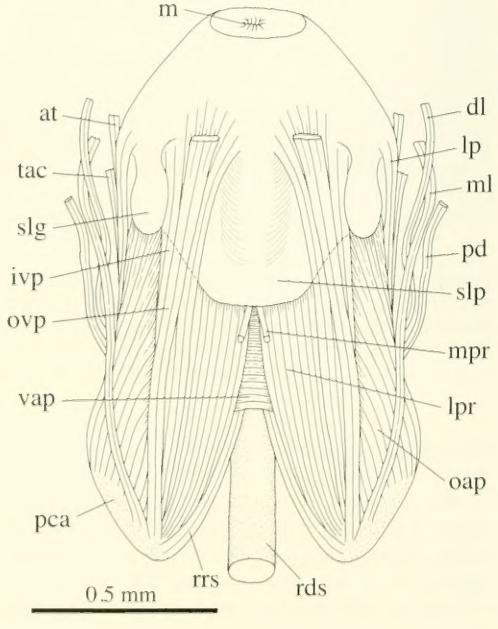

FIG. 7. Ventral view of buccal mass, with oral tube and sublingual pouch intact.

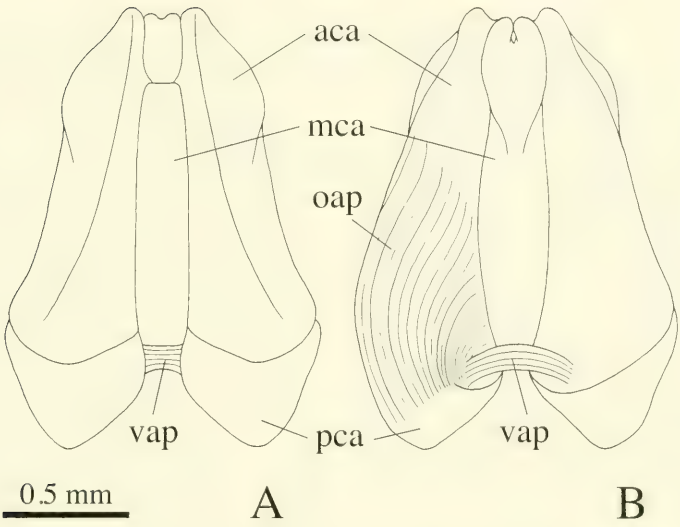

FIG. 8. Odontophoral cartilages. A. Dorsal view. B. Ventral view. Outer approximator muscle (oap) is removed on left side. Anterior part of ventral approximator muscle (vap) is also removed (cf. Fig. 7).

sides of the anterior cartilages are connected by the ventral approximator muscle (vap: Fig. 7). The anterior and posterior cartilages are longitudinally united with the outer approximator muscles (oap: Fig. 7). Sides of anterior cartilages are attached to the body wall by tensor muscles (tac: Figs. 6, 7).

The radula is composed of an ensheathed part of the radular sac posterior to the buccal cavity, the subradular membrane spread over the buccal mass, and functional area exposed into the buccal cavity on the bending plane. The radular sac is extended backwards and not coiled in loops. Its posterior end is smooth and simple. The subradular membrane is attached by the median and lateral protractor muscles (mpr, lpr: Fig. 7) anteriorly, and the retractor muscles (rsr: Fig. 7) laterally and posteriorly. The retractor muscles of radular sac (rrs: Fig. 7) originate from the posterior end of the posterior cartilages, insert on the radular sac ventrally. The radular teeth morphology was described by Sasaki et al. (2003).

The esophagus begins from the posterodorsal part of the buccal mass and comprises two parts, the anterior and posterior esophagi. The anterior esophagus is dorsoventrally depressed, almost consistent in width and curves towards the left over the buccal mass. Inside of the anterior esophagus is divided into the dorsal food channel (dfc) in the center and the lateral esophageal pouches (lep) on both sides by a pair of the dorsal folds (Figs. 9D, E). The inside of the lateral esophageal pouches is glandular, and therefore, it can also be called the anterior esophageal gland. Behind the buccal mass, a main part of the anterior esophagus slightly turns forward to form a very short loop. The posterior part of lateral oesphageal pouches are separated from dorsal food channel, extending ventrally as the posterior esophageal gland, and surrounds the radular sac completely (peg: Fig. 9F). Their epithelia are heavily folded (Fig. 9F). The posterior esophagus is narrower than the anterior esophagus (Fig. 5), and defined by corrugated inner structure (Fig. 9G). It runs below the radular sac and other organs at the deepest level of the visceral cavity.

The stomach is marked by the enlargement in diameter and folded into U shape. The inside has large cuticularized area, short crescent-shaped gastric caecum (gc: Fig. 5), tooth of gastric shield (gst: Figs. 3E, 5), and paired typhlosoles on the ventral side. The digestive glands are well developed around the stomach and connected to the distal part of the stomach through four openings dorsally and two openings ventrally. The ducts of digestive glands are further divaricated complicatedly.

The intestine is circular in cross section, nearly consistent in diameter along its entire length, turns in two sites, posterior to the buccal mass and below the pericardium (Fig. 5).

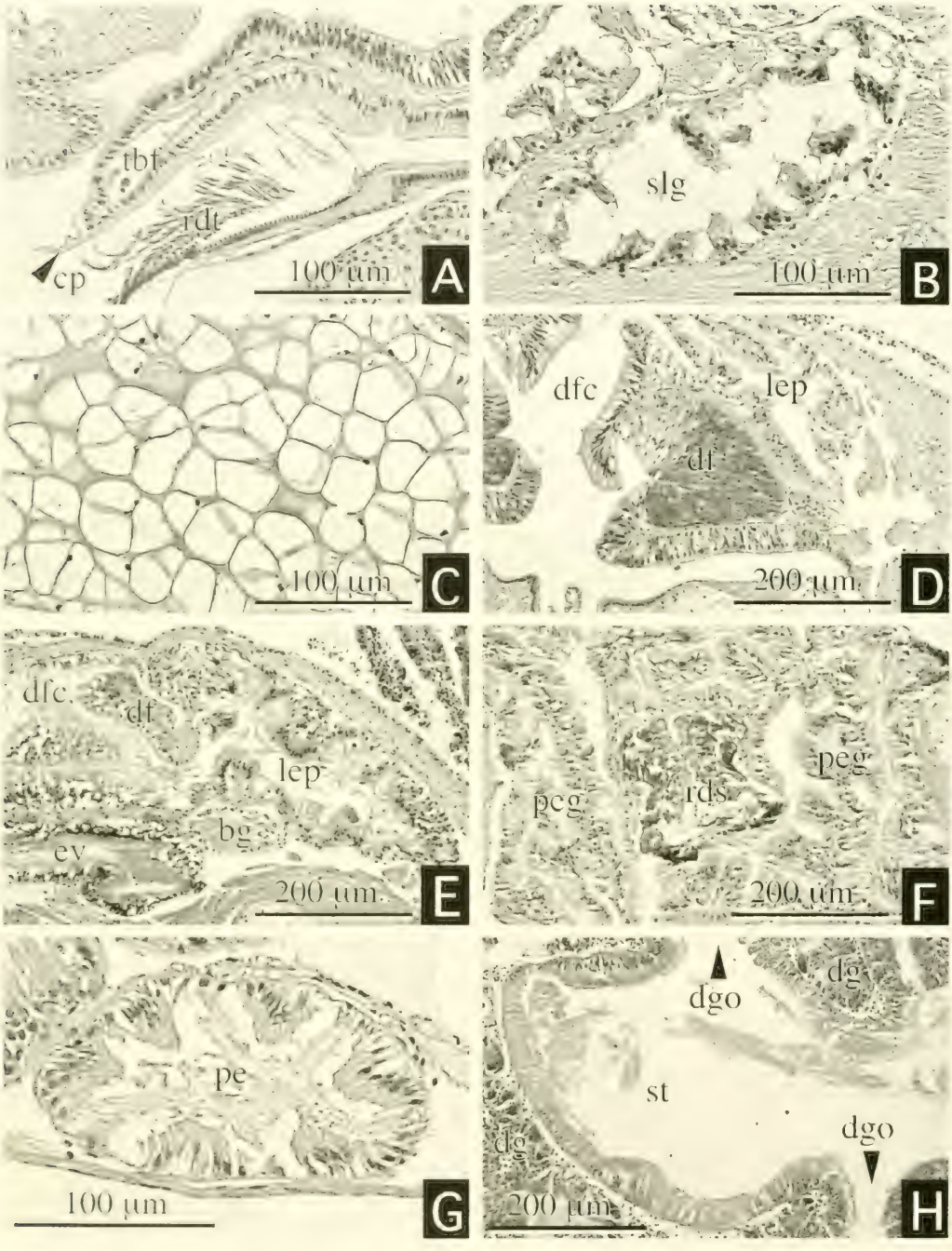

FIG. 9. Histology of digestive system. A. Longitudinal section of transverse buccal fold (tbf), cuticularized plate (cp), and radular teeth (rdt). B. Cross section of sublingual gland (slg). C. Vertical section of anterior odontophoral cartilage. D. Cross section of left half of dorsal fold (df) and lateral esophageal pouch (lep) over anterior part of buccal mass. E. Cross section of left half of anterior esophagus in more posterior part than in Fig. D. F. Cross section of posterior esophageal gland (peg) surrounding radular sac (rds). G. Cross section of posterior esophagus (pe). H. Cross section of stomach, showing openings to digestive glands (dgo) on dorsal and ventral (upper and lower in figure) sides. C, H. UMUT RM28647. B, D–F. UMUT RM28648. A, G. UMUT RM28651.

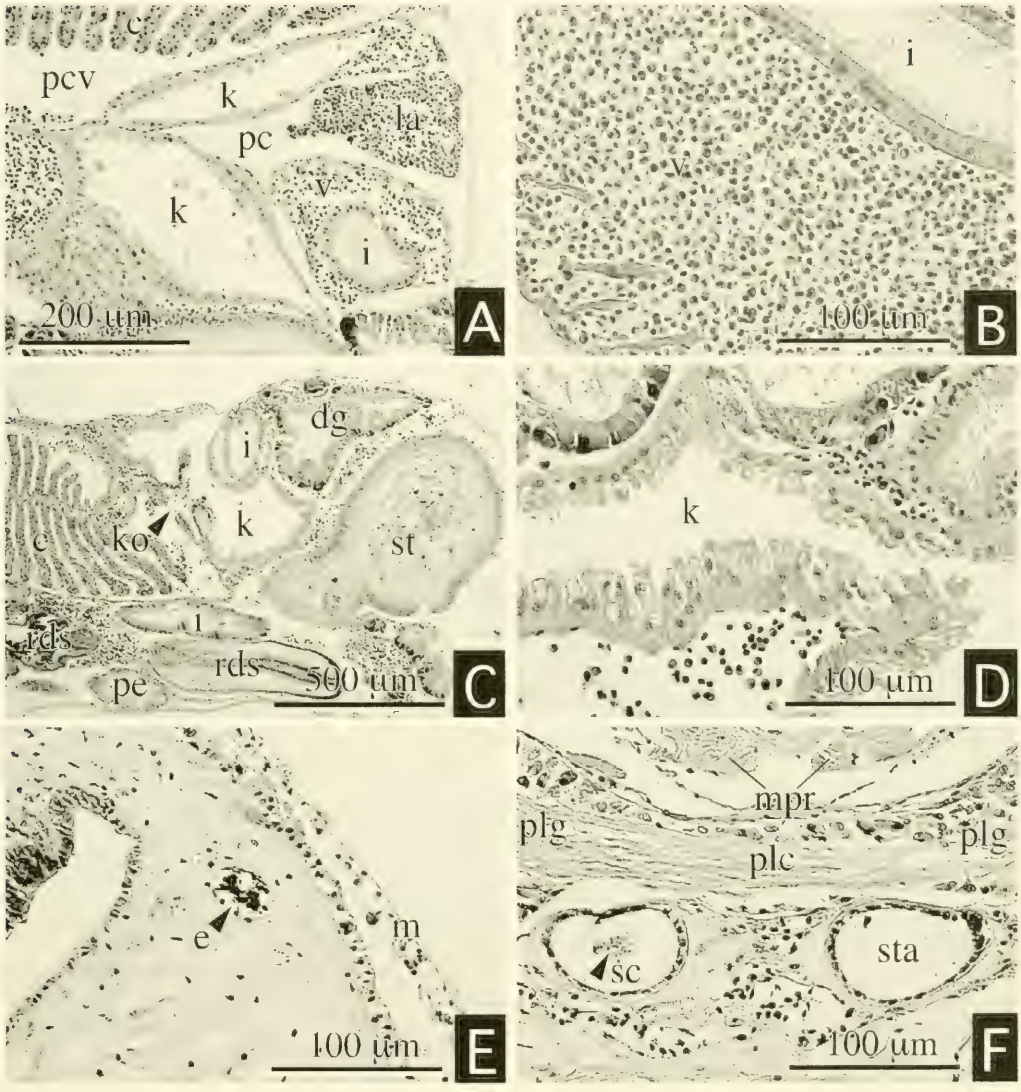

FIG. 10. Histology of circulatory and excretory systems and sense organs. A. Horizontal section of kidney (k) and pericardium (pc). B. Enlarged view of ventricle (v) penetrated by intestine (i). C. Longitudinal section of kidney (k), its opening (ko) and adjacent organs. D. Enlarged view of epithelium of kidney (k). E. Cross section of left eye (e). F. Cross section of statosysts (sta) containing statoconia (sc) below pleural commissure (plc). D. UMUT RM28647. E–F. UMUT RM28648. A–C. UMUT RM28651.

Its epithelium is densely ciliated and composed of a layer of prismatic cells (Fig. 10B). Around the second turn, the intestine penetrates the ventricle and the pericardium. No anal (rectal) gland was found near the distal part of the intestine. The anus opens on the right anterior side of the pallial cavity (Fig. 1).

Circulatory System

The heart is enclosed in the pericardium and consists of paired auricles of unequal size and a median ventricle (Figs. 1, 3F, 10A). The left auricle is much larger than the right and connected to blood vessels from the ctenidium and

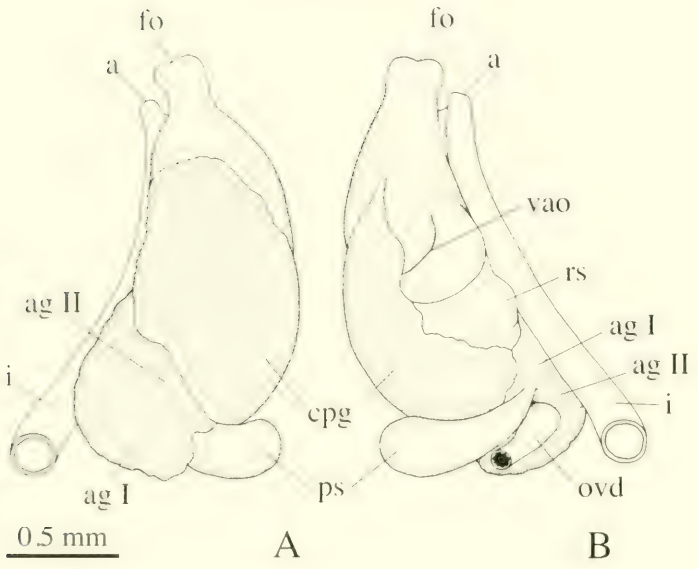

FIG. 11. Pallial oviduct of female, with intestine intact. A. Dorsal view. B. Ventral view.

the mantle. The margin of the left auricle is rugose and constricted to be separated into a few chambers (Fig. 3F). The right auricle is vestigial and situated posterior to the ventricle (Fig. 1). The ventricle is voluminous, penetrated by the intestine, stiffened by cardiac muscles inside (Fig. 10B). An aorta from the ventricle is short and opens into haemocoel of the body. An aortic bulb is not formed. Large sinuses are developed among visceral organs and around the buccal mass. Considerable blood sinuses are also found near the lateral wall of the pedal musculature. Blood space adjacent to the kidney is connected anteriorly to the afferent ctenidial vessel.

Excretory System

The excretory organ consists of a single kidney and the pericardium. The kidney is on the posterior side of the ctenidium; its excretory opening is located below ctenidial base (ko: Fig. 10C). The inside of the kidney is partly partitioned into two branches on the anterior and right sides of the pericardium, respectively (Fig. 10A), but both are indistinguishable in histology. The epithelium of the kidney is not lamellated throughout the entire area and consists of single-layered papillate cells with basal nuclei (Fig. 10D). The renopericardial connection is extended between the two branches of the kidney.

Reproductive System

The sexes are separate, and their reproductive organs exhibit striking sexual dimorphism. Large part of the reproductive organ is occupied by the gonad and pallial gonoduct. In both sexes, the gonad develops dorsal to the digestive gland, the gonoduct is not connected to the kidney, and gonopericardial connection is not present.

The female reproductive system consists of the ovary, a thin oviduct connecting the ovary and the pallial oviduct, a complex of pallial oviduct and associated glands, and the vaginal duct. The ovary and dorsal surface of the pallial oviduct are visible on the dorsal side of the animal through the mantle. The oviduct is thin and circular in cross section, arises from anteroventral part of the ovary, extends forward, and enters the pallial oviduct from its ventral side (Figs. 11B, 12, 15C).

The pallial oviduct is surrounded by two differently stained glands, a posteriorly situated albumen gland, and an anteriorly located capsule gland. The albumen gland covers posterior area of the pallial oviduct and is further subdivided into two portions, albumen gland I and II (agI, agII: Fig. 11). Both parts are not stained darkly, and the albumen gland I is obviously more translucent than the albumen gland II (Fig. 15C). The pallial oviduct opens into the pallial cavity through its anterior tip (fo: Fig. 11).

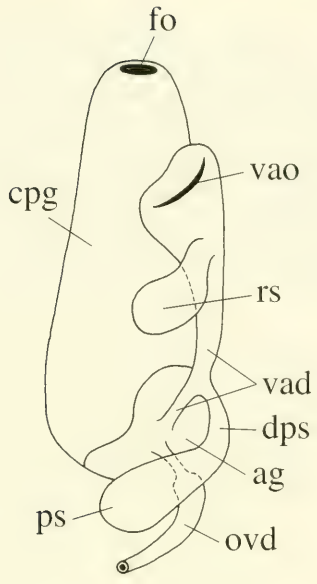

FIG. 12. Schematic drawing of ventral view of pallial oviduct, showing connection of various ducts and associated structures.

The vaginal duct is ventral to the pallial oviduct, covered entirely with epithelium of pallial oviduct, elongated, and connected to the pallial oviduct posteroventrally. The terminal of the vaginal duct is expanded and opens as an oblique slit (vgo: Fig. 11B). Posterior to the vaginal opening, the receptaculum seminis is branched off from the vaginal duct. Spermatozoa are oriented in the receptaculum seminis towards its epithelium (Fig. 15A). The distal end of the receptaculum seminis is visible in ventral view (Fig. 11B). Near its connection to the pallial oviduct, the vaginal duct gives off another pouch-like structure. It is tentatively named "posterior sac" because of unknown function and uncertain homology with other reproductive structures. Its inside is characteristically roughened by many folds (ps: Figs. 15B–E), and no spermatozoa was found there. A groove or ovipositor is not developed on the right side of the neck of the female.

The male reproductive organ is composed of the testis (Fig. 15F), vas deference, seminal vesicle, and pallial male gonoduct (Fig. 13). The testis contains many cylindrical sectors in which growing sperms are arranged radi-

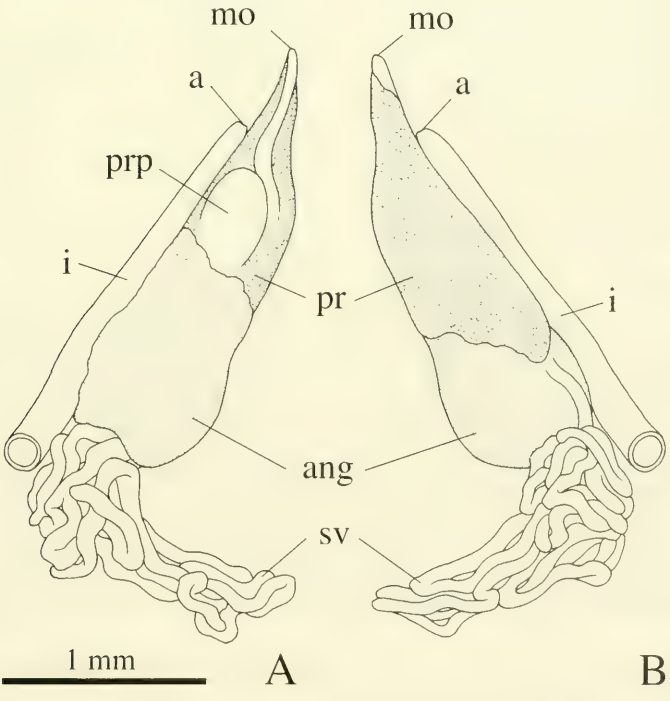

FIG. 13. Pallial gonoduct of male, with intestine intact. A. Dorsal view. B. Ventral view.

ally. A thin vas deference originates from the ventroanterior side of the testis and extends anteriorly. The distal part of the vas deference is complicatedly folded to form the seminal vesicle, which is filled with filamentous, completed spermatozoa oriented in parallel (Figs. 14C–D, 15G). The spermatophore was not observed in any section of vas deference.

The pallial male gonoduct is greatly enlarged and surrounded by two different glands, the annex gland posteriorly and the prostate gland anteriorly (Figs. 13, 15H). In dorsal view, a part of the prostate pouch is visible on the outer surface (Fig. 13A).

The penis arises from inner side of right cephalic tentacle, dorsoventrally depressed, lobate with a tapered tip (Fig. 1). Its dorsal surface is smooth, the right margin is grooved throughout, and the ventral side has a single ciliated papilla (Figs. 14A, B). A ciliated groove was not found between the gonoduct opening and the penis.

Nervous System

The nervous system consists mainly of the circumesophageal nerve ring, pedal cords, and visceral nerve. The circumesopahgeal nerve ring is formed by pairs of three major ganglia, namely, pleural, pedal, and cerebral ganglia. Their configuration is of hypoathroid type, namely pleural and pedal ganglia are more adjacent than cerebral ganglia (Fig. 16).

The cerebral ganglia are located on the bases of the cephalic tentacles and send nerves to the cephalic tentacles and oral area. The paired ganglia are connected to each other by the cerebral commissure over the oral tube and the labial commissure below the sublingual pouch. Labial ganglia are not formed on the labial commissure. The buccal ganglia are obliquely extended at the base of the anterior esophagus and connected to the cerebral ganglia through thin connectives.

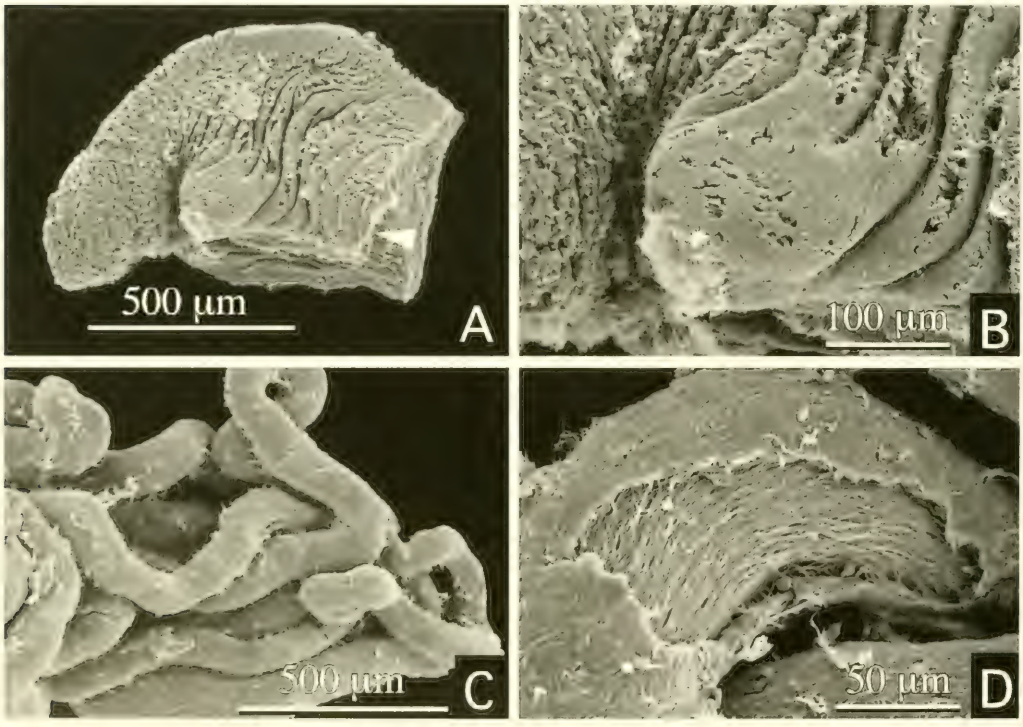

FIG. 14. Scannning electron micrographs of male reproductive organs. A. Ventral view of penis removed from head. Arrowhead indicates groove along right margin. B. Enlarged view of ciliated papilla on right ventral side. C. Seminal vesicle in convoluted part of vas deference. D. Spermatozoa contained in seminal vesicle. Part of epithelium of seminal vesicle is removed to show inside. A–D. UMUT RM28652.

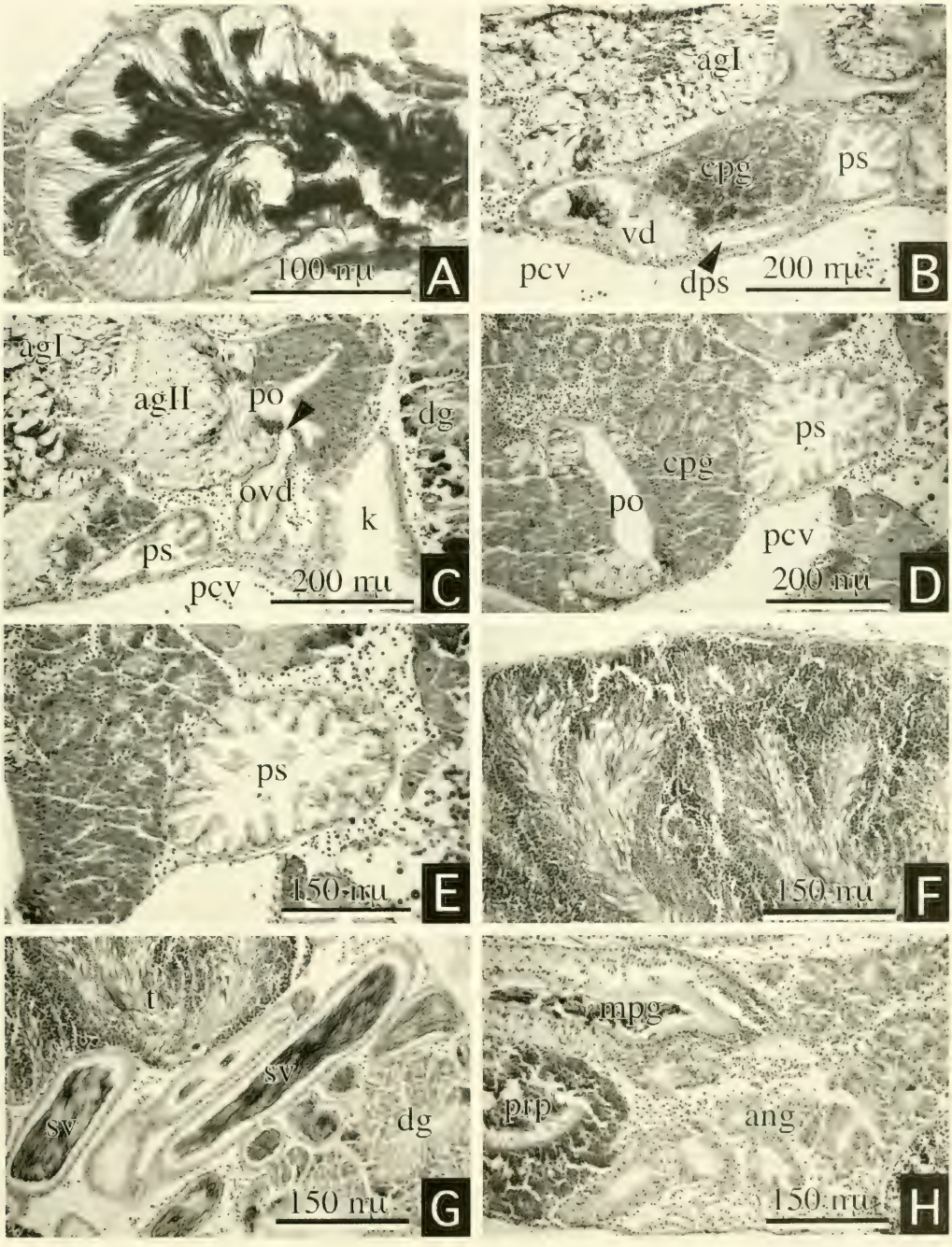

FIG. 15. Histology of reproductive organs of female (A–E) and male (F–H). A. Cross section of seminal receptacle. B. Longitudinal section of posteroventral part of pallial oviduct. C. Longitudinal section of pallial oviduct where oviduct (ovd) is connected to lumen of pallial oviduct. D. Longitudinal section of posterior sac (ps) of vaginal duct and surrounding glands. E. Enlarged view of longitudinal section of posterior sac (ps) of vaginal duct. F. Vertical section of testis. G. Vetical section of seminal vesicle (sv) containing completed spermatozoa. H. Horizontal section of glands of pallial gonoduct of male. A. UMUT RM28648. F–H. UMUT RM28650. B–E. UMUT RM28651.

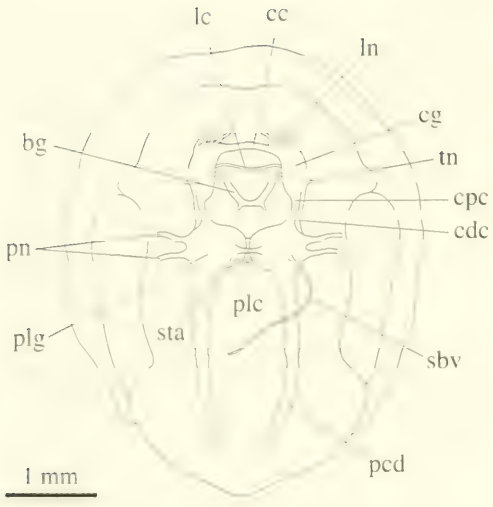

FIG. 16. Configuration of nervous system in dorsal view.

The pleural ganglia are situated below the posterior part of the buccal mass, and they are the largest among all ganglia and connected by a rather thin pleural commissure. Two distinct nerves are extended from the pleural ganglia to the body wall musculature.

The pedal ganglia underlie pleural ganglia and are connected by the pedal commissure. The pleural and pedal ganglia are juxtaposed closely, forming almost fused complexes, but they receive separate cerebropleural connectives and cerebropedal connectives, respectively, from the cerebral ganglia. The pedal ganglia give off thin nerves anteriorly and thick pedal cords posteriorly. Pedal cross connection was not detected in the present sections.

The visceral nerve is not a closed loop or streptoneurous and represented only by the subesophageal part, which arises from the right pleural ganglion toward base of ctenidium along the pallial cavity wall. The supraesophageal part is totally missing.

Sense Organs

Possible sensory organs include cephalic tentacles, oral lappets, papillae on the outer lip of the mouth, posterior epipodial fold, osphradium, statocysts, and eyes. The most of external structures are described above. Epipodial sense organs, and subradular organs are absent.

The osphradium is elongated as a vermiform ridge along the left shell muscle, weakly developed, and two-folded with a longitudinal central groove (os: Fig. 4E). Eyes are rudimentary without cornea and lens, simply represented by pigmented cells, and sunken in connective tissue of eye stalks. Statocysts are attached to the dorsal side of pedal ganglia and below pleural commissure (plc: Fig. 10F) and contain several statoconia (sc: Fig. 10F).

DISCUSSION

Comparative Morphology

The morphology of neritopsine gastropods, in which *Shinkailepas* is included, is highly diverse in both shell and soft parts. Their similarity and dissimilarities have been employed for systematics at various taxonomic levels (e.g. Sasaki, 1998) and also used as characters for phylogenetic analysis (Ponder & Lindberg, 1997; Sasaki, 1998). The results of observations on *Shinkailepas myojinensis* in this study are compared with descriptions of relatively well-investigated genera (Tables 1, 2).

Shell: The shell of *Shinkailepas* is characterized by (1) a limpet form with the apex on the posterior center, (2) a multispiral globular protoconch with growth lines, (3) the septum extended between the visceral mass and foot, and (4) microscopic canal structures with crowded openings inside and sparse outside. Among these, (1) and (3) are also found in *Phenacolepas* and *Septaria*. (2) is shared by aquatic neritopsines in general (Sasaki, 1998). The shell of another vent-associated neritopsine limpet, *Olgasolaris*, differs from that of *Shinkailepas* in a more centrally situated apex, many regular and fine radial riblets, and thick periostracum overhanging the shell margin. *Phenacolepas* and related shallow-water forms are most similar to *Shinkailepas* among Neritopsina in above characters, except (4).

It is well known that numerous thin canals and corresponding pores are produced independently in the shells or valves of all polyplacophorans, part of bivalves and gastropods, and brachiopods (reviewed by Reindl & Haszprunar, 1996). In Gastropoda, canals and mantle processes (caeca) are found in the Fissurellidae (Reindl & Haszpruanr, 1994), Neritiliidae (Kano & Kase, 2002), *Shinkailepas* (Beck, 1992; Sasaki et al., 2003; this study), and *Olgasolaris* (Beck, 1992). Shell canals of *Shinkailepas* (and possibly also *Olgasolaris*)

TABLE 1. Comparison of character states in *Shinkailepas* and non-phenacolepadid neritopsine genera.

Family	Phenacolepadidae	Neritiliidae	Neritidae	Neritidae	Helicinidae
Genus	*Shinkailepas*	*Pisulina, Neritilia*	*Septaria*	*Nerita*	*Waldemaria*
Reference	this study	Kano & Kase (2002)	Sasaki (1998)	Sasaki (1998)	Sasaki (1998)
shell form	limpet	coiled	limpet	coiled	coiled
canal structures of shell	partly penetrating shell completely	opening only inside of shell	absent	absent	absent
operculum shape	trapezoidal	semicircular	trapezoidal	semicircular	semicircular
apophysis of operculum	absent	present	present	present	absent
epipodial flap	present	absent	absent	absent	absent
cephalic lappet	present	absent	absent	absent	absent
ctenidium	well-developed	vestigial	well-developed	well-developed	absent
vestigial gill on right side	absent	absent	absent	present	absent
hypobranchial gland	absent	present	present	present	present
anterior pallial gland	present	?	?	?	?
post-tentacular gland	present	?	?	?	?
sublingual gland	present	present	present	present	absent
central tooth of radula	present	absent	present	present	present
lateral teeth of radula	fourth teeth longitudinal	outer (third) teeth oblique	fourth teeth transversely shield-like	fourth teeth transversely shield-like	fourth teeth oblique
lateromarginal plate	absent	present	absent	absent	absent
radular sac	short	long	short	short	short
anterior esophageal glands	not elongated	elongated posteriorly	not elongated	not elongated	not elongated
floor gland in anterior esophagus	absent	present	absent?	absent?	absent?
posterior esophageal glands	tightly enclosing radular sac	loosely enclosing radular sac	lateral to radular sac	lateral to radular sac	lateral to radular sac

(*continues*)

(*continued*)

Family	Phenacolepadidae	Neritiliidae	Neritidae	Neritidae	Helicinidae
Genus	*Shinkailepas*	*Pisulina, Neritilia*	*Septaria*	*Nerita*	*Waldemaria*
Reference	this study	Kano & Kase (2002)	Sasaki (1998)	Sasaki (1998)	Sasaki (1998)
opening(s) between stomach and digestive glands	paired	single	paired	paired	paired
glandular and non-glandular regions of kidney	undifferentiated	undifferentiated	differentiated	differentiated	differentiated
right auricle	present	present	present	present	absent
erythrocytes	possibly present	absent	absent	absent	absent
female reproductive openings	diaulic	diaulic	triaulic	diaulic	diaulic
vaginal opening	slit-like, anterior	slit-like, posterior	small pore, anterior	small pore, anterior	small pore, posterior
receptaculum seminis	posterior to vaginal opening	part of spermatophore sac	connected to dorsal albumen gland	connected to dorsal albumen gland	absent?
posterior sac of vaginal duct	present	absent	absent	absent	absent
crystal sac in female	absent	absent	present	present	absent
neck furrow in female	absent	present	absent	absent	absent
female flap	absent	present	absent	absent	absent
seminal vesicle	entangled	double, uncoiled	entangled	entangled	simple
cephalic penis	present	rudimentary or absent	present	present	absent
seminal groove on penis	lateral	?	?	?	-
visceral nerve loop	incomplete	incomplete	complete	complete	complete?
eyes	vestigial, closed	vestigial, open	well-developed, closed	well-developed, closed	well-developed, closed
osphradium	present	present	present	present	absent
central zone of osphradium	longitudinally grooved	?	?	simple	-
statocyst position relative to pedal ganglia	posterodorsal	anterior	posterodorsal	posterodorsal	posterodorsal
statocyst content	statoconia	statolith	?	?	?

are different from those of above-mentioned taxa in two points: (1) The diameter, cross-sectional form, and density of the canals are more prominently variable in *Shinkailepas* (Sasaki et al., 2003: fig. 12C), and (2) some of canals penetrate the shell completely, but many of them do not. In the Fissurellidae, all canals penetrate the shell at least in an early ontogenetic stage with a rather constant diameter (e.g., Sasaki, 1998: fig. 40d). In the Neritiliidae, canals never reach the outer surface of the shell (Kano & Kase, 2002). Similar canals are not found in any section of the shell in other neritopsines, such as *Nerita* and *Cinnalepeta* at microstructural level (Sasaki, 2001).

Operculum: Most neritopsines are operculate, except the Ceresidae, Proserpinidae (Thompson, 1980) and shell-less *Titiscania*. The common features of the opercula of *Shinkailepas* (and *Olgasolaris*) include (1) its position on the foot musculature below the visceral mass, (2) a trapezoidal, nail-like shape in outline, (3) two-layered structure, namely, calcified and corneous parts, (4) the nucleus located on the left side relative to animal's longitudinal axis, (5) the division into initially spiral and subsequently non-spiral parts, possibly of pre- and post-metamorphic stages, by different modes of growth line formation, and (6) the absence of an apophysis (Okutani et al., 1989: fig. 12; Beck, 1992: pl. 1, fig. 4, pl. 5, fig. 4; Sasaki et al., 2003: fig. 12D). Among neritopsines, the opercula of *Shinkailepas* and *Olgasolaris* are most similar to that of *Septaria* in (1) and (2), but different in the remaining features. The demarcation of nucleus (possibly of pre-meta-

TABLE 2. Comparison of character states among four phenacolepadid genera.

Genus Reference	*Shinkailepas* this study	*Olgasolaris* Beck (1992)	*Phenacolepas* Fretter (1984)	*Cinnalepeta* Sasaki (1998)
canal structures of shell	partly penetrating shell completely	penetrating shell	absent	absent
operculum shape	trapezoidal	subtrapezoidal	semicircular, vestigial	absent
apophysis of operculum	absent	absent	?	-
circumpallial microtentacles	absent	absent	present	present
epipodial flap	present	present	absent	absent
cephalic lappet	present	present	absent	absent
posterior esopahgeal glands	tightly enclosing radular sac	?	lateral to radular sac?	lateral to radular sac
intestine	2 loops	2 loops	3 loops?	5 loops
glandular and non-glandular regions of kidney	undifferentiated	?	differentiated	differentiated
erythrocytes	spherical?	?	biconcave	biconcave
vaginal opening	transversely slit-like, anterior	transversely slit-like, anterior	small pore	small pore, anterior
receptaculum seminis	posterior to vaginal opening	?	present	connected to dorsal albumen gland
posterior sac of vaginal duct	present	?	absent	absent
female flap	absent	present?	present as ovipositor?	absent?
seminal groove on penis	lateral	dorsal	?	?
eyes	vestigial, closed	?	well-developed, closed	well-developed, closed

morphic part) is also known in the Neritiliidae (Kano & Kase, 2000, 2003, in press; Kano et al., 2003), *Bathynerita* (Warén & Bouchet, 1993), and *Phenacolepas* (Kimura & Kimura, 1999: fig. 7C–D), and therefore it is not characteristic of *Shinakailepas* and *Olgasoralis*. The possession of apophysis in the operculum is rather common throughout neritopsines except the Helicinidae, *Shinkailepas*, and *Olgasolaris* (cf. Sasaki, 2001). The number of microstructual layers varies from two to four in neritopsine opercula (Suzuki et al., 1991; Sasaki, 2001), but its phylogenetic or adaptive significance is uncertain.

External Anatomy: The mantle margin is normally simple without projective or massive glandular structure in neritopsines, but it is modified in shallow-water phenacolepadids. The inner fold of the mantle margin is characteristically fringed with retractile microtentacles in *Phenacolepas* and *Cinnalepeta* (Fretter, 1984: fig. 5; Sasaki, 1998: fig. 85b). In addition, a thick layer of glandular tissue is developed on ventral surface in *Phenacolepas* (Fretter, 1984: fig. 5). But, in contrast, such a specialization does not occur in *Shinkailepas*. The mantle margin morphology is, therefore, a distinctive character between shallow-water and deep-water phenacolepadids.

Various forms of folds or tentacles are developed in gastropod epipodium as in vetigastropods, cocculinifom limpets, "vent-taxa," deep-sea phenacolepadids, and part of cerithioideans (Ponder & Lindberg, 1997). In *Shinkailepas* and *Olgasolaris*, the epipodial fold arises from the latero- to postero-dorsal part of the foot in *Shinkailepas* (Okutani et al., 1989; Beck, 1992; Warén & Bouchet, 2001: fig. 33; this study), whereas comparable structure is totally absent in other neritospines including shallow-water phenacolepadids (Tables 1, 2). Because the degree of epipodial fold development is different from species to species, it is a useful taxonomic character at species level in *Shinkailepas* (see below).

The cephalic lappets are small flaps projected on the inner side of the cephalic tentacles. They are found in part of the vetigastropods and neritopsines and may be all regarded as homologue in terms of position and innervation. It is a common feature that the lappets are enlarged and used as a penis in male in neritopsines if present. The cephalic lappets are present in *Shinkailepas* (Fig. 1; Okutani et al., 1989: fig. 10), *Olgasolaris*, and *Bathynerita* (Warén & Bouchet, 2001), but absent in the Neritiliidae, shallow-water phenacolepadids, and the Neritidae excluding *Bathynerita* (Table 1).

Pallial Organs: The pallial cavity of *Shinkailepas* contains a set of pallial organs common to the aquatic Neritopsina, namely the ctenidium, osphradium, anus, pallial gonoduct with genital opening(s), and excretory pore, but a hypobranchial gland is absent.

The ctenidium of *S. myojinensis* has typical neritopsine elements: (1) a single ctenidium is situated on the left side, (2) ctenidial lamellae are bipectinate in opposing arrangement on either side of ctenidial axis, (3) ctenidial lamellae are centrally ridged, (4) skeletal rods and (5) sensory pockets are absent. These features are not distinguishable from those of other neritopsines, except for a greatly reduced ctenidium of the Neritiliidae (Kano & Kase, 2002) and its total absence of in terrestrial groups. A wart-like structure termed "vestigial gill" on the right side of the pallial cavity (Fretter, 1965: fig. 1c; Sasaki, 1998: fig. 77c) is lacking in *S. myojinensis*. The occurrence of the structure is restricted to members of the genus *Nerita* and not universal to neritopsines.

The hypobranchial gland is present on the right pallial roof in some neritopsines, for example, the Neritidae (Fretter, 1965; Berry et al., 1973; Sasaki, 1998), Neritiliidae (Kano & Kase, 2002), and Helicinidae (Thompson, 1980; Sasaki, 1998). However, it is absent in corresponding position in *S. myojinensis*, *Phenacolepas* (Fretter, 1984), and *Cinnalepeta* (Sasaki, 1998).

In *S. myojinensis*, two glandular zones (the post-tentacular gland and anterior pallial gland) are observed on the right anterior corner of the pallial cavity. These glands may be analogous to the hypobranchial gland of other gastropods, but probably do not fulfill the function as a normal hypobranchial gland due to their restricted position at the anterior right. The true hypobranchial gland develops in deeper position in the pallial cavity in other neritoideans. The two glandular zones in *S. myojinensis* possibly have a function related to reproduction, since the glands are developed near the gonoduct opening in both sexes.

Digestive System: Its has been generally accepted that neritopsines lack true jaws (Fretter, 1965). However, paired cuticularized plates apparently develop on the inner wall of the oral tube in *S. myojinensis* (Fig. 9A). Identical plates are also present generally in other

neritopsines. Sasaki (1998) described these plates as jaws, because they are located at a position corresponding to those of other gastropods. They may not be regarded as the jaws in that they lack scaly microelements, but such microelements are also lacking in the jaws of *Cocculina* and Patellogastropods (Sasaki, 1998). Although the term "jaw" was not used in the present description, it is also possible that they represent a reduced state of the jaws. Homology can be established between jaws and cuticularized plates under positional criterion but is uncertain under structural criterion. More extensive comparison should be made among gastropod jaws for further discussion.

Buccal mass structure is remarkably useful character to define higher taxa in basal groups of gastropods (Sasaki, 1998). For example, patellogastropods, vetigastropods, and neritopsines each have their own composition of musculature and cartilages. The buccal musculature of *Shinkailepas* belongs to neritoidean type described for *Nerita, Septaria*, and *Cinnalepeta*, and is partially different from that of *Waldemaria* (Sasaki, 1998: table 5).

The configuration of odontophoral cartilages is invariable throughout the Neritopsina (Sasaki, 1998; Kano & Kase, 2002). Large anterior and small posterior cartilages are paired and connected by ventral approximator muscle, and the median cartilage is situated between the anterior cartilages. The structure of odontophoral cartilages of *S. myojienensis* conforms entirely to this pattern.

The presence of a pair of sublingual glands beside the sublingual pouches is a distinctive character of neritoideans, including the Neritidae, Neritiliidae, and Phenacolepadidae (Sasaki, 1998: Kano & Kase, 2002). They are absent in the Helicinidae (Sasaki, 1998) or unknown in the rest of neritopsine members. The salivary glands are absent throughout the Neritopsina without exception. Probably their absence is compensated by the development of sublingual glands and anterior esophageal glands.

The radular formula of *Shinkailepas* is the same as that of most members of neritoideans. The radula of *Shinkailepas* and that of *Olgasolaris* (Beck, 1992) are typified by the following six features: (1) the central tooth is present, (2) the first lateral teeth are obliquely elongate, (3) the second and third lateral teeth are small, (4) the fourth lateral teeth are longitudinally elongate without serration in their cusps, (5) lateromarginal plates are absent, and (6) the marginal teeth have small triangular projection below their cusps (Sasaki et al., 2003: figs. 12, 14).

Major differences in radular characters exist mainly in the central and lateral teeth among Neritopsina. In contrast to most neritopsines, including *Shinkailepas*, the central tooth is absent in the Neritiliidae (Kano & Kase, 2002, 2003, in press; Kano et al., 2003), *Neritopsis* (Warén & Bouchet, 1993: fig. 3D), and *Titiscania* (Bergh, 1890; Taki, 1955). The lateral teeth morphology is considerably variable among neritopsines and it is difficult to generalize. For example, in the Neritidae, the first laterals are transversely elongate, and the fourth laterals are thickened in shield-like form. The Neritiliidae (Kano & Kase, 2002, 2003, in press; Kano et al., 2003) and most helicinoideans (e.g., Thompson, 1980; Sasaki, 1998; Richling, 2004) have obliquely elongate outermost lateral teeth with sharp serrations, and lateral teeth are more reduced in *Neritopsis*, *Titiscania*, and the Hydrocenidae (Ponder, 1998: fig. 15.76). The presence of prominence below cusps in the marginal teeth (Kano & Kase, 2002: fig. 8) is known in the Neritiliidae, *Neritopsis*, and *Titiscania* (Kano & Kase, 2000, 2002), but not in others.

The configuration of the radular sac has not hitherto been focused in the studies of the Neritopsina. Recently, Kano & Kase (2002) pointed out that in the Neritopsina the length of radular sac can be categorized into two groups. A long, looped radular sac is typical of the Neritiliidae, *Neritopsis* and *Titiscania*; by contrast, a short straight type occurs in the Neritidae, Phenacolepadidae, and Helicinidae (Kano & Kase, 2002). *Shinkailepas myojinensis* (Fig. 5), *S. kaikatensis* (Okutani et al., 1989: fig. 15), and *Olgasolaris tollmanni* (Beck, 1992: fig. 3B) have the latter type.

The esophagus of the Neritopsina exhibits similar structure throughout the group (Sasaki, 1998): (1) It consists of the anterior and posterior esophagi only, lacking the differentiation of a mid-esophagus, (2) the anterior esophagus is sectioned into a centrally situated, dorsal food channel and lateral esophageal pouches with the anterior esophageal glands inside, and (3) the posterior part of the lateral esophageal pouches are separated as the posterior esophageal glands. The esophagus of *S. myojinensis* matches this generalization well.

A peculiar shaped esophagus was recently described in the Neritiliidae by Kano & Kase (2002). The anterior esophageal glands are

extremely elongated posteriorly as separated pouches and overlie the posterior esophageal glands. This double structure of esophageal glands is not known in other neritopsines including *Shinkailepas*.

The posterior esophageal glands in *S. myojinensis* are complicatedly infolded and tightly enclose the radular sac with a narrow interstitial space (Fig. 9F). This morphology apparently differs from that of other neritopsines, including the Neritiliidae (Kano & Kase, 2002: fig. 5D). Hence, the structure of this part may be of sufficient systematic value, but cross-sectional morphology of the glands have not been described for comparison in the rest of neritopsines.

Another distinctive character of the Neritiliidae is the presence of the floor glands arising from anterior esophageal floor (Kano & Kase, 2002). The glands are paired blind sacs that open from the posterior side of the floor of the anterior esophagus and consist of two glandular cells ciliated differently and stained differentially with haematoxylin (Kano & Kase, 2002: fig. 3C). The identical glands were not found in sections of equivalent position in *S. myojinensis*.

The stomach of the Neritopsina consists of a cuticularized area, gastric shield with a short reflected tooth, paired (major and minor) typholosoles on the ventral side, a ciliated intestinal groove between the typhlosoles, and a short gastric caecum (Fretter, 1965; Sasaki, 1998; Kano & Kase, 2002). Differences in the stomach structure among the Neritopsina is not very conspicuous. The Neritiliidae have only a single connection between stomach and digestive glands (Kano & Kase, 2002), whereas two or more openings of digestive glands occur near sorting area in the stomach of other neritopsines (e.g., Bourne, 1909, 1911; Fretter, 1965, 1984; Sasaki 1998). It is uncertain at present whether the number of the openings is dependent on body size or phylogentetically fixed.

Excretory System: Neritopsine excretory system is composed of the auricle with podocytes as an ultrafiltration site, renopericardial duct as a conduit of primary urine, and a single left kidney for osmoregulation and excretion (Estabrooks et al., 1999). Within the Neritopsina, two types of kidneys are known to date: (1) In the Neritidae, Phenacolepadidae, and Helicinidae, the kidney is composed of glandular region, non-glandular bladder, and short ureter (Little, 1972; Sasaki, 1998; Estabrooks et al., 1999). (2) In the Neritiliidae, the wall of kidney is simple, not specialized into glandular and non-glandular areas (Kano & Kase, 2002). The kidney of *S. myojinensis* belongs to the latter type. Functional differences of these two types are not clear, though development of infoldings in the glandular area of the former type is obviously related to functional advantage to increase its surface area.

Circulatory System: In the heart of gastropods the ventricle is always single, but it is attached by paired or unpaired auricle(s), depending on taxa. In *S. myojinensis*, the pericardium contains two (right and left) auricles and a single median ventricle. The right auricle is obviously present in *S. myojinensis* but greatly reduced. A vestigial auricle is also present in *Cinnalepeta* (Sasaki, 1998) and *Phenacolepas* (Fretter, 1984). In other neritopsines, the right auricle is present in the Titiscanidae and Cerisidae of the Helicinidae but absent in the Hydrocenidae, Proserpininae, and Helcinidae (Sasaki, 1998). The ventricle is penetrated by the rectum in the Neritopsina, except the Hydrocenidae and Helicinidae (Sasaki, 1998).

Although details have not been studied hematologically, the presence of erythrocytes is a possible general feature of phenacolepadids. They are discoidal and biconcave in *Phenacolepas* (Fretter, 1984) and *Cinnalepeta* (Sasaki, 1988: fig. 85d). The animals of these two genera are red in fresh live condition, but immediatedly turned pale after the death by fixation. In *Shinkailepas* aff. *kaikatensis* from the Mariana Back-arc Basin, the red color is also very vivid only while living (Hasegawa et al., 1997). Hence, species of *Shinkailepas* are presumed to have erythrocytes. The form of haemocytes in *S. myojinensis*, however, do not seem discoidal but spherical (Fig. 10B).

Female Reproductive System: The female organs of neritopsines consist mainly of the ovary, oviduct, pallial oviduct with albumen and capsule glands, and vaginal duct with two sac-like appendages.

It is well known that the number of reproductive opening(s) is different in female among neritopsine taxa. In *S. myojinensis*, pallial oviduct and vaginal duct have their own openings (diaulic). In contrast, female reproductive system is triaulic with an additional enigmatic duct in *Septaria* (Sasaki, 1998) and monaulic in *Titiscania* (Marcus & Marcus, 1967) (neritiliids do not have a monaulic system as pre-

viously believed (Kano & Kase, 2002). A diaulic reproductive system is most common among Neritopsina.

Separation of the vaginal duct from the pallial oviduct is a common feature in most neritopsines, and it is also true of *S. myojinensis*. Characteristically, the vaginal duct in *S. myojinensis* is associated with three structures: (1) a transverse slit of the vaginal opening below the anterior part of pallial oviduct, (2) the receptaculum seminis near the vaginal opening, and (3) the "posterior sac" below the posterior part of the pallial oviduct.

A slit-like vaginal opening in the anterior position is also described in *Olgasolaris* (Beck, 1992). In the Neritiliidae, the vaginal opening is also a slit but located posteriorly (Kano & Kase, 2002). It is a small pore, not a long slit, in *Phenacolepas* (Fretter, 1984), *Cinnalepeta*, the Neritidae, and Helicinidae (Sasaki, 1998).

An anteriorly situated receptaculum seminis near the vaginal opening in *S. myojinensis* is unique among the Neritopsina. In other groups, receptaculum seminis has been identified in various positions (e.g., Sasaki, 1998), but it has not always been verified on histological basis. The presence of oriented spermatozoa in its epithelium (Fig. 15A) is the most reliable criterion for this identification.

The "posterior sac" of the vaginal duct may also be peculiar to *S. myojinensis*. Its inner wall is heavily folded characteristically. Because no sperm or egg was contained in sectioned specimens, its actual function in reproduction could not be detected. An equivalent structure is unknown in *Olgasolaris* (Beck, 1992) and *Phenacolepas* (Fretter, 1984), or lacking in *Cinnalepeta* (Sasaki, 1998: fig. 84). The spermatophore sac in the Neritidae and *Cinnalepeta* (Sasaki, 1998: figs. 76, 84) cannot be homologized due to the differences in topological relationships with other reproductive organs.

It is uncertain whether *S. myojinensis* produces spermatophores or not. The spermatophores are generally known to occur in neritopsine gastropods (Robertson, 1989, reviewed gastropod spermatophores). In the Neritidae, intact spermatophores are often contained in the spermatophore sac in female (Sasaki, 1998: figs. 76c–d), and the formation of the spermatophore sheath is also observable in the seminal vesicle of the male. In this case, the formation of spermatozoa is undoubted. But in *S. myojinensis*, no spermatophore was found in any section of female and male reproductive systems.

The pallial oviduct of *S. myojinensis* is enclosed by two kinds of glands that correspond to the albumen and capsule glands, as is generally found in gastropods that produce egg capsules. The albumen gland is further divisible into two parts in *S. myojienesis*. The similar division is also known in the Neritiliidae, Neritidae, and shallow-water phenacolepadids (Sasaki, 1998; Kano & Kase, 2002), but not in non-neritopsine gastropods.

Some neritopsines are known to have a mineral-containing "crystal sac" and cover egg capsule with minerals from the sac. The absence of the crystal sac was verified in *S. moyojinensis* in this study and in the Neritiliidae by Kano & Kase (2002). Meanwhile, the sac is distended with calcified grains in the Neritidae (Sasaki, 1998: fig. 77h). Marcus & Marcus (1967) reported the crystal sac in *Titiscania*, but it is questionable (Kano & Kase, 2002). Probably the possession of the crystal sac is restricted to the family Neritidae.

Internally fertilizing gastropods may develop a particular structure conveying eggs from the female opening to the foot through the neck region. The Neritiliidae have the neck furrow and female flap on the right side in the female (Kano & Kase, 2002: figs. 2B, 15B), and presumably eggs are conveyed along a ciliated furrow in oviposition. The female flap in Neritiliidae is possibly homologous to the structure identified as the ovipositor in *Phenacolepas* by Fretter (1984) (Kano & Kase, 2002). In *S. myojinensis*, a corresponding structure was not found in the right pedal region. Because actual behavior of egg deposition has never been observed in phenacolepadids, functional significance of right neck-foot morphology is unclear.

Male Reproductive System: Male reproductive organs of neritopsines generally comprise the testis, vas deference, seminal vesicle, pallial male gonoduct with prostate, and penis. All of these organs represent a common element of the male reproductive system possessed universally by internally fertilizing gastropods.

The seminal vesicle is formed in convoluted part of vas deference in some Neritopsina, for example, Neritidae including *Bathynerita* (Warén & Bouchet, 1993), shallow-water phenacolepadids (Sasaki, 1998), and *Shinkailepas*. By contrast, in the Neritiliidae, the seminal vesicle is double and different from tightly convoluted type (Kano & Kase, 2002). In the Helicinidae, it is simple, not en-

tangled (Thompson, 1980; Sasaki, 1998). Thus, the configuration of vas deference is a useful character defining some higher taxa in Neritopsina.

It is common pattern in Neritopsina that the male pallial gonoduct is covered with the annex gland posteriorly and the prostate anteriorly. The formation of the prostate pouch on the dorsal side in *Shinkailepas* is a distinctive character not known in other neritoideans.

The position and structure of copulatory organ in male is greatly variable across various groups of gastropods. In Neritopsina, the penis arises unexceptionally from inner side of the right cephalic tentacle, if present. The seminal groove in the penis extends along right lateral margin in *S. myojienesis* (Fig. 14A) but on dorsal surface in *Olgasolaris tollmanni* (Beck, 1992: pl. 5, fig. 6).

The penis in male arises in a position equivalent to the right cephalic lappet of female. This may suggest that the penis has arisen as a modified cephalic lappet. The development of the penis is, however, independent of that of the cephalic lappets, because neritids and *Cinnalepeta* lacking cephalic lappets have the penis in a similar position (Sasaki, 1998). In the Neritiliidae, which entirely lack the cephalic lappets, the penis is rudimentary in *Pisulina* or absent in *Neritilia* (Kano & Kase, 2002).

A ciliated papilla on the ventral side of the penis (Figs. 14A–B) is unique to *S. myojinensis* among Neritopsina. But, the presence or absence of equivalent structure in other neritopsines is actually uncertain, because the penis has not been observed from its ventral side in the previous studies. Its function remains entirely unidentified.

Nervous System: The nervous system of *S. myojinensis* consists of a hypoathroid circumesophageal nerve ring, non-streptoneurous visceral nerve with characteristic configuration, a pair of thick pedal cords, and other thin peripheral nerves.

The circumesophageal nerve ring of *S. myojinensis* is rather concentrated for that of neritopsines, compared to other anatomically examined species (Fretter, 1984; Sasaki, 1998; Kano & Kase, 2002). Especially, pleural and pedal ganglia are closely situated and almost fused with each other. The positions of cerebral and buccal ganglia are similar to those of various rhipidoglossate gastropods. Pleural ganglia have their own commissure, which is a general character peculiar to neritopsine gastropods.

The presence of labial commissure below sublingual pouch is also a common feature throughout the Neritopsina. A similar commissure also exists in the Patellogastropoda in general, but it is different from that of neritopsines in that the labial ganglia are developed on the commissure. The labial commissure without ganglia also occurs in the Ampullariidae (Berthold, 1991), which shows a comparable state in a different clade of gastropods.

It is interesting that the visceral part of nervous system does not form a complete loop in some neritopsines. Such a condition is also known in *Phenacolepas* (Fretter, 1984), neritiliids (Kano & Kase, 2002), and *S. myojinensis*. In the Neritidae, it is complete, and the supraesophageal loop can be traced over the anterior esophagus from the right pleural ganglion (e.g., Sasaki, 1998: fig. 79d). The incomplete visceral loop is possibly secondary reduction rather than primary condition, because remaining gastropods and other molluscs generally have a complete visceral/lateral nerve loop.

Sense Organs: The eyes are often reduced or secondarily lost in various organisms living in such dark environments as caves and the deep sea. *Shinkailepas myojinensis* is distinct from other neritopsines in that the eyes are certainly present but markedly vestigial. They are represented only by pigmented cells, lacking lens, and deeply embedded below epithelium of the eye stalks. In contrast, the eyes of shallow-water and terrestrial neritopsines are filled with the lens and covered with the cornea. Another exception to this generalization is the Neritiliidae which have open eyes without lens and cornea (Kano & Kase, 2002). They are considered to have been modified due to adaptation to cryptic habitat. The eyes in *Shinkailepas* are probably non-functional and reduced in the dark deep-sea environment.

There seems to be some differences in the structure of osphradium among Neritopsina. In aquatic neritopsines, the osphradium is located along the left shell muscle, and defined by central zone, paired lateral zones, and pigment bodies at ultrastructural level (Haszprunar, 1985). However, it is two-folded with a longitudinal central groove and devoid of clearly ciliated lateral zones in *S. myojinensis*. A similar longitudinal groove on the central zone is also present in *Bathynerita* (Warén & Bouchet, 1993), but obviously absent in other neritopsines, such as *Nerita* (Haszprunar, 1985; Sasaki, 1998: fig. 77d).

The position of the statocysts is somewhat variable among neritopsines. They are located on the posterodorsal side of the pedal ganglia in *S. myojinensis,* and also the Neritidae (Sasaki, 1998) and Helicinidae (Bourne, 1911). In the Neritiliidae, their position is shifted more anteriorly (Kano & Kase, 2002).

Each statocyst contains either single statolith or many statoconia, depending on taxa in gastropods (Ponder & Lindberg, 1997). Even within Neritopsina, there are both of these two types. At least two examples are known: statocysts in the Neritiliidae (Kano & Kase, 2002) and statoconia in *S. myojienesis*. Their condition in other taxa have not been clearly described based on histological observations. The systematic and functional significance of statocyst contents is still unclear throughout neritopsines.

Systematic Implications

The allocation of *Shinkailepas* to Neritopsina was corroborated by sufficient numbers of anatomical characters. The features shared with other neritopsines in general (Sasaki, 1998: 220–221) are: (1) a multispiral globular protoconch with growth lines in aquatic members, (2) a single left ctenidium lacking skeletal rods and bursicles, (3) a single osphradium along the left shell muscle, (4) three (anterior, posterior, and median) elements of odontophoral cartilages, (5) the dorsal levator muscles of odontophore, (6) the tensor muscles of anterior cartilages, (7) the absence of the salivary gland, (8) the esophageal glands separated from the esophagus posteriorly, (9) a small crescent-shaped gastric caecum, (10) the labial commissure without labial ganglia, (11) one-side origin of visceral loop from right side, and (12) the pleural commissure. Some more characters were previously regarded as general neritopsine features (Sasaki, 1998), but at least two, eye and kidney structure, were rejected as revealed in recent studies. As discussed above, closed eyes with vitreous body is not found in Neritiliidae and *Shinkailepas*. The kidney in neritopsines is not always clearly differentiated into glandular and non-glandular sections.

Within Neritopsina, anatomical comparison suggests that *Shinkailepas* is included in the superfamily Neritoidea, which currently includes the Neritidae and Phenacolepadidae. The Neritoidea is mainly diagnosed by characters of digestive and reproductive organs, such as (1) a well-developed oral lappet, (2) the sublingual glands, (3) the median levator muscles of odontophore, (4) the radular formula n-4-1-4-n, (5) two auricles with right one smaller, (6) the capsule and albumen glands in female, (7) the annex gland in male gonoduct, and (8) the penis from the inner side of right cephalic tentacle. This definition of the superfamily with above characters is revised from that of Sasaki (1998).

Concerning the relationships with other neritopsine families, *Shinkailepas* shares no anatomical characters uniquely with three helicinoidean families (Bourne, 1911; Thomson, 1980; Sasaki, 1998) or Neritiliidae (Kano & Kase, 2002). The remaining families, the Hydrocenidae, and the Neritopsidae (including Titiscanidae: Kano et al., 2002), have not been described sufficiently for comparison. In molecular characters, Kano et al. (2002) revealed the relationships (Neritopsidae (Hydrocenidae (Helicinidae + Neritiliidae) (Neritidae + Phenacolepadidae))) based on 28S rRNA sequences. Therefore, it is highly likely that neritoidean groups including *Shinkailepas* are phylogenetically distinct from non-neritoidean families within the Neritopsina.

The Neritoidea is currently divided into the Neritidae and Phenacolepadidae, and *Shinkailepas* is assigned to the latter (Beck, 1992; Warén & Bouchet, 2001). Phenacolepadids, including shallow-water and vent/seep-endemic groups, share a rather limited number of unique characters compared to other neritopsine families. Their common characters are: (1) transversely elongated first lateral teeth of the radula, (2) longitudinally tall fourth lateral teeth, and possibly (3) erythrocytes. The absence of hypobranchial gland may be another general character of the family, but its state is not certain in *Olgasolaris*. There are some more similarities, but in fact, they are not specific to phenacolepadids. For example, the cephalic lappets are also described in neritid *Bathynerita* (Warén & Bouchet, 1993), a cephalic penis also occurs in the Neritidae, two shell muscles without division are found in *Septaria* (Sasaki, 1998).

Within phenacolepadids, two major groups, deep-sea and shallow-water ones, can be clearly diagnosed. Two deep-sea vent/seep-associated genera, *Shinkailepas* and *Olgasolaris*, have similar characters in common (Table 2): (1) shell canals and pores penetrated by the mantle processes, (2) a trapezoidal, non-spiral, calcified operculum, (3) the cephalic lappets, (4) the epipodial fold, (5) the absence of circumpallial tentacles, (6) the intestine

with two loops only, and (7) an anteriorly positioned, slit-like vaginal opening. Most of these similarities are distinctive of these two genera, suggesting their close phylogenetic relation.

In contrast to two deep-sea genera, shallow-water phenacolepadids (*Phenacolepas* + *Cinnalepeta*) are united by (1) the absence of shell pores and mantle processes, (2) a weakly developed, spiral operculum with apophysis, if present, (3) the absence of cephalic lappets, (4) the absence of epipodial folds, (5) the circumpallial tentacles, (6) complex loops of intestine, and (7) a small pore-like vaginal opening.

At the species level, there are some anatomical differences among four described species of *Shinkailepas*. The comparison with *S. myojinensis* revealed that in *S. briandi* Warén & Bouchet, 2001, (1) the eye stalks ("eye-lobes") are very weakly developed, (2) the epipodial fold in neck region is prominent on the left and right sides, (3) the penis is more elongated, and (4) the posterior margin of the epipodial fold is not divided into triangular tentacles. Likewise, in *S. kaikatensis* Okutani, Saito & Hashimoto, 1989, (1) the eye stalks are not developed, (2) the penis is more acutely pointed, and (3) the number of tentacles on the epipodial folds ("pedal papilla") is smaller (11 in *S. kaikatensis*, 11–19 in *S. myojinensis*). In *S. tufari* Beck, 1992, the number of tentacles on epipodial folds is largest (20–22) among known species, but other characters have not been described in detail. Thus, eye stalks, epipodial folds in neck and posterior pedal regions, and penis are useful species-level taxonomic characters in the external features of the soft part. For shell, radular, and opercular characters at species level, see Sasaki et al. (2003).

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APPENDIX: Abbreviations used in descriptions

a = anus
aca = anterior cartilage of odontophore
acv = afferent ctenidial vein
ag = albumen gland
ag I = albumen gland I
ag II = albumen gland II
al = anterior levator muscle of odontophore
ang = annex gland
apd = anterior pedal gland
apg = anterior pedal groove
apl = anterior pallial gland
at = anterior tensor muscle of odontophore
bcv = buccal cavity
bg = buccal ganglion
c = ctenidium
cc = cerebral commissure
cdc = cerebropedal connective
cg = cerebral ganglion
clp = cephalic lappet

cp = cuticularized plate
cpc = cerebropleural connective
cpg = capsule gland
ct = cephalic tentacle
dbt = dorsal buccal tensor muscle
df = dorsal fold of esophagus
dfc = dorsal food channel of anterior esophagus
dg = digestive gland
dgo = opening of digestive gland to stomach
dl = dorsal levator muscle of odontophore
dps = duct of posterior sac
e = eye
ecv = efferent ctenidial vein
ef = epipodial fold
es = eye stalk
ev = esophageal valve
f = foot
fo = female opening of gonoduct
gc = gastric caecum
gst = tooth of gastric shield
i = intestine
if = inner fold of mantle margin
ivp = inner ventral protractor muscle of odontophore
k = kidney
ko = kidney opening
la = left auricle
lca = labial cartilage
lep = lateral pouch of anterior esophagus
ln = labial nerve
lp = lateral protractor muscle of odontophore
lpr = lateral protractor muscle of subradular membrane
lsm = left shell muscle
m = mouth
mca = median cartilage of odontophore
ml = median levator muscle of odontophore
mm = mantle margin
mo = male opening of gonoduct
mp = mantle process
mpg = male pallial gonoduct
mpr = median protractor muscle of subradular membrane
mt = mantle
oap = outer approximator muscle of cartilages
of = outer fold of mantle margin
ol = oral lappet
os = osphradium
ot = oral tube
ovd = oviduct
ovp = outer ventral protractor muscle of odontophore
p = penis
pc = pericardium
pca = posterior cartilage of odontophore
pcd = pedal cord
pcv = pallial cavity
pd = posterior depressor muscle of odontophore
pds = pedal sole
pdt = postdorsal buccal tensor muscle
pe = posterior esophagus
pg = periostracal groove
plc = pleural commissure
plg = pleural ganglion
pls = pallial sinus
pn = pallial nerve
po = pallial oviduct
pr = prostate
prp = prostate pouch
ps = posterior sac of vaginal duct
ptg = post-tentacular gland
ra = right auricle
rds = radular sac
rdt = radular teeth
rev = retractor muscle of esophageal valve
rrs = retractor muscle of radular sac
rs = seminal receptacle
rsm = right shell muscle
rsr = retractor muscle of subradular membrane
sbv = subesophageal part of visceral loop
sc = statoconia
slg = sublingual gland
slp = sublingual pouch
sn = snout
srm = subradular membrane
st = stomach
sta = statocyst
sv = seminal vesicle
t = testis
tac = tensor muscle of anterior cartilage
tbf = transverse buccal fold
tn = tentacular nerve
v = ventricle
vad = vaginal duct
vao = vaginal opening
vap = ventral approximator muscle of cartilages

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EFFECTS OF DISSOLVED LEAD AND COPPER ON THE FRESHWATER PROSOBRANCH *LANISTES CARINATUS*

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ABSTRACT

Lead and copper bioconcentration and toxicity to the freshwater prosobranch *Lanistes carinatus* (Olivier, 1804) were examined after single and combined exposure. Metal bioaccumulation in the digestive gland of adult individuals was investigated after 21 days exposure to 100 µM lead nitrate, 10 µM copper sulphate, and 0.002 X (1 X = 36 µM lead: 1 µM copper; a ratio matching that recorded in the snail's aquatic habitat). Lead was bioaccumulated higher than copper in the group exposed to the metal solution mixture. Elevated lead or copper concentrations were demonstrated in combined solution group relative to single metal solution examined individuals. Both metals accumulated over 50 fold in single solution examined groups and more than 800 times in combined solution tested individuals. Acute toxicity experiment showed lower 24 hour LC_{50} for snails exposed to metal mixtures rather than single solutions studied individuals. Chronic toxicity study demonstrated more histopathological damage in the digestive tubules of individuals 21 days exposed to combined metal solution relative to dissolved lead or copper examined snails. The results revealed synergistic toxic effect of both metals on *L. carinatus*. Further investigations are currently going on to examine the potential value of that snail as biomonitor for aquatic pollutants.

Key words: *Lanistes*, lead, copper, bioconcentration, toxicity levels, histopathology.

INTRODUCTION

Several authors have addressed heavy metal bioconcentration in aquatic molluscs (Sholz, 1980; Simkiss et al., 1982; Phillips & Rainbow, 1993; AbdAllah et al., 2003). The uptake of metals in freshwater bodies is a function of different variables as membrane permeability and physiological status of the organisms, pH, water temperature, water hardness, and acid radical of the metal salt. The concentration of a substance within the accumulator organism is the difference between the amount taken in and the amount released (Ravera, 2001). A mechanism operated in the digestive gland to detoxify metal pollutants, binding them with metallothionein (a sulphhydryl-rich protein with low molecular weight) or with some other agent and storing them in the lysosomes (George, 1982; Simkiss & Mason, 1983).

Heavy metal pollution has become the cause of serious concern and has attracted the attention of governmental authorities. Lasheen (1987) has described heavy metal pollution in Egypt. Generally, the ratio of heavy metals in the freshwater bodies is a function of the anthropogenic spill and natural input. However, most of the available information regarding their accumulation and toxicity were based on single metal solution experiments. Parott & Sprague (1993) showed that combination of low copper concentrations with high concentrations of zinc resulted in antagonistic effect on fathead minnows. He reported that heavy metals might interact antagonistically or synergistically depending on the type of metals and species affected. Harrahy & Clements (1997) observed that removal of zinc from a synthetic sediment contaminated with a mixture of lead, copper, zinc, and cadmium resulted in a pronounced decrease in growth and egg laying rate and an increase in the survival rate of the midge *Chironomus tentans*. Lead is considered of the most toxic heavy metals to human health, affecting nervous and excretory systems (Hutton, 1987). Also, it affects the haem synthesis mainly through inhibiting the conversion of α-aminolevulinic acid to porphobilinogen (Berry et al., 1974). Copper is a

trace element needed in minute amounts by aquatic molluscs to synthesize haemocyanin (Ghiretti & Ghiretti-Magaldi, 1975; Simkiss & Mason, 1983). Increase of the copper content within the molluscan tissues resulted in toxic effects at the target organs (WHO, 1989; AbdAllah, 2000).

The toxicity studies are needed to establish the water pollution standards necessary to protect the aquatic life. Also, this kind of study can supply information about the effect of sudden discharge of pollutants on the aquatic organisms. In addition, it supplies information about their sensitivity to contaminants, determining thereby the maximum permissible concentrations for aquatic life (Clubb et al., 1975).

An extensive literature has appeared recently documenting the use of molluscs as successful sentinel organisms screening the aquatic environment for metal contaminants (Simkiss et al., 1982; Cossa, 1989; AbdAllah et al., 1999, 2003). *Lanistes carinatus* is a widely distributed gastropod in Egyptian freshwater ecosystems (Brown, 1995). It has a large enough size to provide sufficient tissue for metal analysis. Investigations concerning its storing capability of various metal pollutants, their histopathological changes, and influences on different biological activities of that species are required to set up its efficiency as biomonitor to freshwater contaminants.

The present work aims to investigate the bioaccumulation and toxicity of lead and copper for the freshwater snail *Lanistes carinatus* when examined singly and to depict the nature of toxicity of both metals together, whether synergistic or antagonistic, when mixed in a ratio resembles that in the inhabitant area. Also, the histopathological change in the digestive gland as a result of prolonged exposure to sublethal levels of these metals is described.

MATERIALS AND METHODS

Sampling

The freshwater prosobranch *Lanistes carinatus* was collected from El-Mansoureya Canal, Abou-Rawash, Giza. Before any treatment, the snails were washed in running water to remove any debris and maintained in three-liter aquaria for one week to be adapted to the laboratory conditions and to release their internal metal contents. The aquaria were aerated with electric air pumps, and the snails were fed every other day with fresh romain lettuce.

Preparation of Test Solutions

Test solutions for lead nitrate and copper sulphate were prepared in terms of molar concentrations as mentioned by AbdAllah et al. (2000). Additionally, a mixture of both metals was made in the ratio of 18 : 0.5 for lead and copper respectively, to match their observed proportion in the native habitat (18 µm lead and 0.5 µm copper).

Uptake of Lead and Copper

Groups of 30 adult and healthy snails each were exposed to 100 µM lead nitrate, 10 µM copper sulphate, and 0.002 X for 21 days in three-liter aquaria. Snails were fed fresh lettuce every other day. A space of 50 ml/snail was allowed to prevent competition of snails and to minimize the effect of snails' secretions (Thomas & Benjamin, 1974). The aquaria were continuously aerated using electric air pumps. The solutions were changed twice a week. Twenty-one days later, ten snails were collected from each group and were prepared for subsequent digestion.

Sample Preparation for Heavy Metal Analysis

The snails collected at the end of the previous experiment were crushed in a petri plate. Shell pieces were removed and the soft tissues were dissected out to isolate the digestive gland using fine scissors. The dissected organ was rinsed in pure water and weighed to the nearest 0.005 mg using a Mettler balance. Then, the excised organ was frozen at -70°C for 24 h and digested according to McDaniel (1991) and AbdAllah et al. (2003).

Determination of Heavy Metals in the Digested Tissues

Lead and copper were determined in the digested tissue using the graphite furnace spectroscopy, employing a Perkin-Elmer spectrometer with a specific-hollow cathode lamp for each metal (McDaniel, 1991; Pip, 1992; Kraak et al., 1993). The metal concentration was calculated in µg/mg wet weight.

Statistical Analysis

Two-way ANOVA followed by Student's t-test comparison of least square means were done to test the significance of metal accumulation in the different examined groups using Super-

TABLE 1. Two-way ANOVA examining the effect of lead and copper interaction on bioconcentration of metals in the digestive gland of *L. carinatus*.

Source	df	Sum of squares	Mean squares	F-value	P-value
Metal	1	3891381	13890000	16.253	0.0003
Treatment	1	38575511	38580000	45.134	0.0001
Metal*Treatment	1	13583318	13580000	15.893	0.0003
Residual	36	30768878	854691		

ANOVA software computer program, Abacus Concept, Inc., Berkeley, California. Possible correlation relationship between lead and copper levels in the digestive gland of snails exposed to snail mixture was examined. Also, regression analysis was conducted to determine the relationship between metal concentration within the digestive gland and organ weight.

Determination of Toxic Levels

Preliminary experiments were conducted to set the appropriate concentrations of each metal and the metal mixture for the toxicity studies. The lead nitrate concentrations tested were 100, 250, 500 µM, 1 mM, and 5 mM, while the examined copper sulphate concentrations were, 20, 30, 50, 100, 500, and 1,000 µM. The toxicity of lead and copper interaction was studied employing a mixture of lead nitrate and copper sulphate (1 X = 18 µM : 0.5 µM respectively). The concentrations selected for the toxicity study were 0.001 X, 0.005 X, 0.01 X, 0.05 X, and 0.1 X. Groups of ten adult healthy snails were exposed to each examined concentration for 24 h. The number of dead snails was counted. Failure to respond to needle touch was considered as sign of death. The experiment was repeated three times. LC_{25}, LC_{50}, LC_{75}, and LC_{95} were determined according to Finney (1971).

Histopathological Study of Long-Term Toxicity

The effects of chronic exposure for a period of three weeks to sublethal levels of lead nitrate (100 µM), copper sulphate (10 µM), and a mixture of these metals (0.002 X) were investigated histologically in the digestive gland. Moreover, normal histological features of control snails were described.

Following the exposure period, the exposed and control individuals were dissected and the examined organ was isolated. Paraffin blocks of that organ were prepared according to Bancroft & Stevens (1996). Five-µm thin sections were made using a rotary microtome, stained with Haematoxylin and Eosin, dehydrated in an ascending series of ethyl alcohol, cleared in xylene, and mounted in Canada balsam. The permanent preparations of digestive gland of exposed and control individuals were photographed using a 35 mm camera attached to a Zeiss light microscope.

RESULTS

Bioconcentration of Lead and Copper in *Lanistes*

Lead and copper concentrations were compared in the digestive gland of the freshwater prosobranch *Lanistes carinatus* using a two-way analysis of of variance (ANOVA) (Table 1). Significant differences ($P < 0.001$) were found between metal concentrations in the digestive glands of snails that underwent single and mixed exposure and between lead and copper levels. Also, the interaction of metal type and treatment had a significant effect on metal concentration in the examined organ ($P < 0.001$). Comparison of least square of means (Table 2) showed significant difference between lead ($P < 0.05$) or copper ($P < 0.01$) concentrations of snails exposed to single and combined metals and also demonstrated significant difference ($P < 0.01$) between capability of lead and copper bioconcentration in the digestive gland of snails exposed to metal mixture. Lead showed higher bioaccumulation factor than copper (Table 3) even in presence of mixed metals. In all cases, lead and copper are concentrated over 50 fold in the digestive gland of the snails singly exposed and more than 800 fold for snails that exposed to combined metals compared to the surrounding water. Significant negative correlation relation-

TABLE 2. Student's t-test (t-values) comparing the least square of means of lead and copper concentrations in the freshwater prosobranch *L. carinatus* after single and mixed exposure. (* P < 0.05, ** P < 0.01)

		Single exposure		Mixed exposure	
		Cu	Pb	Cu	Pb
Single exposure	Cu	–	-0.032	2.132*	ND
	Pb		–	ND	7.569**
Mixed exposure	Cu			–	-5.670**
	Pb				

ship (r = -0.95) was demonstrated between lead and copper uptake in the digestive gland of snails after 21 days exposure to combined metals. Regression analysis between metal concentration and weight of the examined organ showed a significant relationship r = -0.893, P < 0.05 for lead (Fig. 1) and r = -0.877, P < 0.02 for copper (Fig. 2).

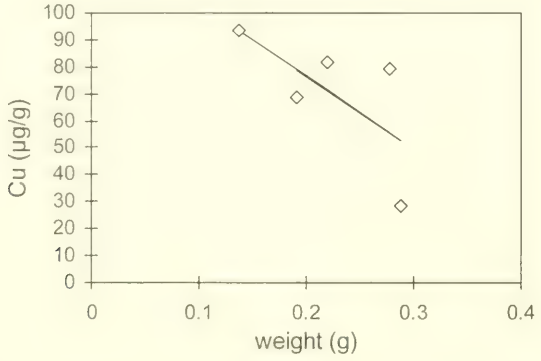

FIG. 1. Linear regression relationship between copper concentration (µg/g) and weight of the digestive gland of *L. carinatus* (r = -0.877, P < 0.02).

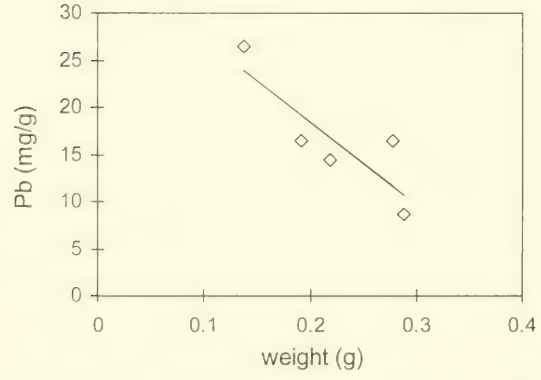

FIG. 2. Linear regression relationship between lead concentration (mg/g) and weight of the digestive gland of *L. carinatus* (r = -0.893, P < 0.05).

TABLE 3. Bioaccumulation factor of lead and copper in the digestive gland of *L. carinatus* after single and mixed long-term exposure.

	Single exposure	Mixed exposure
Copper	55.811	854.401
Lead	68.953	3198.493

Acute Toxicity of Lead and Copper

Toxicity levels: LC_{25}, LC_{50}, LC_{75}, and LC_{95} of lead, copper, and a mixture of them are demonstrated in Table 4. The data demonstrated that copper was more toxic than lead. The mixture of both metals was highly toxic relative to single metals.

Histological Structure of the Digestive Gland of *Lanistes carinatus*

The digestive gland of control snails (Fig. 3) consists of ovoid to cylindrical shaped digestive tubules. Each tubule is composed of columnar basophil cells with darkly stained granules and digestive secretory cells that exhibit the absorptive phase where the cells are partially disintegrated. These cells are rested on a basement membrane or the integument.

Histopathological Effect of Chronic Exposure to Metal Treatment

Chronic exposure to 100 µM lead nitrate (Fig. 4) resulted in presence of necrotic digestive and basophil cells in a wide tubular cavity with

FIG. 3. Light structure of the digestive gland of *L. carinatus*; digestive cell (dc), digestive tubule (dt). Scale bar = 50 µm.

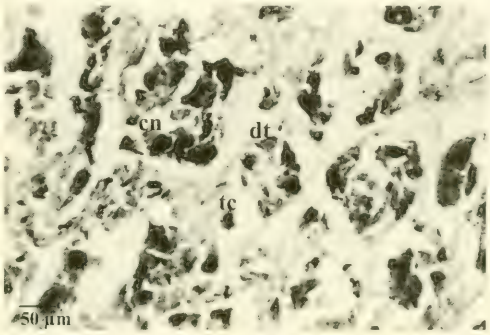

FIG. 5. Transverse section of the digestive gland of *L. carinatus* exposed to 10 µM copper sulfate for three weeks; cell necrosis (cn), digestive tubule (dt). Scale bar = 50 µm.

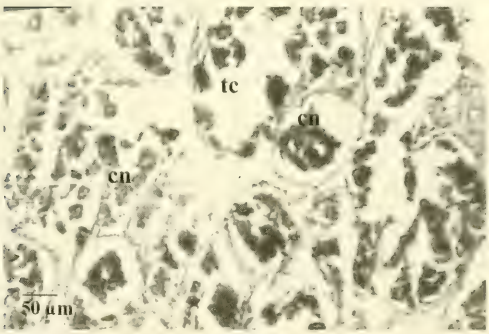

FIG. 4. Transverse section through the digestive gland of *L. carinatus* 3 weeks exposed to 100 µM lead nitrate; tubular cavity (tc), cell necrosis (cn). Scale bar = 50 µm.

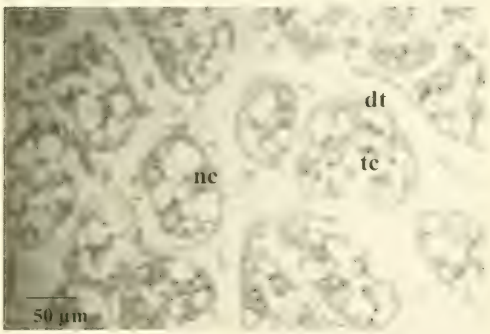

FIG. 6. Light micrograph of the cross-sectioned digestive gland excised from *L. carinatus* three weeks exposed to 0.002 X; digestive tubule (dt), tubular cavity (tc), residual necrotic cells (nc). Scale bar = 50 µm.

TABLE 4. Toxicity levels of 24 h single and combined exposure to lead, copper for the freshwater snail *L. carinatus*.

	LC_{25}	LC_{50}	LC_{75}	LC_{95}
Lead	562.34 µM	1.995 mM	6.761 mM	21.379 mM
Copper	39.81 µM	141.25 µM	251.19 µM	1.778 mM
Lead-Copper	0.0035 X	0.018 X	0.079 X	0.708 X

slight tubular deterioration. Digestive tubules of snails exposed to 10 µM copper sulphate for three weeks were almost filled with batches of damaged digestive and basophil cells. Deterioration of the digestive tubules was obvious (Fig. 5). Destructive effect as a result of three weeks exposure to a mixture of both metals (0.002 X) was clearly illustrated (Fig. 6), with the digestive tubules appearing almost vacuolated and enclosing rudiments of the disintegrated basophil and digestive cells.

DISCUSSION

The long-term toxicity data of metal pollutants can supply valuable information about the sensitivity of exposed organisms to such pollutants. In addition, the combined effect of metals is a subject worthy of study, as the metals naturally exist together in the aquatic environment in variable ratios, depending on various input sources (Clubb et al., 1975). The present investigation demonstrated a variable response of the examined prosobranch snail toward the short-term exposure to sublethal levels of lead nitrate, copper sulphate and a mixture of both metals (18 µg/l lead nitrate: 0.5 µg/l copper sulphate). The results are in agreement with the observations of Mathur et al. (1981), who found a variable acute toxicity effect of zinc, copper, and mercury on the freshwater pulmonate *Lymnaea luteola*. Recorded toxicity levels revealed that the combined effect of the two metals was more toxic than that of individual metals. This finding is in accordance with the observations of Harrahy & Clements (1997), who found that the removal of zinc from a synthetic sediment incorporated with mixture of cadmium, copper, lead, and zinc resulted in increasing the survival rate of *Chironomus tentans*. However, the results are in contrast with those of Parrott & Sprague (1993), who showed that the combination of low copper concentrations with high concentrations of zinc resulted in antagonistic effect on fathead minnows.

It is well documented that the digestive gland is the major site of metal storage in molluscs (Simkiss et al., 1982; Simkiss & Mason, 1983; AbdAllah, 1999; AbdAllah & Moustafa, 2002). A mechanism of metal detoxification was successfully operated in that organ to phagocytoze heavy metals after being chelated with a proper agent, specifically metallothionein for copper and cadmium, and carbonate or lipofucsin for lead (George, 1982; Simkiss & Mason, 1983; Philips & Rainbow, 1993). However, this mechanism has a maximal threshold, at which the toxic signs started to be manifested in that organ at higher dosages. In the present work, the uptake studies demonstrated higher capability of lead to store in the digestive gland tissues even in the presence of low copper concentrations and a significant negative correlation between lead and copper concentrations, which indicates an inversely relationship between their bioaccumulation in the gland tissues. This finding is in accordance with results of a previous study (AbdAllah & Moustafa, 2002).

Histological studies are effective as a biomarker tool indicating the pathological effect of a toxicant upon living organisms (Landis & Yu, 1995; AbdAllah, 2003). Necrosis, lesions, in addition to the appearance of disorganized, and vacuolated cell masses are the prominent features of the histopathological influence of a specific toxicant (Sullivan & Cheng, 1976; Sunila, 1984; AbdAllah, 2000). The chronic toxicity of copper and lead followed similar pattern, in which necrotic cells appeared filling the tubular cavity and being detached from the tegument in *Lanistes*. The finding is in agreement with observations of Tolba et al. (1999) for the effect of chronic exposure of the schistosome vectors *Biomphalaria alexandrina* and *Bulinus truncatus* to copper sulphate. Other studies defined the toxicity status as the increase in diameter of the digestive tubule

that accompanied the reduction in cellular length (George, 1990). The effect of long-term exposure to sublethal concentrations of mixed concentrations of lead nitrate and copper sulphate on the digestive gland of *Lanistes carinatus* was more toxic compared to that shown for the single metals, with degeneration of tubular cells, expansion of the tubular cavity and detachment of tubular tegument observed. This supports the toxicity data of previous studies (Harrahy & Clements, 1997; AbdAllah et al. 2000), and indicates that the interaction of lead and copper is more toxic, compared to that of each metal singly. It is worth mentioning that the findings of this experimental study might not be valid for field investigations, because in the water canal other organic and inorganic substances are present. The interaction of these compounds with lead and copper might be antagonistic, minimizing or abolishing their toxicities. Also, the concentrations used are $^1/_{10}$ of the calculated LC_{50} and are fairly higher than that recorded in the freshwater body (18 µm lead and 0.5 µm copper). The results are also consistent with that of Wong (1987), who described this type of effect as more than an additive effect and recommended the use of metal mixtures for both chronic and acute toxicity studies rather than single metal solutions, because they supply more valuable and realistic information about the nature of heavy metal toxicities in the aquatic ecosystem. Further studies on *L. carinatus* are currently going on concerning its storage capability and sensitivity to other heavy metals and organic pollutants to investigate its potential value as biological monitor capable of screening the Egyptian freshwater ecosystem for various contaminants.

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PHYLOGENETIC ANALYSIS OF THE PERI-HYDROTHERMAL VENT BIVALVE *BATHYPECTEN VULCANI* BASED ON 18S rRNA

Suzanne C. Dufour[1], Gerhard Steiner[2] & Peter G. Beninger[3]

ABSTRACT

The species *Bathypecten vulcani* (Schein-Fatton, 1985), found at the periphery of hydrothermal vents at the East Pacific Rise, possesses primitive shell microstructures, which have led to its characterization as a living fossil. The shell-based classification of *B. vulcani* within the Pectinoidea has been difficult, the species bearing similarities to both the Pectinidae and the Propeamussiidae; as a result, interpretations of the anatomy and biology of the species in an evolutionary and taxonomic context have been hindered. Here, an 18S rRNA-based molecular phylogeny is used to compare *B. vulcani* with other pectinoids. The molecular trees group *B. vulcani* with the propeamussiid *Parvamussium undisonum*, in a clade distinct from all pectinids. These results support the inclusion of *B. vulcani* within the propeamussiid clade, making it the most well-studied representative of this poorly known group.

Key words: *Bathypecten vulcani*, Propeamussiidae, phylogeny, 18S, hydrothermal.

INTRODUCTION

Several faunal species believed to be endemic to hydrothermal vents possess anatomical characters described as primitive or archaic (Newman, 1985). Among these is the bivalve *Bathypecten vulcani*, which has been found at the periphery of hydrothermal vents at the East Pacific Rise, at 9°N and 13°N. In its original description, *B. vulcani* was classified as a pectinid, having shell structural and ultrastructural characters reminiscent of Paleozoic pectinoids (Schein-Fatton, 1985). Based on these shell characters, the species was deemed a living fossil.

An examination of the gill of *Bathypecten vulcani* revealed a simple, homorhabdic organization, which is primitive in comparison to the heterorhabdic gills of all other described pectinids (Le Pennec et al., 1988), including *Hemipecten forbesianus*: the specimens originally described by Yonge (1981) as having homorhabdic gills were recently found to have heterorhabdic gills (Beninger, pers. obs). Structural similarities between the gills of *B. vulcani* and those of early developmental stages of pectinids suggested that an evolutionary transition from homorhabdic to heterorhabdic gills had occurred within the Pectinidae (Beninger et al., 1994).

Schein-Fatton (1988) re-evaluated the phylogenetic position of *Bathypecten vulcani*, as well as that of its newly renamed congener, *B. eucymatus* (Dall, 1898), collected at abyssal depths from the Bay of Biscay. A reexamination of the shell microstructure of both *Bathypecten* species showed differences between the two species, with *B. vulcani* having more archaic features, and characters that could not be reconciled with either the Pectinidae or the Propeamussiidae. According to Waller (1972, 1984), the major character allowing distinction between both groups is the ctenolium: it is present, at least in early stages, in all pectinids, but absent in propeamussiids. In *B. vulcani*, the ctenolium is lacking (Schein-Fatton, 1988).

The genus *Bathypecten* was eventually placed within the Pectinidae, in the subfamily Propeamussiinae (Schein, 1989, from the family-group name Propeamussiidae Abbott, 1954), a sister-group to the subfamily Pectininae. Diagnostic characters for the subfamily Propeamussiinae are the same as those for the family Propeamussiidae, *sensu* Waller (1978). It is important to note that in the classification of Schein (1989), as in other classi-

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fications (e.g., Waller, 1978, 1984), both groups are considered to be sister taxa. In this paper, we will refer to these taxa by their family names, Pectinidae and Propeamussiidae, according to common usage, and to avoid confusion; this does not imply familial rather than subfamilial status.

More recently, it was found that the ultrastructure of the spermatozoa of *Bathypecten vulcani* differed significantly from that of pectinids, given that it could not be classified into either of the pectinid structural categories of Le Pennec et al. (2002). Unfortunately, due to the absence of information on spermatozoan ultrastructure in propeamussiids (Healy et al., 2000), it is unknown whether the spermatozoa of *B. vulcani* resemble those of propeamussiids. Similarly, a more detailed analysis of the anatomy, ciliation, and mucocyte types and distribution of the gill in *B. vulcani* has shown that it is substantially different from that of adult pectinids; however, it shows a number of similarities with the limited information available for propeamussiid gills (Beninger et al., 2003).

In the end, the conclusions stemming from observations of *Bathypecten vulcani* anatomy (e.g., the gill of *B. vulcani* represents the ancestral pectinid condition – Beninger et al., 1994) could not be confidently interpreted in a taxonomic and evolutionary context, due to the uncertain phylogeny of this species based on shell characters alone. In order to better classify *B. vulcani* within the Pectinoidea, the 18S rRNA sequence is here obtained and compared with that of other pectinids and propeamussiids.

MATERIALS AND METHODS

Sample Collection

Two specimens of *Bathypecten vulcani* were collected in May 2000 from the periphery of hydrothermal vents at 9°N along the East Pacific Rise, at the sites Tubeworm Pillar (9°49.6'N, 104°17.38'W, depth: 2,540 m) and Marker 141 (9°49.8'N, 104°17.4'W, depth: 2,530 m). At Tubeworm Pillar, the bivalves were within about 10 m from active smokers; at Marker 141, they were about 350 m from the closest smokers, which were at Tubeworm Pillar. Upon arrival at the surface, the bivalves were immediately fixed in absolute ethanol.

Ethanol-preserved specimens of *Parvamussium undisonum* (Dijkstraw, 1995) were obtained from the Muséum National d'Histoire Naturelle, Paris (Norfolk 1 expedition, station DW 1699, coll. M. Boisselier).

DNA Extraction and Amplification

The ethanol-preserved animals were washed in distilled water prior to DNA extraction. Genomic DNA was extracted from the adductor muscle and gills with the "DNeasy Tissue Kit"® (Qiagen). The near-complete 18S rRNA gene was amplified using the primers 18A1 (5'- CCT ACC TGG TTG ATC CTG CCA G - 3') and 1800r (5'- ATG ATC CTT CCG CAG GTT CAC C - 3'). The PCR-reactions were made on a Robocycler 96 (Stratagene) in a 30 µl reaction mix (1.5 mM $MgCl_2$, each dNTP at 250µM, each primer at 0.5 µM, 0.6 units Biotaq Red polymerase [Bioline] and the supplied reaction buffer at 1 x concentration). The PCR cycle conditions were: initial denaturation step of 2 min at 94°C, 36 cycles of 30 sec denaturation at 94°C, 45 sec annealing at 50°C, and 2 min primer extension at a 72°C, followed by a final primer extension step of 10 min at 72°C. PCR products were purified with the Concert Rapid PCR Purification System (Life Technologies) and sequenced with a range of primers (Steiner & Dreyer, 2003) on an ABI 3700 at VBC-Genomics Bioscience Research GmbH, Vienna.

Choice of Taxa, Alignment and Phylogenetic Analysis

The 18S rRNA sequences of *Bathypecten vulcani* and *Parvamussium undisonum* were aligned with those of all available species of Pectinidae, Spondylidae, Limidae (excluding the species of *Limatula* because of their divergent sequences), Anomiidae, and Plicatulidae (Table 1). According to Steiner & Hammer (2000), Giribet & Wheeler (2002), and Matsumoto (2003), the latter three family-groups comprise the closest relatives to the Pectinoidea. Additional outgroup taxa were selected from the Pinnidae and Arcoidea (Table 1). The computer-aided alignment of these 34 sequences produced by CLUSTAL X 1.8 (Thompson et al., 1997) using default parameters and subsequent manual corrections is available from the authors (GS).

Unweighted heuristic parsimony (MP) searches were made with PAUP* 4.0b10 (Swofford, 1998) on a PC with 50 random addition sequences and TBR branch swapping. Bootstrap support (BP) was assessed by 1,000 replicates, each with three random sequence additions. The program MODELTEST 3.06 (Posada & Crandall, 1998) determined the GTR+I+Ã model as most suitable for maximum-likelihood analyses (ML). The param-

eters estimated from the data were set for a ML search submitting the parsimony strict consensus tree to SPR branch swapping with rearrangements limited to cross four branches in PAUP*. We tested the phylogenetic signal and the robustness of the ML tree with the quartet-puzzling program TREE-PUZZLE 5.0 (Schmidt et al., 2002) under the same model as the ML analysis and parameters estimated by the program and with 100,000 puzzling steps. In addition, we analyzed phylogenetic relationships with Bayesian inference implemented in MRBAYES 3.0b4 (Huelsenbeck & Ronquist, 2001). We ran six chains through 200,000 generations under the GTR+I+Ã model starting with random trees. The first 300 trees were discarded as burn-in for the calculation of posterior probabilities.

TABLE 1. Systematic list of species used in the phylogenetic analysis, with the GenBank accession number of the 18S rRNA sequences.

Systematic position	Species	Accession Number
Arcoidea		
Arcidae	*Arca noae* (Linné, 1758)	X90960
	Acar plicata (Dillwyn, 1817)	AJ389630
	Barbatia virescens (Reeve, 1844)	X9197
Noetiidae	*Striarca lactea* (Linné, 1758)	AF120531
Glycymerididae	*Glycymeris pedunculus* (Linné, 1758)	AJ389631
	Glycymeris sp.	X91978
Pinnoidea		
Pinnidae	*Pinna muricata* (Linné, 1758)	AJ389636
	Atrina pectinata (Linné, 1767)	X90961
Anomioidea		
Anomiidae	*Anomia ephippium* (Linné, 1758)	AJ389661
	Pododesmus caelata (Reeve, 1859)	AJ389650
	Pododesmus macrochisma (Deshayes, 1839)	
Plicatuloidea		
Plicatulidae	*Plicatula plicata* (Linné, 1767)	AJ389651
	Plicatula australis (Lamarck, 1819)	AF229626
Limoidea		
Limidae	*Lima lima* (Linné, 1758)	AJ389652
	Limaria hians (Gmelin, 1791)	AF120534
	Ctenoides annulatus (Lamarck, 1819)	AJ389653
Pectinoidea		
Spondylidae	*Spondylus crassisquamatus* (Lamarck,1819)	AJ389646
	Spondylus hystrix (Röding, 1798)	AJ389647
	Spondylus sinensis (Schreibers, 1793)	AF229629
Propeamussiidae	*Bathypecten vulcani* (Schein-Fatton, 1985)	AY557608
	Parvamussium undisonum (Dijkstra, 1995)	AY557607*
Pectinidae	*Pecten maximus* (Linné, 1758)	L49053
	Placopecten magellanicus (Gmelin, 1791)	X53899
	Adamussium colbecki (E. A. Smith, 1902)	AJ242534
	Flexopecten glaber (Linné, 1758)	AJ389662
	Argopecten irradians (Lamarck, 1819)	L11265
	Argopecten gibbus (Linné, 1758)	AF074389
	Chlamys islandica (Müller O. F., 1776)	L11232
	Chlamys hastata (Sowerby, 1843)	L49049
	Mimachlamys varia (Linné, 1758)	L49051
	Crassadoma gigantea (Gray, 1825)	L49050
	Exellichlamys spectabilis (Reeve, 1853)	AJ389648
	Pedum spondyloideum (Gmelin, 1791)	AJ389649

*The partial 28S sequence of *Parvamussium undisonum* is deposited under the accession number AY557609.

RESULTS AND DISCUSSION

18S Sequence and Molecular Phylogeny

The alignment resulted in a data matrix with 1,973 characters, of which 215 are parsimony-informative. The parsimony search yielded 112 shortest trees of 517 steps (CI = 0.594, RC = 0.478). The topology of the resulting strict consensus tree (Fig. 1) differs only slightly from the single maximum-likelihood tree (-lnL = 6386.38877) (Fig. 2). All analyses firmly support the taxa Propeamussiidae (*Parvamussium* + *Bathypecten*), Pectinidae, and the Spondylidae. The monophyly of the Pectinoidea is always recovered, albeit with varying branch support. The Propeamussiidae and Pectinidae always appear as sister taxa with low support. This distinction is corroborated by the analysis of the mitochondrial gene, cytochrome-oxidase-I (Matsumoto, 2003), which supports pectinoid monophyly but yields a sister group relationship of Propeamussiidae to the clade (Spondylidae + Pectinidae). The two propeamussid species have similar and highly divergent sequences and, accordingly, an extremely long common branch. Although the limid species have similarly long branches, there is no indication of a long-branch attraction effect.

The molecular information is therefore consistent with the inclusion of *Bathypecten vulcani* in the propeamussiid group, rather than with the pectinid group.

Soft Anatomical and Spermatozoan Characters

Comparisons of new and published data concerning anatomical and spermatozoan characteristics of *Bathypecten vulcani*, pectinids, propeamussiids, and spondylids reveals that *B. vulcani* shares more affinities with the Propeamussiidae. Some of these anatomical characters may be apomorphies of propeamussiids, others are likely to be plesiomorphies, as discussed below.

The gill structure of *Bathypecten vulcani* is much simpler than that of pectinids (Beninger

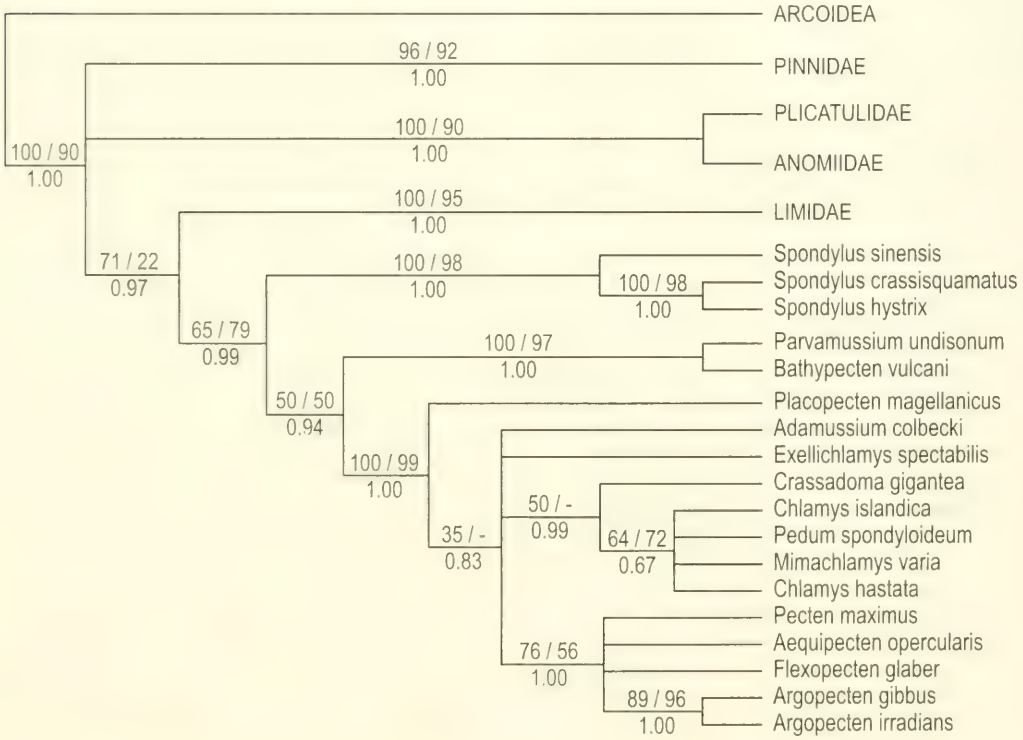

FIG. 1. Strict consensus of 112 most parsimonious trees. Bootstrap and ML-puzzling supports are above, posterior probabilities below branches.

& Le Pennec, 1991), spondylids, and limids (Ridewood, 1903; Dakin, 1928): it is non-plicate, homorhabdic, has a non-reflected outer demibranch, and lacks latero-frontal cilia, inter-filamentar junctions, and interlamellar junctions (Beninger et al., 2003). The relatively poorly known propeamussiid gills have many similar features. The organization of *Bathypecten vulcani* gill filaments does not correspond to the inverted arrangement reported for *Propeamussium lucidum*, in which the frontal ciliary tracts were deemed to be located in the suprabranchial chamber (Morton & Thurston, 1989). However, this atypical organization could easily have been misinterpreted, given that filaments without junctions are easily disorganized and entangled during fixation (Morton & Thurston, 1989).

Further examinations of propeamussiid gills would be needed to determine how the *B. vulcani* gill organization compares to other members of this family. If other propeamussiids are found to share the simple gill structure of *B. vulcani*, then some character-states (homorhabdy, lack of plicae, and lack of filamentar and lamellar junctions) are likely to be plesiomorphic for the Propeamussiidae; similar character-states are found in anomiids, plicatulids, and arcids, with some variation in the extent of interfilamentar and interlamellar junctions (Ridewood, 1903; Yonge, 1973). In addition, the small labial palps and non-arborescent lips observed in *B. vulcani* (Beninger et al., 2003), and described in some propeamussiids (Yonge, 1981), may be plesimorphies for the Propeamussiidae (as compared to the condition in the Pectinidae and Spondylidae – Dakin, 1928; Yonge, 1973; Beninger & Le Pennec, 1991). The lack of laterofrontal cilia, as described in *B. vulcani* (Beninger et al., 2003) and in *Propeamussium lucidum* (Morton & Thurston, 1989), is likely to be apomorphic for propeamussiids, as it has been described for no other pteriomorph to date. Also, the unique spermatozoan type described for *B. vulcani* (Le Pennec et al., 2002) may be

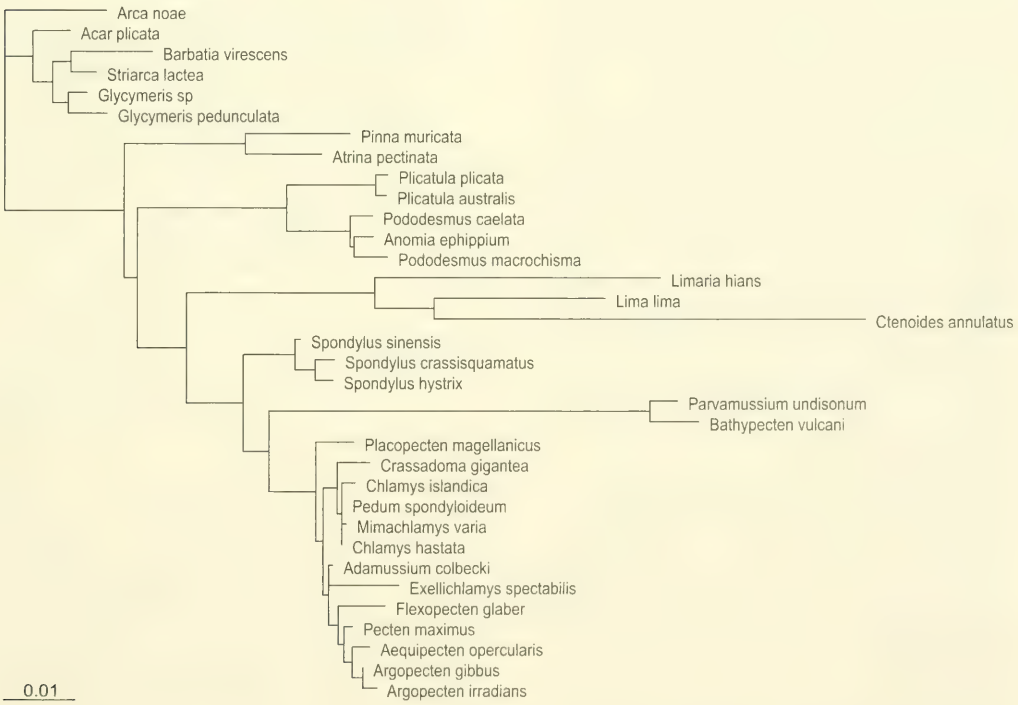

FIG. 2. Maximum likelihood tree (-ln L = 6386.38877) found under the GTR+I+Γ model. Model parameters estimated by MODELTEST: substitution rate matrix A-C = 2.2511, A-G = 2.9955, A-T = 1.6583, C-G = 1.3161, C-T = 4.9707, G-T = 1.00); nucleotide frequencies A = 0.2472, C = 0.2213, G = 0.2724, T = 0.2591; assumed proportion of invariable sites, pinvar = 0.5835; gamma distribution of rates at variable sites in four categories with shape parameter, alpha = 0.6141.

apomorphic for propeamussiids, if a similar structure was found in this group. Further anatomical observations are required to confirm the evolutionary status of these characters.

The presence of prismatic calcite on the left valve, such as found in *Bathypecten vulcani*, is only known from a group of Paleozoic fossils ancestral to the Propeamussiidae, the Pterinopectinidae and the Aviculopectinidae (Newell, 1938). *Bathypecten vulcani* may therefore have retained primitive characters, either by early phyletic divergence from other propeamussiids, or by paedomorphosis.

Propeamussiid Anatomy and Habitat

Several of the anatomical characters of *Bathypecten vulcani* and of other propeamussiids are likely to be related to their deep-sea habitat. As described by Allen (1981), deep-sea bivalves are commonly small in size, and have reduced gills; this miniaturization is thought to be associated with the small amounts of available food at great depths. Most propeamussiids are found at depths greater than 150 m, and were probably deep-sea inhabitants in the Mesozoic and Cenozoic (Waller, 1972).

One of the possible consequences of the deep-sea habitat of propeamussiids, and a possible outcome of their small body size, is the simplification of the gill. At the present state of knowledge, all propeamussiids have homorhabdic gills, with, at least in *Bathypecten vulcani*, few filaments. Due to the size restriction, it might not be possible for a gill with only approximately 50 filaments to become plicate, and by extension, heterorhabdic. Although the gills of developing postlarvae of pectinids are known to develop principal filaments at about 4 mm body size, and plication at about 7 mm (Beninger et al., 1994; Veniot et al., 2003), these growing pectinids contain at least three times as many filaments of the same diameter per gill as *B. vulcani* (Veniot et al., 2003). Although a bivalve the size of a typical propeamussiid can thus have a gill with enough filaments to become plicate and heterorhabdic, this may not be the most efficient organization for an adult bivalve, given the space limitation.

To date, *Bathypecten vulcani* has only been found in the proximity of hydrothermal vents; however, no particular effort has been made to collect this species at other sites. *Bathypecten vulcani* may not be restricted to vent environments, given its feeding regime, which is largely dependent on particulate food originating from surface waters (Le Pennec et al., 2003). Other *Bathypecten* species have been collected from bathyal and abyssal sediments in the Bay of Biscay and in the western Pacific (Schein, 1989), and do not appear to be found at vents. The discovery of *B. vulcani* in environments outside hydrothermal vent sites would confirm that its presence at vents is largely opportunistic; *B. vulcani* may simply be taking advantage of the relatively high amounts of particulate matter that are available at vents (Enright et al., 1981; Gage & Tyler, 1991).

CONCLUSIONS

The results of the present molecular phylogenetic analysis are consistent with *Bathypecten vulcani* being a member of the family Propeamussiidae. This placement is in concordance with the classification of *B. vulcani* based the absence of a ctenolium, this being the major criterion used to distinguish pectinids from propeamussiids (Waller, 1984). Interpretations of the biology of *B. vulcani* should thus be recast in the light of its propeamussiid status, rather than with reference to the pectinids (Beninger et al., 2003; Le Pennec et al., 1988, 2002). Although far from complete, this body of work thus represents the most considerable amount of knowledge concerning any propeamussiid to date.

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THE GENUS *STRICTISPIRA* IN THE WESTERN ATLANTIC (GASTROPODA: CONOIDEA)

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ABSTRACT

The genus *Strictispira* [formerly Turridae, now Strictispiridae] in the western Atlantic area is reviewed. Two new species, ***S. redferni*** and ***S. coltrorum***, are proposed. *Crassispira quadrifasciata* (Reeve, 1843) is reassigned to *Strictispira*. Three additional species – *S. drangai* (Schwengel, 1951), *S. paxillus* (Reeve, 1845), and *S. solida* (C. B. Adams, 1850) – are discussed. *Drillia acurugata* Dall, 1890, regarded as a Recent species as well as fossil and as a *Strictispira*, is shown to be fossil only, with Recent specimens considered to be that species here regarded as *S. redferni*. Similarly, *Drillia ebenina* Dall, 1890, initially a fossil species and often considered to be Recent and a synonym of *S. solida*, is shown to be fossil only. Recent specimens identified as *S. ebenina* are regarded as *S. solida*. Characteristics of the genus and species were studied, and are here described and illustrated, including shell morphology, opercula, and anatomy – especially foregut anatomy and radular structure. Comparisons are made with similar-appearing species, both within the genus and in other genera. The feeding mechanism of strictispirids is probably by ingestion aided by grasping of the prey by extruded radular teeth, followed by rasping and tearing of the prey by the teeth. The protoconch is paucispiral, indicating direct development. The genus has a western Atlantic distribution from the lower eastern Carolinian province to the Caribbean/West Indian province, including both sides of Florida, the Florida Keys, Mexico and Central America, the Greater Antilles, Virgin Islands, Lesser Antilles, lower Caribbean, and the Brazilian province.

Key words: *Strictispira*, taxonomy, shell morphology, radular structure, foregut anatomy.

INTRODUCTION

The genus *Strictispira* was established by McLean (1971a: 125) for crassispirine-like tropical eastern Pacific species bearing a distinctive radular structure and teeth. The genus was placed in a new subfamily Strictispirinae of the family Turridae. The family Turridae has recently been reclassified by Taylor et al. (1993), with some of the subfamilies elevated to family level, the Strictispiridae among them. This classification is used here. McLean (1971a: 123–125) described the subfamily and the genus, described and illustrated the radulae of the eastern Pacific species (1971a: figs. 86, 87), and pointed out the characteristics of the sinus structure of the group.

Discussing Strictispirinae, McLean commented (1971a: 124) that he was "much indebted to Virginia Maes for an exchange of ideas concerning the group, of which she has for some time been aware." The late Virginia Maes had specialized in the family Turridae for some years, and, although she published only sparsely, became one of the authorities on that large group. She meticulously curated the turrid collection at the ANSP. With regards *Strictispira* species, McLean commented (1971a: 125) that *Drillia ebenina* Dall, 1890, a western Atlantic species originally described as fossil, is also a member of the genus. He said (1971b: 730), with regard to the eastern Pacific *Strictispira stillmani* Shasky, 1971, "*Strictispira ebenina* is a related Caribbean species". There has been confusion as to whether *Drillia ebenina* and *Pleurotoma solida* C. B. Adams, 1850, are conspecific. I believe that Recent specimens identified as *S. ebenina* are in fact *S. solida*. Collections at both the USNM and the ANSP show mixing of the two identifications. At the ANSP, Recent material considered *S. ebenina* was maintained separate but following *S. solida*. Review of these shows that they are *S. solida*. It is probable

that Maes had identified *S. solida* as strictispirid, because a specimen (ANSP 282214) from Belize with soft parts had been collected in 1961 by Robert Robinson of the ANSP. Maes's card files contain a card with photographs of the shell and one showing the radula, which bears typical strictispirid teeth. Although now assigned to *Strictispira*, the specimen was without identification originally. It was located in the *S. ebenina* section, probably having initially been considered that on Maes curating this material.

In the course of her studies Maes had synonymized various species. These synonymies were seldom published, but have been listed in Malacolog, the online database of the western Atlantic molluscan fauna created at the ANSP by Gary Rosenberg, and have therefore circulated among malacologists. *Pleurotoma solida* with *Drillia ebenina* as a synonym is an example.

Further, Maes (1983) identified *Pleurotoma paxillus* (Reeve, 1845) as a *Strictispira* and demonstrated other significant strictispirid anatomical features, including the lack of a poison gland and bulb. She also pointed out that the characteristic sinus structure ("turrid notch", on the shoulder slope in this case, not to be confused with the "stromboid notch" on the lower lip) of the group is not restricted to the strictispirids but also occurs in some western Atlantic crassispirine species. She further commented about the difficulty differentiating the shells of *S. paxillus* and *S. solida*, plus such other similar-appearing species as *Crassispirella fuscescens* (Reeve, 1843) and *Crassiclava apicata* (Reeve, 1845). Kantor et al. (1997), in a cladistic study based on considerable foregut research of crassispirine species, suggested that the conventional subgenera of *Crassispira* be raised to generic level. This is followed here, thus *Crassiclava* and *Crassispirella* are assigned generic level, *Crassispira* remaining at generic level but without subgenera.

Taylor et al. (1993) reviewed the foregut anatomy of strictispirids, illustrating the radular structure and teeth, noting absence of a poison apparatus, and showing that the buccal mass is positioned at the anterior end of the proboscis, the buccal tube being short, and they discussed the feeding mechanism. Kantor & Taylor (1994) reviewed *S. paxillus* in the light of a study of Maes's material, including analysis of serial sections, pointing out and illustrating details of the foregut anatomy (compared here with the present findings).

In the tropical eastern Pacific, *Strictispira* contains two species, *S. ericana* (Hertlein & Strong, 1951) and *S. stillmani*; the sister genus *Cleospira* contains only *C. ochsneri* (Hertlein & Strong, 1949). The western Atlantic species, listed in Malacolog (*fide* Maes), are *Strictispira acurugata* (Dall, 1890), *S. drangai* (Schwengel, 1951), *S. paxillus*, and *S. solida*, with *Drillia ebenina* as a synonym. The Recent material in the ANSP collection considered to be *S. acurugata* by Maes is here shown to be the new species *S. redferni*. *Drillia acurugata* is restricted to fossil forms only, and is herein considered a probable member of the cochlespirine genus *Pyrgospira*. With the addition of two new taxa, *Strictispira redferni* and *Strictispira coltrorum*, reassignment of *Crassispira quadrifasciata,* which has been determined to be strictispirid, *S. paxillus*, *S. solida*, and possibly *S. drangai,* the number of *Strictispira* species in the western Atlantic is tentatively six. No members of *Cleospira* are yet known in this area.

Fossil taxa considered to belong in the genus on the basis of shell morphology are: *S. acurugata* (Dall, 1890), from the Upper Pliocene-Lower Pleistocene Caloosahatchee Formation of Florida; *S. aurantia* (Olsson, 1922) from the Late Miocene Gatun formation in Costa Rica; *S. ebenina* (Dall, 1890) from Upper Pliocene-Lower Pleistocene Caloosahatchee Formation and from the Middle Pliocene Pinecrest Beds of Florida; *S. lomata* (Woodring, 1928) and *S. ponida* (Woodring, 1928), from the Upper Pliocene Bodwen Formation of Jamaica; *S. proebenina* (Gardner, 1937) from the Upper Middle Miocene Shoal River Formation of Florida – Maes ms notes; McLean (1971a: 125) included *ponida* and *lomata* on the basis of sinus structure – and *Clavus* (*Crassispira*) *zizyphus* Berry, 1940, from the Lower Pleistocene Hilltop Quary of San Pedro, California (pers. comm., McLean). As stated, *S. acurugata* and *S. ebenina* have been thought to be Recent as well as fossil, but are here considered fossil only and are further discussed below. Analysis of the other fossil taxa are outside the scope of this paper.

MATERIALS AND METHODS

Specimens of the genus *Strictispira*, some with soft parts, from various geographic localities, were examined as to shell morphology, and, where possible, as to anatomy, especially foregut and radular morphology. Photographs were made of representative shells. SEM

preparations were made of protoconchs, opercula, and, in one instance, of a radular ribbon. Preserved specimens were dissected; dry specimens were treated with KOH and dissected as possible. Drawings of individual teeth were made from radular preparations, which had been slide mounted and stained. Serial sections were made in one instance. Type material and voucher specimens were deposited at the USNM, MORG, and other institutions. Radular preparations were deposited at the USNM and the ANSP.

Institutional abbreviations used:

AMNH = American Museum of Natural History, New York, U.S.A.
ANSP = Academy of Natural Sciences, Philadelphia, U.S.A.
DMNH = Delaware Museum of Natural History, Wilmington, Delaware, U.S.A.
FMNH = Field Museum of Natural History, Chicago, Illinois, U.S.A.
LACM = Los Angeles County Museum of Natural History, Los Angeles, California, U.S.A.
MCZ = Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, U.S.A.
MNHN = Muséum National d'Histoire Naturelle, Paris, France
MORG = Museu Oceanografico do Rio Grande, Rio Grande, Brazil
NHM = The Natural History Museum, London, England
NM = Natal Museum, Pietermaritzburg, South Africa
USGS = United States Geological Survey, Washington, D.C., U.S.A.
USNM = National Museum of Natural History, Smithsonian Institution, Washington, D.C., U.S.A.

Other abbreviations:

spec. = specimen(s)
colln. = collection
Exp. = Expedition
Stn. = Station

SYSTEMATICS

Strictispiridae McLean, 1971
Genus *Strictispira* McLean, 1971
Type species: *Crassispira ericana* Hertlein & Strong, 1951

Description

Shells of small size (approximately 10–25 mm), drilliiform, dark colored, sculptured with axial ribs and spiral cords or threads; concave sulcus with subsutural cord; laterally directed, U-shaped sinus with projecting parietal tubercle; protoconch smooth, of approximately two whorls; operculum ovoid, with terminal nucleus; animal with large radular ribbon bearing numerous rows of paired marginal teeth of pistol shape, with median flange; lacking poison apparatus.

Strictispira coltrorum, new species

Figures 1–3, 19, 25, 36

Description

Shell small (to approximately 11 mm), drilliiform, elongate, turreted, moderately high spired; body whorl about half shell length; anterior canal short, open, unnotched. Color medium brown overall to color form with variably lighter spiral banding of shell periphery, lighter outer lip and parietal tubercle. Protoconch (Fig. 19) of two smooth whorls, tip protruding; teleoconch 6–6½ whorls, moderately strong subsutural cord, occasionally lighter colored than rest of shell; shoulder sulcus sharply concave; shoulder tabulate; axial ribs, with blunt posterior ends, extending anteriorly, forming flat whorl profile to following whorl. Body whorl with flat peripheral region, ribs curving around moderately convex base, disappearing at moderately concave junction with canal. Suture rising slightly onto preceding whorl at end of body whorl. Ribs rounded, slightly less in width than interspaces, slightly opisthocline, 13–14 to varix on body whorl, 18–20 on penultimate whorl and spire whorls. Last rib enlarged to form modest varix ¼ whorl or less back from thin, curved lip edge. Occasional specimens with varix formed of two joined ribs. Spiral cords rounded, evenly spaced, 4–5 on spire whorls, not crossing ribs or doing so only faintly until below periphery, 6–7 forming slightly laterally elongate beads on crossing axials, beads becoming stronger anteriorly, 5–6 strong cords on canal. Moderately deep, U-shaped sinus on sulcus, apex at mid point, projecting parietal tubercle narrowing sinus entrance. Sinus tracks present on sulcus. Three distinct spiral threads always present on sulcus. Very shallow stromboid notch always present.

FIGS. 1–9. Shells of *Strictispira* spp. FIGS. 1–3: *Strictispira coltrorum*, holotype, MORG 43415, Escavalda Id., Guarapari, Espirito Santo, Brasil, 10.9 x 4.0 mm; FIGS. 4–6: *Strictispira redferni*, holotype, USNM 1010771, Abaco Id., Bahamas, 9.3 x 3.6 mm; FIG. 7: *Strictispira redferni* variety, ANSP 221823, Vaca Key, Florida Keys, 14.1 x 4.6 mm; FIG. 8: *Strictispira redferni* variety, ANSP 355797, Exuma, Bahamas, 10.9 x 4.0 mm; FIG. 9: *Drillia acurugata*, holotype, USNM 97320, Caloosahatchee Riv., Upper Pliocene, Florida, 21.0 x 7.8 mm. Scale bar = 10 mm.

Anatomy

One specimen containing dried animal with operculum available. Animal with foot, head and mantle/siphon mottled black. Foot elongate. Head small, with two tentacles bearing eyes distally and laterally. Large siphon on left continuous with thin mantle. Mantle edge behind tentacle bases dorsally, bearing sinus indentation on right. Mantle semitransparent; gills and osphradium visible on left and penis on right, originating behind right tentacle, reflected backwards under mantle. Foregut anatomy difficult to discern but showing large rhynchodeum and moderate sized proboscis, both with circular internal folding due to retraction. Structure of buccal tube and cavity could not be determined. Massive odontophore dominating body cavity. No poison gland or bulb present. No salivary gland seen. Odontophore of paired cartilages, strong subradular membrane and paired, marginal radular teeth present. Partial radular ribbon with approximately 80 pairs of teeth. Teeth (Fig. 36) approximately 190 µm, solid, pistol-shaped, with pointed anterior end and median flange. Operculum (Fig. 25) ovate, elongate, with pointed anterior end and terminal nucleus.

Type Material and Locality

Holotype, MORG 43415, Escavalda Id., Guarapari, Espirito Santo, Brasil (20°42'S, 40°25'W), dredged at 25–30 m, on bryozoans, Dec. 1993, A. Bodart!; paratypes, same data as holotype: 2 spec., USNM 1011351; 1 spec., USNM 1011352; 1 spec. each at AMNH, ANSP, DMNH, FMNH, LACM, MCZ, MNHN, MORG, NHM, NM (material *ex* author's colln.).

Distribution

Known only from the type locality.

Discussion

This is a very uniform group of shells, undoubtedly a population sample. One specimen has 4 fairly strong spiral lirae inside the outer lip extending back into the shell for about ¼ whorl.

Strictispira coltrorum is nearest *Strictispira redferni*, but is typically smaller (the holotype of *S. redferni*, selected because of its excellent condition, is smaller than the holotype of *S. coltrorum*). It is more elongate, has more and narrower axial ribs than *S. redferni*, has a stronger parietal tubercle of typical strictispirid form, different protoconch – two whorls, with protruding tip vs. 1½ whorls with a partially immersed tip in *S. redferni*, and different color – medium brown vs. black brown for *S. redferni*. The radular structure and radular teeth are essentially the same in both species.

Strictispira coltrorum is similar to *Crassiclava apicata* (Fig. 16) in shell morphology, differing by being smaller, having a strictispirid sinus, having more and closer ribs and more concave sulcus, different protoconch – two whorls with protruding laterally placed tip rather than 2–2½ whorls with flat lateral tip, and of different color-medium brown vs. dark brown.

Etymology

The species is named after José and Marcus Coltro for their kind donation of specimens and their contributions to malacology.

Strictispira drangai (Schwengel, 1951)
Figures 10, 20, 26

Crassispira drangai Schwengel, 1951: 116, pl. 8, fig. 1.

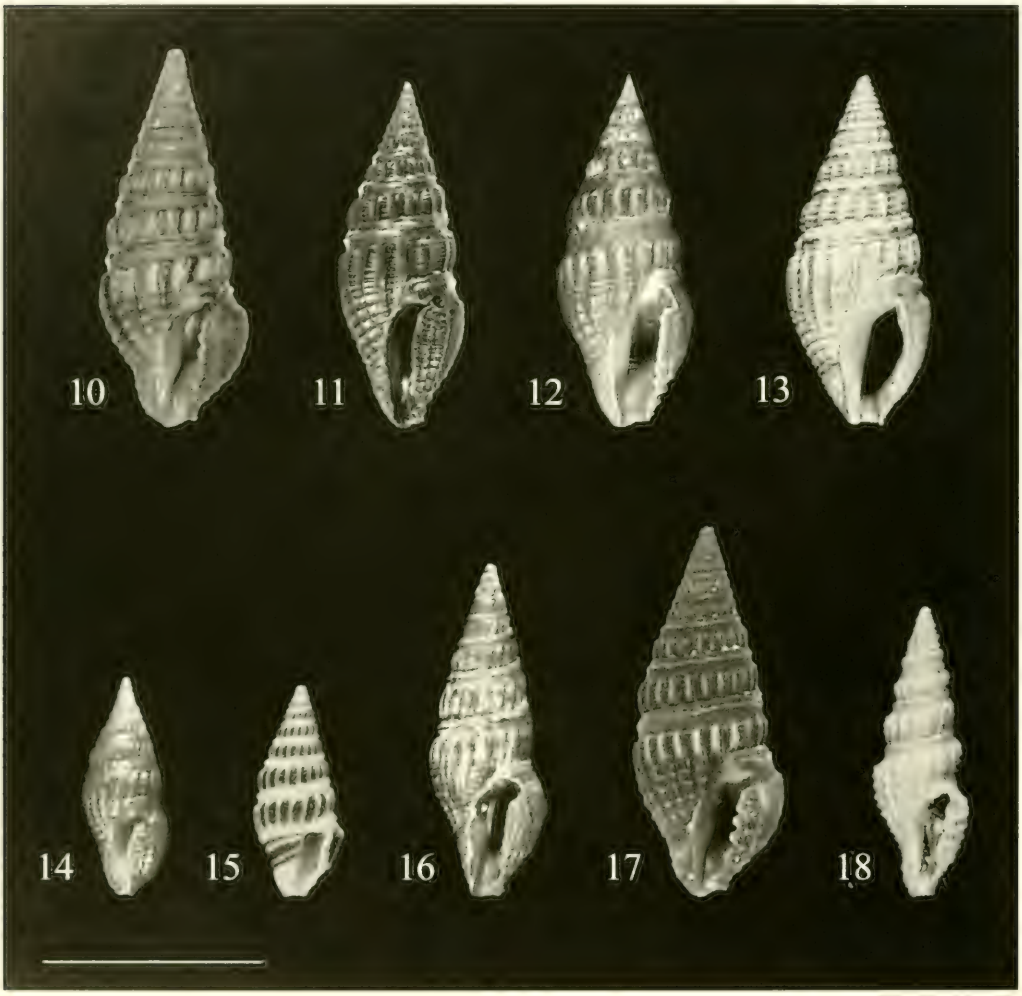
Crassispira (*Crassispirella*) *drangai* (Schwengel, 1951) – Abbott, 1974: 273, species 3056, list ("Very close to *Clathrodrillia solida* C. B. Adams."); Redfern, 2001: 125, species 519, pl. 56.

Strictispira drangai (Schwengel, 1951) – Malacolog, 2004, list.

Description

Shell fusiform, turreted, moderately tall spired, spire angle 32°, length to approximately 25 mm; body whorl somewhat truncate anteriorly, slight basal constriction. Protoconch (Fig. 20) of two smooth, brown whorls; teleoconch approximately 8 whorls. Whorl outline flattish below sulcus. Sulcus narrow, concave, bearing fine spiral striae and curved sinus traces, preceded by strong, sharply crested subsutural cord somewhat distant from suture. Sculpture of narrow axial ribs, 17–22 on penultimate whorl, producing whorl shoulder, interspaces wider, disappearing at bottom of base. Four or five regularly spaced, widely separated spiral cords, crossing axials weakly on periphery, 4–6 more prominent, basal cords below periphery, producing beading on crossing axials, 5–6 cords down

canal. Fine secondary spiral threads between primaries overall. Sculpture forms pattern of rectangular spaces with enclosed spiral threads. Enlarged axial or two forming varix behind outer lip. Aperture parallel-sided, ending in short, open, slightly notched anterior canal bent slightly right. Small stromboid notch. Lip edge fluted. Columellar callus thin, emarginate. Sinus deep, U-shaped, with moderately projecting parietal tubercle, most specimens with vertical groove behind distal end of tubercle (see Discussion below). Color shiny dark brown when fresh, rib interspaces usually lighter colored, especially on body. Operculum (Fig. 26) ovoid, with pointed anterior end and terminal nucleus.



FIGS. 10-18. Shells of *Strictispira, Crassispira, Drillia, Crassiclava, Crassispirella, Pyrgospira* spp. FIG. 10: *Strictispira drangai*, holotype, ANSP 247104, Hastings, Barbados, 17.7 x 6.7 mm; FIG. 11: *Strictispira solida*, USNM 900424, Key West, 16.0 x 5.9 mm; FIG. 12: *Crassispira* sp., ANSP 368728, Bahamas, 16.0 x 6.4 mm; FIG. 13: *Drillia ebenina*, figured syntype, USNM 97318, Caloosahatchee Riv., Florida, Upper Pliocene, 16.5 x 7.0 mm; FIG. 14: *Strictispira paxillus*, specimen figured by Maes (1983: fig. 10), ANSP 342987, White Bay, Guana Id., British Virgin Ids., 10.0 x 4.4 mm; FIG. 15: *Strictispira quadrifasciata*, USNM 902242, Jamaica, 9.6 x 3.9 mm; FIG. 16: *Crassiclava apicata*, specimen illustrated by Maes (1983: fig. 15), ANSP 355011, White Bay, Guana Id., British Virgin Ids., 15.8 x 5.6 mm; FIG. 17: *Crassispirella fuscescens*, USNM 900978, off Stiltsville, Miami, Florida, 16.9 x 6.9 mm; FIG. 18: *Pyrgospira ostrearum*, specimen illustrated by Tryon (1884, pl. 34, fig. 79), ANSP 15470, Boca Ciega Bay, Florida, 13.4 x 4.9 mm. Scale bar = 10 mm.

Type Material and Locality

Holotype, ANSP 247104, Hastings, Barbados, T. Dranga!, 1950, *ex* Schwengel colln. Shell length 17.7 mm, not 12.5 mm, as stated by Schwengel. Measurements: 17.7 x 6.7 x 9.3 (body whorl length) x 5.8 (aperture length) mm.

Distribution

West Florida (site not given), off Miami, Bahamas, Greater Antilles, St. Thomas, Barbados.

Material Examined

ANSP: holotype, 247104, Barbados; 1 spec., 355567, Grand Bahama Id., 26°38'N, 78°25'W, J. Worsfold!, *ex* Worsfold colln.; 1 spec., 298408, reef, NE of North Point, Elbow (Little Guana) Cay, Abaco, Bahama Ids., 7 ft (2 m), under dead *Acropora palmata*, R. Robertson!, 4 Aug. 1953; 1 spec., 193696, off Miami, 27 fms (48 m), rocky, T. L. Moise!, 30 Apr. 1954; 1 spec., 62760, W. Florida, C. W. Johnson!, 1890; 1 spec., 374474, Grand Bahama Id., 26°31'N, 78°46'30"W, J. Worsfold!, *ex* Worsfold colln.

USNM: 1 spec., 64398, Jamaica; 1 spec., 102967a, St. Thomas; 1 spec., 411904, Ensenada de Cochinos, Cuba, J. B. Henderson!; 1 spec., 411908, Cochinos Bay, Cuba, rocky shore, J. B. Henderson!; 1 spec., 900980, Egmont Key, Florida, Gulf of Mexico, 45 ft (13.5 m), P. Williams!, 25 May 1985; 1 spec., 1023063, shoreline NW of Thurstone Bay, Abaco, Bahamas, 26°43'03"N, 77°19'85"W, live collected from underside of rock, 0.5 m, 1 July 1997, C. Redfern!, *ex* Redfern colln. (last two lots *ex* author's colln.).

Discussion

Crassispira drangai was included as a member of the genus *Strictispira* by Maes on the basis of shell morphology, a reasonable location in view of its similarity to *Strictispira solida*, but questionable on the basis of the parietal tubercle, which is crassispirine. A preserved specimen (USNM 1023063, 15.0 x 6.2 mm), that figured by Redfern (2001), and the source of the protoconch and operculum figures here shown, was kindly made available by that author. However, although some animal features could be discerned, a radula was not retrieved. Therefore, the current assignment is tentative, based on shell morphology, and definitive generic assignment must await anatomical study.

Shells of *S. solida* and *S. drangai* are very similar, differing chiefly on the basis of one character, the pattern formed by the peripheral spiral cord structure. In *S. solida* (Fig. 11), there is a variable number of regularly spaced cords, crossing the ribs as well as between them, with no formation of rectangular spaces. In *S. drangai* (Fig. 10), the primary spirals are fewer, narrower, and more widely spaced, and rectangular spaces are produced between them and the axials. Three or four fine secondary spiral threads are present between the primary spiral cords. This formation is absent in *S. solida*. Schwengel noted the fewer spirals on *S. drangai*, with finer secondary spiral threads in the interspaces. On the shell base, there are variably rectangular to square spaces formed in both species, this not being a differentiating feature. All other shell characters are variably present in both *S. drangai* and *S. solida*. *Strictispira drangai* is generally larger, M = 18.2 mm in length for *S. drangai*, 14.8 mm for *S. solid*a, and the body whorl/shell length ratio is smaller for *S. drangai*, 46% vs. 61% for *S. solida*. Overlapping is present though for both measurements. When fresh, *S. solida* has a black shell; *S. drangai* is very dark brown. The lighter intercostal coloring is applicable to both species and is not a differentiating character.

Crassispirella fuscescens (Reeve, 1843) (Fig. 17) is perhaps more likely confused with *S. drangai*, being quite similar to it. It differs in having a stubbier shell, with less basal constriction, and a slightly larger body whorl (56% vs. 51%). The sulcus is less concave. There are more axial ribs with more prominent beading on the basal segment. The peripheral sculpture pattern is less prominent in *C. fuscescens* because the spirals are closer together, but is essentially the same as in *S. drangai*. The axial interspaces are always of lighter color in *C. fuscescens*, although faint in some specimens. It may be absent in *drangai*. Kaicher (1984: card 3906) figured the illustrated syntype of *C. fuscescens*, a worn, faded shell, but the peripheral sculpture is evident in this photo, a hand lens being necessary to see the fine threads. De Jong & Coomans (1988: 109, species 582, pl. 43) report specimens of *C. fuscescens* from Curaçao, reaching 24 mm. Their illustration is excellent.

A lot in the ANSP (368728, 13 specimens, including 4 preserved, from the Bahamas) that had been considered to be *S. solida*, although

closer to *S. drangai*, turns out to be a *Crassispira* (Fig. 12); anatomical study of the preserved specimens revealed a radula of the duplex type similar to that of *Crassispira* (Kantor et al., 1997). The shell has a moderately extended canal and a general form similar to *Crassiclava apicata*. There is a vertical groove behind the forefront of the parietal tubercle, as seen in *Crassispira*, therefore assignment to *Crassispira* is likely. The shells share the rectangular peripheral sculptural pattern of *S. drangai* and *C. fuscescens*, differentiation being based on the shell form and extended canal. This form is apparently undescribed. It is not further considered here, rather being included for differentiation from the present taxa.

Etymology

Named after Mr. Ted Dranga, the discoverer of the type specimen.

Strictispira paxillus (Reeve, 1845)
Figures 14, 21, 27, 37

Pleurotoma paxillus Reeve, 1845: pl. 31, species 285.

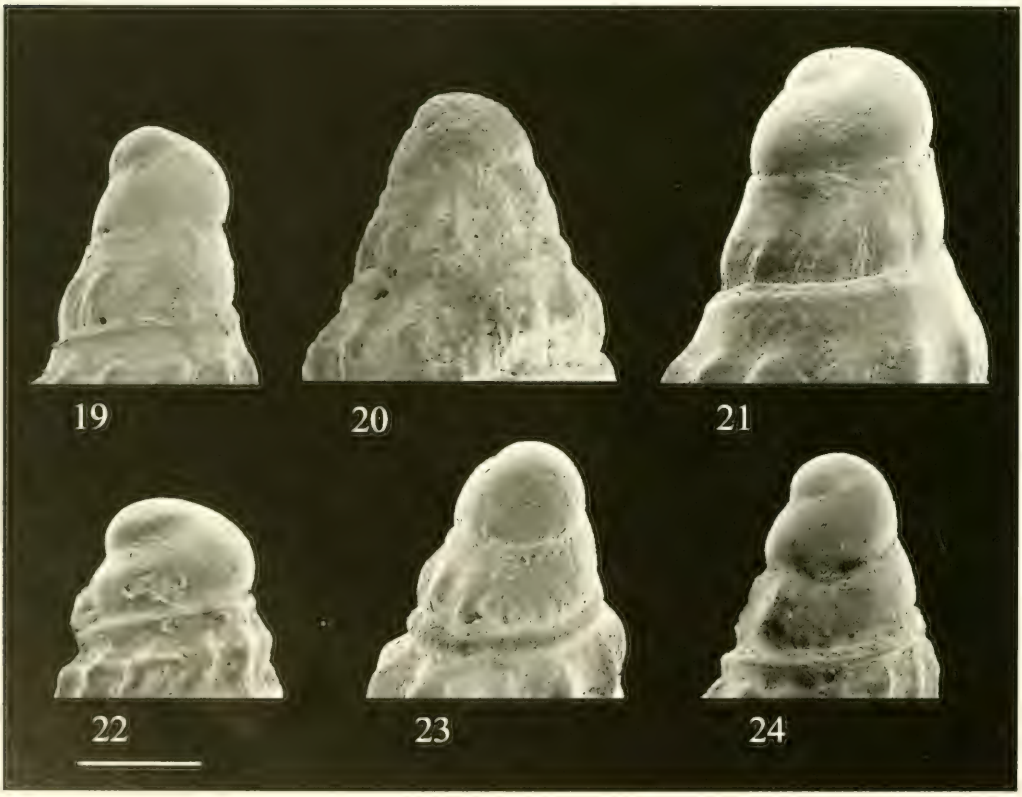
Drillia (*Crassispira*) *paxillus* (Reeve, 1845) – Tryon, 1884: 194, pl. 14, fig. 92 [repetition of Reeve's fig.].

Crassispira paxillus (Reeve, 1845) – de Jong & Coomans, 1988: 109, species 581, description and figure.

Strictispira paxillus (Reeve, 1845) – Maes, 1983: 318, figs. 10, 21, 29, 43, 47; Redfern, 2001: 127, species 526, pl. 57; Malacolog, 2004, list.

Clathrodrillia solida (C. B. Adams, 1830 [sic]) – Rios, 1975: 130, pl., 39, fig. 583 [a misidentification, *fide* Maes, 1983: 318, "The Brasilian shell figured is *S. paxillus*"].

Pleurotoma nigrescens Reeve, 1845, *ex* Gray MS: pl. 26, species 235.



FIGS. 19–24. Protoconchs of *Strictispira* spp. FIG. 19: *Strictispira coltrorum*, USNM 1011352; FIG. 20: *Strictispira drangai*, USNM 1023063; FIG. 21: *Strictipira paxillus*, specimen, one of two, juvenile, shell 7.1 x 3.3 mm, Redfern colln.; FIG. 22: *Strictispira quadrifasciata*, USNM 902243; FIG. 23: *Strictispira redferni*, USNM 1010773; FIG. 24: *Strictispira solida*, USNM 900428. Scale bar = 0.5 mm.

Pleurotoma jamaicensis Guppy, 1866: 290, pl. 16, fig. 6.

Drillia jamaicensis (Guppy) – Pilsbry, 1922: 320, list and text [synonymized *Drillia ebenina* Dall, 1890].

Material Examined

2 spec., one mature, one juvenile, Chub Rocks, Abaco, Bahamas, live collected on underside of rocks, 9 m, C. Redfern!, 10 Oct., 1982, Redfern colln.

ANSP: 1 spec., 342987, White Bay, Guana Id., British Virgin Ids., 2–3 m, in drifted sand on rocks, V. O. Maes!, 15–28 Feb., 1975 (specimen in Maes, 1983: fig.1); 1 spec., 15317, no locality data, *ex* R. Swift colln.; 3 spec., 15487, "St. Thomas, W. I. (Krebs)" R. Swift; 1 spec., 249182, Jack Bay, Anegada, Virgin Ids., 0–8 ft (0–2.4 m), sand, stones, coral, Stn. 770, A. J. & J. C. Ostheimer!, 18 Mr., 1960; 1 spec., 249316, 0.25 to 2 mi. SE of East Point, Anegada, Virgin Ids., 6–20 ft (1.8–6 m), mostly sand, Stn. 774, A. J. & J. C. Ostheimer!, 20 Mr., 1960; 1 spec., 313121, Guantanamo Bay, Cuba, outer beaches, R. T. & S. Abbott!, May 1967; 1 spec., 331166, 0.5–1 mi. SSW of The Bluff, Beef Id., British Virgin Ids., 12–14 fms (21.6–25.2 m), R. Robertson!, 11 Dec. 1973; 4 spec., 350580, Reef south of Bellamy Cay, Trellis Bay, Beef Id., British Virgin Ids., 1–5 m, R. Robertson & V. O. Maes!, 16–21 Feb. 1973; 1 spec., 350784, Pointe des Chateaux, Grande Terre Id., Guadeloupe, R. A. & V. O. Maes!, Feb. 1967; 1 spec., 355363, Enmedio Reef, Vera Cruz, Mexico, J. W. Tunell!, 17 June 1973; 1 spec., 355364, Isla de Lobos Reef, Vera Cruz, Mexico, J. W. Tunell!, 9 June 1973.

Paleontological colln.: 6 spec., ANSP 3773, Jamaica, H. Vendryes!, *ex* Guppy colln.

USNM: 2 spec., 161147, Mayaguez Harbor, Puerto Rico, U.S. Fish Comm.; 1 spec., 502569, off Falmouth, Antigua, beach, University of Illinois Exp., J. B. Henderson!, 1918; 1 spec., 702318, Van Thiel, Curaçao, 10 ft (3 m), underside of rocks at low tide, *ex* Mrs. D. Meyer colln., 20 Jan. 1981; 2 spec., 900416, Curtain Bluff, Antigua and Barbuda, 5–15 ft (1.5–4.5 m), Sept. 1981; 2 spec., 900417, Curtain Bluff, Antigua and Barbuda, 20 ft (6 m), S. Jazwinski!; 2 spec., 900418, Samana, Las Galeras, Dominican Republic, 4–7 ft (1.2–2 m), G. Duffy!, Aug. 1994; 2 spec., 900419, Cabo Rojo, Bahia Salinas, Puerto Rico, 18 ft (5.5 m), night collected, G. Duffy!,18 May 1996 (last four lots *ex* author's colln.).

Distribution

Guantanamo, Cuba, east to Dominican Republic, Puerto Rico, Virgin Ids., Guadeloupe in Leeward Ids.; Mexico, Atlantic coast of Costa Rica (Robinson & Montoya, 1987: 391, list); Curaçao, Aruba, Bonaire area (de Jong & Coomans, 1988: 109); Brazil (Rios, 1975: 130, 583, pl. 39, as *Clathrodrillia solida*, *fide* Maes, 1983: 318); Colombia (Diaz & Puyana, 1994: 222, 875, description and fig., as *Crassispira* (*Strictispira*) *paxillus*).

Description

Shell broadly biconic fusiform, spire angle 39°, length to approximately 10 mm (reported to 15 mm by de Jong & Coomans, 1988: 109); spire outline slightly concave; body whorl large, truncate anteriorly with little basal constriction; anterior canal absent. Protoconch (Fig. 21) of two smooth whorls, teleoconch approximately seven whorls. Whorls slightly rounded below sulcus on later whorls. Subsutural sulcus flattish, subsutural cord projecting little, finely doubled. Sculpture of approximately 20 slightly opisthocline axial ribs on body whorl, forming a shoulder below sulcus, fading at base, and evenly spaced spiral threads between axials, becoming stronger and crossing axials with beading below shell periphery. Fine spirals and curved sinus traces on sulcus. Varix behind outer lip. No stromboid notch. Sinus U-shaped, deep, with protruding parietal tubercle somewhat constricting sinus entrance. Color uniformly shiny black to dark brown, with rib interspaces same color in fresh shells.

Animal, according to Maes, with head and foot similar to crassispirines, covered with sooty blotches, with a muscular foregut, lacking a poison apparatus, and with characteristic radular teeth that protrude "from the buccal mass-like a pair of ice-tongs". Radular teeth (Fig. 37) pistol-shaped, slender for genus, with flange slightly posterior from midpoint. Operculum (Fig. 27) semitransparent, reddish-orange, ovoid, with pointed anterior end and terminal nucleus.

Discussion

Described from an unknown locality, *Strictispira paxillus* was not identified as western Atlantic until Maes's work, although Tryon had thought that it was in all likelihood a synonym of the western Atlantic *Drillia* (*Crassispira*) *fuscescens* (Reeve, 1843). Maes

examined Reeve's NHM *paxillus* material. On the type label, "West Indies" had been written in. She recognized it as the same as certain western Atlantic specimens, these therefore being *paxillus*. A note with *S. paxillus* ANSP 15317 states, "agrees with type BM. V. O. M. 7/3/68". Identified as "*D.* (*Drillia*) *paxillus*", Maes had penciled over this "*Crassispira*", showing she was not thinking of *Strictispira* at that time. Her later Guana Id. anatomical material clearly identified the species as strictispirid. It is worth noting how similar Reeve's excellent illustration of the species is to *S. paxillus* specimens in the USNM and ANSP collections, including Maes' Guana Id. material.

Maes (1983: 318f) described *S. paxillus* briefly, figured it, including the shell, protoconch, and a radular section, plus foregut anatomy, stomach and male reproductive system, reference to which is here made for details. She considered *Pleurotoma nigrescens* Reeve, 1845, and *P. jamaicensis* Guppy, 1866, the latter from the Upper Pliocene of Jamaica, as synonyms, these both being high-spired forms. Pilsbry (1922) discussed *Drillia jamaicensis* from the Guppy collection at the ANSP, and these six specimens were examined (Paleo, colln. 3773). They are clearly *S. paxillus*. The illustrated specimen from Maes (1983) is shown in Figure 14. Maes pointed out that there are a number of species of similar general appearance, both within the strictispirids, as well as in other families, such as *Crassispirella fuscescens* and *Crassiclava apicata*. Thus, literature records are not reliable unless voucher material is available.

FIGS. 25–30. Opercula of *Strictispira* spp. FIG. 25: *Strictispira coltrorum*, USNM 1011351; FIG. 26: *Strictispira drangai*, USNM 1023063; FIG. 27: *Strictispira paxillus*, as with Fig. 21; Fig. 28: *Strictispira quadrifasciata*, USNM 902243; FIG. 29: *Strictispira redferni*, USNM 1010775; FIG. 30: *Strictispira solida*, USNM 411922. Scale bar = 1.0 mm.

Differentiation from other species, as she points out, is based on the broad shell, flat sulcus, and numerous ribs in *paxillus*. Additionally, the slightly concave spire outline, absence or near absence of an anterior canal, flat basal profile, and doubled subsutural cord are characteristic. The radula readily distinguishes *S. paxillus,* and other strictispirids, from similar species in other genera, such as *Crassispirella fuscescens* (Fig. 17) and *Crassiclava apicata* (Fig. 16).

Distinguishing shell features include larger size for both *C. fuscescens* and *C. apicata*. *Crassispirella fuscescens* has a sculptural pattern on the shell periphery of rectangular spaces, as described above with *S. drangai*, stronger beading on the base, and lighter color between the axials. *Crassiclava apicata* has a narrower shell with a higher spire, longer anterior canal with stronger basal constriction, and axial ribs curving onto preceding sulcus. For differentiation from other strictispirids, see following.

Strictispira quadrifasciata (Reeve, 1845)
Figures 15, 22, 28, 38

Pleurotoma quadrifasciata Reeve, 1845, pl. 28, species 251.

Drillia (*Crassispira*) *quadrifasciata* (Reeve, 1845) – Tryon, 1884: 195, pl. 14, fig. 82 [repeat of Reeve's fig.].

Crassispira quadrifasciata (Reeve, 1845) – Kaicher, 1984: card 3896; Leal, 1991: 189, pl. 24, fig. G.; Rosenberg, 1992: 105, illustrated; Malacolog, 2004, list.

Crassispira (*Crassispirella*) *quadrifasciata* (Reeve, 1845) – Humfrey, 1975: 183, pl. 22, fig. 12.

Material Examined

1 spec., Curtain Bluff, Antigua, 5–15 ft (1.5–4.5 m), Sept. 1981, sacrificed to obtain radula.

USNM: 1 spec., 19046, no locality, U.S. Exploring Exp.; 1 spec., 86869, Samana Beach, Santo Domingo, 16 fms (29 m), Blake Exp.; 1 spec., 367064, no locality, *ex* T. L. Casey colln.; 1 spec., 502561, Pelican Id., Barbados, shallow, on coral, Southern University of Illinois Exp., 1918; 20 spec., 598487, E side Buccoo Reef, Tobago, R. W. Foster!, Apr. 1951; 2 spec., 682194, Buccoo Reef, Tobago, Smithsonian Bredin (SBI) Exp., Stn. 8, 5 Apr. 1959, 9:30 AM–12:30 PM; 25 spec., 682219, Buccoo Reef, Tobago, SBI Exp. Stn. 15, middle portion of reef, off high ground, dry at low tide, 6 Apr. 1959, 7–9 AM; 10 spec., 682294, Buccoo Reef, Tobago, SBI Exp. Stn. 26, shallow, 9 Apr. 1959; 1 spec., 682318, Buccoo Reef, Tobago; 6 spec., 902240, Curtain Bluff, Antigua, 5–15 ft (1.5–4.5 m), Sept. 1981; 1 spec., 902241, off Cat Id., Bahamas, 3–6 ft (0.5–1.8 m); 1 spec., 902242, between Montega Bay and Tryall, Jamaica, 20–40 ft (6–12 m), Dec. 1989; 2 spec., 902243, south coast of Dominican Republic, 1–3 m, G. Duffy!; 1 spec., 902244, Roatan Id., Honduras, 10 ft (3 m), P. Williams!, 1985 (last five lots *ex* author's colln.).

ANSP: 8 spec., 195808, Buccoo Reef, Tobago, label reads "compared with type in BM, V. O. M., 4 July 1968"; 1 spec., 240097, off Morro de Pto. Moreno, Isla de Margarita, Venezuela, 4–50 ft (1.2–15 m), W. M. Hellman!, 4 Feb. 1959, Stn. 21; 1 spec., 291178, 1 mi. N of Holetown, Barbados, 3–20 ft (1–6 m), reef and sand, R. & V. O. Maes!, Dec. 1963 (figured in *Encyclopedia of Seashells*, G. Rosenberg, 1992: 105); 1 spec., 300152, Genipabú, Natal, Rio Grande do Norte, Brazil, dry to 3 ft (to 1 m), sand, rock outcrop, grass, G. & M. Kline!, 3 Dec. 1963, Stn. 582; 2 spec., 313113, outer beaches, Guantanamo Bay, Cuba, R. T. & S. Abbott!, May 1967; 10 spec., 1 mi. N of Pointe des Chateaux, Guadeloupe, 3–10 ft (1–3 m), weed on coral rock, V. O. Maes!, live animal photographed; 1 spec., 351090, Kralendijk, Bonaire, 12°09'N, 68°18'W, 25 Feb. 1970.

Distribution

Bahamas, Greater Antilles, Lesser Antilles, Tobago, Venezuela, Brazil, Honduras.

Description

Shell small (to approximately 12 mm), elongate-biconic, turreted, gradually narrowing below periphery with little basal constriction to truncate, open, anterior canal. Body whorl half shell length. Prominent subsutural cord, narrow, concave shoulder sulcus. Protoconch (Fig. 22) shiny chestnut colored, low and squat, 1½ smooth whorls, followed by ¼ whorl with quickly enlarging axial riblets blending into adult sculpture. Teleoconch whorls 5½–7. Numerous (approximately 20 on penultimate whorl, 15 to varix on body whorl), rounded, narrow, straight, slightly opisthocline axial ribs with wider interspaces, extending from bottom of sulcus to following suture on spire and, of

decreasing strength below periphery on body whorl, to junction with anterior canal. Regularly spaced spiral cords, 3–4, weak on spire, stronger on later whorls. On body whorl a fifth below periphery, followed by 2–3 more; on anterior canal, 4–5 more or less "packed", close-set, strong cords, appearing set-off from sculpture above. Beads formed on spirals crossing ribs. Fine secondary spiral threads overall. Aperture ovoid with U-shaped sinus at upper end and projecting parietal tubercle that may narrow entrance to sinus. Low tooth-like swelling may be present below sinus inside outer lip. Varix of one or two enlarged ribs behind outer lip. Shallow stromboid notch in some specimens. Distinctive color pattern, white base, variable chestnut banding, typically producing prominent white banding on sulcus, on fifth spiral cord region below periphery, the anterior canal tip, and on the beads on ribs or entire rib white. Some material with no white on sulcus or on beads. Variable pattern down shell base.

Radular teeth (Fig. 38) pistol-shaped marginals, approximately 180 µm, pointed anterior end, flange about $^1/_3$ forward from spatulate posterior end. Thirty-five pairs of teeth on fragmented radula sections on slide. Operculum (Fig. 28) amber, roundly ovoid with moderately pointed anterior end and terminal nucleus.

Discussion

Shells rather uniform in appearance, differing mainly in color patterning as noted, otherwise occasional specimens lack defined primary spirals on the shell periphery. It is not likely to be confused with any other species.

Conventionally considered crassispirine, availability of a specimen with the soft parts permitted radular study showing that *S. quadrifasciata* is strictispirid, the teeth being characteristic of the family. Whereas the other members of the genus are all somewhat similar in appearance, this species has pronounced color patterning, plus a protoconch and an operculum that differs significantly from the others – protoconch squat with axial riblets terminally, operculum broader with anterior end broad and rounded rather than pointed. Yet there is no difference in radular tooth structure from other strictispirids. I considered proposing a new genus for this species, but a conservative position seems best, assigning it to *Strictispira* pending study of further material.

Strictispira redferni, **new species**

Figures 4–8, 23, 29, 31–35

Strictispira sp. – Redfern, 2001: 127, species 528, pl. 57.

Strictispira acurugata (Dall, 1890) – Malacolog, 2004, list.

Description

(Based on type material, except shell length, which includes all material examined.) Shell small (to approximately 17.5 mm), drilliiform, turreted, body whorl about 60% shell length, anterior canal short, open, unnotched. Color light chestnut, fading to medium brown in beach specimens (the majority of the material), axial ribs slightly lighter at upper ends. Protoconch (Fig. 23) 1½ smooth whorls with partially immersed tip, 6–6¾ teleoconch whorls with moderately strong subsutural cord, which is occasionally somewhat darker than rest of shell, followed by strongly concave shoulder sulcus, shoulder tabulate, axial ribs to following suture forming flat whorl profile. On body whorl, after flat peripheral region, ribs curving around moderately convex base and end at moderately concave junction with canal. Ribs blunt posteriorly, rounded, slightly opisthocline, of equal width to interspaces, 9–13 to varix on body whorl, 12–15 on penultimate whorl. Last rib or two enlarged forming moderate-sized varix ¼ whorl back from thin, curved lip edge. Spiral cords rounded, evenly spaced, 5–7 on spire whorls, not crossing ribs until below periphery, 5–9 across base, and 5–7 strong cords on canal. Slightly laterally elongate beads on spirals crossing basal axials, becoming stronger anteriorly. Moderately deep U-shaped sinus on sulcus, apex at mid point, upper edge forming slightly projecting parietal tubercle on joining body whorl. Sinus tracks present on sulcus. Spiral threads 3–5 on sulcus, always present but varying from moderately strong to faint. Occasional fine spiral lirae extending somewhat back into shell below sinus inside outer lip. No stromboid notch, except slight curvature occasionally on mature specimens.

Anatomy

Animal whitish overall or with black mottled foot, head, and mantle/siphon complex. Foot elongate. Head small, bearing two tentacles with eyes distally and laterally. Mantle edge behind tentacle bases dorsally, bearing sinus indentation on right. Mantle thin, semitrans-

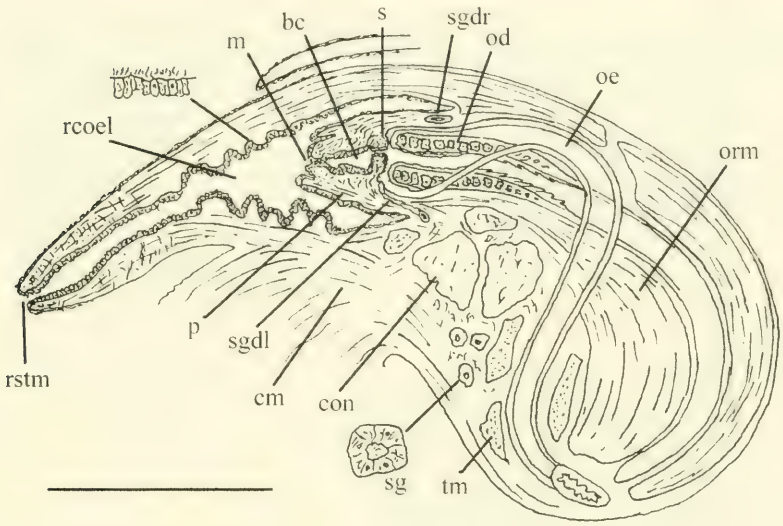
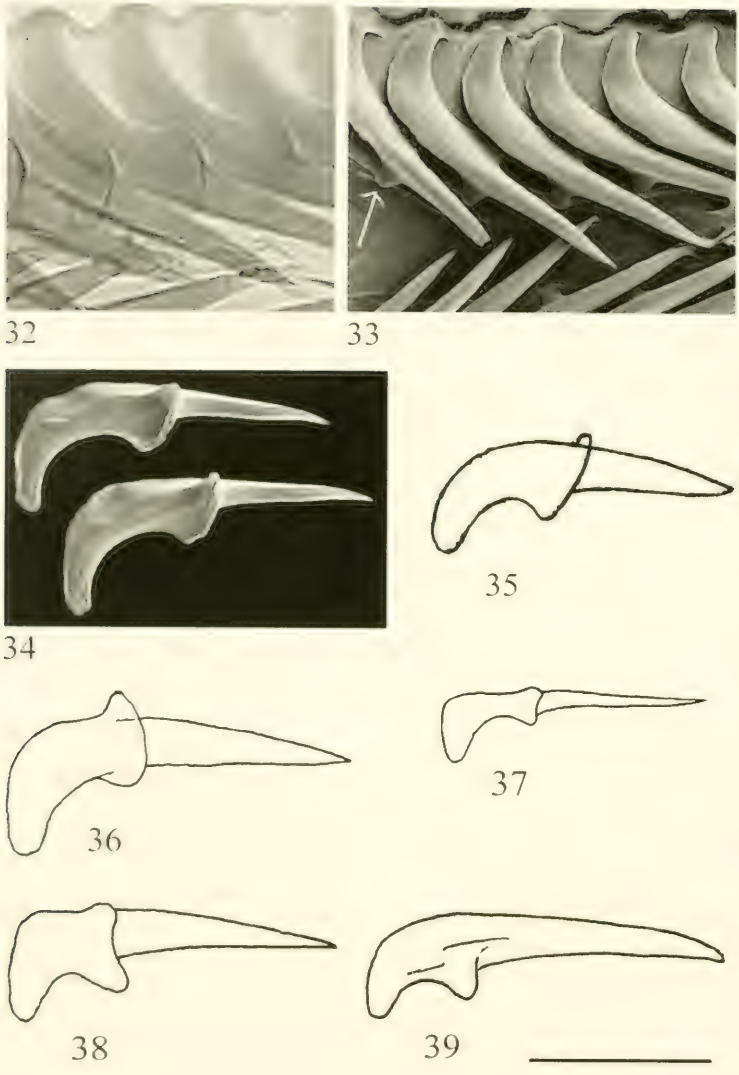


FIG. 31. *Strictispira redferni*. Semidiagrammatic sagital section of head and foregut, from serial section. Shell 10.1 x 3.8 mm, sacrificed: bc = buccal cavity, cm = columellar muscle, con = circumoral nerve ring, m = mouth, od = odontophore, oe = oesophagus, orm = odontophoral/radular retractor muscle, p = proboscis, rcoel = rhynchocoel, rstm = rhynchostome, s = buccal septum, sg = salivary gland, sgdl = left salivary gland duct, sgdr = right salivary gland duct, tm = transverse muscle bundle. Scale bar = 1mm.

parent, gills and osphradium visible on left and penis on right, originating behind and lateral to right cephalic tentacle, reflected backwards beneath mantle in male. Foregut anatomy (Fig. 31) showing rhynchostome medial, just below tentacle bases. Rhynchodeum large, with walls compressed longitudinally from retraction, producing strong circular, folding interiorly. Heavy longitudinal musculature throughout length, continuous with columellar muscle ventrally and extending posteriorly in body cavity. Radial and circular musculature interspersed in rhynchodeal walls, especially anteriorly, but no distinct rhynchostomal sphincter. High columnar rhynchodeal epithelium becoming flat cuboidal posteriorly. Moderately sized, muscular proboscis with strong folding due to retraction and with circular fold around mouth opening. Mouth opening into short buccal tube, which enlarges rapidly forming buccal cavity demarcated posteriorly from opening to oesophagus by muscular septum. Epithelium of proboscis same as rhynchodeal. Massive odontophore and radular structure dominating body cavity. Radula opening into proximal oesophagus, curving from ventrally and right. Strong radular membrane with doubled odontophoral cartilages curve posteriorly through body cavity. Radula of approximately 120 pairs of solid, pistol-shaped, pointed marginal teeth with median flange, measuring approximately 200 µm (Figs. 34, 35). Radular and odontophoral muscle heavy, extending posteriorly, joining with rhynchodeal, proboscis, and columellar muscle, interspersed with prominent transverse muscle bundles. Coiled salivary gland composed of single layer of ciliated cuboidal cells ventral to anterior odontophore and oesophagus, splitting into two ducts, left curving around oesophagus and opening into oesophagus just posterior to buccal septum. Right duct termination not seen due to slide defect. Poison gland or bulb absent. Oesophagus circular initially, becoming flattened due to compression between bundles of circumoral nerve ring (not shown in figure), lined by single layer of ciliated cuboidal cells. Operculum (Fig. 29) ovate, elongate, with flat columellar side, narrowed anteriorly and pointed, with terminal nucleus.

Type Material & Locality

Holotype, USNM 1010771, lee side of Guana Cay, Abaco Id., Bahamas (26°41'50"N, 77°9'35"W), dredged live, 12 ft (3.6 m), 9 July



FIGS. 32–39. Radular ribbons and teeth of *Strictispira* spp. FIG. 32: *Strictispira redferni*, USNM 1010773, slide preparation, light-transmitted, ribbon section; FIG. 33: *Strictispira redferni*, USNM 1010775, SEM preparation, ribbon section; FIG. 34: *Strictispira redferni*, USNM 1010775, SEM preparation, radular teeth, ventral view; FIG. 35: *Strictispira redferni*, ANSP A9421, Tavernier Key, Florida Keys; FIG. 36: *Strictispira coltrorum*, USNM 1011351; FIG. 37: *Strictispira paxillus*, drawing of tooth from Kantor & Taylor (1994: fig. 2C, using Maes's material), data as with Fig.14; FIG. 38: *Strictispira quadrifasciata*, Antigua, shell 7.9 x 4.1 mm, sacrificed; FIG. 39: *Strictispira solida*, USNM 411922. Scale bar = approximately 50 µm (Fig. 32), 100 µm (Figs. 33–39).

1994, C. Redfern!; paratypes: 38 spec., USNM 1010772, sandbank, lee side Guana Cay, Abaco, Bahamas, 9 July 1992, C. Redfern!, and 11 spec., USNM 1010773, spoil bank, Guana Cay, Abaco, Bahamas, 14 Aug. 1989, C. Redfern!; 1 spec., with data as per 1010772, at each of the following: AMNH, ANSP, DMNH, FMNH, LACM, MCZ, MNHN, MORG, NHM, NM (material *ex* author's colln.).

Additional Material Examined

USNM: 2 spec., 53452, No Name Key, Florida Keys, in grass below 2 m, H. Hemphill!; 1 spec., 27650, Lower Matecumbe Key, Florida, H. Hemphill!; 1 spec., 1021270 [*ex* 272674], Newfound Harbor Key, Florida Keys, P. Bartsch!; 12 spec., 1021140 [*ex* 411865], N shore Key West, Florida, beach, J. B. Henderson!; 3 spec., 1021137 [*ex* 411870], Upper Matecumbe Key, Florida, beach, J. B. Henderson!; 2 spec., 411953, Key West, Florida, 4.5 fms (8 m), J. B. Henderson!; 1 spec., 412158, Tortugas, Florida, 16 fms (29 m), J. B. Henderson!; 1 spec., 668097, off Dog Id., Florida, Gulf of Mexico, near Clearwater, 4–6 fms (7–11 m), in *Astropecten articulata* stomach, Oct. 1962, G. Radwin!; 2 spec., 601681, Jamaica. (USNM specimens were separated from large suite of *Pyrgospira ostrearum* specimens.)

ANSP, as *Strictispira acurugata*: approximately 400 spec., 221823, Boot Key Harbor, Vaca Key, Florida Keys, B. R. Bales!, Jan.–March 1945, *ex* Schwengel colln. (originally identified as *Crassispira tampaensis*); approximately 75 spec., 313080 (*ex* 221702), Bonefish Key, Florida Keys, B. R. Bales!, *ex* Schwengel colln.; 1 spec., 314456, 0.5 mi. SE of Burnt Point, Crawl Key, Florida Keys, in sand pockets among weed and rock, 2–4 ft (0.5–1.25 m), V. O. Maes!, 27 April 1968 (originally identified as *Crassispira* sp.); 1 spec., 313084 (ex 264988), Boca Ciega Bay, near St. Petersburg, Florida, *ex* J. D. Parker colln.; 3 spec., 368733, Hotel, W end Grand Bahama Id., Bahama Ids., 26°42'15"N, 78°59'50"W, J. Worsfold!, *ex* Worfold colln.; 1 spec., 368499, McLean Town, Grand Bahama Id., Bahama Ids., 26°38'45"N, 77°57'30"W, 3 ft (1 m), J. Worsfold!, *ex* Worsfold colln.; 1 spec., 355797, Wardwick Wells Key, Exuma, Bahama Ids., 24°22'N, 76°36'W, intertidal sand, D. Cosman!, *ex* Cosman colln.; 5 spec., White Sound, Elbow Cay, Great Abaco Id., Bahama Ids., 26°32'N, 76°58'W, W. G. Lyons!, 1972, *ex* Lyons colln.; 1 spec. 355798, Whale Cay, Abaco Id., Bahama Ids., 26°43'N, 77°14'W, D. Cosman!, Aug. 1979, *ex* Cosman colln.; 1 spec., 329768, Bimini Lagoon, near Bailey Town, Bimini Ids., R. Robertson!, 1957–58; 6 spec., 370553, North Hawksville Creek, Bahama Ids., 26°32'N, 78°45'W, 1–3 ft (0.3–1 m), J. Worsfold!, *ex* Worsfold colln.; 2 spec., 374473, Grand Bahama Id., Bahama Ids., J. Worsfold!, *ex* Worsfold colln.; 4 spec., alcohol preserved, A9421, between Tavernier Key and channel to Tavernier Creek, Florida Keys, 25°2'N, 80°30'W, on Thalassia, 18 June 1971, *ex* Florida Marine Research Lab.

Drillia acurugata examined: USNM: holotype, 97320, Caloosahatchee Riv., Florida; 1 spec., 113153, Shell Creek, Florida; 1 spec., unnumbered, rock pit 3.5 mi. W of La Belle, Florida, N side of Caloosahatchee Riv. Author's colln: 1 spec., Caloosahatchee Riv.

Distribution

Lower west coast of Florida, Florida Keys to Tortugas, Bahamas, Bimini, Jamaica.

Discussion

Although a common, even abundant, species judging by its frequency at Abaco and its having been collected at other, rather widely separated sites, often in large numbers, *Strictispira redferni* has not been recognized as a separate species, generally being identified as small specimens of *Pyrgospira ostrearum* (Stearns, 1872), or as *Strictispira acurugata*. *Strictispira redferni* differs from *P. ostrearum* firstly by the shell of *P. ostrearum* (Fig. 18) having no parietal tubercle (although old specimens may have an accumulation of gerontic callus at this site) or varix, secondly by *P. ostrearum* being more strongly beaded, the spirals crossing more numerous and narrower ribs, being larger, taller, narrower, and by a beaded subsutural cord. However, immature specimens of *S. redferni* lacking a varix and parietal tubercle can be difficult to differentiate, although the ribs are usually wider and lack beading in *redferni*. *Pyrgospira tampaensis* (Bartsch & Rehder, 1939: 136, pl. 17, figs. 5, 13), which I consider to be a form of *P. ostrearum*, differs from *P. ostrearum* mainly in fewer axials, and subdued beading. It intergrades with *P. ostrearum*.

Maes segregated 12 lots of shells and one lot of alcohol-preserved specimens in the ANSP under the name *Strictispira acurugata* (Dall, 1890), and this was subsequently carried in Malacolog under that name. She apparently considered them living representatives of the Florida Pliocene fossil, and strictispirids on the basis of shell morphology. As her identifications have circulated, collectors have identified specimens as *S. acurugata*. Examination shows that these are not that species, but rather *S. redferni*, including a large form of that species. As seen in

Figure 9, true *S. acurugata* from the Upper Pliocene/Lower Plesitocene is larger, has a nearly flat, broad shoulder sulcus, spirals that are flat, wide bands separated by grooves rather than rounded cords, and there is no varix or parietal tubercle. There is a distinct stromboid notch, and the subsutural cord is weak, hugs the suture, and undulates with the previous ribs. The Recent "*S. acurugata*" specimens do not share these features but correspond with *S. redferni*, some being identical to the type material and of the same size, others larger, reaching 13–14 mm in length. A few (Fig. 8) resemble *S. acurugata* superficially. A large form (Fig. 7, see below) is narrow and reaches 17.5 mm. However, there is complete intergrading of forms. It is noted that Maes considered them all to be the same species.

It is worth noting that the generic position of the fossil species, assigned to *Drillia* by Dall, is in fact uncertain, appearing on the basis of the available material to more likely be of a group, such as the subfamily Cochlespirinae, which lacks a varix or an elaborated sinus at maturity. The genus *Pyrgospira* is a likely assignment.

Review of Recent ANSP material segregated as "*S. acurugata*" permits its being divided into two groups. The first consists of two lots with many specimens, 221823 and 313080. ANSP 221823 is composed of shells of rather uniform morphology (Fig. 7), mature specimens being larger (largest specimen 15.7 mm, yet an 11.5 mm specimen is still juvenile) than the type series of *S. redferni*. They are narrower, have a shallowly concave sulcus, less pronounced ribs with a tendency to intercalary axial ribs or enlarged growth markings on the body whorl. They are considered a variety of *S. redferni*. In this lot, and to a greater degree in ANSP 313080, there is intergrading with the type series. ANSP 313080 contains a number of these large forms, one of 17.5 mm, plus others of sizes to that of the type lot, all showing intergrading with the types of *S. redferni*.

Maes separated a number of specimens in good condition from each of the two lots as representative. Random selection of a number of specimens from these forms group 1. The second group consists of a number of lots showing a full range of intergrading between the first group and the type series, a number of the shells being identical to the type lot. The second group is combined with additional USNM material to form a transition grouping from the type lot to the large variety. These groups plus *Pyrgospira acuruguta* specimens were examined for possibly significant shell morphology differences, as show in Table 1.

Although the statistics for the uncommon *P. acurugata* are of limited reliability due to the low N and the fact that two, perhaps the third also, of the four specimens are immature, thus skewing shell measurements, nevertheless the findings tend to substantiate the differentiation of *S. redferni* and *Pyrgospira*? *acurugata*. *Pyrgospira acurugata* is larger, with a lower body length/shell length ratio (the 21 mm holotype is larger than the mean, 55%, but still smaller than *S. redferni*), more axials usually, fewer spirals. Qualitative rather than quantitative features are more important in differentiating these taxa, the differentiating features being noted above. (The number and character of axials is the same on the early whorls as on the mature whorls, and this is applicable to all taxa noted here.)

With regards the species generally, *S. redferni* shows a weakly defined sinus structure for the genus in that the parietal tubercle does not protrude markedly so as to narrow the sinus opening as seen in other species of the genus. However, occasional specimens of the large varietal group have more extended parietal tubercle roofs.

Maes (1983) and Kantor & Taylor (1994), who restudied Maes's material, including serial sections, described and discussed the foregut anatomy of *Strictispira paxillus*. *Strictispira redferni* can be compared with their findings. The two species are basically the same, their major features agreeing – absence of poison apparatus, large odontophore with corollary large retractor muscles, same radular tooth structure and salivary duct structure. Different is the presence of a buccal cavity area, followed by a septum, separating it from the oesophagus in *S. redferni*, as opposed to the large proboscis and essentially absent buccal tube and cavity in *S. paxillus,* in which the odontophore and radular ribbon occupy the entirety of the proboscis. In the serial-sectioned specimen of *S. redferni*, the radular structure curves from below the oesophagus, ending posterior to the buccal region behind the septum at the beginning of the oesophagus. However, in a dissected Abaco specimen, the radula was positioned at the proboscis mouth. It must be assumed that the arrangement in the specimen of *S. redferni* that was serial sectioned represents a further retracted state than that in the described specimen of *S. paxillus,* rather than an anatomical differ-

TABLE 1. *Strictispira redferni* and *Pyrgospira*? *acurugata* shell characters. N = number of specimens, M = mean, ± = standard deviation. Lower line is observed range.

	Length (mm)	Width (mm)	Body (mm)	Teleowhorls	Axials Penultimate Whorl	Spirals Body Whorl	Length/Width (%)	Length/Body (%)
Strictispira redferni (types)	N = 10 M = 10.6 ± 0.9 9.3–12.3	N = 10 M = 4 ± 0.4 3.6–4.4	N = 10 M = 6.6 ± 0.6 5.8–7.6	N = 10 M = 6.3 ± 0.3 6.0–6.75	N = 10 M = 13.5 ± 0.7 12–15	N = 10 M = 18.9 ± 1.3 16–22	N = 10 M = 37.5 ± 0.6 36–40	N = 10 M = 6.3 ± 0.9 61–69
Strictispira redferni (variety)	N = 7 M = 13.4 ± 0.6 12.4–14.8	N = 7 M = 4.8 ± 0.4 4.4–5.4	N = 7 M = 8.1 ± 0.5 7.5–9.0	N = 7 M = 7.1 ± 0.2 6.75–7.5	N = 7 M = 14.7 ± 0.8 13–16	N = 7 M = 18.4 ± 0.9 17–21	N = 7 M = 35.4 ± 0.5 33–37	N = 7 M = 60 ±0.5 57–62
Strictispira redferni (additional)	N = 14 M = 11.7 ± 1.3 9.8–14.5	N = 14 M = 4.3 ± 0.7 3.6–5.2	N = 14 M = 7.0 ± 0.8 6.3–8.1	N = 14 M = 6.7 ± 0.7 6–8	N = 14 M = 14.8 ± 1.5 12–18	N = 14 M = 18.0 ± 1.3 16–22	N = 14 M = 37 ± 0.7 35–39	N = 14 M = 60 ± 1.4 55–65
Pyrgospira acurugata	N = 4 M = 17 ± 1.3 14.3–21.0	N = 4 M = 6.6 ± 0.7 5.7–7.8	N = 4 M = 8.2 ± 1.5 6.0–11.6	N = 4 M = 8.5 ± 0.3 8–9	N = 4 M = 16.3 ± 0.2 16–17	N = 4 M = 16.0 ± 0.6 15–18	N = 4 M = 38.5 ± 0.5 37–41	N = 4 M = 45.5 ± 1.8 39–55

ence. The alimentary musculature of these animals is obviously very powerful. The shortened proboscis in *S. redferni*, in contrast to the larger organ noted in *S. paxillus*, suggests heightened retraction.

Radular studies of *S. redferni* show findings very similar to *S. paxillus*. The large, paired odontophore, robust ribbon with strong membrane are equivalent. Teeth are the same, those of *S. redferni* (Figs. 34, 35), showing only minor differences from *S. paxillus* (Fig. 37), as seen in Kantor & Taylor (1994), that of *S. paxillus* being more slender.

Of interest is the seeming discrepancy between SEM and light-transmitted images of the teeth. As seen in Figure 32, light transmitted slide preparations show the flange strongly, giving the impression that it wraps around the shaft, "collar-like". However, the SEM preparation (Fig. 33) shows the flange simply protruding slightly from underneath the shaft (arrow). Thus, the flange is shown to attach on the lower/ventral side of the tooth. McLean recognized this, as indicated by his comment that the projecting collar-like structure was on the inside (ventral side) of the tooth (1971b: 729). The attachment is sturdy, and extends from the tooth base to the flange. The depressed region on the underside of the tooth at the bend might be noted (also seen by Kantor & Taylor, 1994: fig. 2C). It appears to result from the pressure of the adjacent tooth's flange.

Etymology

The species is named for Mr. Colin Redfern, who collected the type material, and has been both generous and extremely helpful in assisting the author in this work.

Strictispira solida (C. B. Adams, 1850)
Figures 11, 24, 30, 39

Pleurotoma solida C. B. Adams, 1850: 61; Clench & Turner, 1950: 342, pl. 29, fig. 8 [lectotype designated].

Strictispira solida (C. B. Adams, 1850) – Maes, 1983: 320, text with *Strictispira paxillus*; Kaicher 1984: card 3917; Redfern, 2001: species 527, pl. 57; Malacolog, 2004, list.

Crassispira (*Crassispirella*) *fuscescens* (Reeve, 1843) – Abbott, 1958: 94 [list and description plus text; synonyms: *Pleurotoma solida* C. B. Adams, 1850, *Drillia ebenina* Dall, 1890].

Not *Drillia ebenina* Dall, 1890: 33, pl. 2, fig. 8; Abbott, 1974: 270, species 2997 [reprint of Dall's 1890 figure], as a synonym of "*Drillia* (*Clathrodrillia*) *solida*"; Malacolog, 2004, list, as synonym of *S. solida*.

Not "*Clathrodrillia solida*" (C. B. Adams, 1830 [sic]) – Rios, 1975: 130, pl., 39, fig. 583 [a misidentification, *fide* Maes, 1983: 318, "The Brasilian shell figured is *S. paxillus*"].

Not *Drillia solida* (C. B. Adams, 1850) – Bandel, 1984: 166, fig. 309, pl. 20, fig. 8.

?*Clathrodrillia solida* C. B. Adams, 1830 [sic] – Rios, 1985: 136, species 621, pl. 46 [Dall's figure of *ebenina*]; Rios, 1994: 159, species 712, pl. 53 [uncertain whether this is *S. solida* or not].

Description

Shell broadly biconic, fusiform, spire angle 37°, length approximately 19 mm, body large, somewhat truncate anteriorly, little basal constriction. Protoconch (Fig. 24) two smooth whorls, teleoconch approximately eight whorls. Sulcus narrow, concave, bearing fine spiral striae and curved sinus traces, preceded by a strong, sharply crested subsutural cord somewhat distant from suture. Whorl outline flattish below sulcus. Body whorl riding up variably on preceding whorl terminally. Sculpture of approximately 18 narrow axial ribs extending slightly onto preceding sulcus, producing a shoulder of variable strength, with wider interspaces, disappearing on base, and 7–16 regularly spaced spiral threads between axials, more prominent and wider spaced below shell periphery, producing some weak beading on crossing the axials. Enlarged axial or two forming a varix behind outer lip. Aperture parallel-sided, ending in short, open, slightly notched anterior canal bent slightly right. Lip broken back, usually healed, just following varix in about half of the specimens. Weak stromboid notch. Sinus deep, U-shaped, with parietal tubercle projecting as flat roof-like structure nearly closing opening. Color shiny black when fresh.

Animal with conventional structures externally – foot, head and siphon grayish-amber, mottled with sooty black. Tentacles with eyes placed laterally half way to tips. Rhynchostome below and midway between tentacles. Rhynchocoel large, muscular walls folded transversely and irregularly, large proboscis folded on itself. Body cavity dominated by large radular ribbon. No poison apparatus. Section

of ribbon has approximately 80 pairs of marginal teeth. Teeth (Fig. 39) approximately 190 µm, pistol-shaped, with flange near tooth base, "pistol grip" short. Operculum (Fig. 30) ovoid, with pointed anterior end and terminal nucleus.

Material Examined

Lectotype, MCZ 186005, Jamaica.

USNM: 2 spec., 27644, Lower Matecumbe Key, H. Hemphill!, identified as "*ebenina*", "type" penciled in on label (see Dall's comment concerning these specimens under Discussion below); 1 spec., 95943, Abrolhos Ids., off east Brazil; 4 spec., 102967, St. Thomas; 1 spec., 130465, Antilles, *ex* Lea colln.; 1 spec., 214978, St. Thomas, *ex* Carnegie Institute colln.; 1 spec., 366729, Jamaica?, Vendryes!, *ex* Orcutt colln.; 1 spec., 383177, Jeremie, Haiti, Orcutt colln.; 1 spec., 411903, Key West, 2 fms (3.5 m), J. B. Henderson! (identified as *Drillia ebenina*); 1 spec., 411910, Tortugas, 16 fms (29 m), J. B. Henderson!, Eolis Stn. 33, 1911 (identified as *Drillia ebenina*); 1 spec., 411911, Tortugas, 15 fms (27 m), J. B. Henderson!, Eolis Stn. 34, 1911; 1 spec., 411913a, off Miami, 10 fms (18 m), J. B. Henderson!, Eolis Stn. 70, 1913; 1 spec., 411914, Key West, J. B. Henderson!, Eolis Stn. 73 (identified as *Drillia ebenina*); 1 spec., 411915, off Government Cut, Miami, Florida, 3 fms (5.5 m), J. B. Henderson!, Eolis Stn. 83, 1913; 1 spec., 411918, Santa Lucia, Cuba, 2–4 fms (3.5–7 m), Barrera Exp., Stn. 200; 1 spec., 411920, Cabanas Harbor, 25 fms (45 m), Barrera Exp., Stn. 202; 1 spec., 411921, Cabanas Harbor, Cuba, 3–12 fms (5.5–21.5 m), Barrera Exp., Stn. 203: 5 spec., 411922, Santa Rosa, Cuba, 3–6 fms (5.5–11 m), Barrera Exp., Stn. 209; 10 spec., 411923, Esperanza, Cuba, 2–3 fms (3.5–5.5 m), Barrera Exp., Stn. 210; 1 spec., 411924, Cape San Antonio, Cuba, Barrera Exp., Stn. 224; 1 spec., 843357, off west Florida (Naples), 26°03'11"N, 82°27'27"W, 17 m, Continental Shelf Associates for MMS/BLM, scuba, 1 June 1983; 1 spec., 900421, Peanut Id., Lake Worth, Florida, 3 May 1969; 1 spec., 900422, SW of Key West, 114 fms (205 m), R. Black!, 1975; 2 spec., Finger Channels, off Stiltsville, Miami, Florida, 2–3 ft (0.5–1 m); 1 spec., 900424, W side of Fleming Id., Key West, Florida, 16 ft (4.8 m), 22 Sept. 1995; 1 spec., 900425, E side Marquesas Keys, Florida Keys, 12 ft (3.5 m), scuba at night, 12 July 1991; 1 spec., 900426, Tourmaline Reef, Mayaguez, Puerto Rico, 40 ft (12 m), 10 March 1993; 1 spec., 900427, Isla Morro, Pelotas, Venezuela, 24 ft (7 m); 1 spec., 900428, Cayo Levisa, Oriente, Cuba, 15 ft (4.5 m), scuba at night, 7 Aug., 1995; 1 spec., 900429, Isla Coche, Venezuela, 50 ft (15 m), scuba at night, 16 July 1993; 3 spec., 1004124, W side Fleming Id., Key West, Florida, 20 ft (6 m), scuba at night, 20 Dec., 1995; 3 spec., 1004125, Tambor Cay, Atlantic Panama, 40 ft (12 m), scuba at night, 11 Oct. 1992 (last ten lots *ex* author's colln.)

ANSP: 1 spec., 84478, St. Johns, Antigua, Silas L. Schumo!, 1903; 1 spec., 194117, off Garden Cove, Key Largo, Florida, 3 fms (5.4 m), T. L. Moise!; 1 spec., 198968, NW of Water Pt., North Sound, Grand Cayman Id., A. J. Ostheimer 3rd!, Stn. D31; 1 spec., 232571, off Palm Beach, Florida, J. S. Schwengel!, 24 April 1940; 1 spec., 281650, SE end of McBride Cay, Belize, Stn. 106, R. Robertson!, 25 Aug. 1961; 1 spec., 282214, mouth of Monkey Riv., Belize, 12 ft (3.5 m), coarse quartz sand, 16°21'45"N, 88°29'00"W, R. Robertson!, 21 Aug. 1961; 4 spec., 284033, off mouth of Mullins Riv., Belize, Stn. 62, R. Robertson!, 1–2 Aug. 1961; 1 spec., 313036, outer beaches, Guantanamo Bay, Cuba, R. T. & S. Abbott!, May 1947; 1 spec., 313083 (*ex* 221702, split from lot of *Crassispira cubana*), Bonefish Key, Florida, J. S. Schwengel!; 1 spec., 320964, St. Thomas, W. I., R. Swift!; 1 spec., 337481, Key West, Florida, C. L. Richardson!; 1 spec., 368352, Tamarind, Grand Bahama Id., 26°30'45"N, 78°36'01"W, J. Worsfold!, *ex* Worsfold colln.; 2 spec., 368588, Settlement Pt., W end, Grand Bahama Id., 1 ft (0.3 m), live, at night, J. Worsfold!; 13 spec., 368728, hotel, W end, Grand Bahama Id., 2–4 ft (0.5–1.2 m), live, on sand and rocks, at night, J. Worsfold!; 4 spec., 374475, Grand Bahama Id., J. Worsfold!

Drillia ebenina examined: USNM: figured syntype , 97318, plus 10 further syntypes of same lot, one larger than figured specimen, Caloosahatchee Riv., Florida, Pliocene; 3 spec., 23983, Caloosahatchee Riv., Pliocene; 5 (of 9) spec., 113150, Shell Creek, Florida, Pliocene; ANSP: large batch, 18058, N. St. Petersburg, Florida, Pliocene, W. G. Fargo!, 8 Oct. 1946, *ex* Fargo colln.; 1 spec., 58371, no locality, 21 Mr. 1984.

Author's colln.: 2 spec., Pinecrest beds, Sarasota, Florida, Middle Pliocene.

Distribution

Palm Beach to Miami, Florida, to Florida Keys and Tortugas; off Naples, west Florida, Florida Bay (Tabb & Manning, 1961: 581, list, as *Crassispira ebenina*); Bahamas, Cuba, Grand Cayman, Jamaica, Puerto Rico, St. Thomas, Antigua; Belize, Colombia (Diaz & Puyana, 1994: 222, species 875, description and fig., as *Crassispira* (*Strictispira*) cf. *solida*, and Diaz, 1994: 40, list, as *Strictispira solida*), Venezuela, Brazil.

Discussion

Crassispira ebenina has been confused with *S. solida* for many years. It is probable that literature records of Recent specimens of the fossil *C. ebenina* are in all likelihood *S. solida*, and that position is adopted here. *Drillia ebenina* was described by Dall from the Upper Pliocene-Lower Pleistocene of Florida, and was considered by him as Recent also. He noted it found in shallow water in the Florida Keys by Hemphill, and gave it a distribution of Gulf of Mexico from Florida to Vera Cruz. Except for reporting one specimen from Puerto Rico (Dall & Simpson, 1901: 387), Dall did not mention *S. solida*. Other authors (e.g., Mazyck, 1913: 8; Abbott, 1954: 268; Tabb & Manning, 1961: 581) continued this identification, considering *D. ebenina* as Recent, listing it from S. Carolina, E. Florida, the West Indies. For whatever reason, *S. solida* was not considered a valid or important species. It was listed only (Krebs, 1864: 12; Simpson, 1887: 54), or considered a synonym of *Crassispirella fuscescens* (Reeve, 1843) (Tryon, 1884: 193; Abbott, 1958: 94; Warmke & Abbott, 1962: 134). Finally, Abbott (1974: 270) considered *S. solida* a valid species, nevertheless considering *C. ebenina* a synonym. Abbott's 1958 misidentification of *S. solida* as *C. fuscescens* is based upon ANSP 198968 from Grand Cayman Island. Abbott included a slip with the shell stating, [it] "matches *solida* CBA OK". Maes indicated this was written approximately 1957. The shell is *S. solida*, and was determined as that by Maes (5 Oct. 1977). It measures 14.6 x 6.2 mm, and is a typical specimen. It appears Abbott recognized the shell as *S. solida*, but through some error reported it as *S. fuscescens* in the publication. Comparison of *S. solida* and *C. ebenina* (Figs. 11, 13) shows that they are not conspecific. Although similar in appearance, the sinus of *C. ebenina* is not strictispirid but crassispirine, probably a member of the genus *Glossispira*, at least on the basis of the sinus and parietal tubercle structure (McLean, 1971a: 121; 1971b: 720) (see previous comment about conventional crassispirid subgenera). The shell of *Glossispira ebenina* is broader (although there are narrower forms, otherwise identical), there are more axial ribs, the spirals are more robust, and the subsutural cord is somewhat weaker, slightly rounded, and weakly beaded.

For separation of *S. solida* from *C. fuscescens*, see features for *C. fuscescens* noted above with *S. drangai* and *S. paxillus*. Additionally, *C. fuscescens*, has a non-crested subsutural cord. Separation from *S. drangai* is also discussed above.

Strictispira solida may be differentiated from *S. paxillus* by its larger size, narrowly concave sulcus, fewer and more robust, orthocline axial ribs, and stronger, sharply crested subsutural cord a bit distant from the suture. A common species, *Pleurotoma solida* was assigned to the genus *Strictispira* by Maes (1983: 320) in the text with *Pleurotoma paxillus*, which she had discovered to be strictispirid by virtue of its radular teeth, although the radula of *S. solida* was not mentioned. However, her identification card includes a photograph of a radular slide preparation of a specimen of *S. solida* showing pairs of marginal teeth of strictispirid form (ANSP 282214). The radular study shown here confirms this assignment, the radular teeth being typical of the genus. The basal segment is slightly less flexed and a bit shorter than typical of the genus. This is seen in Maes's slide figure also. This is not to the degree seen in the eastern Pacific sister genus *Cleospira*, as illustrated in McLean (1971a: fig. 88), wherein the flexing is still less and the flange less prominent. Bandel's figures of what is purported to be *S. solida* do not conform to the present findings, but rather show teeth very much like those of *Cleospira*. At this time, no representatives of *Cleospira* are known in the western Atlantic. Bandel does not figure or describe the shell(s) identified as *S. solida*, consequently in view of his radular findings, it is possible that there is a form of the genus *Cleospira* in this region.

CONCLUSIONS

The discovery of the existence of five or six species of *Strictispira* in the western Atlantic demonstrates that the genus is more common than realized, and more morphologically diverse. It is likely that further members of the group will be recognized upon availability and study of the animals.

The strictispirid radular structure findings further suggest, as stated by Taylor et al. (1993), that the feeding mechanism of the strictispirids involves rasping and tearing of prey by a protruding radula. In the present species, the radula can be protruded through the buccal cavity to the anterior proboscis, as with *S. paxillus* (Maes, 1983: 320; Kantor & Taylor, 1994: 343), thereby obtaining access to the prey. The radula could serve as a grasping organ, assisting in propelling food to the buccal cavity and oesophagus by the teeth splaying out after crossing the bending plane and then coming together, like Maes's "ice-tongs" metaphor, in a grasp of the prey on retraction. No food remnants were present in examined specimens.

The protoconch structure indicates direct development, consequently there would probably be no planktonic dispersion. This suggests that there is higher likelihood of different forms having developed from common ancestors.

It is evident that correct systematic assignment of crassispirine-like taxa requires knowledge of the animal, especially radular information. Correct generic location of *S. quadrifasciata*, *S. redferni*, and *S. coltrorum* would not have been suspected without the radula. There is no specific shell morphology that signifies the genus *Strictispira*, although the members do usually share a drilliiform shell with a strictspirid sinus structure. Until the radula is known, generic location can be assigned only on a tentative basis.

There is little available information concerning habitat, shallow, rocky areas with sand and occasionally vegetation being the reported features. Usually of shallow water, *S. solida* was dredged at 200 m.

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GERM CELL DIFFERENTIATION AND SEXUAL MATURATION OF THE FEMALE *NEPTUNEA* (*BARBITONIA*) *ARTHRITICA CUMINGII* (CROSSE, 1862) (GASTROPODA: BUCCINIDAE)

Ee-Yung Chung[1], Sung Yeon Kim[2], Gab-Man Park[3] & Jong Man Yoon[4]

ABSTRACT

Oogenesis, the gonadosomatic index (GSI), reproductive cycle, and first sexual maturation of the female *Neptunea (Barbitonia) arthritica cumingii* have been investigated by light and electron microscope observations. In the early vitellogenic oocyte, the Golgi complex and mitochondria were involved in the formation of glycogen, lipid droplets, and yolk granules. In late vitellogenic oocytes, the rough endoplasmic reticulum and multivesicular bodies were involved in the formation of proteid yolk granules in the cytoplasm. In particular, compared with the results of other gastropods, it differs in that appearances of cortical granules at the cortical layer and microvilli on the vitelline envelope, which is associated with heterosynthetic vitellogenesis, were not observed in vitellogenic oocytes during oogenesis. A mature yolk granule was composed of three components: main body (central core), superficial layer, and the limiting membrane. Monthly changes in the gonadosomatic index in females studied in 2002 and 2003 were closely associated with ovarian developmental phases. Spawning occurred between May and August in 2002 and 2003, and the main spawning occurred between June and July, when the seawater temperature rose to approximately 18–23°C. The female reproductive cycle can be classified into five successive stages: early active stage (September to October), late active stage (November to February), ripe stage (February to June), partially spawned stage (May to August), and recovery stage (June to August). The rate of individuals reaching the first sexual maturity was 53.1% in females of 51.0 to 60.9 mm in shell height, and 100% in those over 61.0 mm.

Key words: *Neptunea (Barbitonia) arthritica cumingii*, oogenesis, germ cell differentiation, sexual maturation.

INTRODUCTION

Neptunea arthritica cumingii (Crosse, 1862) is one of the most important edible gastropods in such East Asian countries as Korea, Japan, China, and Russia (Yoo, 1976; Kwon et al., 1993). This species is especially found in silty sand of the subtidal zone of the west coast of Korea. Recently, as the standing stock of this species gradually decreased due to extensive reclamation projects and reckless overharvesting, it has been designated as one of the important organisms in need of resource management.

There have been some studies on *Neptunea* species on aspects of reproduction, including the reproductive cycle (Takahashi et al., 1972; Takamaru & Fuji, 1981; Fujinaga, 1985; Kawai et al., 1994), and spawning (Miyawaki, 1953; Amio, 1963; Son, 2003), on aspects of ecology including distribution (Ito & Tachizawa, 1981; Ito, 1982; Kwon et al., 1993), growth (MacIntosh & Paul, 1977; Fujinaga, 1987; Suzuki et al., 1996) of *N. arthritica,* and feeding (Pearce & Thorson, 1967) of *N. antiqua.* There has been one study on the spawning season of *N. cumigii* in the East China Sea (Amio, 1963). But, there are still gaps in our knowledge of its reproductive biology. So far, little information is available on ultrastructural study on germ cell differentiation and sexual maturation of *N. arthritica cumingii* in the Korean waters and the Japan Sea (Chung & Kim, 1996). However, there is some information on ultrastructural study of oogenesis in other gastropods (McCann-Coillier, 1977, 1979; Griffond & Gomot, 1979; Griffond, 1980; Hodgson & Eckelbarger, 2000; Pal & Hodgson, 2002).

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Therefore, the results of ultrastructural studies on germ cell differentiation of this species and other gastropods provides important information on the reproductive mechanisms. The reproductive cycles of the local populations in marine gastropods vary with such environmental factors as water temperature and food availability (Chung et al., 2002). Understanding the reproductive cycle and the spawning period of *N. arthritica cumingii* will provide necessary information for natural spat collections or the recruitment period and age determination of this population. In addition, data on first sexual maturity and reproductive strategy of this population are very useful information for natural resource management. The main aim of the present study is to understand germ cell differentiation during oogenesis, the reproductive cycle and first sexual maturity of this species.

MATERIALS AND METHODS

Sampling

Specimens of *Neptunea arthritica cumingii* (Crosse, 1862) were collected monthly at the subtidal zone of Maldo, Kunsan, Korea, from January to December 2002 (Fig. 1). Snails ranging from 41.0 to 106.8 mm in shell height were used for the present study. After the snails were transported alive to the laboratory, shell heights were immediately measured.

Gonadosomatic Index (GSI)

A total of 486 individuals were used for calculation of the GSI. Monthly changes in the mean gonadosomatic index (GSI) were calculated by the following equation (Chung et al., 2002) (Fig. 2):

$$GSI = \frac{\text{Thickness of the gonad} \times 100}{\text{Diameter of posterior appendage including the gonad and digestive gland}}$$

Germ Cell Differentiation by Electron Microscopic Observation

For electron microscopical observations, excised pieces of the gonads were cut into small pieces and immediately fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. After initial fixation, the specimens were washed several times with the same buffer and then

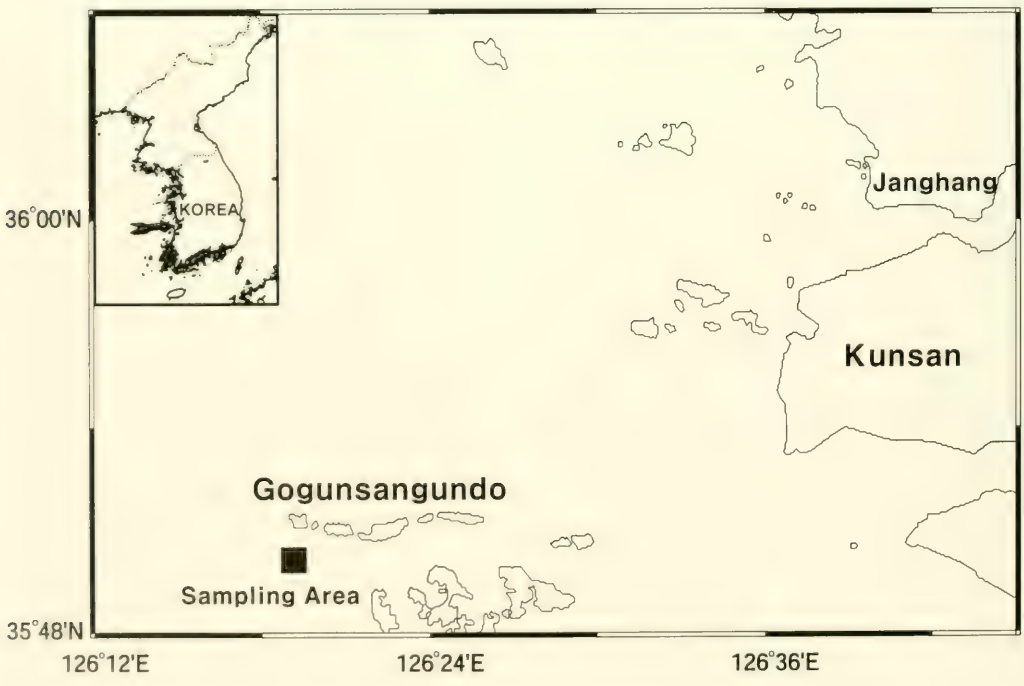

FIG. 1. Map showing the sampling area.

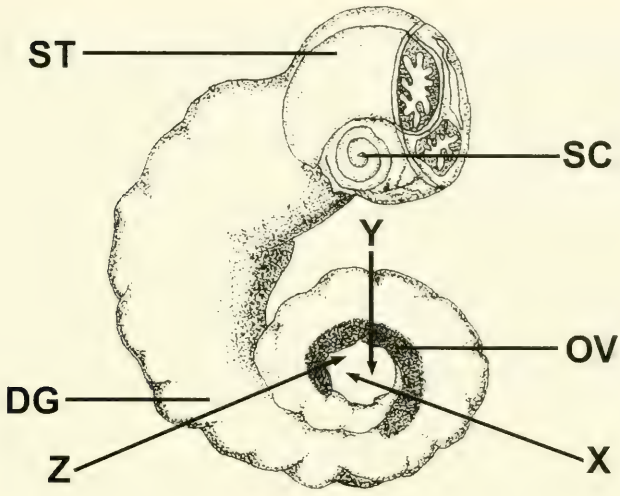

FIG. 2. Anatomy of *Neptunea arthritica cumingii*, removed from its shell. Posterior appendage showing the ovary and digestive gland. X, Y and Z denote the sections for measurement of GSI. Three sections are spaced equally. Abbreviations: DG, digestive gland; OV, ovary; ST, stomach; SC, stomachal caecum.

further fixed in 1% osmium tetroxide dissolved in 0.2 M phosphate buffer solution (pH 7.4) for 1 h at 4°C. Specimens were then dehydrated in a series of increasing concentrations of ethanol, cleared in propylene oxide, and embedded in Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives with a Sorvall MT-2 microtome and an LKB ultramicrotome at a thickness of about 800–1,000 Å. Tissue sections were mounted on collodion-coated copper grids, stained with uranyl acetate followed by lead citrate, and examined with a JEM 100 CX-2 (80 kV) electron microscope.

Gonadal Development by Histological Observations

For light microscopic examination of histological preparations, a total of 456 individuals were used for histological analysis of the gonads from January to December 2002. Gonad tissues were removed from shells and preserved in Bouin's fixative for 24 h and then washed with running tap water for 24 h. Tissues were then dehydrated in alcohol and embedded in paraffin molds. Embedded tissues were sectioned at 5–7 μm thickness using a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, Mallory's triple stain and PAS stain, and examined using a light microscope.

First Sexual Maturity

The first sexual maturation of a total of 187 female individuals (31.4–90.5 mm in shell height) were investigated histologically in order to determine the shell heights of snails reaching maturation and participating in reproduction from May (ripe stage) to late August (after spawning).

RESULTS

Position and Morphology of the Gonads

Neptunea arthritica cumingii is a dioecious species composed of well-defined female and male individuals. The ovary is located on the surface of the digestive gland in the spiral posterior region of the shell (Fig. 2). The ovary is composed of numerous oogenic follicles. As the ovary matured, it extended to the outer part of the digestive gland. As maturation progresses, the sex of the snail can be distinguishable easily by color: the ovary being pale yellow and testis yellowish-brown. At this time,

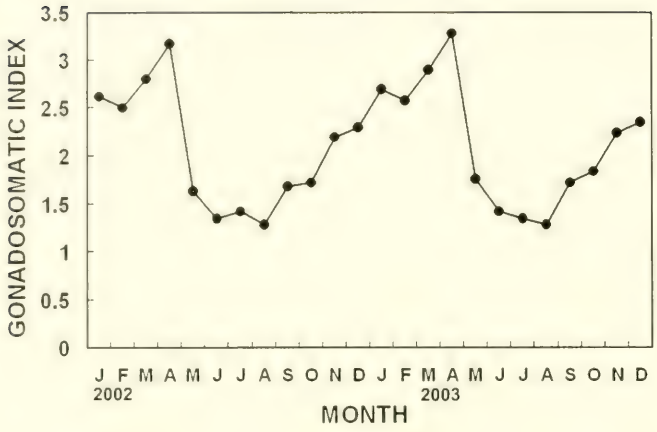

FIG. 3. Monthly changes in the gonadosomatic index of female *Neptunea arthritica cumingii*, for two years from January 2002 to December 2003.

if it was slightly scratched with a razor, ripe eggs readily discharged from the ovary. But after spawning, the ovary degenerated, and it became difficult to distinguish their sexes by external color or dissection.

Monthly Changes in the Gonadosomatic Index (GSI)

Monthly GSI changes in females were showed in Figure 3. In 2002, the GSI slowly increased from September and reached the maximum (mean 3.11) in April when seawater temperature rapidly increased. The GSI rapidly decreased after May, and the values reached the minimum in August, when spawning was completely finished. Monthly changes in the GSI in 2003 showed similar patterns with those in 2002.

Germ Cell Differentiation in the Ovary by Electron Microscopic Observations

Ultrastructural observations allow the germ cell developmental phases during oogenesis can be divided into 4 phases: (1) oogonial phase, (2) previtellogenic phase, (3) vitellogenic phase, and (4) mature phase. Characteristic features in each stage were as follows:

Oogonial Phase: Oogonia in the oogonial phase, which propagated on the germinal epithelium (follicular wall), were oval and 15 μm in diameter. They commonly were single or formed a cluster on the germinal epithelium. Each oogonium had a large nucleus with chromatin, several mitochondria, and the endoplasmic reticulum, vacuoles in the cytoplasm (Fig. 4A).

Previtellogenic Phase: Previtellogenic oocytes were 25–90 μm in diameter. With cytoplasmic growth, several small mitochondria, a well-developed endoplasmic reticulum and several vacuoles were concentrated around the nucleus in the cytoplasm of the previtellogenic oocyte. The number of Golgi complexes, scattered from the perinuclear region to the cortical region of the oocyte, increased. At this time, many vacuoles formed by the Golgi complex appeared around the endoplasmic reticulum, several mitochondria, and large vesicles were present in the cytoplasm of the previtellogenic oocyte (Fig. 4B).

Vitellogenic Phase: In the early vitellogenic oocyte, especially, well-developed endoplasmic reticulum and vacuoles in the cytoplasm were concentrated around the nucleus having nucleoli. At this time, the follicle cell, which lied adjacent to the early vitellogenic oocyte, had an elongated nucleus. In particular, electron-dense granules and several lipid droplets were accumulated in the cytoplasm of the follicle cell (Fig. 4C). With the initiation of yolk formation, lipid droplets were accumulated in the vacuoles formed by the Golgi complex in the perinuclear region. Lipid droplets diffused toward the cortical layer, and then glycogen particles appeared around the mitochondria at the cortical region of early vitellogenic oocytes (Fig. 4D). At this time, after electron-dense materials were accumulated in the Golgi complex (Golgi sac, Golgi vacuoles, and Golgi vesicles), lipid droplets were formed by secre-

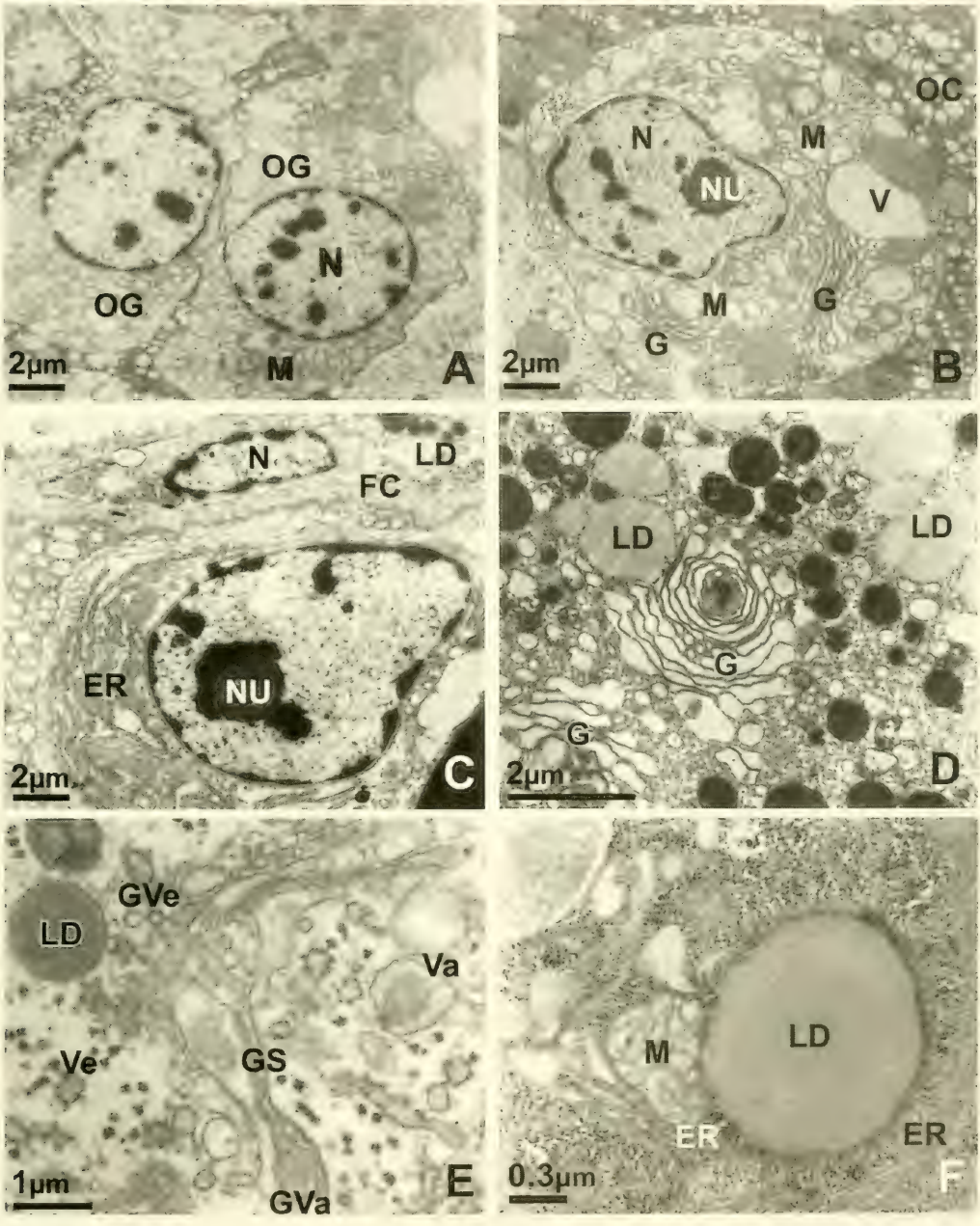

FIG. 4. Electron micrographs of the previtellogenic and early vitellogenic phases during oogenesis of *Neptunea arthritica cumingii* (A–F). A: Oogonia in the oogonial phase, with a large nucleus and several mitochondria in the cytoplasm; B: A previtellogenic oocyte, with a large nucleus with a few nucleolus and several mitochondria, the Golgi complex, and vacuoles in the cytoplasm; C: An early vitellogenic oocyte attached to a follicle cell, with a large nucleus containing chromatin and a number of vacuoles and well-developed endoplasmic reticulum in the cytoplasm; D: An early vitellogenic oocytes, with well-developed Golgi complex, glycogen particles and lipid droplets, E: An early vitellogenic oocyte, with lipid droplets formed by secretions in vacuoles and vesicles; F: An early vitellogenic oocyte, with a lipid droplet surrounded by the endoplasmic reticulum and the mitochondria. Abbreviations: CR, chromatin; ER, endoplasmic reticulum; G, Golgi complex; GS, Golgi sac; GVa, Golgi vacuole; GVe. Golgi vesicle; LD, lipid droplet; M, mitochondrion; N, nucleus; NO, Nucleolus; NU, nucleolus; OC, oocyte; OG, oogonium; ER, Endoplasmic reticulum; Va, vacuole; Ve, vesicle.

tion of electron-dense materials in the large vacuoles and small vesicles, which were formed by the Golgi vacuoles and Golgi vesicles (Fig. 4E). On the other hand, relatively large lipid droplet was surrounded by the endoplasmic reticulum, the mitochondria and glycogen particles in the cytoplasm of the early vitellogenic oocyte (Fig. 4F). In the late vitellogenic oocyte, lots of yolk granules appeared between the rough endoplasmic reticulum and the mitochondria at the cortical layer in the cytoplasm (Fig. 5A). At this time, the multivesicular bodies, which were formed by the modified cristae of the mitochondria, appeared near the nuclear envelope of the nucleus in the late vitellogenic oocyte. Yolk precursors, such as glycogen particles, lipid droplets, yolk granules and multivesicular bodies, were accumulated in the cytoplasm (Fig. 5B). Eventually, proteid yolk granules were formed by yolk granules and multivesicular bodies (Fig. 5C).

Mature Phase: Mature oocytes were about 180–250 x 300–450 μm in diameter. In the mature oocyte, various sizes of proteid yolk granules were intermingled with small lipid yolk granules, and it became a small mature yolk granule (Fig. 5C). Relatively small mature yolk

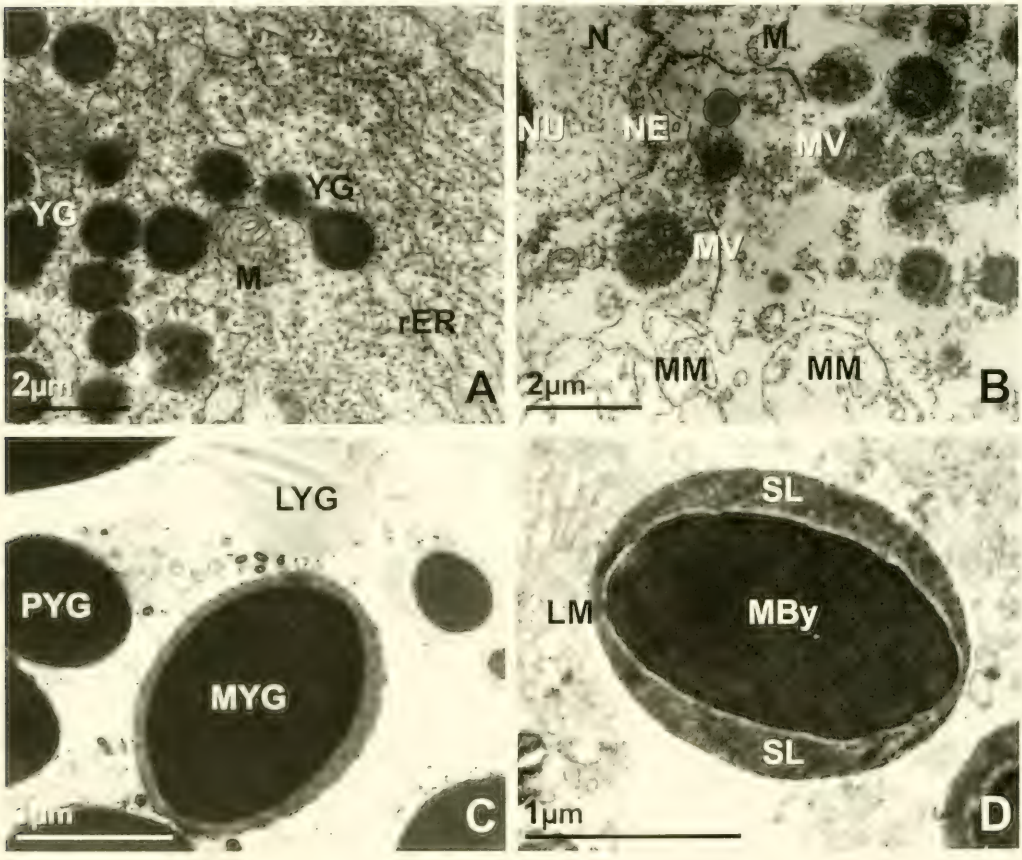

FIG. 5. Electron micrographs of late vitellogenic and mature phases during oogenesis of *Neptunea arthritica cumingii* (A–D). A: A late vitellogenic oocyte, with yolk granules between the rough endoplasmic reticulum and the the mitochondria; B: A late vitellogenic oocyte, with a number of multivesicular bodies formed by modified mitochondria; C: A late vitellogenic oocyte, with proteid yolk granules formed by yolk granules and multivesicular bodies; D: A mature oocyte, with a mature yolk granule being composed of the main body (central core), superficial layer and a limiting membrane of a yolk granule. Abbreviations: LD, lipid droplet; LM, limiting membrane; LYG, lipid yolk granule; M, mitochondrion; MBy, main body; MM, modified mitochondrion; MYG, mature yolk granule; MV, multivesicular body; N, nucleus; NE, nuclear envelope; NU, nucleolus; PYG, proteid yolk granule; rER, rough endoplasmic reticulum; SL, superficial layer.

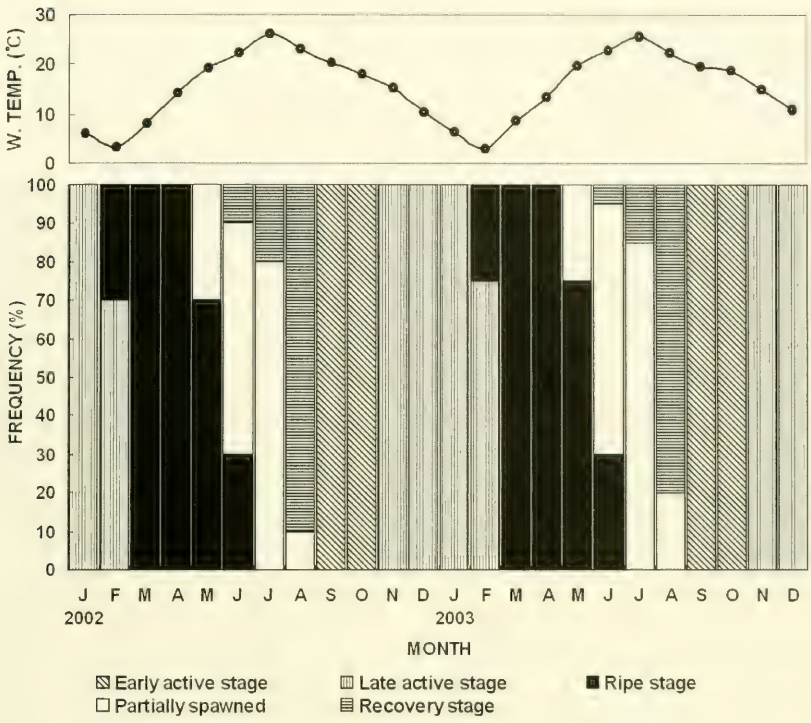

FIG. 6. Frequency of gonadal phases of *Neptunea arthritica cumingii* and the mean water temperatures, for two years, from January 2002 to December 2003.

granules were continuously mixed with each other and became large mature yolk granules in the cytoplasm. A fully mature yolk granule is composed of three components: (1) main body, (2) superficial layer, and (3) a limiting membrane (Fig. 5D).

Reproductive Cycle with the Gonad Developmental Stage

Based on the morphological features and sizes of germ cells and the tissue cells around them, the reproductive cycle with gonadal phases can be classified into five stages in females. Especially, the reproductive cycle and monthly changes in water temperatures showed similar patterns in 2002 and 2003 (Fig. 6). The criteria in defining of each stage are as follows:

Early Active Stage: The gonadal volume was small, and the follicles occupied approximately 25% of the gonad. The follicular walls were relatively thick. Oogonia and previtellogenic oocytes propagated along the oogenic follicular walls and mesenchymal tissues of the ovary. The oogonia and previtellogenic oocytes are about 15–25 μm in size, respectively. At this time, early vitellogenic oocytes of 25–50 μm in diameter formed an egg-stalk attached to the walls (Fig. 7A). The individuals in the early active stage were found from September to October when seawater temperatures were gradually decreasing.

Late Active Stage: This stage is characterized by the presence of developing early vitellogenic oocytes. Follicular walls (germinal epithelium) were thin. A number of early vitellogenic oocytes of 100–140 μm in diameter were attached to the follicular walls through each egg-stalk. With the initiation of yolk formation, there were numerous yolk granules in the cytoplasm of late vitellogenic oocytes of 150–200 x 250–300 μm in diameter. Some fully mature oocytes were free in the lumen of the follicle (Fig. 7B, C). The individuals in the late active stage appeared from November to February.

Ripe Stage: In females, the majority of oocytes grew to 160–180 μm in diameter, occupied over 70% of the gonad, and follicular walls became very thin. Mature oocytes growing up to 180–250 x 300–450 μm in diameter became

tetragonal or polygonal in shape, and contained a number of mature yolk granules (Fig. 7D). Mature or ripe ovaries were found in February through June, when seawater temperatures gradually increased.

Partially Spawned Stage: Since about 50–70% of the oocytes in the follicles were discharged, the lumen of the follicles emptied. Spawned ovaries were characterized by the presence of a few undischarged vitellogenic

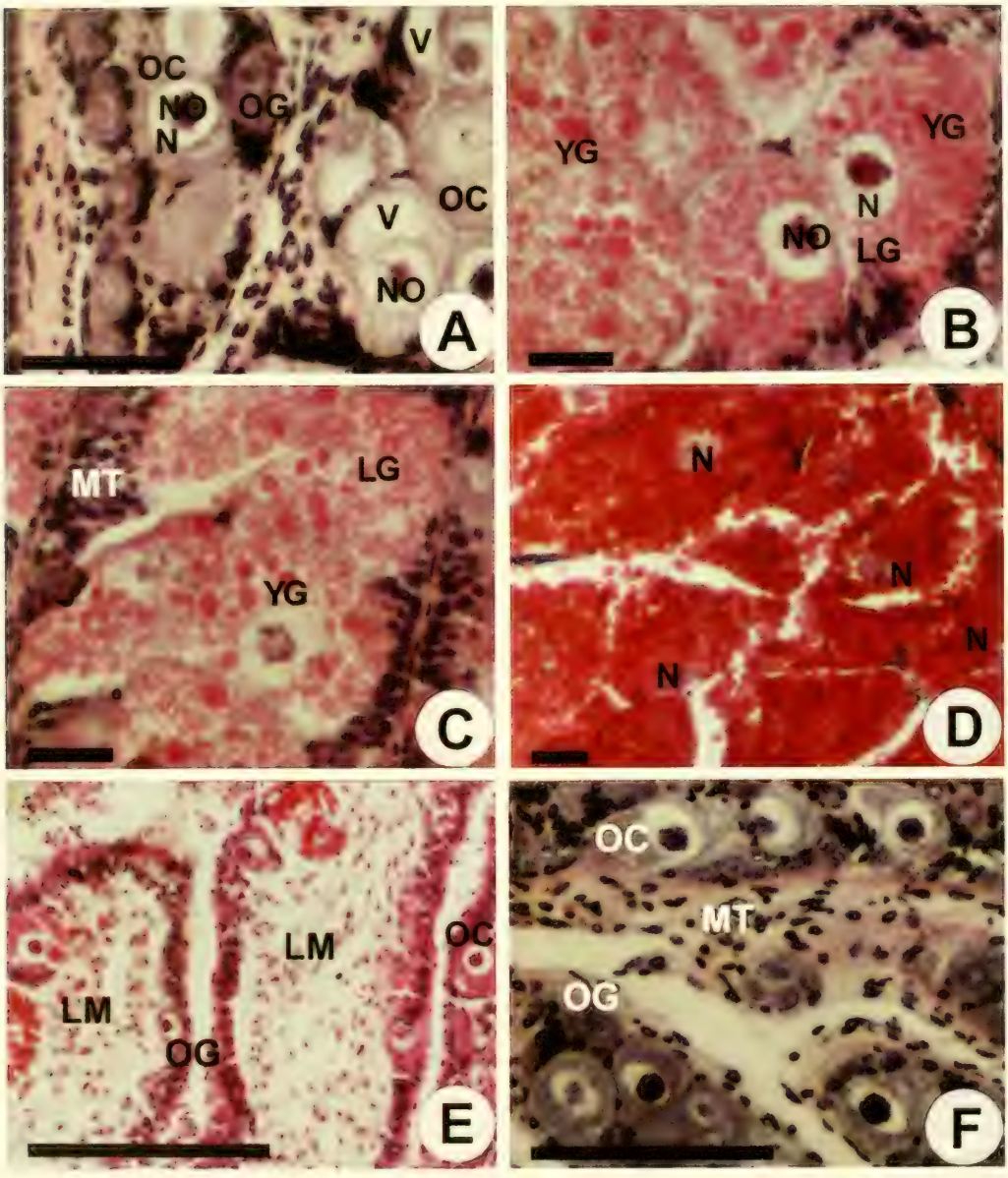

FIG. 7. Photomicrographs of the gonadal phases of female *Neptunea arthritica cumingii*. A: Transverse section of oogenic follicles in the early active stage; BC: Section of follicles in the late active stage; D: Section of ripe oocytes in the ripe stage; E: Section of follicles in the partially spawned stage; F: Section of the follicles in the recovery stage. Scale bars = 50 μm. Abbreviations: LG, lipid granule; LM, lumen; MT, mesenchymal tissue; N, nucleus; NO, nucleolus; OC, oocyte; OG, oogonium; V, vacuole; YG, yolk granule.

TABLE 1. The shell height and first sexual maturity of female *Neptunea* (*Barbitonia*) *arthritica cumingii* from May to August, 2002.

Shell height (mm)	Gonadal developmental stage						
	EA	LA	RI	PS	RE	Total	Mature (%)
31.4–40.9	34					34	0.0
41.0–50.9	26	2	2			30	13.3
51.0–60.9	15	2	10	5		32	53.1
61.0–70.9		3	21	6		30	100.0
71.0–80.9		2	22	9		33	100.0
81.0–90.5			16	12		28	100.0
Total						187	100.0

Abbreviations: EA, early active stage; LA, late active stage; RI, ripe stage; PS, partially spawned stage; RE, recovery stage.

oocytes, as well as previtellogenic oocytes in the follicles (Fig. 7E). The individuals in this stage appeared from May to August, and the main spawning occurred between June and July when the seawater temperature rose to approximately 16–23°C.

Recovery Stage: After spawning, the undischarged vitellogenic oocytes in the lumen of the follicle undergo cytolysis, and each follicle was contracted, and then degeneration or resorption of undischarged vitellogenic or mature oocytes occurred. Thereafter, the rearrangement of newly formed connective tissues, a few oogonia and previtellogenic oocytes appeared on the newly formed follicular walls (Fig. 7F). The individuals in the recovery stage appeared from June to August.

First Sexual Maturity

Before and after spawning, a total of 187 female individuals (31.4–40.9 mm in shell height) were histologically examined to certify whether they reached maturity and participated in reproduction. The rate of shells of different sizes that reached first sexual maturity is summarized in Table 1. The breeding season of this species was from May to August. In the case of some individuals with gonad developmental stage in the late active stage in May through August, it is supposed that they reach maturity, except for individuals in the early active stage during the breeding season. First sexual maturity was 0% in female snails of 31.4–40.9 mm in shell height if they were at the early active stage during the breeding season.

The percentage of first sexual maturity of the female snail of 41.0 to 50.9 mm in shell height was 13.3%. The percentages of first sexual maturity of the female individuals of 51.0 to 60.9 cm in shell height were over 50%, all of which were at the late active, ripe or partially spawned stages. First sexual maturity was 100% for snails over 61.0 mm in height.

DISCUSSION

Germ Cell Development and Vitellogenesis

As vitellogenesis commences, nuclei of the oocytes increased in size. Early vitellogenesis is characterized by proliferation of endoplasmic reticulum and mitochondria, both of which are closely associated with lipid droplets. According to our electron microscope observations of early vitellogenic oocytes of *N. arthritica cumingii*, the Golgi apparatus is thought to be involved in a number of vacuoles and small vesicles in the perinuclear region in the cytoplasm, with carbohydrate (glycogen) particles filling the vacuoles. Lipid droplets and lipid yolk granules are then added to the vacuoles and vesicles formed by the Golgi complex (referred as autosynthetic by Taylor & Anderson, 1969), as in *Ilyanassa obsoleta* (Taylor & Anderson, 1969), *Biomphalaria glabrata* (deJong-Brink et al., 1976), *Mytilus edulis* (Reverberi, 1971), *Rapana venosa* (Chung et al., 2002), *Siphonaria capensis* (Pal & Hodgson, 2002), *Patella barbara*, *P. argenvillei*, *P. granularis*, *P. oculus*, *P. miniata*, and *Helcion pectunculus* (Hodgson & Eckelbarger, 2000). This study suggests that the Golgi complex and various sizes of vacuoles are involved in the formation of lipid droplets in the early vitellogenic

oocytes. From our observations of oogenesis, it is assumed that the mitochondria and the endoplasmic reticulum near lipid droplets are involved in the formation of lipid droplets in the early vitellogenic oocyte. However, we did not find pinocytotic tubules, which are thought to be involved in yolk production as seen in the vitellogenic oocytes of *Agriolimax reticulatus* (Hill & Bowen, 1976; Dohmen, 1983). In the late vitellogenic oocyte, we also did not observe microvilli on the vitelline envelope, which is thought to be involved in helping in absorption, transportation and secretion of egg envelopes (Nørrevang, 1968), as seen in *Mactra chinensis* (Chung, 1997), *M. veneriformis* (Chung & Ryou, 2000), and *Siphonaria serriata* (Pal & Hodgson, 2002).

Formation of cortical granules is a prominent feature of late vitellogenic oocytes in most bivalves, such as *Mactra chinensis* (Chung, 1997) and *M. veneriformis* (Chung & Ryou, 2000). Regarding formation of cortical granules during oogenesis, Hodgson & Eckelbarger (2000) described that Golgi complexes appeared predominately in the cortical region of the ooplasm and secrete electrone-dense, cortical granule-like organelles in the vitellogenic oocytes of *Patella barbara*, and they stated that Golgi complexes synthesize cortical granules. In the present study, however, such structures were not observed in the vitellogenic oocytes, as in *Iliana obsoleta* (Taylor & Anderson, 1969) and *Rapana venosa* (Chung et al., 2002). Compared with *Patella barbara*, the lack of these structures is a prominent characteristic during oogenesis, representing a significant difference in *N. arthritica cumingii*. In the present study, proteid yolk granules, which appeared near the rough endoplasmic reticulum and modified mitochondrial structure (multivesicular bodies), as seen in *Hypselodoris tricolor* and *Godiva banyulensis* (Medina et al., 1986), were observed at the cortical region of the cytoplasm. Accordingly, it is assumed that the endoplasmic reticulum and multivesicular bodies are involved in the formation of proteid yolk granules (Taylor & Anderson, 1969) as yolk precursor. In the present study, although the follicle cell, which lies adjacent vitellogenic oocyte, contains electron-dense granules and lipid droplets, we could not observe clear evidence of secretion into the vitellogenic oocyte. Therefore, it is assumed that *N. arthritica cumingii* synthesize yolk autosynthetically, as in the majority of gastropods, exceptions being some gastropods (*Planorbarius corneus*, *Lymnaea stagnalis*, *Hypselodoris tricolor, Godiva banyulensis*, *Siphonaria capensis*, and *S. serrata*) that synthesize yolk autosynthetically and heterosynthetically (Bottke et al., 1982; Medina et al., 1986; Pal & Hodgson, 2002).

Gonadal Development and Maturation

We observed that gametogenesis of *N. arthritica cumingii* begins at a temperature of about 3°C, with maximum gonadal maturation occurring in April 2002 and 2003, when water temperatures rose (Fig. 7) and phytoplankton was very abundant. Periods of high food abundance and gonad development were nearly coincident. In Korean coastal waters, growth and production of *Meretrix lusoria* and *Ruditapes philippinarum* are very high in the spring and early summer seasons (Kim et al., 1977; Chung et al., 1994; Lee, 1995) due to the abundant phytoplankton that occurs with increasing water temperatures. *Ruditapes philippinarum*, *Meretrix lusoria*, and other clams are commonly used as food by *N. arthritica cumingii*. At this time, abundant food can be supplied to *N. arthritica cumingii* during the period of gonadal development and maturation. Therefore, it is suggested that gonadal development and maturation of *N. arthritica cumingii* is closely related to water temperature and food availability.

TABLE 2. Comparisons of the spawning season of Buccinidae in each locality.

Species	Spawning season	Locality	Author
Neptunea arthritica cumingii	May–August	Kunsan, Korea	Present study
N. cumingii	July–August	East China Sea, China	Amio, 1963
N. arthritica	May–June	Usu Bay, Hokkaido, Japan	Fujinaga, 1985
N. arthritica	May–August	Saroma, Hokkaido, Japan	Kawai et al., 1994
N. constricta	December	East Sea, Korea	Son, 2003
Siphonalia assidariaeformis	December	East China Sea, China	Habe, 1960

Breeding Pattern

As shown in Table 2, our histological observations show that spawning of *N. arthritica cumingii* on the west coast of Korea occurs from late May to August in 2002 and 2003 when sea water temperatures were high. The spawning season of *N. cumingi* collected by the trawl net in the East China Sea occurs between July and August (Amio, 1963). *Neptunea arthritica* in Japan has been reported to spawn once a year between May and June in Usu Bay, Japan (Fujinaga, 1985).

Therefore, it is assumed that the spawning period of *N. arthritica cumingii* on the west coast of Korea occurred somewhat earlier than that in the East China Sea. On the whole, *N. arthritica cumingii* in Korea is a summer breeder, based on the criteria outlined by Boolootian et al. (1962) for marine mollusks. In general, it is assumed that spawning of *N. arthritica cumingii* and *N. arthritica* in Korea and Japan occurs between May and August. However, spawning of *N. constricta* and *Siphonalia assidariaeformis* (Buccinidae) occurs during December, these species being winter breeders (Table 2). Therefore, the slight discrepancy in the spawning period between these studies might be related to geographic differences in water temperature and food availability (Chung et al., 2002).

First Sexual Maturity with the Gonad Developmental Stage

From the result of histological observations, we found that although the specimens were collected during the breeding season, the gonadal development of smaller individuals ranging from 31.4 to 40.9 mm in shell height were in the early active stage as small number of oogonia and the previtellogenic oocytes were present in the follicle of the ovary. Judging from histological observations, it is supposed that the size of the oocyte could not have reached maturity until late August, when spawning ended. Snails of 51.0–60.9 mm high were in the late active, ripe and partially spawned stages, and more than 50% reached first sexual maturity. However, all snails in the late active, ripe, or partially spawned stages spawned if they were larger 61.0 mm. This means that larger individuals can reach maturity earlier than smaller individuals. In the aspect of natural resources management, the present study suggests that because harvesting snails < 51.0 mm can potentially cause a drastic reduction in recruitment, a prohibitory measure should be taken for adequate resource management. Henceforth, age determination by size of the individuals should be investigated in detail for natural resources management of this species.

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REVISION OF THE GENUS *ISLAMIA* RADOMAN, 1973 (GASTROPODA, CAENOGASTROPODA, HYDROBIIDAE), ON THE IBERIAN PENINSULA AND DESCRIPTION OF TWO NEW GENERA AND THREE NEW SPECIES

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ABSTRACT

The presence of the genus *Islamia* Radoman, 1973, on the Iberian Peninsula is confirmed based on the detailed study of a group of species, of which three were previously included in the genus *Neohoratia* Schütt, 1961. These species are most abundant in the south-southeastern Mediterranean region but also inhabit the northern Mediterranean areas of the peninsula, with scattered populations in central and western Spain. Iberian *Islamia* currently includes *I. globulus* (Bofill, 1909), *I. lagari* (Altimira, 1960), and *I. ateni* (Boeters, 1969), plus two new species, *I. pallida* and *I. henrici*, the latter with two subspecies *I. h. henrici* and *I. h. giennensis*. Two new genera are also described, *Milesiana* and *Josefus*, each of which contains one species: *M. schuelei* (Boeters, 1981), which was previously assigned to *Neohoratia*, and most recently to *Islamia*, and a new species, *Josefus aitanica*, respectively. Histological study of the female genitalia confirmed the presence of two seminal receptacles and the absence of a bursa copulatrix in all species belonging to the three genera. In *Islamia*, the distal receptacle was once considered to be a reduced bursa copulatrix. We also confirm that there is no trace of glandular tissue on the penial lobe in any of the *Islamia* species for which histological evidence is available.

Key words: Caenogastropoda, Hydrobiidae, *Neohoratia*, *Islamia*, *Milesiana*, *Josefus*, taxonomy, Spain, Iberian Peninsula.

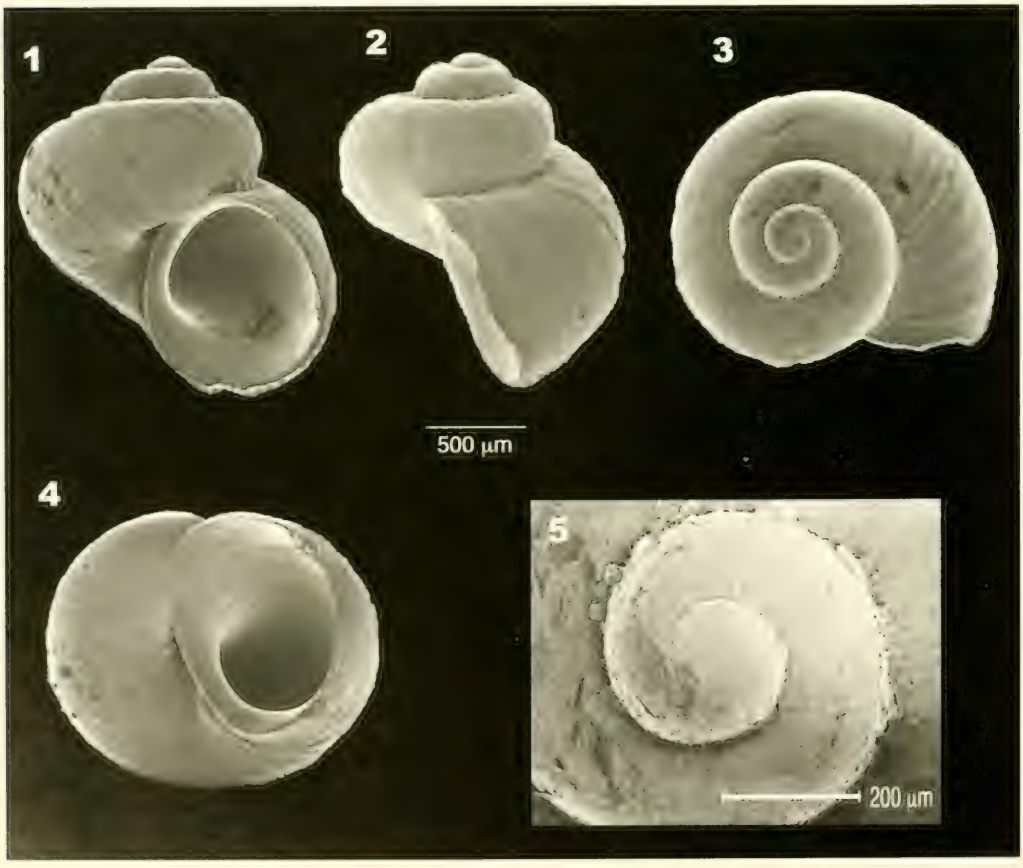
INTRODUCTION

The European fauna of hydrobiids is particularly rich in valvatiform species. However, their morphological study is challenging because of their minute size. Many new genera and species have been described on the basis of shell features, which are known to be highly convergent. Sometimes other anatomical characters, which are frequently non-diagnostic, are used in these descriptions. Data on character variability are absent or very rare. The result has been a much confused taxonomic picture that was recently reviewed and partially clarified by Bodon et al. (2001), who redescribed the type species of most of the European valvatiform genera based on new anatomical studies and data in the literature.

Preliminary studies on Iberian Peninsular valvatiform hydrobiids (Ramos et al., 1992, 1995; Arconada et al., 1996) have shown considerable morphological diversity and high endemicity. Boeters (1988) recognized that species of two genera, *Horatia* Bourguignat, 1887, and *Neohoratia* Schütt, 1961, inhabited this geographical area. An in-depth taxonomic review of the two genera is currently unravelling a very complex situation. Four new genera and several new species have been described in recent papers (Ramos et al., 2000; Arconada & Ramos, 2001, 2002). Some of the species in the new genera were previously included in the above-mentioned genera. We continue these studies by revising the taxonomy of another group of species previously assigned to *Neohoratia* by Boeters (1988).

It has been difficult to distinguish the species of the genera *Neohoratia* Schütt 1961, and *Islamia* Radoman, 1973, given their morphological similarities (Bodon & Giovanelli, 1994; Bodon et al., 1995; Manganelli et al., 1998). The type species of *Neohoratia* is *Valvata* (?) *subpiscinalis* Kuscer 1932 (Figs. 1–5, paratypes from the Biological Institute,

Scientific Research Centre of Ljubljana, N 1862, leg. Dr. J. Bole). This genus has undergone several changes in its taxonomic status. It has been regarded as a subgenus of *Hauffenia* Pollonera, 1898, and of *Horatia* Bourguignat, 1887 (Schütt, 1961; Boeters, 1974; Bodon & Giovanelli, 1994), and as a distinct genus (Bole & Velkovrh, 1986; Boeters, 1988; Bole, 1993). *Neohoratia* is characterised by having a rather short, flat, blunt or slightly pointed penis with 1–3 small, knob-like lateral lobes on its left side near the apex. The female genitalia include a pin-like bursa copulatrix and one proximal, small seminal receptacle (Bole, 1993; Bodon et al., 2001). Boeters (1988) and Boeters & Rolán (1988) overlooked these diagnostic characters while including several species from the Iberian Peninsula in this genus (*Amnicola globulus* Bofill, 1909; *Microna ateni* Boeters, 1969; *Valvata coronadoi* Bourguignat, 1870; *Hauffenia* (*Neohoratia*) *coronadoi schuelei* Boeters, 1981; *Valvata* (*Tropidina*) *fezi* Altimira, 1960; *Hauffenia* (*Neohoratia*) *gasulli* Boeters 1981; and *Neohoratia azarum* Boeters & Rolán, 1988). However, according to Boeters (1988), these Iberian species, apart from having a narrowing ('Einschnürung') of the outer side of the female oviduct glands (capsule + albumen glands), lacked a bursa copulatrix and had a renal oviduct with two seminal receptacles. This combination of characters, in addition to a male genitalia with a penis usually having one glandular lobe on its left side, has been described as typical of the genus *Islamia* (Bodon et al., 1995; Bodon et al., 2001). *Islamia* is attributed to a wide geographical distribution in the Mediterranean area [species are claimed to be from: Turkey (Schütt, 1964; Radoman, 1973b); the Balkanic



FIGS. 1–5. Shell of *Valvata subpiscinalis* (IBCICL paratype nº 1862).

Peninsula (Radoman, 1973a, b, 1978, 1983); Italy (Giusti & Pezzoli, 1981; Bodon et al., 1995, 1996, 2001; Bodon & Cianfanelli, 2002); Israel (Schütt, 1991; Bodon et al., 1995); Greece (Radoman, 1973b, 1978); and France (cited as *Hauffenia* Pollonera, 1898) (Bernasconi, 1984)].

It was thus feasible that the species listed above from the Iberian Peninsula could be attributed to the genus *Islamia* (type species *Hydrobia valvataeformis* Möllendorff, 1873) or even to new genera. In fact, two of them, *Hauffenia* (*Neohoratia*) *gasulli* [*N.* (?) *gasulli*, *sensu* Boeters, 1988] and *Valvata* (*Tropidina*) *fezi* [*N.* (?) *fezi*, *sensu* Boeters, 1988] were recently allocated to two new genera, *Tarraconia* Ramos & Arconada, 2000 (in Ramos et al., 2000), and *Spathogyna* Arconada & Ramos, 2002, respectively.

Here we describe three new species and redescribe the morphological characters (including previously unknown characters) of the above-mentioned species using a multidisciplinary approach based on type specimens and a vast amount of recently collected material. Additionally, histological studies of these species provide evidence that the two sac-like structures on the renal oviduct are seminal receptacles and demonstrate the nonglandular nature of the penial lobe.

We conclude that two of the "*Neohoratia*" species (*sensu* Boeters, 1988) from the Iberian Peninsula (*Amnicola globulus* and *Microna ateni*) actually belong to the genus *Islamia*, as hypothesized by Bodon et al. (2001). Two other species, one of them with two subspecies, are described as new and placed into *Islamia*. Another species, *Hauffenia* (*Neohoratia*) *coronadoi schuelei*, reported as *N. schuelei* (in Boeters, 1988) and as *Islamia schuelei* (in Bodon et al., 2001), is redescribed and placed into a new genus *Milesiana*, and a third new species is described and placed in a new genus, *Josefus*. *Neohoratia azarum* has not been included here because still unpublished data (Arconada, 2000) clearly demonstrate that its anatomy is differs considerably from the genera and species described here.

This paper increases the number of species and expands the distribution area of *Islamia* (Schütt, 1961; Radoman, 1973a, b; Giusti et al., 1981; Bernasconi, 1984; Bodon et al., 1995) in Europe and reinforces the hypothesis that the Iberian Peninsula is one of the richest hydrobioid (*sensu* Davis, 1979) diversity areas in the Mediterranean Basin (Arconada & Ramos, 2003).

MATERIAL AND METHODS

Field collections, anatomical studies, histological protocols, and morphometric measurements are described in Ramos et al. (2000) and Arconada & Ramos (2001). The number of specimens studied for histology and morphometry, localities and sampling dates for each species are indicated in the corresponding section in the text. The morphological descriptions are based on terminology from Hershler & Ponder (1998). Scanning Electron Microscope (SEM) photographs were made with a Philips XL20 following the methodology described in Ramos et al. (2000). Type material of *Islamia globulus* was photographed with a Environmental Scanning Electron Microscope (ESEM) Philips Quanta 200 SEM at low vacuum mode, after being cleaned with ultrasound (Figs. 18, 20, 23, 25, 27, 30, 31, 33, 34) or the periostracum removed by immersion in 5% sodium hypochlorite (Figs. 19, 28).

Paratypes of *Islamia cianensis* Bodon, Manganelli, Sparacio & Giusti, 1995 (n° 6732), and *I. gaiteri* Bodon, Manganelli, Sparacio & Giusti, 1995 (n° 6733), from the Museo Zoologico "La Specola" collection were used for comparisons.

Localities are listed according to the code: stream or spring, municipality, province, UTM co-ordinates, sampling date, collector's initials, museum catalogue number and preservation conditions (see abbreviations below). Locality names and UTM co-ordinates were obtained from the official Army Geographical Service map (1:50.000 series).

Statistical Analyses

All statistics (mean value, standard deviation and coefficient of variation) were calculated using STATVIEW for Macintosh, and standardized in order to avoid the effect of the measurement scale.

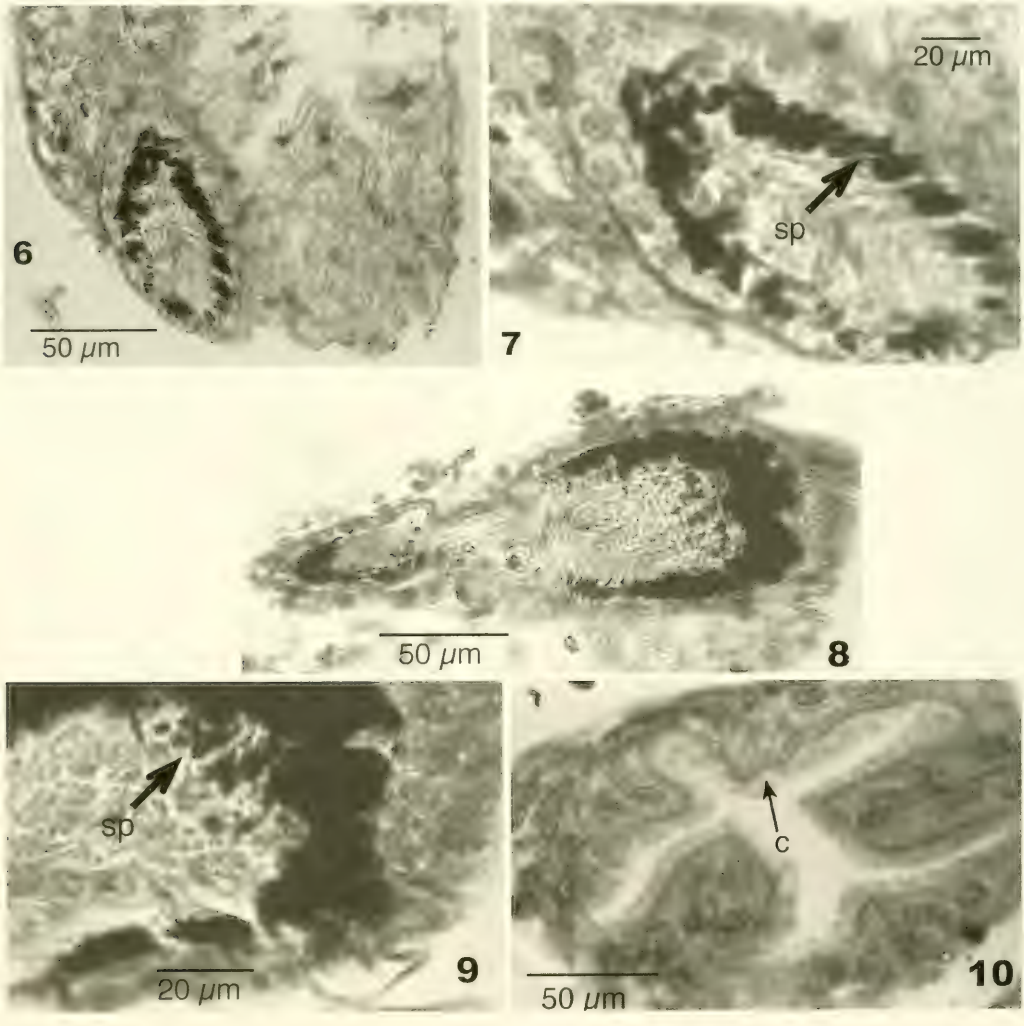
A discriminant funcion analysis (DFA) was performed on nine shell measurements (no ratios) with STATISTICA v.6 for Windows in order to identify the morphological characters that best differentiated species when no or few anatomical data were available. There were no missing data. The effects of violating assumptions are minimized taking into account the robustness of the *F* test (Lindman, 1974). The significance of the overall discriminatory power of the analysis was tested using Wilk's Lambda. Canonical correlation was used to

measure interspecific variation. Classification functions were computed for each group (population) to determine, with the highest probability, which case belonged to which population. Cases were assigned to the group with the highest classification score.

Abbreviations Used in the Text, Tables and Figures

Shell and Operculum Characters: AH: aperture height; AL: aperture length; AW: aperture width; LBW: length of body whorl; NL: length of opercular nucleus; NW: width of opercular nucleus; NSW: number of spire whorls; OL: operculum length; OW: operculum width; OLWL: length of the last whorl of the operculum; OLWW: width of the last whorl of the operculum; SL: shell length; SW: shell width; WAW: width of the antepenultimate whorl; WBW: width of the body whorl; WPW: width of the penultimate whorl; CV: coefficient of variation; SD: standard deviation.

Anatomical Characters: Ag: albumen gland; Bc:



FIGS. 6–10. Histological sections of the anterior female genitalia of *Milesiana schuelei* showing the position of the spermatozoids inside the seminal receptaculum. Note the heads of the spermatozoids attached to the ciliated epithelial cells of the seminal receptacles. FIGS. 6, 7: Proximal seminal receptaculum; FIGS. 8, 9: Distal seminal receptaculum; FIG. 10: Inner epithelium of the widened renal oviduct. Abbreviations: c: cilia; sp: spermatozoids.

bursa copulatrix; Cg: capsule gland; DBC: duct of the bursa copulatrix; Os: osphradium; P: penis; Pl: penial lobe; Po: pallial oviduct; Pp: pseudopenis; Pr: prostate; Ro: renal oviduct; SR1: distal seminal receptacle; SR2: proximal seminal receptacle; Ss: style sac; St: stomach; Vc: ventral channel of capsule gland; L: length; W: width. The concentration of the nervous system was determined by the "RPG" ratio (Davis et al., 1976): length of pleuro-supraesophageal connective divided by the sum of the lengths of right pleural ganglion, pleuro-supraesophageal connective and supraesophageal ganglion. Following several studies, a synthesis of RPG ratios from diverse hydrobioid taxa indicates: dorsal nerve ring concentrated (≤ 0.29); moderately concentrated (0.30–0.49); elongated (0.50–0.67); extremely elongated (≥ 0.68) (Davis et al., 1984, 1986, 1992).

Collections: MNCN: Museo Nacional de Ciencias Naturales, Madrid, Spain; MZB: Museu de Zoologia, Barcelona, Spain; NNM: Nationaal Natuurhistorisch Museum, Leiden, Naturalis, The Netherlands; MHNG: Muséum d'Histoire Naturelle, Genève, Switzerland; SMF: Forschungsinstitut und Natur-Museum Senckenberg, Frankfurt, Germany; MZUF: Museo Zoologico "La Specola", Università di Firenze, Italy; IBCICL: Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia; NHMW: Naturhistorisches Museum, Wien, Austria.

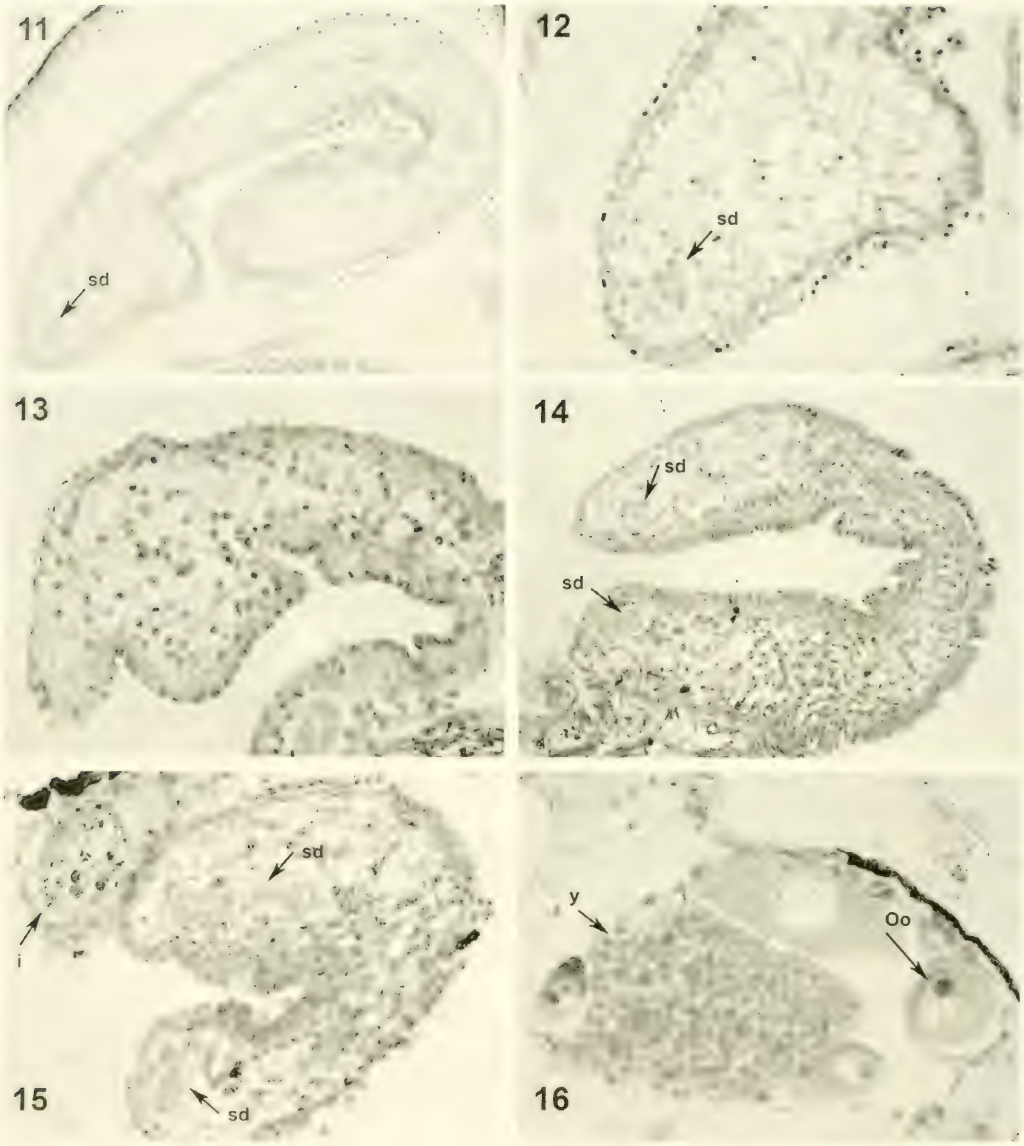
Collectors: R. A.: R. Araujo; B. A.: B. Arconada; J. A.: J. Astigarraga; A. B.: A. Bertrand; D. B.: D. Buckley; A. C.: A. Camacho; J. E.: J. Escobar; S. J.: S. Jiménez; N. M.: N. Martín; D. M.: D. Moreno; C. N.: C. Noreña; J. P.: J. I. Pino; J. M. R.: J. M. Remón; J. R.: J. Roca; E. R.: E. Rolán; G. T.: G. Tapia.

GENITAL HISTOLOGY

Histological studies of 4 µm serial sections were conducted with special focus on female and male genital systems. For each species, the number and sex of specimens investigated are indicated in the corresponding texts.

Considering the female genitalia of *Islamia globulus*, *I. henrici henrici*, *Milesiana schuelei*, and *Josefus aitanica*, histological evidence of "oriented sperm" in the two sac-like structures on the renal oviduct was obtained. The spermatozoa are arranged with their heads anchored to the cell surface among the cilia of the epithelial cells lining the lumen of the seminal receptacle (Figs. 6–9). This is the typical method for sperm storage in a molluscan seminal receptacle (Thompson & Bebbington, 1969; Giusti & Selmi, 1985; Fretter & Graham, 1994: 303–306) and is morphologically responsible for the whitish-pearly refringence characteristic of this structure. On the other hand, the bursa copulatrix (gametolytic gland) does not contain spermatozoa or contains few, non-oriented spermatozoa (its content is centrally located and never refringent) (see also Ramos et al., 2000; Bodon et al., 2001). Therefore, morphological refringence can be used to distinguish bursa copulatrix from seminal receptacles or even to infer the possible role of sperm storage deposit in widened parts of the renal oviduct (Davis et al., 1992; Ramos et al., 2000, and papers cited therein) when histological evidence is not available. The widened portion of the renal oviduct has a thick, more developed inner epithelium in relation to the portion between proximal and distal seminal receptacles, giving rise to a stretched lumen where the spermatozoids move (Fig. 10). Histological differences along the renal oviduct epithelium are similar to those described for *Tarraconia gasulli* (Ramos et al., 2000) and suggest that the widened part of the oviduct may act as an additional sperm storage. However, we are not able to confirm this hypothesis, because we have not had evidence of oriented spermatozoa in any of the species studied.

Careful analysis of serial sections of males belonging to *I. globulus*, *I. pallida*, *M. schuelei*, and *J. aitanica* reveals that the penis and penial lobe are made up of a thick layer of external muscles beneath the outer epithelium (Figs. 11–15). The inner structure consists of numerous vascular spaces of reticulated connective tissue, denser along the periphery of the penis, with muscle fibres running between them. There was no indication of any glandular tissue either on the penial lobe or on any other part of the penis. This structure is similar to that described for other molluscs (Fretter & Graham, 1994: 302). The undulating penial duct can also be observed throughout the different sections of penis until it enters the nuchal area. Females of several species have a nuchal node or a pseudopenis located on the right side of the head, in a position similar to that of the male penis. These females have fully functional genitalia with mature oocytes in the ovary (Fig. 16).



FIGS. 11-16. Histological sections of the penis and its non-glandular lobe. FIGS. 11, 12: *Islamia globulus* from Sopeira population; FIG. 13: *Milesiana schuelei* from Turrillas population; FIGS. 14, 15: *Josefus aitanica* from Torremanzanas population (type locality); FIG. 16: Female gonad of *I. henrici henrici* from Guadalora River population (Hornachuelos), showing oocytes and yolk. Abbreviations: sd: sperm duct; i: rectum; Oo: oocytes; y: yolk.

SYSTEMATIC DESCRIPTION

Islamia Radoman, 1973

Adriolitorea Radoman, 1973a: 234.
Mienisiella Schütt, 1991: 134–136.

Type Species

Islamia valvataeformis (Möllendorff, 1873: 59) = *Horatia servaini* Bourguignat, 1887 (by original designation). *Horatia servaini* is a junior synonym of *Hydrobia valvataeformis* Möl-

lendorff according to Radoman, 1983, and accepted by Bodon et al., 2001.

Diagnosis

Shell small or very small, ovoid or planispiral, rarely ovate-conic; operculum without peg; central tooth with one or two basal cusps on each side; penis with a well-developed non-glandular lobe on its left side; female genitalia with two seminal receptacles, proximal (SR2) larger and longer than distal (SR1); seminal receptacles located on opposite sides (or positions) on unpigmented renal oviduct; they can arise either close to or rather distant from each other; proximal seminal receptacle (SR2) usually with evident duct and distal (SR1), usually without a duct evident; bursa copulatrix absent.

Islamia globulus (Bofill, 1909)

Amnicola globulus Bofill, 1909: 205; 1915: 57, 58, pl. 6, fig. 6; 1917: 35.
Amnicola anatina globulus (Bofill, 1909) – Bofill & Haas, 1920: 50, 57, pl. III, figs. 19, 20.
Amnicola similis (Draparnaud) – Haas, 1929: 408, 409, fig. 163.
Pseudamnicola similis globulus (Bofill, 1909) – Altimira, 1960: 10; 1963: 16.
Neohoratia globulus globulus (Bofill, 1909) – Boeters, 1988: 214, figs. 137–144, 151–155, 163–170, pl. 2, fig. 22; Bech, 1990: 61.
Islamia globulus globulus (Bofill, 1909) – Bodon et al., 2001: 179, figs. 195–200; Bodon & Cianfanelli, 2002: 20.

Type Locality

Font del Sot del Pinell, close to Portellet del Montsech, Lérida, U.T.M.: GC16.

Material Examined

Type material: A lot containing 41 syntypes (dried) of *A. globulus* collected by Artur Bofill at type locality were deposited in the MZB (Bofill, 1917), catalogue number: 80-1589. The specimen illustrated in Figs. 18, 23, 25, 27, 30, 33, is here designated lectotype (ICZN, 1999: Art. 74.7). The remaining syntypes are therefore paralectotypes. Lectotype (MZB 80-1589a) and 29 paralectotypes from this lot are in the MZB collections and 9 in the MNCN collections with n° MNCN 15.05/46546. The second lot with around 1,000 syntypes (dried) is in the MZB collections (MZB 80-1628).

Other populations examined: This species is widely distributed in the provinces of Lérida and Huesca (Fig. 17). Boeters (1988) also

FIG. 17. Map of localities of the genera *Islamia*, *Milesiana* and *Josefus* in the Iberian Peninsula.

cited it from Gerona, although we cannot confirm these data so far. One lot from Font La Figuereta (Lérida) population kept in the MZB (80-1629) was also examined and compared with that from the same locality kept in the MNCN (15.05/46540). Five specimens (ethanol) from Laguarta population were donated to the MZB (n° 2002-0537).

Localities

Spring in Amargosa, Aristot, Lérida, UTM: 31TCG871948, 14 March 1999, B. A., MNCN 15.05/46527 (ethanol and frozen material); Blanca spring, Vilanova de Meya, Lérida, UTM: 31TCG371551, 25 Feb. 1986, J. R., MNCN 15.05/46528 (ethanol, SEM preparation and frozen material); La Argentería spring, Baix Pallars, Lérida, UTM: 31TCG381842, 2 Oct. 1986, J. R., MNCN 15.05/46529 (ethanol and SEM preparation); El Regué spring, Vilanova de Meya, Lérida, UTM: 31TCG304539, 27 Feb. 1986, J. R., MNCN 15.05/46530 (ethanol); La Fayeda spring, Abella de la Conca, Lérida, UTM: 31TCG475668, 10 Oct. 1986, J. R., MNCN 15.05/46531 (ethanol); Fontanet spring, Abella de la Conca, Lérida, UTM: 31TCG4269, 14 March, 1999, B.A., MNCN 15.05/46593 (ethanol and frozen material); Les Greixes spring, Sant Esteve de La Sarga, Lérida, UTM: 31TCG126635, 8 May 1986, J. R., MNCN 15.05/46532 (ethanol); Blanca spring, Gabet de la Conca, Lérida, UTM: 31TCG301658, 13 May 1986, J. R., MNCN 15.05/46533 (ethanol); D'Arcallo spring, Baix Pallars, Lérida, UTM: 31TCG482818, 29 Sept. 1986, J. R., MNCN 15.05/46534 (ethanol); La Sarga spring, Gabet de La Conca, Lérida, UTM: 31TCG375567, 26 Feb. 1986, J. R., MNCN 15.05/46535 (ethanol); Freda spring, Abella de la Conca, Lérida, UTM: 31TCG473677, 10 May 1986, J. R., MNCN 15.05/46536 (ethanol), 14 March 1999, B. A., MNCN 15.05/46616 (ethanol and frozen material); Freda spring de Casa Pallas, Arén, Lérida; UTM: 31TCG065908, 28 March 1987, J. R., MNCN 15.05/46537 (ethanol); Bordons spring, Arén, Huesca, UTM: 31TCG085881, 31 March 1987, J. R., MNCN 15.05/46538 (ethanol); Adraén, Cadí mountains, Lérida, UTM.: 31TCG767817, 15 Feb. 1998, A. B., MNCN 15.05/46539 (ethanol and SEM preparation); 15 March 1999, B. A., MNCN 15.05/46541 (ethanol and frozen material); La Figuereta spring, Alós de Balaguer, Lérida, UTM: 31TCG253439, 11 March 1986, J. R., MNCN 15.05/46540 (ethanol); Les Bulles spring, Isona, Lérida, UTM: 31TCG371667, 8 May 1986, J. R., MNCN 15.05/46594; Laguarta, Huesca, UTM: 30TYM374998, 12 April 1995; B. A., MNCN 15.05/46542 (ethanol and SEM preparation); 26 Oct. 1995, B. A. & E. R., MNCN 15.05/46543 (ethanol, SEM preparation and frozen material); Grima spring, Gistain, Huesca, UTM: 31TBH799184, 13 April 1995, B. A., MNCN 15.05/46544 (ethanol); Sopeira spring, Huesca, UTM: 31TCG1487, 24 July 1991, R. A., D. M., J. M. R., MNCN 15.05/46545 (ethanol and SEM preparation).

Material Examined for Morphometry and Histology

Shell and anatomical measurements (Tables 1, 3–7) correspond to populations from Lérida and Huesca: Operculum and radular measurements (Tables 2, 4) to Huesca (see table captions). Male and females studied and measured were collected in the following months: Feb., March, April, May, July, and Oct. For histology, four females and three males were studied from a spring in Sopeira, Huesca (July 1991), and one female from Laguarta, Huesca (Oct. 1995).

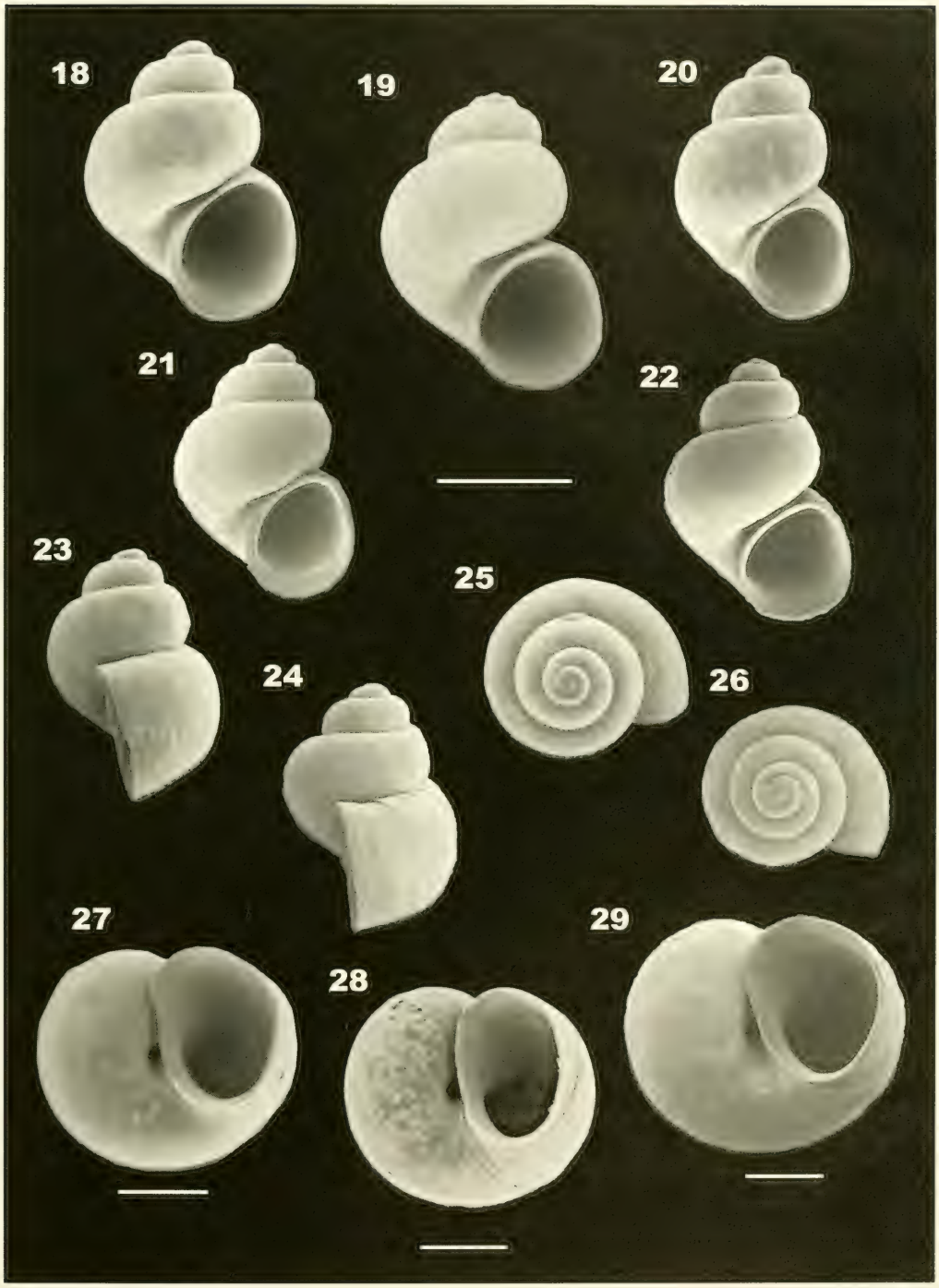
Diagnosis

Shell ovate-conic, body whorl narrow; operculum ovate; central tooth of radula with a single basal cusp on each side; ctenidium well developed; short pleuro-subesophageal connective; esophagus running straight underneath cerebral commissure; bean-shaped prostate gland; big penis, usually black pigmented, with one large, unpigmented non-glandular lobe, commonly protruding from the tip of penis; pyriform and pedunculated proximal seminal receptacle (SR2) and small, elongated, sessile distal seminal receptacle (SR1); receptacles emerge distinctly separated from each other.

Description

(Figs. 18–29, 30–35, 42–49; Tables 1–7; Bodon et al., 2001: figs. 195–200)

Shell: Shell ovate-conic, 4.1 whorls; sutures deep, aperture oval, slightly prosocline; peristome complete, slightly thickened at columelar margin, slightly reflected at lower and columelar margin; body whorl very narrow, over $^{5}/_{7}$ of the total shell length; protoconch consisting of 1.5 whorls; protoconch width and width of the nucleus are 380 µm and 140 µm, respectively (Figs. 30–35); protoconch pitted; umbilicus narrow, 130 µm



FIGS. 18–29. Shells of *Islamia globulus*. FIGS. 18, 23, 25, 27: Lectotype (MZB 80-1589a); FIG. 19: Paralectotype (MZB 80-1589b); FIG. 20: Paralectotype (MNCN 15.05/46546); FIG. 28: Paralectotype (MZB 80-1589c); FIGS. 21, 24, 26, 29: Shells from Laguarta; FIG. 22: Shell from Sopeira population. Scale bar = 1 mm (FIGS. 18–26); 500 µm (FIGS. 27–29).

TABLE 1. Shell measurements (in mm) of type localities of several *Islamia* Iberian species, except for *I. globulus* populations (1–4). 1 - Sopeira spring, Sopeira, Huesca; 2 - Vilanova de Meia, Lérida; 3 - Sant Esteve de La Sarga, Lérida; 4 - Alós de Balaguer, Lérida. *I. lagari* (5). *I. ateni* (6). *I. pallida* (7). *I. h. henrici* (8) and *I. h. giennensis* (9).

	1 Mean ± SD; CV (Max-Min) (n = 30)	2 Mean ± SD; CV (Max-Min) (n = 19)	3 Mean ± SD; CV (Max-Min) (n = 8)	4 Mean ± SD; CV (Max-Min) (n = 7)	5 Mean ± SD; CV (Max-Min) (n = 6)	6 Mean ± SD; CV (Max-Min) (n = 9)	7 Mean ± SD; CV (Max-Min) (n = 8)	8 Mean ± SD; CV (Max-Min) (n = 15)	9 Mean ± SD, CV (Max-Min) (n = 7)
SL	1.93 ± 0.12; 0.06 (2.26-1.68)	1.94 ± 0.10; 0.05 (2.26-1.68)	2.18 ± 0.11; 0.05 (2.32-2.02)	1.85 ± 0.14; 0.08 (2.00-1.56)	1.38 ± 0.06; 0.05 (1.43-1.28)	1.70 ± 0.20; 0.12 (2.20-1.54)	1.10 ± 0.19; 0.17 (1.41-0.81)	0.84 ± 0.07; 0.09 (1.00-0.73)	0.85 ± 0.08; 0.09 (1.00-0.75)
SW	1.39 ± 0.09; 0.06 (1.56-1.26)	1.44 ± 0.09; 0.06 (1.60-1.32)	1.47 ± 0.08; 0.06 (1.64-1.36)	1.39 ± 0.11; 0.08 (1.60-1.26)	1.21 ± 0.09; 0.07 (1.35-1.08)	1.09 ± 0.11; 0.10 (1.37-0.97)	1.26 ± 0.21; 0.16 (1.58-1.04)	1.07 ± 0.12; 0.11 (1.28-0.88)	1.09 ± 0.06; 0.06 (1.15-0.97)
SL/SW	1.38 ± 0.07; 0.05 (1.56-1.26)	1.35 ± 0.07; 0.05 (1.47-1.27)	1.37 ± 0.07; 0.05 (1.47-1.22)	1.34 ± 0.10; 0.07 (1.45-1.18)	1.1 4 ± 0.05; 0.04 (1.19-1.05)	1.56 ± 0.06; 0.04 (1.64-1.45)	0.88 ± 0.09; 0.11 (1.03-0.72)	0.78 ± 0.04; 0.05 (0.84-0.72)	0.78 ± 0.06; 0.08 (0.91-0.71)
AH	0.97 ± 0.06; 0.06 (1.10-0.86)	0.99 ± 0.05; 0.05 (1.08-0.90)	1.04 ± 0.07; 0.07 (1.16-0.96)	0.93 ± 0.04; 0.04 (1.00-0.88)	0.79 ± 0.06; 0.07 (0.85-0.70)	0.87 ± 0.08; 0.09 (1.05-0.80)	0.68 ± 0.10; 0.15 (0.84-0.54)	0.61 ± 0.04; 0.07 (0.70-0.53)	0.59 ± 0.02; 0.04 (0.61-0.55)
LBW	1.51 ± 0.08; 0.05 (1.72-1.38)	1.54 ± 0.07; 0.05 (1.66-1.36)	1.75 ± 0.14; 0.08 (1.94-1.56)	1.45 ± 0.12; 0.08 (1.60-1.20)	1.18 ± 0.06; 0.05 (1.23-1.08)	1.39 ± 0.15; 0.11 (1.77-1.24)	0.90 ± 0.17; 0.19 (1.16-0.62)	0.72 ± 0.05; 0.08 (0.81-0.61)	0.74 ± 0.07; 0.10 (0.87-0.63)
WBW	1.17 ± 0.07; 0.06 (1.04-0.78)	1.22 ± 0.07; 0.06 (1.32-1.08)	1.29 ± 0.06; 0.05 (1.42-1.20)	1.21 ± 0.04; 0.03 (1.28-1.16)	0.95 ± 0.04; 0.05 (1.00-0.88)	0.96 ± 0.10; 0.10 (1.20-0.87)	0.96 ± 0.18; 0.18 (1.26-0.71)	0.73 ± 0.08; 0.11 (0.88-0.58)	0.74 ± 0.05; 0.06 (0.80-0.65)
AL	0.92 ± 0.06; 0.06 (1.04-0.78)	0.96 ± 0.06; 0.06 (1.10-0.86)	1.00 ± 0.06; 0.06 (1.12-0.94)	0.90 ± 0.07; 0.08 (1.00-0.80)	0.59 ± 0.03; 0.05 (0.62-1.55)	0.70 ± 0.07; 0.11 (0.83-0.59)	0.59 ± 0.12; 0.21 (0.78-0.45)	0.56 ± 0.05; 0.09 (0.65-0.46)	0.50 ± 0.03; 0.07 (0.57-0.47)
AW	0.78 ± 0.06; 0.07 (0.96-0.68)	0.84 ± 0.06; 0.07 (0.96-0.72)	0.87 ± 0.05; 0.06 (0.96-0.82)	0.79 ± 0.04; 0.06 (0.84-0.72)	0.72 ± 0.03; 0.04 (0.75-0.68)	0.66 ± 0.07; 0.11 (0.83-0.60)	0.58 ± 0.10; 0.18 (0.71-0.43)	0.51 ± 0.04; 0.09 (0.58-0.41)	0.46 ± 0.02; 0.05 (0.50-0.42)
WPW	0.7 4± 0.04; 0.05 (0.84-0.66)	0.76 ± 0.04; 0.06 (0.84-0.68)	0.81 ± 0.05; 0.06 (0.88-0.74)	0.76 ± 0.04; 0.05 (0.80-0.70)	0.95 ± 0.04; 0.05 (1.00-0.88)	0.62 ± 0.07; 0.11 (0.77-0.57)	0.48 ± 0.09; 0.19 (0.61-0.34)	0.52 ± 0.03; 0.06 (0.55-0.47)	0.34 ± 0.03; 0.10 (0.37-0.30)
WAW	0.39 ± 0.04; 0.10 (0.50-0.32)	0.39 ± 0.04; 0.10 (0.44-0.32)	0.41 ± 0.03; 0.07 (0.46-0.38)	0.39 ± 0.04; 0.10 (0.44-0.32)	0.59 ± 0.03; 0.05 (0.62-1.55)	0.30 ± 0.05; 0.15 (0.40-0.26)	0.21 ± 0.05; 0.23 (0.28-0.13)	0.21 ± 0.02; 0.11 (0.23-0.17)	0.14 ± 0.01; 0.08 (0.15-0.12)
NSW	4.10 ± 0.25; 0.06 (4.75-3.50)	4.42 ± 0.17; 0.05 (4.50-4.00)	4.28 ± 0.21; 0.05 (4.50-4.00)	4.18 ± 0.19; 0.05 (4.50-4.00)	0.72 ± 0.03; 0.04 (0.75-0.68)	4.03 ± 0.08; 0.02 (4.25-4.00)	3.50 ± 0.20; 0.06 (3.75-3.25)	3.43 ± 0.25; 0.07 (3.75-3.00)	3.42 ± 0.12; 0.03 (3.50-3.25)

TABLE 2. Operculum measurements (in mm) of *Islamia* Iberian species. All populations from type locality except specimens of *I. globulus* (1) belonging to Laguarta population (Huesca). *I. ateni* (2), *I. pallida* (3), *I. h. henrici* (4) and *I. h. giennensis* (5).

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3 Mean ± SD; CV (Max-Min)	4 Mean ± SD; CV (Max-Min)	5
OL	0.88 ± 0.04; 0.05 (0.96-0.82) (n = 9)	0.58 ± 0.02; 0.03 (0.61-0.56) (n = 4)	0.45 ± 0.03; 0.07 (0.47-0.42) (n = 2)	0.52 ± 0.01; 0.02 (0.54-0.50) (n = 5)	0.55 (n = 1)
OW	0.41 ± 0.01; 0.06 (0.73-0.61) (n = 9)	0.41 ± 0.02; 0.06 (0.44-0.39) (n = 4)	0.38 ± 0.01; 0.02 (0.39-0.38) (n = 2)	0.43 ± 0.02; 0.06 (0.48-0.41) (n = 5)	0.45 (n = 1)
OLWL	0.41 ± 0.01; 0.03 (0.43-0.40) (n = 4)	0.32 ± 0.01; 0.03 (0.32-0.31) (n = 2)	0.16 (n = 1)	0.17 ± 0.02; 0.13 (0.20-0.15) (n = 5)	0.15 (n = 1)
OLWW	0.28 ± 0.04; 0.03 (0.33-0.24) (n = 4)	0.20 ± 0.04; 0.19 (0.22-0.17) (n = 2)	0.10 (n = 1)	0.13 ± 0.01; 0.08 (0.14-0.11) (n = 5)	0.13 (n = 1)
NL	0.33 ± 0.06; 0.20 (0.40-0.24) (n = 4)	0.17 ± 0.02; 0.12 (0.19-0.16) (n = 2)	?	0.27 ± 0.02; 0.10 (0.29-0.23) (n = 5)	0.27 (n = 1)
NW	0.38 ± 0.02; 0.07 (0.42-0.36) (n = 4)	0.20 ± 0.02; 0.12 (0.22-0.18) (n = 2)	0.25 (n = 1)	0.29 ± 0.02; 0.08 (0.32-0.27) (n = 5)	0.30 (n = 1)
OL/OW	1.31 ± 0.06; 0.04 (1.40-1.22) (n = 4)	1.43 ± 0.09; 0.06 (1.51-1.32) (n = 4)	1.17± 0.05; 0.05 (1.21-1.13) (n = 2)	1.20 ± 0.08; 0.06 (1.28-1.08) (n = 5)	1.22 (n = 1)

in diameter (Figs. 27–29). In apical view, shell growth is quite regular and, consequently, the general shell shape is also regular.

Operculum: Pale yellowish, ovate, submarginal nucleus (Figs. 36–38), with a muscle attachment area rounded or oval.

Body: Head scarcely pigmented, with scattered pigment cells around the eye-spots (Fig. 46). External body pigmentation very dark, except last body whorl.

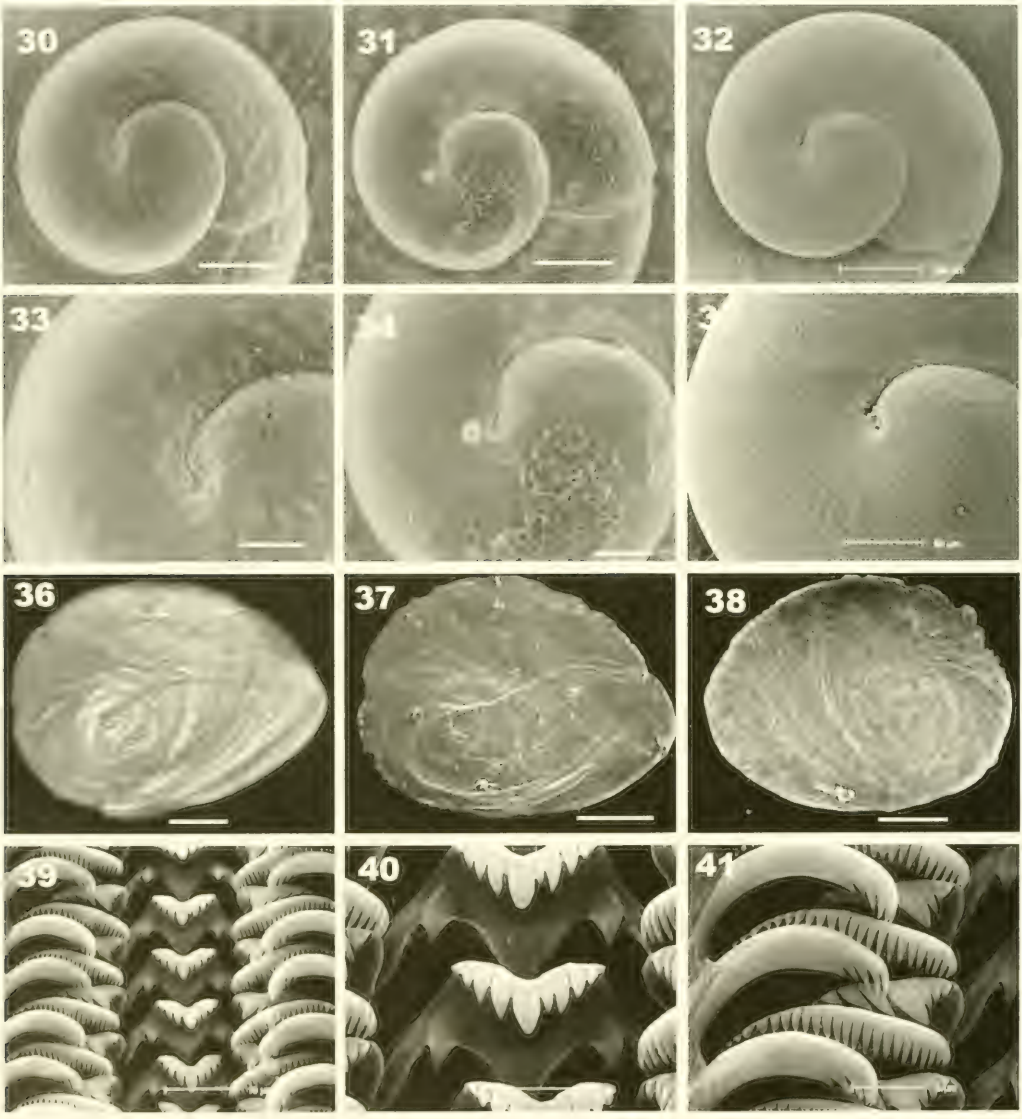
Nervous System: With long pleuro-supraesophageal and short pleuro-subesophageal connectives; RPG ratio is 0.43 (moderately concentrated). Esophagus running straight underneath cerebral commissure (Fig. 42).

Ctenidium: With 12–13 well-developed lamellae (Fig. 43). Occupying nearly entire length of pallial cavity. Osphradium length two to three times longer than its width (Table 3).

TABLE 3. Osphradium measurements (in mm) of several *Islamia* Iberian species. All populations from type localities except for *I. globulus* (1–2): 1 - Gabet de la Conca (La Sarga spring), Lérida; 2 - Sopeira spring, Huesca. *I. ateni* (3), *I. pallida* (4), *I. h. henrici* (5) and *I. h. giennensis* (6).

	1 Mean ± SD; CV (Max-Min) (n = 2)	2 Mean ± SD; CV (Max-Min) (n = 2)	3 Mean ± SD; CV (Max-Min) (n = 13)	4 Mean ± SD; CV (Max-Min) (n = 3)	5 Mean ± SD; CV (Max-Min) (n = 4)	6 Mean ± SD; CV (Max-Min) (n = 3)
Os L	0.19 ± 0.04; 0.23 (0.22-0.16)	0.33 ± 0.01; 0.03 (0.34-0.32)	0.15 ± 0.01; 0.07 (0.16-0.14)	0.12 ± 0.01; 0.05 (0.13-0.12)	0.13 ± 0.02; 0.12 (0.16-0.12)	0.17 ± 0.04; 0.22 (0.21-0.14)
Os W	0.07 ± 0.01; 0.11 (0.07-0.06)	0.08 ± 0.01; 0.07 (0.08-0.07)	0.08 ± 0.01; 0.11 (0.09-0.07)	0.06 ± 0.01; 0.20 (0.07-0.05)	0.08 ± 0.01; 0.07 (0.08-0.07)	0.08 ± 0.02; 0.24 (0.11-0.07)

Stomach – Radula: Stomach length greater than width (Table 5); style sac protruding anteriorly into the intestinal loop (Fig. 44); rectum U-shaped, sometimes bending towards anterior portion of body (Fig. 45). Radula (Table 4) small (17%) relative to maximum shell dimension; central tooth (Figs. 39, 40) with a single basal cusp on each side; distance between cusps is approximately 11 µm; central denticle long and wide, followed on each side by four small denticles in decreasing order of size; lateral teeth with 3–4 denticles on each side of a central one (Fig. 41).



FIGS. 30–41. Protoconch, operculum and radula of *Islamia globulus*. FIGS. 30, 33: Lectotype (MZB 80-1589a); FIGS. 31, 34: Paralectotype (lost specimen); FIGS. 32, 35, 36, 39–41: Shells, opercula and radula from Laguarta population; FIG. 38: Operculum from Sopeira population; FIGS. 30–35: Protoconch and microsculpture; FIGS. 36, 37: Inner side of the operculum; FIG. 38: Outer side of the operculum; FIG. 39: Transverse rows; FIG. 40: Central teeth; FIG. 41: Lateral, outer and inner marginal teeth. Scale bar = 100 µm (FIGS. 30–32); 50 µm (FIGS. 33–35); 200 µm (FIGS. 36–38); 10 µm (FIG. 39); 5 µm (FIGS. 40, 41).

TABLE 4. Radula formulae and measurements (in mm) of *Islamia* Iberian species. *I. globulus* (1) from Laguarta population. *I. ateni* (2) and *I. h. henrici* (3) populations from type localities.

	1	2	3
Central teeth	4+C+4/1-1	5+C+4(5)/1-1	4+C+4/2-2
Central teeth width	~ 9 µm	~ 7 µm	~ 5.6 µm
Left lateral teeth	4+C+3	6+C+3	5+C+3
Inner marginal teeth	~ 24 cusps	~ 24 cusps	~ 24 cusps
Outer marginal teeth	~ 6 cusps	~ 10 cusps	~ 9 cusps
Radula length	~ 345 µm	~ 364 µm	~ 193 µm
Radula width	~ 58 µm	~ 59 µm	~ 46 µm
Number of rows	~ 50	~ 62	?

Male Genitalia: With bean-shaped prostate gland (Table 6) leaning towards the posterior part of the rectal loop (Fig. 45); approximately $^1/_3$ of prostate gland extending into pallial cavity; first lobes of testis spilling over onto posterior chamber of stomach and sometimes reaching anterior chamber; penis large, usually darkly pigmented, with one large unpigmented glandular lobe located in medial position (Figs. 46, 47); penial duct in central position, at base, then running straight to penis tip.

Female Genitalia: Renal oviduct makes a wide circle that overlies the albumen gland (Fig. 48); almost $^2/_3$ of the oviduct glands (albumen + capsule glands) lie inside pallial cavity; oviduct glands (albumen + capsule glands) usually are not narrow, although some females have a discrete narrowing at their outer edge; albumen gland larger than capsule gland (Fig. 48); proximal seminal receptacle (SR2) generally pyriform, pedunculated (Fig. 49); distal seminal receptacle (SR1) smaller, elongated, sessile; both a good distance from each other on opposite positions on renal oviduct; renal oviduct widening posterior to SR2.

Discussion

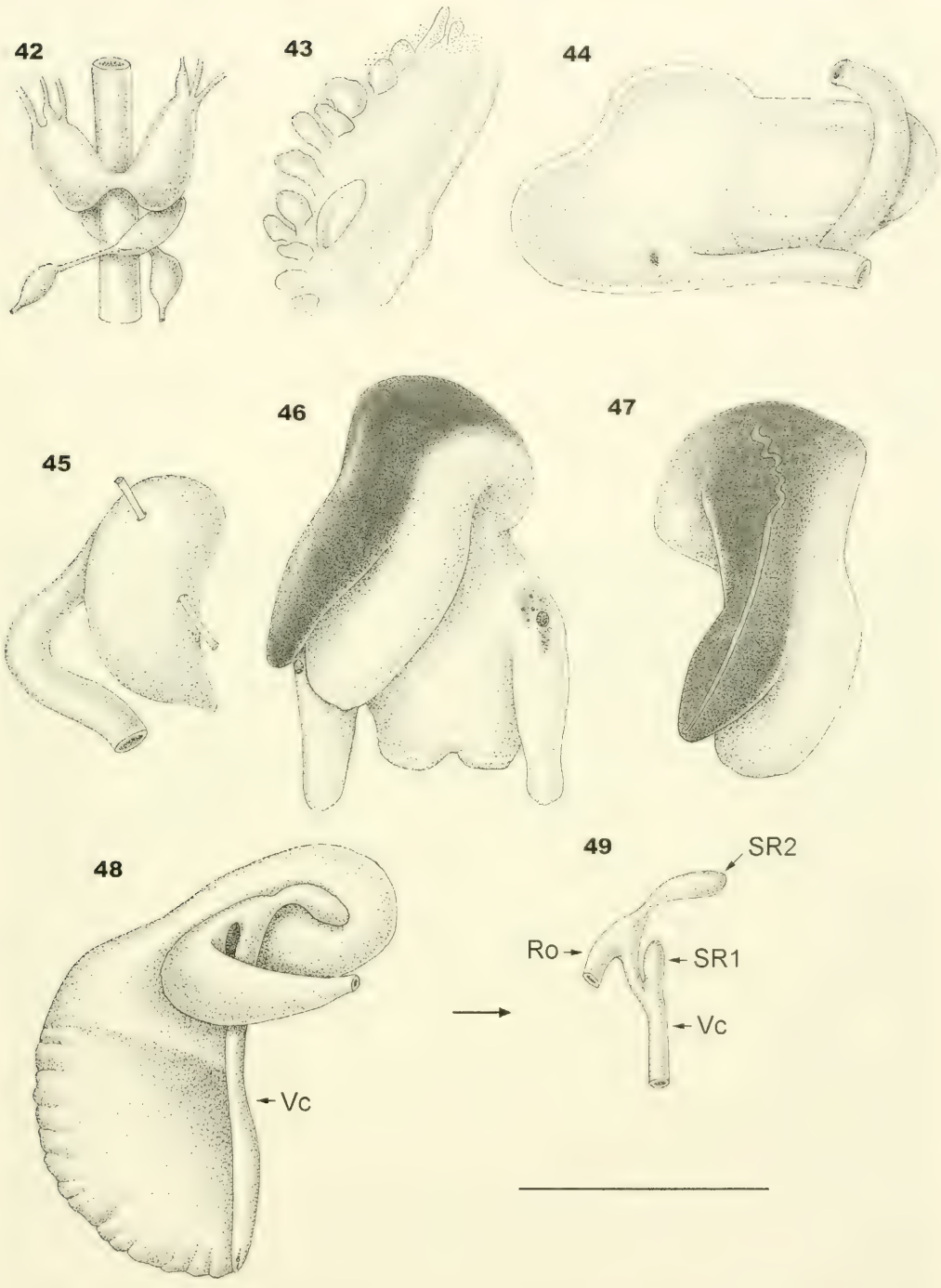
Until now, no lectotype of *Amnicola globulus* has been designated. Since 1920 (Bofill & Haas, 1920), the type material of this species has been referred to in the literature as "unknown". We traced the type material in the MZB collection and found it consists of two lots, one containing 41 specimens (MZB 80-1589) and the other over 1,000 specimens (MZB 80-1628). In the species description (Bofill, 1909) and in later papers, he mentions that "this species was extremely abundant". The first lot contains the original label and is

TABLE 5. Digestive system measurements (in mm) of *Islamia* Iberian species. All populations from type localities, except for *I. globulus* (1) (Sopeira spring, Huesca). *I. ateni* (2), *I. pallida* (3), *I. h. henrici* (4) and *I. h. giennensis* (5).

	1 Mean ± SD; CV (Max-Min) (n = 2)	2 Mean ± SD; CV (Max-Min) (n = 3)	3 Mean ± SD; CV (Max-Min) (n = 3)	4	5 Mean ± SD; CV (Max-Min) (n = 3)
Ss L	0.42 ± 0.01; 0.02 (0.42-0.41)	0.27 ± 0.01; 0.02 (0.28-0.27)	0.19 ± 0.02; 0.11 (0.21-0.17)	0.29 (n = 1)	0.27 ± 0.01; 0.05 (0.29-0.27)
Ss W	0.33 ± 0.04; 0.11 (0.36-0.31)	0.24 ± 0.01; 0.04 (0.25-0.23)	0.18 ± 0.02; 0.12 (0.20-0.16)	0.28 (n = 1)	0.21 ± 0.03; 0.16 (0.25-0.19)
St L	0.46 ± 0.17; 0.37 (0.58-0.34)	0.40 ± 0.07; 0.18 (0.45-0.32)	0.29 ± 0.04; 0.15 (0.34-0.26)	0.26 (n = 1)	0.33 ± 0.02; 0.07 (0.36-0.32)
St W	0.47 ± 0.04; 0.09 (0.50-0.44)	0.41 ± 0.01; 0.02 (0.42-0.40)	0.28 ± 0.04; 0.14 (0.31-0.23)	0.19 (n = 1)	0.28 ± 0.04; 0.16 (0.33-0.24)

TABLE 6. Male genitalia measurements (in mm) of *Islamia* Iberian species: *I. globulus* (1–10) from the following localities: 1 - Sopeira spring, Huesca; 2 - San Esteve de la Sarga (Les Greixes spring), Lérida; 3 - Baix Pallars (La Argenteria spring), Lérida; 4 - Gabet de la Conca (La Sarga spring), Lérida; 5 - Abella de la Conca (La Fayeda spring), Lérida; 6 - Vilanova de Meya (Blanca spring), Lérida; 7 - Alós de Balaguer (La Figuereta spring), Lérida; 8 - Arén (Bordons spring), Lérida; 9 - Huesca, Huesca; 10 - Laguarta, Huesca. The rest of the populations from type localities: *I. ateni* (11), *I. pallida* (12), *I. h. henrici* (13) and *I. h. giennensis* (14).

	1 Mean ± SD; CV (Max-Min)	2	3	4 Mean ± SD; CV (Max-Min)	5	6	7	8	9	10	11	12 Mean ± SD; CV (Max-Min)	13 Mean ± SD; CV (Max-Min)	14 Mean±SD; CV (Max-Min)
r L	0.44 (n = 1)			0.39 (n = 1)			0.46 (n = 1)				0.32 (n = 1)	0.29 (n = 1)	0.50 (n = 1)	0.51 ± 0.12; 0.23 (0.59-0.42) (n = 2)
Pr W	0.27 (n = 1)			0.18 (n = 1)			0.27 (n = 1)				0.27 (n = 1)	0.19 (n = 1)	0.21 (n = 1)	0.23 ± 0.03; 0.13 (0.25-0.21) (n = 2)
P L	0.70 ± 0.17; 0.25 (0.82-0.58) (n = 2)	0.65 (n = 1)	0.87 (n = 1)	0.62± 0.16; 0.26 (0.74-0.51) (n = 2)	0.76 (n = 1)	0.67 (n = 1)	0.77 (n = 1)	0.55 (n = 1)	0.75 (n = 1)	0.78 (n = 1)	0.96 (n = 1)	0.47 ± 0.06; 0.14 (0.52-0.42) (n = 2)	0.66 ± 0.23; 0.24 (0.82-0.50) (n = 2)	0.76 ± 0.19; 0.24 (1.13-0.57) (n = 7)
P W	0.16 ± 0.04; 0.27 (0.19-0.13) (n = 2)		0.24 (n = 1)	0.28 ± 0.06; 0.20 (0.32-0.24) (n = 2)	0.24 (n = 1)	0.15 (n = 1)	0.27 (n = 1)	0.21 (n = 1)	0.15 (n = 1)		0.18 (n = 1)	0.18 ± 0.01; 0.04 (0.09-0.05) (n = 2)	0.16 ± 0.01; 0.09 (0.17-0.15) (n = 2)	0.18 ± 0.04; 0.22 (0.24-0.13) (n = 7)
PL. L	0.32 ± 0.04; 0.12 (0.34-0.29) (n = 2)		0.26 (n = 1)	0.29 ± 0.12; 0.42 (0.37-0.20) (n = 2)	0.38 (n = 1)	0.20 (n = 1)	0.37 (n = 1)	0.37 (n = 1)	0.25 (n = 1)	0.36 (n = 1)	0.35 (n = 1)	0.07 ± 0.03; 0.37 (0.09-0.05) (n = 2)	0.11 ± 0.03; 0.25 (0.13-0.09) (n = 2)	0.15 ± 0.05; 0.03 (0.24-0.08) (n = 7)
Pl. W	0.12 ± 0.05; 0.43 (0.15-0.08) (n = 2)	0.21 (n = 1)	0.15 (n = 1)	0.14 ± 0.04; 0.31 (0.17-0.11) (n = 2)	0.17 (n = 1)	0.10 (n = 1)	0.19 (n = 1)	0.17 (n = 1)	0.12 (n = 1)	0.12 (n = 1)	0.10 (n = 1)	0.10 ± 0.01; 0.10 (0.1-0.10) (n = 2)	0.07 ± 0.02; 0.32 (0.08-0.05) (n = 2)	0.12 ± 0.03; 0.2 (0.15-0.08) (n = 6)
Head length	0.57 ± 0.06; 0.11 (0.62-0.52) (n = 2)	0.61 (n = 1)	0.87 (n = 1)	0.62 (n = 1)	0.60 (n = 1)	0.70 (n = 1)	0.58 (n = 1)	0.64 (n = 1)	0.62 (n = 1)		0.57 (n = 1)	0.39 (n = 1)	0.51 ± 0.09; 0.17 (0.57-0.45) (n = 2)	0.71 ± 0.21; 0.29 (1.14-0.55) (n = 7)
PL./ Head length	1.25 ± 0.44; 0.35 (1.56-0.94) (n = 2)	1.07 (n = 1)	1.00 (n = 1)	0.83 (n = 1)	1.27 (n = 1)	0.95 (n = 1)	1.27 (n = 1)	0.87 (n = 1)	1.21 (n = 1)		1.67 (n = 1)	1.08 (n = 1)	1.35 ± 0.68; 0.50 (1.83-0.87) (n = 2)	1.16 ± 0.33; 0.28 (1.55-0.59) (n = 7)



FIGS. 42–49. Anatomy of *Islamia globulus*. FIG. 42: Partial nervous system; FIG. 43: Osphradium and ctenidium; FIG. 44: Stomach; FIG. 45: Prostate and rectum loop; FIGS. 46, 47: Head of a male and penis; FIG. 48: Anterior female genitalia; FIG. 49: Detail of the seminal receptacles; Abbreviations in text. Scale bar = 500 µm (FIGS. 42–48).

probably made up of a selection of the largest specimens; Bofill's descriptions and illustrations were likely based on this lot. After having determined that the specimens of both lots were conspecific, we realised it would be impossible to identify the illustrated specimens (Bofill, 1915; Bofill & Haas, 1920). We selected a lectotype from this first lot.

Islamia globulus is clearly distinguished from the other *Islamia* species by a combination of characters. Its ovate-conic shell easily distinguishes it from both valvatiform (*I. piristoma*, *I. trichoniana*, etc.) and trochiform species (*I. anatolica*, *I. bunarbasa*). Other important character states include a radula with only one basal cusp on each side and two separated seminal receptacles (SR2 large and pedunculated and SR1 small, elongated and sessile). Differences and similarities with *I. ateni* and between *I. globulus* and *I. lagari* are discussed below.

Islamia lagari (Altimira, 1960)

Pseudamnicola lagari Altimira, 1960: 10, fig. 2.
Neohoratia globulus lagari (Altimira, 1960) – Boeters, 1988: 216, figs. 145, 146, 156, 164, pl. 2, fig. 23; Bech, 1990: 61.
Islamia globulus lagari (Altimira, 1960) – Bodon et al., 2001, 43: 179, figs. 201–206; Bodon & Cianfanelli, 2002: 20.

Type Locality

Sot de Can Parés, Gavá, Barcelona, U.T.M.: 31TDF120720 (Fig. 17).

Material Examined

Type material: Lectotype (shell) of *N. globulus lagari* from the NNM (N° 56466/1) (Figs. 50–54). Five dried specimens in the

FIGS. 50–54. Shells of *Islamia lagari* (NNM 56466/1). Scale bar = 1 mm (FIGS. 50–53).

NHMW (Vienna) (Coll. W. Klemm) (NHMW 79000/K 45087) had a label with the same handwriting as that of lectotype. The text in both labels is the same "*Pseudamnicola lagari* Alt. Can Parés. Gavá. Barcelona. 11–59". In addition, the label in NHMW has number "7" also handwritten, thus suggesting that Altimira probably collected seven specimens in Nov. 1959, one of which has not yet been located. Therefore, the specimens at the NHM should be paralectotypes after designation of the lectotype by Boeters.

Material Examined for Morphometry

Shell measurements (Table 1) correspond to the lectotype and paralectotypes.

Diagnosis

Shell ovate-conic with large and inflated body whorl; operculum ovate; central tooth of radula with a single basal cusp on each side; ctenidium well developed; big penis, black pigmented, with one unpigmented non-glandular lobe located in a subterminal position not protruding from penial tip; pin-like proximal seminal receptacle (SR2) with a long stalk and small, elongated, sessile distal seminal receptacle (SR1); receptacles emerge distinctly separated from one another.

Description
(Figs. 50–54; Table 1)

Shell: Ovate-conic with 3.5 whorls; sutures deep, aperture oval to roundish, slightly prosocline, peristome complete, reflected at lower and columellar margin; body whorl large and inflated, over $^{6}/_{7}$ of the total shell length; protoconch consisting of 1.7 whorls; protoconch width and width of nucleus are 370 µm and 130 µm, respectively (Fig. 54); protoconch pitted; umbilicus narrow, about 80 µm in diameter (Fig. 53), partially covered by reflected columellar lip. In apical view, shell growth is rapid, especially body whorl, which has an inflated appearance.
No specimens were available for anatomical study. Anatomical data are shown in Bodon et al. (2001: figs. 201–206).

Discussion

Islamia globulus and *I. lagari* have been considered both good species and subspecies. The last treatment has prevailed since Boeters (1988) considered both to be subspecies of *Neohoratia globulus*. Based on morphological differences, we propose species status for both taxonomic entities. A detailed anatomical description of *I. globulus* is given here. No ethanol-preserved specimens of *I. lagari* were available for study. Therefore, only dried type material and illustrations from literature have been used to compare this species with *I. globulus*. We used the anatomical descriptions provided by Boeters (1988: figs. 156, 164) and Bodon et al. (2001: figs. 201–206). Morphological differences between *Islamia globulus* and *I. lagari* (Boeters, 1988; Bodon et al., 2001) are based on shell shape, penis size and size and shape of the glandular penial lobe and seminal receptacles.

Shells of *I. globulus* are more compressed laterally and, consequently, are taller and narrower than those of *I. lagari*. The body whorl of *I. globulus* is proportionally smaller (shorter and narrower) than in *I. lagari* (Altimira, 1960). The latter species has an inflated body whorl and a relatively lower spire. The penis of *I. globulus* is larger and has a slightly flatter penial lobe. The free part of penis towards the tip is also flatter, narrower and longer than in *I. lagari*. *Islamia lagari* has a smaller distal seminal receptale (SR1) with a short stalk, which is not evident in *I. globulus*. Proximal seminal receptacle (SR2) of *I. lagari* is less developed than in *I. globulus* and has a longer and more slender stalk.

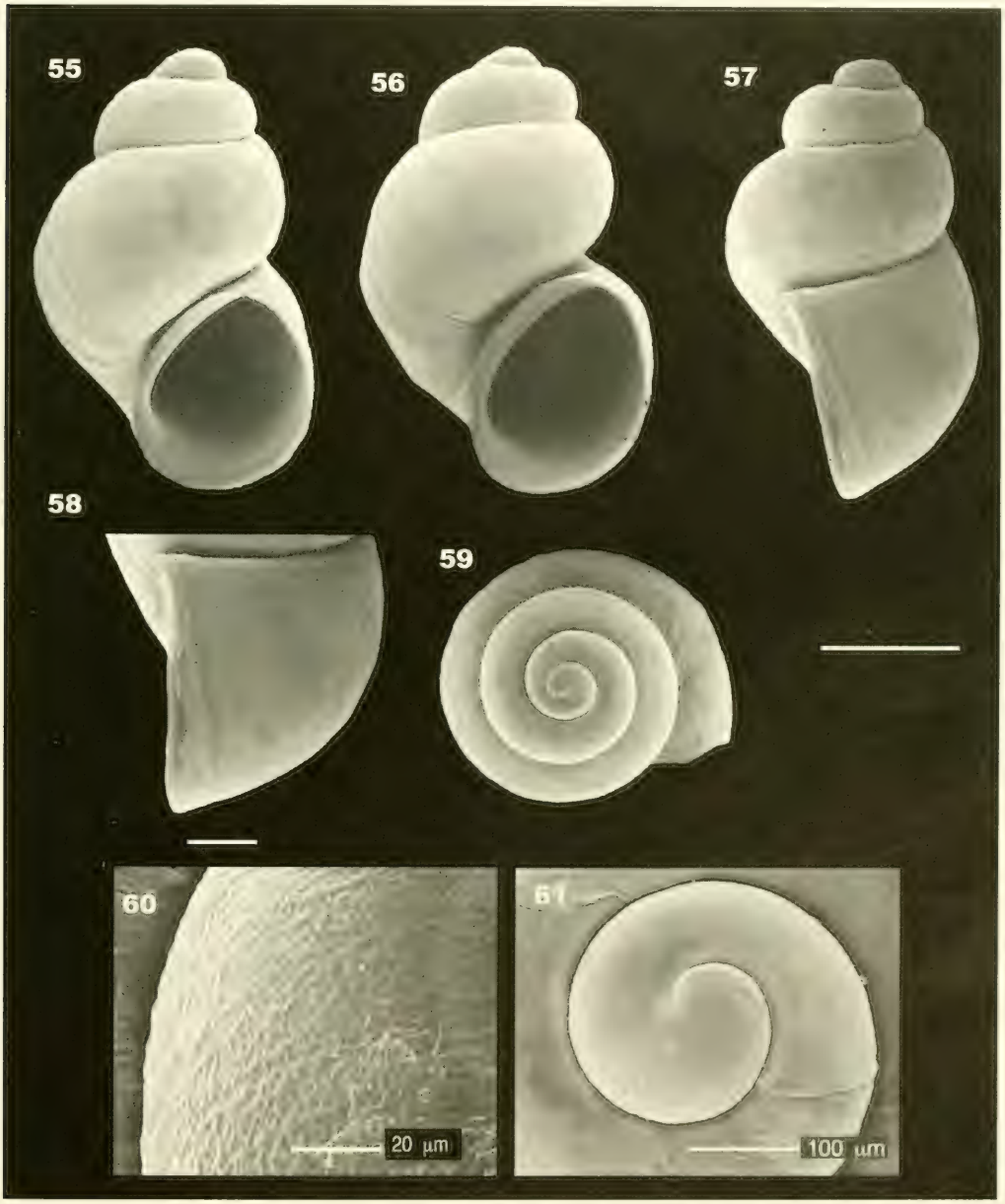
The DFA also confirmed differences between the two species. We analysed nine standard shell measurements from the four *I. globulus* populations and from the *I. lagari* type material. Of the 70 individuals classified, all of the *I. lagari* were correctly classified (100%) and perfectly discriminated from the rest by two highly significant functions (Wilk's lambda = 0.039, F (36.211), $p < 0.0001$). The remaining *I. globulus* individuals were grouped into four overlapping clusters. For the first function, the characters that contributed most of the 83% explained variance were (in order): AL and AH. For the second function the order was: AW, LBW and WBW. Another DFA using all *Islamia* species studied herein yielded similar results. All of the *I. lagari* specimens were correctly classified and definitively discriminated from all the other species (see "Statistical Analysis of *Islamia* species" below and Fig. 138).

Both taxa are allopatric, which does not help clarify their taxonomic status. However, both species are found in two different mountain chains that differ in geological origin and soil

TABLE 7. Female genitalia measurements (in mm) of several *Islamia* Iberian species: *I. globulus* (1–8) from the following populations: 1 - Sopeira spring, Huesca; 2 - Laguarta, Huesca; 3 - Baix Pallars (La Argenteria spring), Lérida; 4 - Gabet de la Conca (La Sarga spring), Lérida; 5 - Abella de la Conca (La Fayeda spring), Lérida; 6 - Vilanova de Meya (Regué spring), Lérida; 7 - Alós de Balaguer (La Figuereta spring), Lérida; 8 - Arén (Bordons spring), Lérida. The rest of the populations from type localities: *I. ateni* (9), *I. pallida* (10), *I. h. henrici* (11) and *I. h. giennensis* (12).

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3	4	5	6	7	8	9 Mean ± SD; CV (Max-Min)	10 Mean ± SD; CV (Max-Min)	11 Mean ± SD; CV (Max-Min)	12 Mean ± SD; CV (Max-Min)
Op L	0.72 (n = 1)	0.81 ± 0.14; 0.17 (0.94-0.67) (n = 3)	0.77 (n = 1)	0.77 (n = 1)	0.90 (n = 1)	0.43 (n = 1)	0.92 (n = 1)	0.56 (n = 1)	0.62 ± 0.02; 0.04 (0.64-0.61) (n = 2)	0.37 ± 0.07; 0.20 (0.42-0.32) (n = 2)	0.56 ± 0.10; 0.18 (0.67-0.47) (n = 3)	0.80 ± 0.10; 0.12 (0.89-0.69) (n = 3)
Op W	0.35 (n = 1)	0.40 ± 0.04; 0.09 (0.43-0.38) (n = 2)	0.28 (n = 1)	0.32 (n = 1)	0.34 (n = 1)	0.23 (n = 1)	0.29 (n = 1)	0.28 (n = 1)	0.25 ± 0.01; 0.03 (0.26-0.24) (n = 2)	0.20 ± 0.02; 0.12 (0.21-0.18) (n = 2)	0.22 ± 0.05; 0.21 (0.28-0.20) (n = 3)	0.21 ± 0.03; 0.17 (0.24-0.17) (n = 3)
Ag. L			0.46 (n = 1)	0.37 (n = 1)	0.42 (n = 1)				0.21 ± 0.03; 0.14 (0.23-0.19) (n = 2)	0.20 (n = 1)	0.22 ± 0.06; 0.26 (0.27-0.15) (n = 3)	0.30 ± 0.03; 0.10 (0.33-0.28) (n = 2)
Cg. L			0.31 (n = 1)	0.39 (n = 1)	0.48 (n = 1)				0.41 ± 0.05; 0.13 (0.45-0.37) (n = 2)	0.12 (n = 1)	0.34 ± 0.05; 0.15 (0.40-0.31) (n = 3)	0.45 ± 0.12; 0.27 (0.53-0.36) (n = 2)
SR1 L	0.18 ± 0.04; 0.23 (0.21-0.15) (n = 2)	0.09± 0.09; 1.07 (0.15-0.02) (n = 2)		0.05 (n = 1)	0.12 (n = 1)		0.06 (n = 1)		0.05 ± 0.01; 0.38 (0.07-0.04) (n = 2)	0.03 (n = 1)	0.06 ± 0.00; 0.10 (0.06-0.05) (n = 3)	0.05 ± 0.01; 0.31 (0.07-0.04) (n = 3)
SR2 L	0.23 ± 0.04; 0.19 (0.26-0.20) (n = 2)	0.18 ± 0.01; 0.05 (0.18-0.17) (n = 2)		0.18 (n = 1)	0.17 (n = 1)		0.18 (n = 1)		0.09 ± 0.00; 0.00 (0.95-0.95) (n = 2)	0.03 (n = 1)	0.09 ± 0.01; 0.11 (0.10-0.08) (n = 3)	0.11 ± 0.05; 0.45 (0.18-0.07) (n = 4)

composition. *Islamia globulus* has a wide geographical distribution in the provinces of Lérida and Huesca (cites in Gerona could not be confirmed). This area is situated in the "Depresión del Ebro". It is of Oligocene origin and is composed of marls and sands on calcareous substrate. At a great distance away, more than 150 km (Fig. 17), *I. lagari* is restricted to a small area in Sierra de Can Parés in the Garraf Massif (Barcelona), on Lower Triassic soils, where limestone, marls and sandstones predominate.



FIGS. 55–61. Topotypes of *Islamia ateni* (MNCN 15.05/46547). FIGS. 55, 56: Frontal view; FIGS. 57, 58: Lateral view; FIG. 59: Spire whorls; FIGS. 60, 61: Protoconch microsculpture. Scale bar = 500 µm (FIGS. 55–57, 59); 200 µm (FIG. 58).

Islamia ateni (Boeters, 1969)

Microna ateni Boeters, 1969: 70, figs. 6–8.
Neohoratia ateni (Boeters, 1969) – Boeters, 1988: 216, figs. 147, 148, 157, 158, 163, 288, pl. 2, fig. 24; Bech, 1990: 62, fig. 11.
Islamia ateni (Boeters, 1969) – Bodon et al., 2001, 43: 178, figs. 189–194; Bodon & Cianfanelli, 2002: 20.

Type Locality

Balneario de San Vicente, Lérida, U.T.M.: CG89 (Fig. 17).

Type Specimens

Holotype in NNM and paratypes NNM/37, SMF 194371/2 and BOE 205 and 206.

Material Examined

The description of this species was made possible by studying topotypical material, kindly provided and deposited in MNCN by H. D. Boeters. There were 13 specimens in alcohol [leg. Boeters coll. 514, 11/9/1972 (Figs. 55–61) MNCN 15.05/46547 (ethanol and SEM preparation)].

Morphometry

All measurements correspond to specimens from the type locality.

Diagnosis

Shell ovate-conic; operculum ovate; central tooth of radula with a single basal cusp on each side; ctenidium well developed; esophagus running straight underneath cerebral commissure; small pear-shaped prostate gland; penis long, unpigmented, with a large, flat, extended. unpigmented non-glandular lobe located near, but not protruding, from its tapered distal end; elongated. pedunculated proximal seminal receptacle (SR2) bending towards distal portion of renal oviduct and small, globular, sessile distal seminal receptacle (SR1); seminal receptacles quite separated from one another.

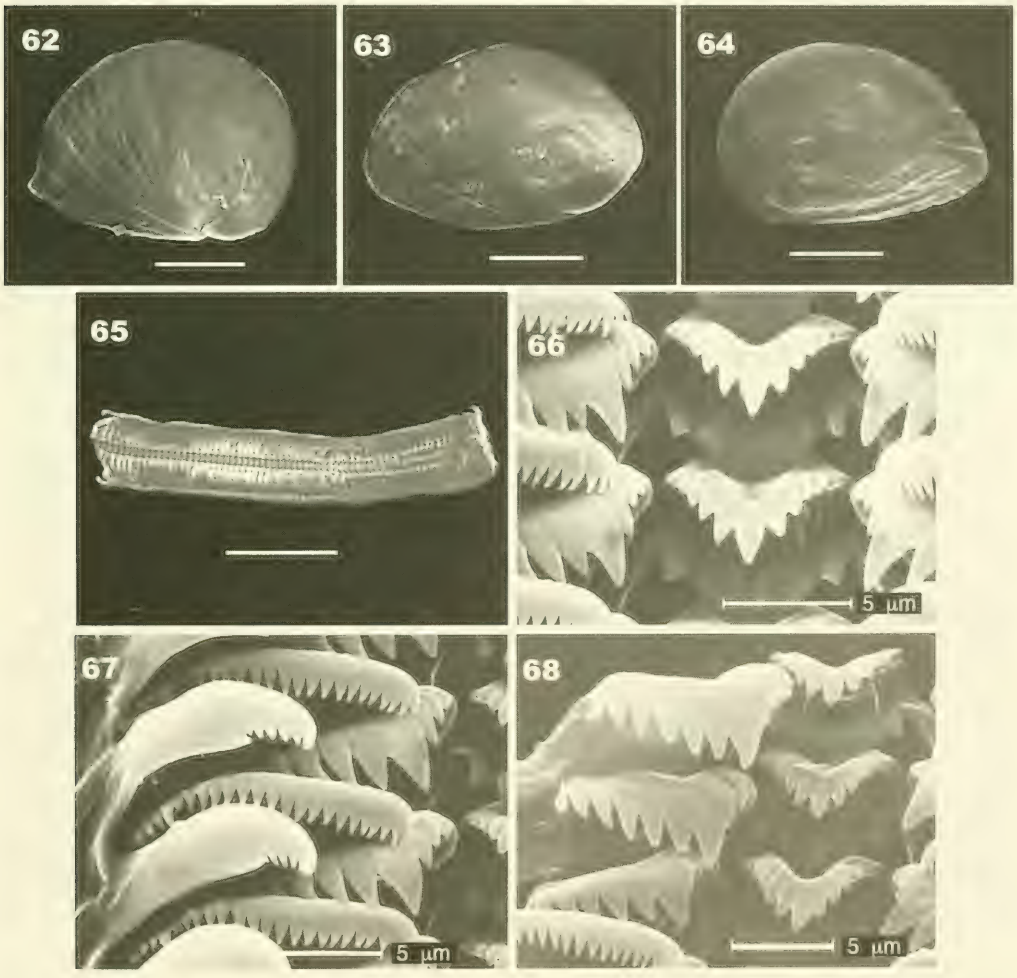
Description
(Bodon et al., 2001: figs. 189–194)

Shell: Ovate-conic, longer than wide, with 4 whorls (Figs. 55–57, 59, Table 1); sutures deep; body whorl occupies more than $^{5}/_{7}$ of total shell length; protoconch pitted (Figs. 60, 61), consisting of 1.5 whorls; protoconch width and width of the nucleus are 280 µm and 120 µm, respectively; last whorl of teleoconch very narrow from apical perspective (Fig. 59); aperture oval, orthocline or slightly prosocline; peristome thin at outer margin and slightly thickened at columellar margin, slightly reflected at lower and columellar margin; umbilicus very narrow; external lip thin (Figs. 57, 58).
Operculum: Yellowish, ovate (Figs. 62–64), with submarginal nucleus; muscle attachment area oval (Fig. 64).
Body: Head dark pigmented from the middle of the tentacles to the eye lobes (Fig. 69); external body pigmentation very dark, except last body whorl.
Nervous System: With long pleuro-supraesophageal connective; no data on pleurosubesophageal connectives were obtained due to the scarcity of specimens available for study; RPG ratio is 0.5 (elongated). Esophagus runs straight underneath the cerebral commissure of the nervous system.
Ctenidium – Osphradium: With approximately 10 lamellae (Fig. 70), occupying $^{3}/_{8}$ of length of pallial cavity. Osphradium oval and intermediate in size (Table 3).
Stomach – Radula: Stomach almost as wide as it is long (Table 5, Fig. 71); style sac protruding anteriorly into intestinal loop; rectum U-shaped (Fig. 70). Radula medium-sized (21%) relative to maximum shell dimension (Table 4, Fig. 65); central tooth with a single basal cusp on each side (Fig. 66); distance between cusps approximately 6.7 µm; central denticle long, sharp, followed on each side by five small denticles in decreasing order of size; cutting edge markedly concave; lateral teeth with 5–6 denticles on each side of central tooth (Figs. 67, 68).
Male Genitalia: Prostate gland small, pear-shaped (Table 6, Fig. 72); vas deferens entering posterior end of prostate, and pallial vas deferens exiting at its middle region, both are relatively close to each other; penis long, unpigmented (Fig. 73), with a large, flat extended, non-glandular lobe near its tapered distal tip; undulating penial duct running along the right portion of penis and becoming straight before opening at penis tip.
Female Genitalia: Distal seminal receptacle (SR1), globular and sessile, smaller than proximal (SR2), which is elongated and pedunculated, bending towards distal portion

of renal oviduct (Fig. 75, Table 7); both seminal receptacles located at a distance from each other on opposite positions of renal oviduct; oviduct glands (albumen + capsule glands) with very weak or no narrowing, capsule gland smaller than albumen gland; renal oviduct forming a wide circle (Fig. 74) overlying albumen gland.

Discussion

Islamia ateni may be differentiated from the remaining European *Islamia* species by its peculiar ovate-conic or bythinelliform shell shape, a very small prostate gland relative to shell length, and by the rather large gap between the two seminal receptacles. A single basal cusp on each side of the central tooth of the radula is a character state shared with *I. valvataeformis*, *I. servaini*, *I. gaiteri*, *I. pusilla* and *I. globulus*. All other species described have two basal cusps. Its morphologically closest species is *I. globulus*. Main characters differentiating both species are related to shell size and shape (that of *I. ateni* are more slender than that of *I. globulus*), shape of the penial lobe (more flattened and less extended in *I. ateni*, never protruding from penis tip), SR2



FIGS. 62–68. Operculum and radula of *Islamia ateni*. FIGS. 62, 63: Outer side of the operculum; FIG. 64: Inner side of the operculum; FIG. 65: Radula; FIG. 66: Central teeth; FIG. 67: Lateral, outer and inner marginal teeth; FIG. 68: Central and lateral teeth. Scale bar = 200 µm (FIGS. 62–64); 100 µm (FIG. 65).

characteristically bending towards distal portion of renal oviduct, and the distance between seminal receptacles, which is longer in *I. ateni*.

Islamia pallida Arconada & Ramos, **n. sp.**

Type Specimens

Holotype MNCN 15.05/46548 (SEM preparation, Fig. 78) and paratypes (Figs. 82, 85, 88, 90, 91) MNCN 15.05/46548, 5 April 1992, D. M. & N. M. (dried material, ethanol and SEM preparation).

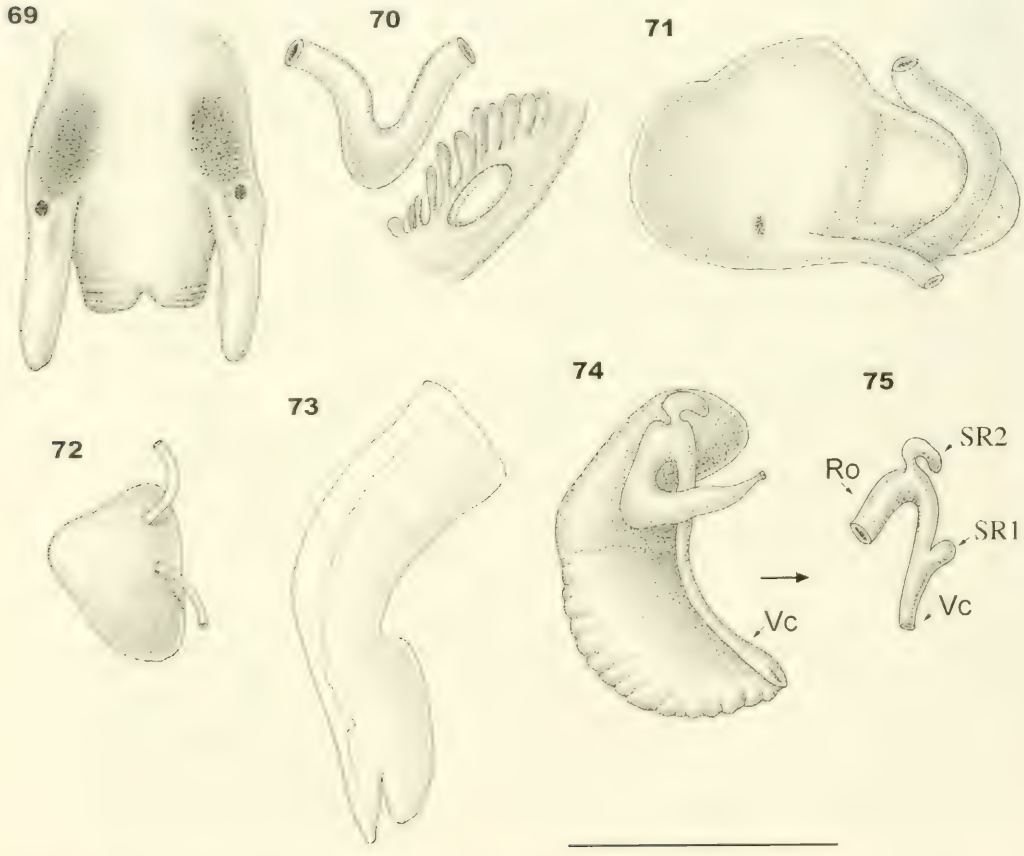
Type Locality

Spring in Patones, Patones de Abajo, Madrid, UTM.: 30TVL603241.

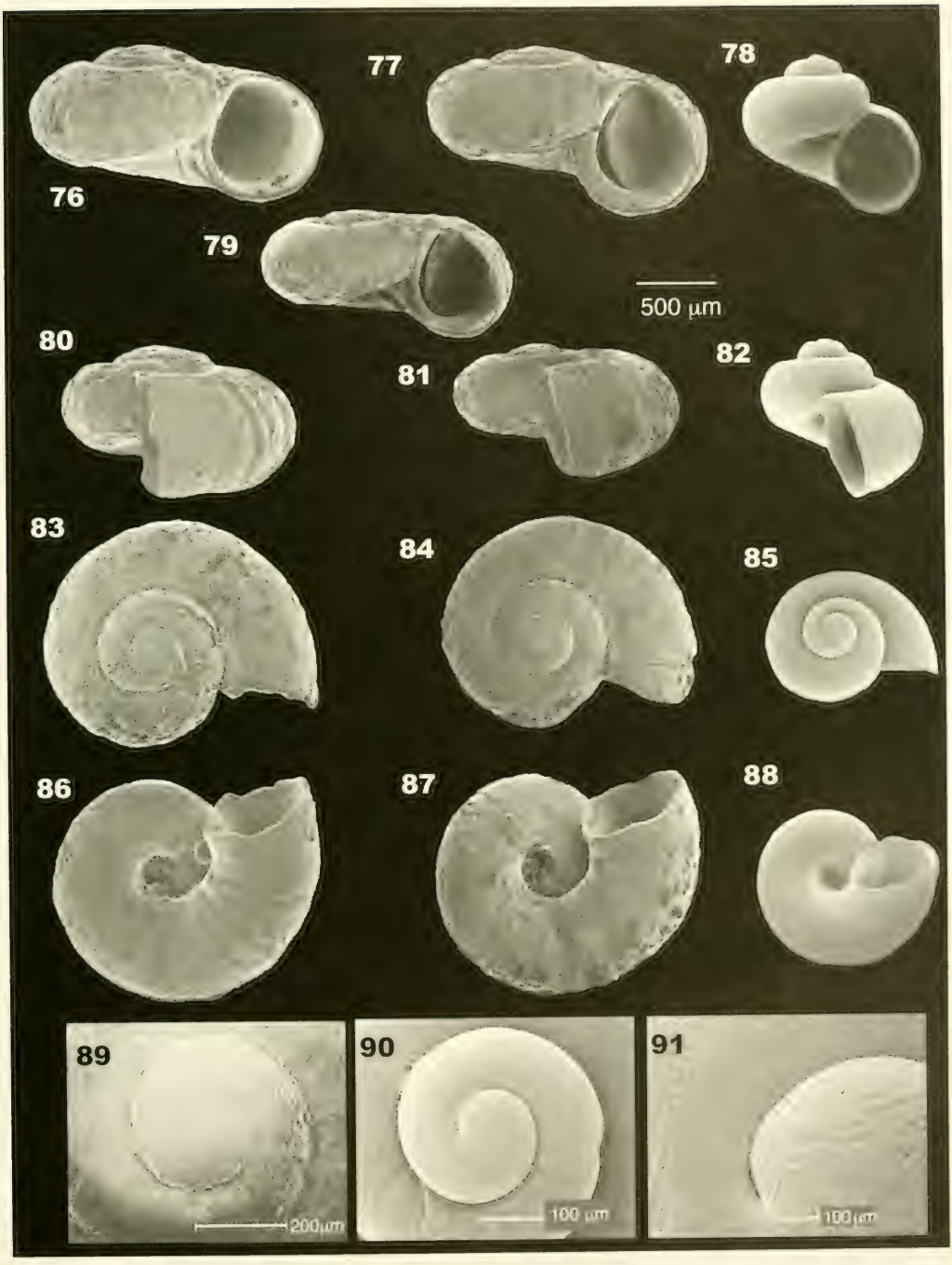
Specimens Examined

The following specimens were also examined for comparative purposes: Lectotype, MHNG (Figs. 76, 80, 83, 86) and paralectotypes, MHNG (Figs. 77, 79, 81, 84, 87, 89) of *Neohoratia* (?) *coronadoi* (Bourguignat, 1870) (originally *Valvata coronadoi*).

Other localities: Province of Madrid (Fig. 17), e.g., Spring in Patones, Patones de Abajo, Madrid (type locality), 29 June 1997, B. A. & D. B., MNCN 15.05/46550 (ethanol material); Jarama River, Patones, Madrid, UTM.: 30TVL5824, 18 Jan. 1989, A. C.; MNCN 15.05/46549 (ethanol); 8 Aug. 1989, A. C.; La Parra channel, Patones, Madrid, UTM.: 30TVL603241, 2 June 1996, B. A. & D. B., MNCN 15.05/46551 (ethanol).



FIGS. 69–75. Anatomy of *Islamia ateni*. FIG. 69: Head pigmentation; FIG. 70: Rectum, osphradium and ctenidium; FIG. 71: Stomach; FIG. 72: Prostate; FIG. 73: Penis; FIG. 74: Anterior female genitalia; FIG. 75: Detail of the seminal receptacles; Abbreviations in text. Scale bar = 500 µm (FIGS. 69–74).



FIGS. 76–91. Shells of *Neohoratia* (?) *coronadoi* and *Islamia pallida*. FIGS. 76, 80, 83, 86: Lectotype of *Neohoratia* (?) *coronadoi* (MHNG); FIGS. 77, 79, 81, 84, 87, 89: Paralectotypes of *Neohoratia* (?) *coronadoi* (MHNG); FIG. 78: Holotype of *I. pallida* (MNCN 15.05/46548); FIGS. 82, 85, 88, 90, 91: Paratypes of *I. pallida*.

Specimens Examined for Morphometry and Histology

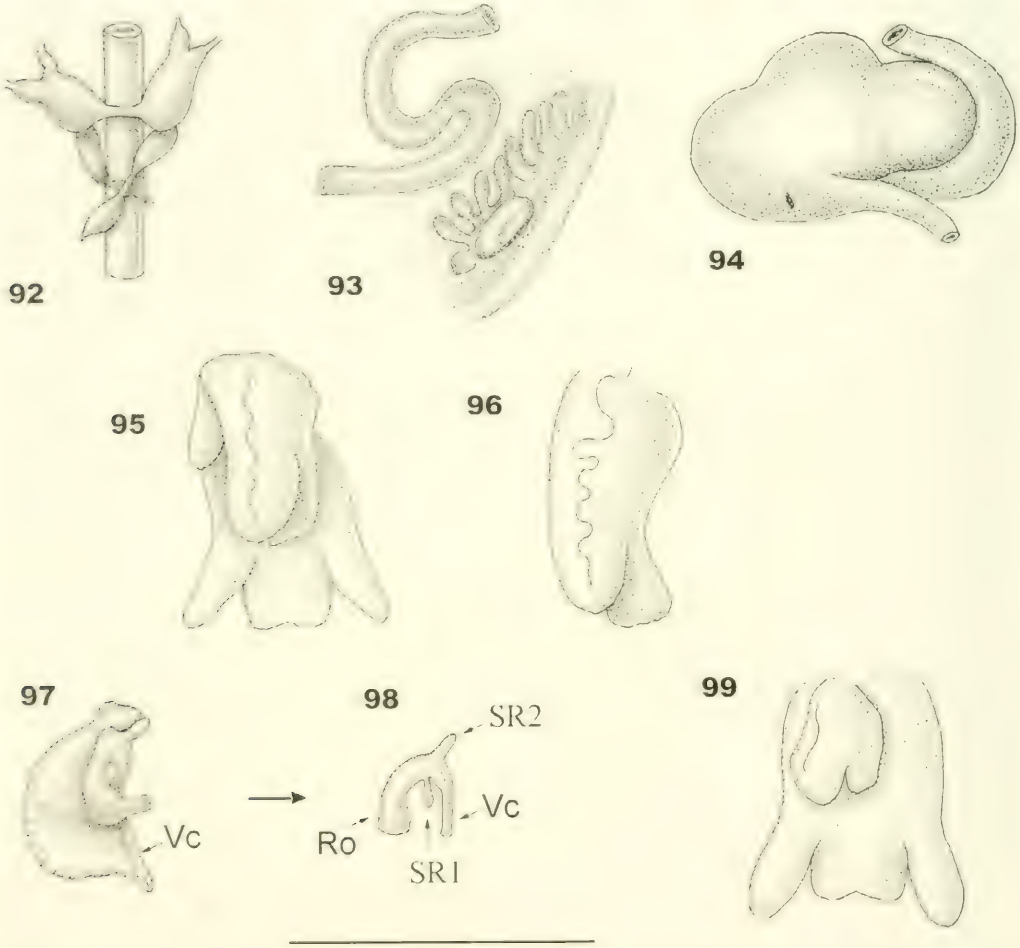
All measurements (Tables 1–3, 5–7) correspond to specimens from type locality (5/4/1992). For histology, one male from type locality (April, 1992) was studied.

Etymology

The name "*pallida*" refers to the fact that body is completely unpigmented.

Diagnosis

Shell depressed-trochiform or valvatiform; operculum circular; head and body unpigmented; ctenidium well developed; short pleuro-subesophageal connective and small subesophageal ganglion; medium size pleuro-supraesophageal connective; esophagus running straight underneath cerebral commissure; penis long, unpigmented, with a rounded and subterminal non-glandular lobe located near its distal end and protruding from penis tip;



FIGS. 92–99. Anatomy of *Islamia pallida*. FIG. 92: Partial nervous system; FIG. 93: Rectum, osphradium and ctenidium; FIG. 94: Stomach; FIGS. 95, 96: Head of a male and penis; FIG. 97: Anterior female genitalia; FIG. 98: Detail of the seminal receptacles; FIG. 99. Head of a female and pseudopenis. Abbreviations as in text. Scale bar = 500 µm (FIGS. 92–97, 99).

penial duct undulates along entire length of central part of the penis; two elongated seminal receptacles located very close to each other on opposite sides of renal oviduct; females with an unpigmented pseudopenis.

Description

Shell: Depressed-trochiform or valvatiform, 3.5 whorls (Figs. 78, 85, Table 1); body whorl occupying more than $^3/_4$ of total shell length; protoconch pitted (Fig. 91), consisting of 1.5 whorls (Fig. 90); protoconch width and width of the nucleus are 350 µm and 120 µm, respectively; aperture prosocline, rounded (Fig. 78); peristome complete, thin (Fig. 82); umbilicus narrow, 0.2 mm in diameter (Fig. 88); shells extremely fragile, some showing marked growth lines in teleoconch microsculpture.

Operculum: Circular, yellowish, with central muscle attachment area on its inner surface (Table 2).

Body: Head and body completely unpigmented (Figs. 95, 99). Eyes absent.

Nervous System (Fig. 92): Medium sized supraesophageal and short pleural-subesophageal connective; subesophageal ganglion very small; RPG ratio is 0.42 (moderately concentrated). Esophagus runs straight underneath cerebral commissure.

Ctenidium – Osphradium: Ctenidium with 9–10 long, narrow lamellae (Fig. 93); osphradium oval, length two times width (Table 3), located in opposite posterior part of ctenidium.

Stomach – Radula: Stomach almost as long as it is wide. Style sac not protruding anteriorly to intestinal loop (Fig. 94). Rectum markedly S-shaped, bending toward anterior portion of body (Fig. 93). Radula: unknown. No data on the radula were available due to its extreme fragility and the scarcity of available specimens.

Male Genitalia: Unpigmented penis almost as long as head (Table 6) with a rounded-trapezoidal, non-glandular, subterminal lobe (Figs. 95, 96) located parallel in ventral position and near its blunt distal tip and protruding beyond tip of penis; penial duct strongly undulating along its length and near central part of penis.

Female Genitalia: Minute with very small oviduct glands (albumen + capsule glands), without narrowing (Fig. 97), located approximately $^1/_3$ inside pallial cavity; renal oviduct making wide circle over albumen gland, which is larger than capsule gland; two elongated seminal receptacles equal in size (Fig. 98, Table 7) very close to one another (almost at the same level) on opposite sides of renal oviduct close to its loop, none of them with a stalk; females have an unpigmented pseudopenis (Fig. 99) measuring approximately 0.20 mm, and occupies almost half length of head.

Discussion

The geographical distribution of this species corresponds to that of *Neohoratia* (?) *coronadoi*, described by Bourguignat (1870) as *Valvata coronadoi* "en los alrededores de Madrid, o, al menos, en algunos manantiales o arroyos de la provincia de Castilla La Nueva" [in Madrid's surroundings or, at least, in some springs or streams of the New Castille Province]. There are no anatomical data available for *Neohoratia* (?) *coronadoi*, which has conchological characters that clearly differ from those of *I. pallida*. The shells of *N.* (?) *coronadoi* are large and planispiral, whereas those of *I. pallida* are small and trochiform. Boeters (1988) dubiously assigned the first species, *V. coronadoi*, to the genus *Neohoratia* [as *N.* (?) *coronadoi*], because of its similarities to *Neohoratia schuelei* (*sensu* Boeters, 1988). After several field samplings, we found no specimen of *Valvata coronadoi*, which is possibly now extinct. The presence of a pseudopenis in all females studied of *I. pallida* is a phenomenon that has also been reported and discussed in another Iberian valvatiform species (*Spathogyna fezi* Arconada & Ramos, 2002). The development of male sexual characters in females has sometimes been related to parasitism (Rothschild, 1938), or even to imposex (Smith 1971; Fioroni et al., 1990). In the case of *I. pallida*, we did not find any sign of parasitism in any of the females studied.

Juvenile specimens kept in an aquarium showed a monthly growth rate of 75% shell length and 87% width. They have a ciliated region in the propodium and at the tip of the tentacles (Figs. 100–103).

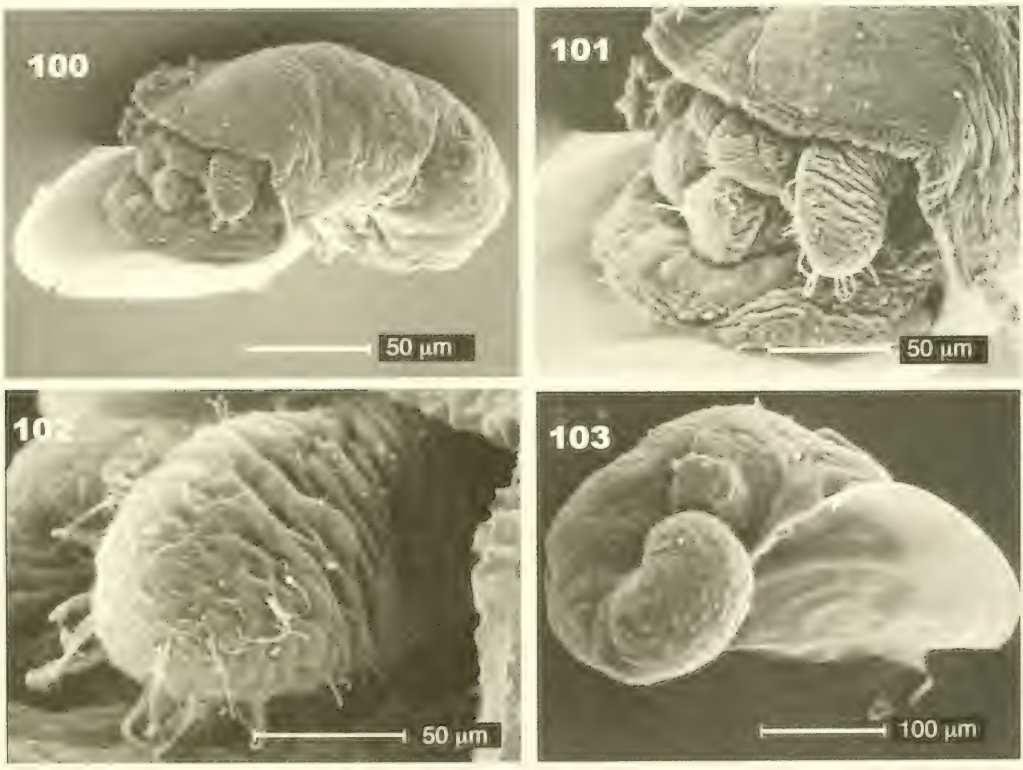
Differences between *I. pallida* and other Iberian *Islamia* species are based on a combination of characters: the absence of head and body pigmentation, a very small subesophageal ganglion, two elongated seminal receptacles, without stalk, very close to one another, located almost at the same level on

opposite sides on the renal oviduct close to its loop and a well-developed female pseudopenis. In relationship to other European *Islamia* species, most of the differences are related with the genitalia. In *I. pallida* the position of seminal receptacles is, in a way, similar to that described for the type species, *Islamia valvataeformis*. However, in *I. pallida* both receptacles are smaller, not pedunculated, similar in size and shape, and are located close to the end of the renal oviduct loop (proximal position), whereas in *I. valvataeformis* (Radoman, 1983: 124, fig. 69A, B; Bodon et al., 2001: 133) both seminal receptacles are "strongly developed" (the proximal one is larger, pyriform, and has an evident stalk), and emerge close to one another from the distal renal oviduct. In *I. pallida*, the penial lobe protrudes beyond the penis tip, similar to that described in species from the Balkan Peninsula. Nevertheless, *I. pallida* has a blunt penis tip. In addition, the penial duct markedly undulates along its length and near the central part of penis. In the Balkan's species, the penial duct runs through the right part of the penis, undulating not so markedly from its base and becoming almost straight at the distal end. As in the Italian *I. gaiteri* and in the French *I. minuta*, *I. globulina*, *I. consolationis* and *I. spirata*, all *I. pallida* specimens studied lack eyes and have a completely unpigmented body. This may be related to living in an interstitial or underground water habitat (Bodon et al., 1995: 47, 51, 52).

Islamia henrici Arconada & Ramos, **n. sp.**

Type Specimens

Holotype MNCN 15.05/46552 (Fig. 15B) (SEM preparation) and paratypes MNCN 15.05/46552, 13 Oct. 1992, E. R. (ethanol and SEM preparation – Figs. 106, 107, 109, 112, 113, 116).



FIGS. 100–103. Juveniles of *Islamia pallida*. FIGS. 100, 103: Complete body and operculum; FIG. 101: Detail of the ciliated propodium; FIG. 102: Detail of the ciliated tentacles.

Type Locality

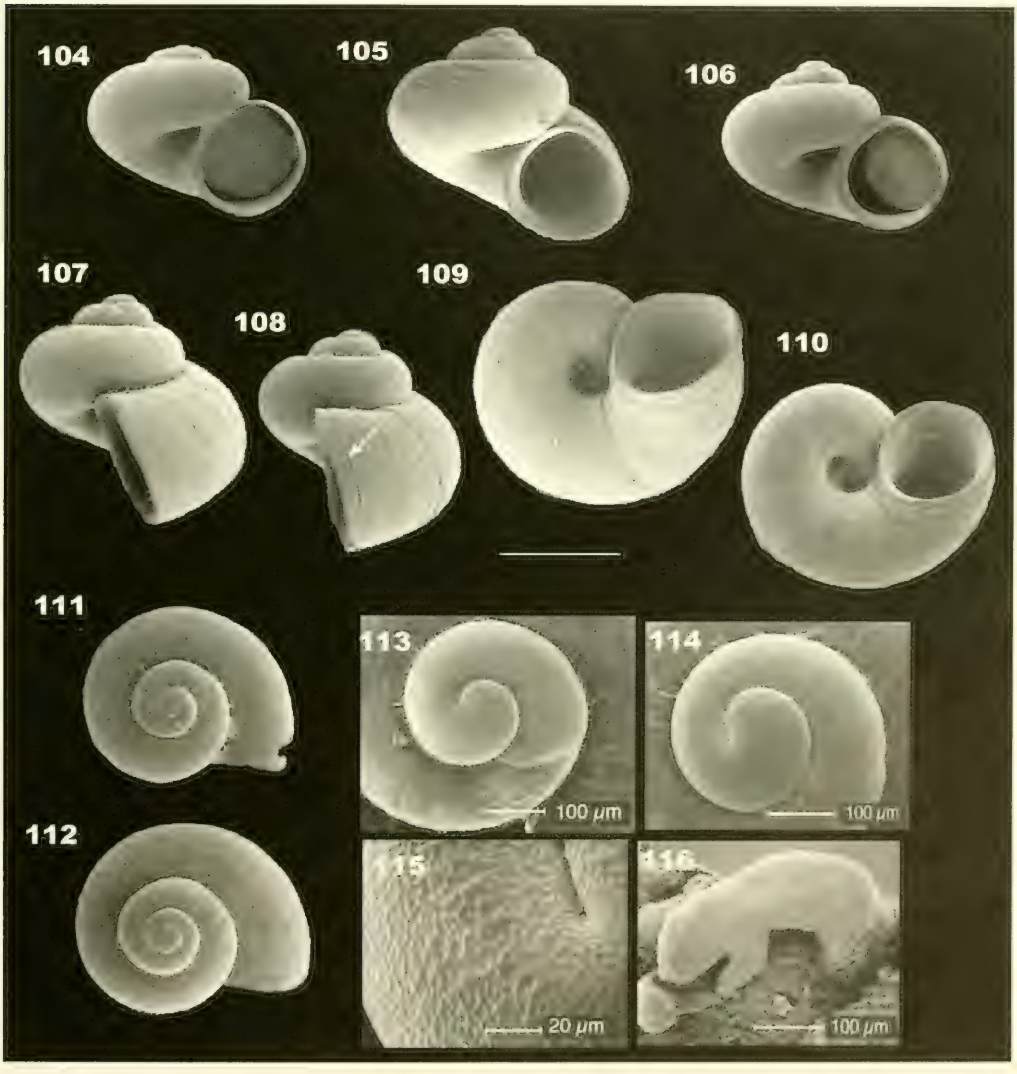
A tributary of the Guadalora River in Parque Natural de Hornachuelos, Córdoba, U.T.M.: 30STG9788.

Etymology

Dedicated to Enrique Arconada, whose given name has been Latinized as Henricus.

Diagnosis

Shell valvatiform or depressed-trochiform; central tooth with two basal cusps on each side; ctenidium scarcely developed or absent; esophagus curving posteriorly to cerebral commissure; long pigmented penis with small nonglandular lobe located near its tip but not protruding from it; proximal seminal receptacle rounded, pedunculated or elongated, with



FIGS. 104–116. Shells and penis of *Islamia henrici*. FIGS. 104, 108, 110, 111, 114, 115: Shells of *I. henrici giennensis* from La Iruela population; FIGS. 105–107, 109, 112, 113, 116: Shells and penis of *I. henrici henrici* from Hornachuelos population; FIG. 104: Holotype of *I. henrici giennensis* (MNCN 15.05/46555); FIG. 105: Holotype of *I. henrici henrici* (MNCN 15.05/46552). Scale bar = 500 µm (FIGS. 104–112).

swollen tip (SR2), bending towards distal portion of renal oviduct and distal seminal receptacle smaller, more or less globular and sessile (SR1).

We consider that this species has two subspecies as follows:

Islamia henrici henrici Arconada & Ramos, **n. subsp.**

Populations Additional to Species Type Material

This subspecies was found in the province of Córdoba (Fig. 17). A tributary of the Guadalora River, Parque Natural de Hornachuelos, Córdoba (type locality), 16 April 1998, B. A., MNCN 15.05/46553 (ethanol and frozen material); La Almarja spring, Parque Natural de Hornachuelos, Córdoba, U.T.M.: 30SUG014869, 16 April 1998, B. A., MNCN 15.05/46577 (ethanol, SEM preparation, and frozen material).

Material Examined for Morphometry and Histology

All measurements of shell, operculum, osphradium, digestive, radular, female and male systems (Tables 1–7) correspond to specimens from the type locality (in Parque Natural de Hornachuelos). Male and females studied and measured were collected in Oct. One female from Guadalora River was studied for histology.

Diagnosis

Long orangish pigmented penis with small non-glandular lobe located in distal position, but not protruding from penis tip; females having a nuchal node.

Description

Shell: Valvatiform or depressed-trochiform, 3.5 whorls (Table 1; Figs. 105, 106, 112); body whorl occupying approximately $^{4}/_{5}$ of total shell length; protoconch pitted consisting of more than 1.5 whorls (Fig. 113); protoconch width and width of nucleus are 290 and 120 µm, respectively; aperture rounded and orthocline or slightly prosocline, sometimes slightly oval descending (Figs. 105–107); peristome complete, thin, slightly reflected at columellar margin; external lip thin, internal lip slightly reflected towards the umbilicus; umbilicus medium-sized, 180 µm in diameter (Fig. 109).

Operculum: Ovate with central nucleus (Figs. 117–118); muscle attachment area rounded.

Body: Head scarcely pigmented with scattered pigment cells at the base of tentacles around the eye-spots (Figs. 124, 129).

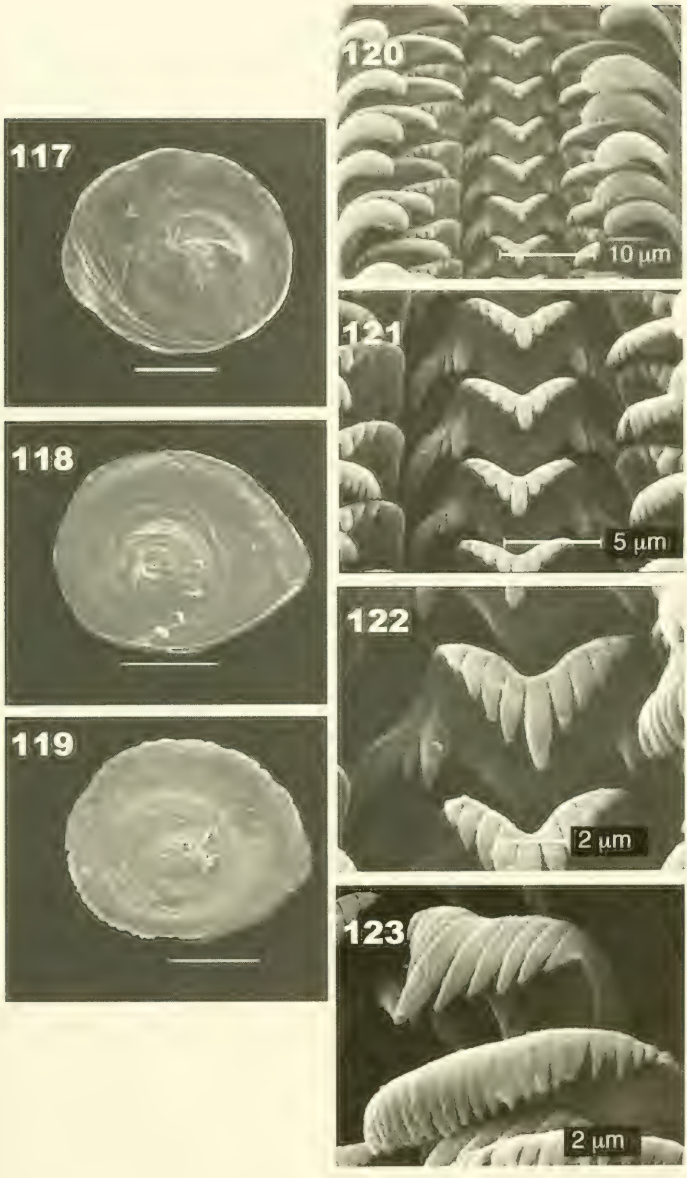
Nervous System (Fig. 125): With a medium-sized pleuro-supraesophageal connective; RPG ratio is 0.3 (moderately concentrated). Esophagus curving posteriorly to cerebral commissure.

Ctenidium – Osphradium: Ctenidium absent or very poorly developed, with 2–6 small lamellae (Fig. 126). Osphradium bean-shaped, length almost two times width (Table 3).

Stomach – Radula: Chambers almost equal in size. Style sac protruding anteriorly into intestinal loop (Table 5, Fig. 127). Rectum forming a marked S-loop and bends towards anterior portion of the body (Figs. 126, 128). Radula medium sized (23%) relative to maximum shell dimension, with two basal cusps on each side of central tooth (Table 5, Figs. 120–122); distance between its internal cusps is approximately 7 µm; its central denticle long, sharp, followed on each side by 4 long denticles in decreasing order of size; cutting edge of central tooth markedly concave; lateral teeth with 5–6 long, sharp denticles on each side of central denticle (Fig. 123).

Male Genitalia: With large bean-shaped prostate gland, narrow anteriorly (Fig. 128); less than 50% of prostate gland extending into pallial cavity; penis very long with small non-glandular lobe located in distal position (Figs. 116, 129), showing a small refringent area; penis orangish pigmented in live specimens; penial duct slightly undulating, close to central part of penis.

Female Genitalia: With renal oviduct that makes a wide circle (Fig. 130); no narrowing of oviduct glands (albumen + capsule glands); capsule gland larger than albumen gland, occupying more than 50% of total pallial cavity length and narrowing at its distal outer margin; proximal seminal receptacle (SR2) oval with a long stalk and slightly bent towards the distal part of renal oviduct (Fig. 131, Table 7); distal seminal receptacle (SR1) smaller than proximal receptacle, globular, sessile; seminal receptacles located relatively far from one another on opposite sides of renal oviduct. Some females have a dark nuchal node on the right side of



FIGS. 117–123. Opercula and radula of *Islamia henrici*. FIGS. 117, 118, 120–123: Opercula and radula of *I. henrici henrici* from Hornachuelos population; FIG. 119: Operculum of *I. henrici giennensis* from La Iruela population; FIG. 117: Outer side of the operculum; FIGS. 118, 119: Inner side of the operculum; FIG. 120: Transverse rows; FIGS. 121, 122: Central teeth; FIG. 123: Lateral and inner marginal teeth. Scale bar = 200 µm (FIGS. 117–119).

head (Fig. 124), which is approximately six times smaller than male penis, occupying 20% of total head length. This nuchal node is usually simple, although it can sometimes be bilobated, similar to the shape of the distal part of male penis.

Islamia henrici giennensis Arconada & Ramos, **n. subsp.**

Type Specimens

Holotype MNCN 15.05/46555 (SEM preparation) (Fig. 104) and paratypes MNCN 15.05/46555 (ethanol and SEM preparation, Figs. 108, 110, 111, 114, 115, 119).

Type Locality

Spring facing the hotel "Sierra Cazorla", La Iruela, Cazorla mountains, Jaén, UTM: 30SWG005969.

Etymology

The subspecific epithet is a Latin adjective related to the province of Jaén (Latin Gienna).

Other Specimens Examined

This species was found in the province of Jaén (Fig. 17). La Toba spring, Jaén, U.T.M.: 30SWH3826, 6 Oct. 1992, E. R., MNCN 15.05/46558 (ethanol); 24 March 1998, B. A., MNCN 15.05/46554 (ethanol); spring facing the hotel "Sierra Cazorla", La Iruela, Cazorla mountains, Jaén, UTM: 30SWG005969, E. R., MNCN 15.05/46556 (ethanol); 30 April 1990, D. M. & N. M., MNCN 15.05/46555 (ethanol); Madera River, La Fresnedilla, Segura mountains, Jaén, UTM.: 30SWH3644, 6 Oct. 1992, E. R., MNCN 15.05/46557 (ethanol and SEM preparation); spring in Cazorla, Jaén, E. R., MNCN 15.05/46559 (ethanol); La Nava de San Pedro, Cazorla, Jaén, UTM: 30SWG094948, 1 May 1990, D. M. & N. M.

Specimens Examined for Morphometry

Shell, operculum, and anatomical measurements – osphradium, digestive, female and male systems (Tables 1–3, 5–7) – correspond to type locality (La Iruela). Male and females studied and measured were collected in April.

Diagnosis

A slight varix near shell aperture in most of the specimens studied from all populations; long black pigmented penis with a small non-glandular lobe located in distal position but not protruding from penis tip, penis tip pointed; females have no nuchal node.

Description

Shell: Valvatiform or depressed-trochiform, with spire consisting of 2.75–3.5 whorls (Table 1; Figs. 104, 111); body whorl occupying approximately $^{4}/_{5}$ of total shell length; protoconch pitted consisting of more than 1.5 whorls (Figs. 114, 115); protoconch width and width of nucleus are 330 and 129 µm, respectively; aperture rounded, orthocline or slightly prosocline, sometimes slightly oval (Figs. 104, 108); peristome complete, thin, slightly reflected at columellar margin; most specimens have a slight varix near shell aperture (Fig. 108); external lip thin; internal lip reflected towards umbilicus; umbilicus medium-sized, 180 µm in diameter (Fig. 110).

Operculum: Ovate yellowish with darker central nucleus (Fig. 119); muscle attachment area rounded.

Body: Head scarcely pigmented with scattered pigment cells at base of tentacles around eye-spots. Mantle with dispersed pigmented areas. Pigmentation quite variable among specimens.

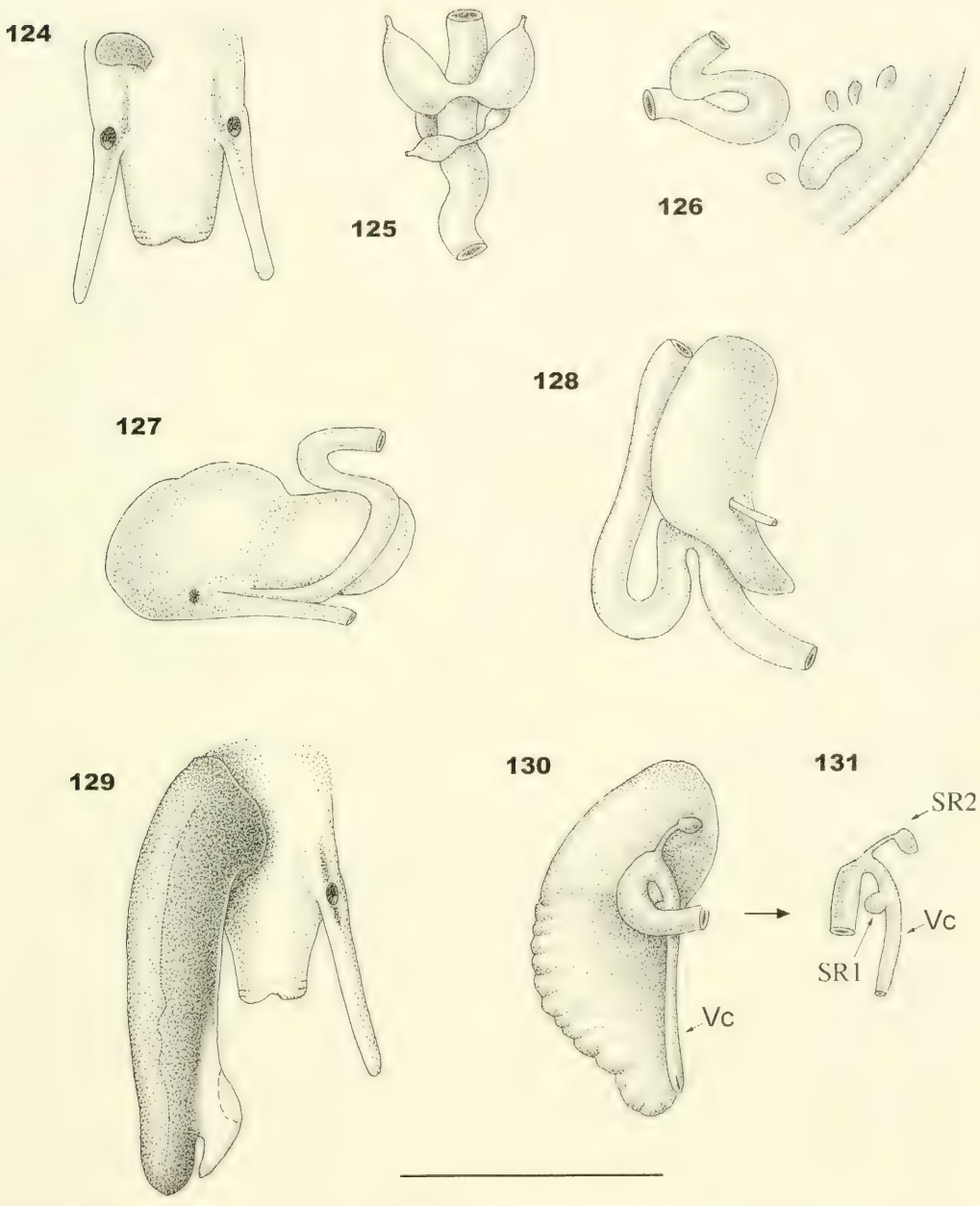
Nervous System (Fig. 132): With a short pleuro-supraesophageal connective; RPG ratio is 0.14 (concentrated). Esophagus frequently making a curve posteriorly to cerebral commissure.

Ctenidium – Osphradium: Ctenidium absent or very poorly developed, with 5–7 small lamellae (Fig. 133). Osphradium oval, length two times the width (Table 3).

Stomach – Radula: Chambers almost equal in size, longer than they are wide. Style sac protruding anteriorly into intestinal loop (Table 5). Rectum forming a marked S-loop, bending toward anterior portion of body. Radula with two basal cusps on each side of central tooth; its central denticle long, sharp, followed on each side by 4 long denticles in decreasing order of size; cutting edge of the central tooth markedly concave; lateral teeth with 4–5 long, sharp denticles on each side of central denticle.

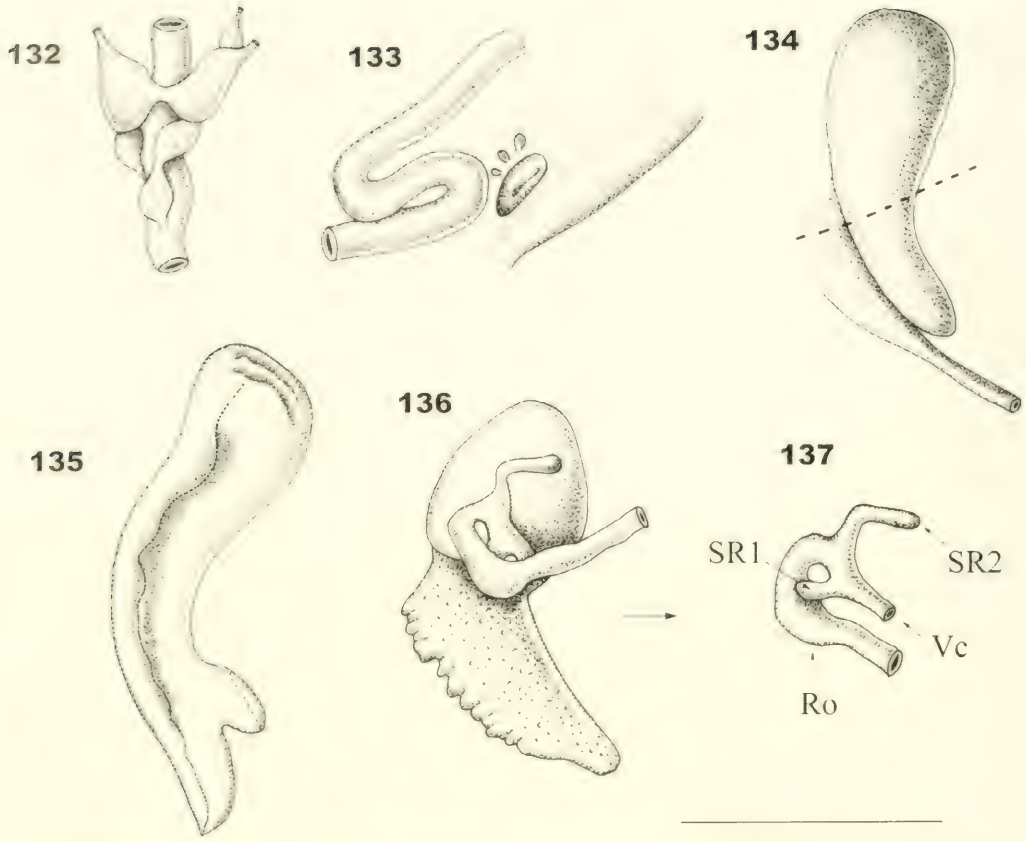
Male Genitalia: With large, long prostate gland, narrowing towards anterior part (Fig. 134); less than 50% of prostate gland extending into pallial cavity; penis very long with a small non-glandular lobe located in a distal position (Fig. 135); penis black pigmented pointed at penis tip; penial duct slightly undulating, running close to central part.

Female Genitalia: With renal oviduct making a wide circle (Fig. 136); oviduct glands (al-



FIGS. 124–131. Anatomy of *I. henrici henrici*. FIG. 124: Head of a female and nuchal node; FIG. 125: Partial nervous system node; FIG. 126: Rectum, osphradium and ctenidium node; FIG. 127: Stomach; FIG. 128: Prostate and rectum; FIG. 129: Head of a male and penis; FIG. 130: Anterior female genitalia; FIG. 131: Detail of the seminal receptacles; Abbreviations in text. Scale bar = 500 µm (FIGS. 124–130).

bumen + capsule glands) sometimes showing a narrowing; capsule gland larger than albumen gland and showing a narrowing at its distal outer margin, occupying more than 50% of total pallial cavity length; proximal seminal receptacle (SR2) elongated, with



FIGS. 132–137. Anatomy of *I. henrici giennensis*. FIG. 132: Partial nervous system; FIG. 133: Rectum, osphradium and ctenidium if present; FIG. 134: Prostate and end of rectum; FIG. 135: Penis; FIG. 136: Anterior female genitalia; FIG. 137: Detail of the seminal receptacles; Abbreviations in text. Scale bar = 500 µm.

swollen tip and bending 90° towards distal part of renal oviduct (Fig. 137); distal seminal receptacle (SR1) much smaller than proximal receptacle (Table 7), elongated or pyriform without evident stalk; seminal receptacles located not far from one another in opposite positions on renal oviduct.

Discussion

All *I. h. henrici* and *I. h. giennensis* populations studied show identical anatomical characters. However, some anatomical differences permit us to distinguish two "groups": one that includes all populations from Córdoba Province, and the other comprising populations from Jaén. The Jaén (*I. h. giennensis*) populations are characterised by a slight varix near the shell aperture in most of the specimens (no varix in *I. h. henrici*), a short supraesophageal connective, RPG ratio = 0.14 (medium-sized in *I. h. henrici,* RPG ratio = 0.30), an oval osphradium (bean-shaped in *I. h. henrici*), a prostate elongated pear-shaped (bean-shaped in *I. h. henrici*), a penial lobe without any refringent area (a small refringent area present in *I. h. henrici*), a black pigmented penis (penis orangish pigmented in *I. h. henrici*), long and slender proximal seminal receptacle (SR2) (elongated with swollen tip in *I. h. henrici*), and the absence of a nuchal node in females. A nuchal node is a constant character in all female specimens from Córdoba (*I. h. henrici*). There is a notable geographic distance between both "groups", which decreases the probabilities of gene flow. The anatomical differences together with the large geographic distances between the "groups", allow us to

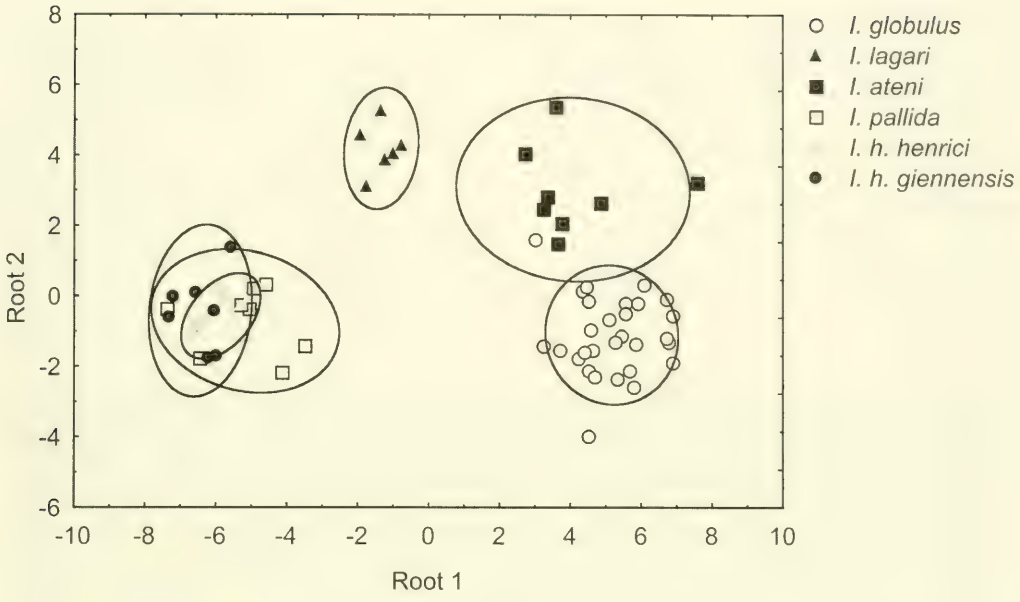


FIG. 138. Plot of discriminant scores on the two canonical axes, obtained from DFA of shell measurements for all Iberian *Islamia* species and subspecies: *I. globulus*, *I. ateni*, *I. lagari*, *I. pallida*, *I.henrici henrici* and *I. h. giennensis*. Confidence interval for ellipses: 0.95.

divide this species into two subspecies, *I. henrici henrici* (Córdoba populations) and *I. henrici giennensis* (Jaen populations). However, more specimens need to be studied to better understand the taxonomical identity of both entities. Unfortunately, due to declining populations, sample sizes were very small.

Islamia henrici can be distinguished from other European *Islamia* species by a group of characters: an under-developed or absent ctenidium (the same character is reported for the Italian *I. gaiteri*, Bodon et al., 1995: 51); a rather long, black or orangish pigmented penis, with a small, pointed lobe, which does not extend the penis tip. This small penial lobe is similar to that described for other species, such as *Islamia gaiteri* (Bodon et al., 1995: 51) and *Islamia* sp. form C, from the population of Monti della Calvana (Giusti et al., 1981: 66). In this latter species, however, the lobe is larger, nearly reaching the tip of the penis. A very small or often indistinguishable area of refringent non-glandular tissue is found at the base of the penial lobe (Fig. 129). The shape of the esophagus posterior to the cerebral commissure is a character that has not previously been described in any *Islamia* species. It slightly curved in *I. henrici*, whereas markedly so in *Josefus aitanica* (see below). Other differences among Iberian *Islamia* are: the reduction or absence of lamellae in the ctenidium, a long stalk on the proximal seminal receptacle, an orangish pigmented penis, and a protuberance on the female heads (the same described for *I. pallida*) of several populations.

Statistical Analysis of *Islamia* species

Conchological differences between *Islamia* species were investigated by a discriminant function analysis using the nine standard shell measurements on Table 1 (all except NSW). For *I. globulus*, the Sopeira population was selected as it had the greatest number of well-classified specimens as well as the highest number of specimens measured (n = 30). Four highly significant discriminant functions were found (Wilk's lambda = 0.0018, F (45, 267) = 18.27, $p < 0.0001$). The variables included in these functions were: SW, WBW, LBW, AL, AW, and WAW. For the first function that accounted for 84.5% of explained variance, the characters that contribute (highest weight) were (in order): SW, WBW and LBW. For the second function, the order was: AL, LBW, SW, WAW and AW. All discriminant functions were highly significant ($p < 0.0001$). Of the 73 individuals classified, all of the *I. ateni*, *I. globulus*,

I. lagari, and *I. h. henrici* were correctly classified (100%); 62.5% of the *I. pallida* individuals and 85.71% of *I. h. giennensis* were also correctly classified. On the scatterplot (Fig. 138), six clusters are observed. Three of them overlap and correspond to the taxa that have the most depressed-trochiform or valvatiform shells and shorter and wider body-whorls (*I. pallida*, *I. h. henrici*, and *I. h. giennensis*).

Milesiana Arconada & Ramos, **n. gen.**

Type Species

Hauffenia (*Neohoratia*) *coronadoi schuelei* Boeters, 1981: 56, figs. 3, 4.

Etymology

This subgenus is dedicated to the musician Miles Davis, for his great contribution to art and pleasure.

Diagnosis

This genus differs from all others by having a proximal receptacle (SR2) sessile and much smaller than distal (SR1), which has a long stalk; the seminal receptacles arise rather close to one another; a big non-glandular lobe is located in medial position of the penis; left pleural and subesophageal ganglia are fused, the pleuro-subesophageal connective is absent in *Milesiana*, whereas it is present in all the other European genera for which information on this character is available (Radoman, 1983), except in the genus *Josefus* described herein. Other features characterizing *Milesiana* are: shell small, ovoid or more usually planispiral; operculum without peg; central tooth with two basal cusps on each side; the two seminal receptacles are located on opposite sides on unpigmented renal oviduct; bursa copulatrix absent.

Milesiana schuelei (Boeters, 1981)

Hauffenia (Neohoratia) coronadoi schuelei Boeters, 1981: 56, figs. 3, 4.
Hauffenia schuelei (Boeters, 1981) – Bernasconi, 1985: 65.
Neohoratia schuelei (Boeters, 1981) – Boeters, 1988: 217, figs. 135–136, 159, 171, 288, pl. 2, fig. 26.
Islamia schuelei (Boeters, 1981) – Bodon et al., 2001: 179; Bodon & Cianfanelli, 2002: 20.
Horatia gatoa Boeters, 1980 – Only paratype in figure 6, which is here re-identified as *M. schuelei*.

Type Locality

"West of two springs between Galera and Orce, Granada" (Boeters, 1981).

Type Specimens

Holotype in SMF 253578/1, paratypes in SMF 253579/1, NNM, Falkner, BOE 222a and 223, *ex* Falkner, 308 and 308b, *ex* Wirth, 548 and 549, *ex* Bou.

In the original description, Boeters (1981) mentioned the type locality but not that of the paratypes. The only available information is: i) that the material was collected by Ulrich Wirth/Bonn (1963), Gerard Falkner/Hörlkofen and Munchen (1967) and Claude Bou/Moulis, Albi (1972), and ii) that species distribution includes: Prov. Granada, Velez-Benaudalla, spring at the road from Motril to Granada (UTM: VF 57), two springs between Galera and Orce (UTM: WG 47). Prov. Teruel, close to Caminreal in ground waters from a tributary of the Jiloca River (UTM: XL 42). Prov. Jaén, between Peal de Becerro and Úbeda, in ground waters of the Guadalquivir River. In his 1988 paper, Boeters confirmed type locality ("west of two springs between Galera and Orce, Prov. Granada", (WG 47) and completed information on paratypes as follows: SMF 253579/1, RMNH, FALK (Galera/Orce), BOE 222a and 223a (Galera/Orce), 308a and 308b (Velez-Benaudalla), 548 (tributary of the Jiloca River) and 549 (tributary of the Fardés River).

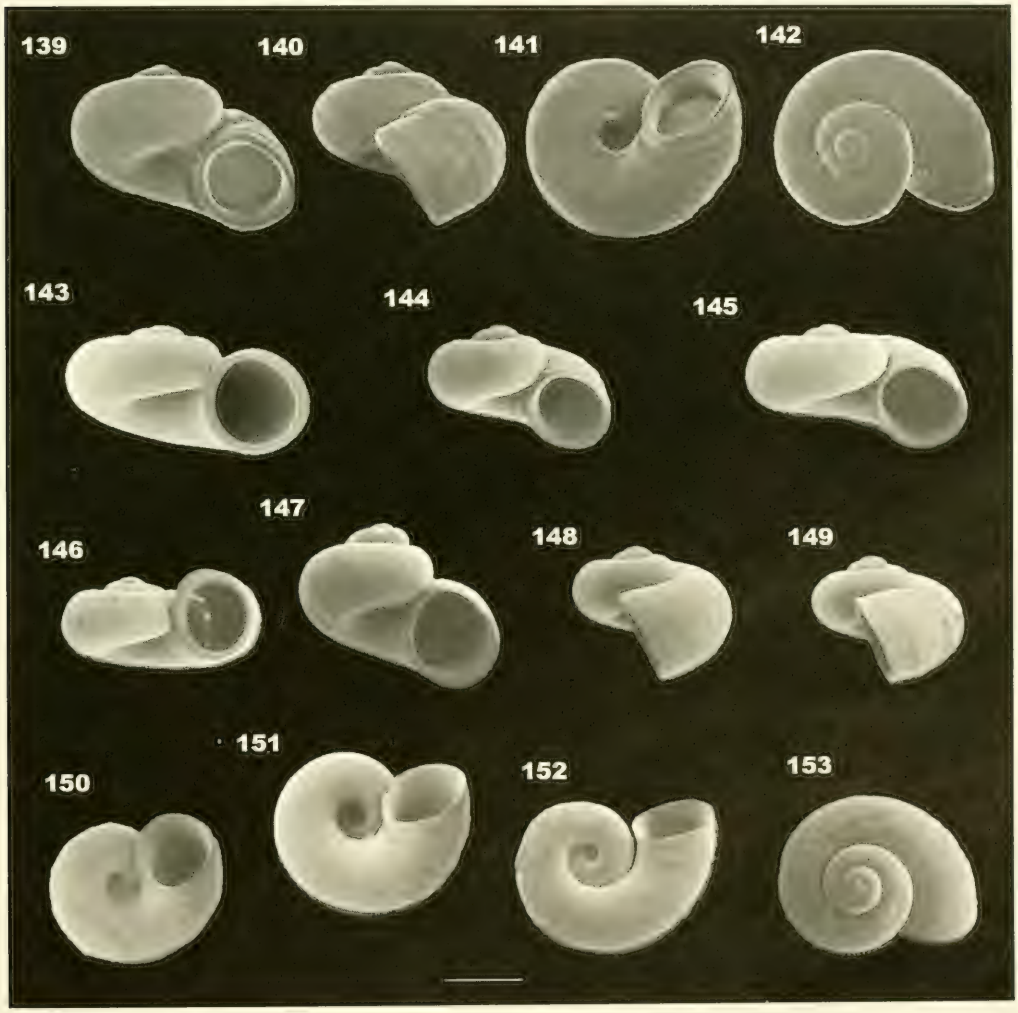
Specimens Examined

Type Material Examined: Holotype in SMF 253578/1 (Figs. 139–142, 154).

Other Populations Examined. This species was found in the provinces of Cádiz, Almería, Granada and Málaga (Fig. 17). A population found far from its distribution range, in the Cáceres province, was provisionally assigned to this species as *M.* cf. *schuelei*. The species has not been found in Teruel Province.

Localities: Algodonales, Cádiz, UTM.: 30STF8584, 19 Oct. 1998, E. R., MNCN 15.05/46495 (ethanol); El Nacimiento spring, Turrillas, Almería, UTM: 30SWF657975, 15 Oct. 1990, D. M., MNCN 15.05/46496 (ethanol, SEM preparation), 10 Oct. 1992, E. R., D. M., MNCN 15.05/46497 (ethanol); Los Minutos spring, Turrillas, Almería, UTM: 30SWF6598, 10 Oct. 1992, D. M., N. M., MNCN 15.05/46591 (SEM preparation); Andarax spring, river and channel, Laujar de Andarax, Almería, UTM: 30SWF0994, 11 Jan. 1992, D. M., N. M.; 11

Oct. 1992, E. R., D. M., MNCN 15.05/46498 (ethanol); Agua spring, Lucainena de Las Torres, Almería, UTM: 30SWF7199, 10 Oct. 1992, E. R., D. M., MNCN 15.05/46499 (ethanol); Vélez Blanco, Almería, UTM: 30SWG7972, E. R., MNCN 15.05/ 46592; Talama spring, Bayarcal, Almería, UTM: 30SWF0098, 26 March 1994, D. M., N. M., MNCN 15.05/46500 (ethanol and SEM preparation), 14 May 1994, D. M., N. M., MNCN 15.05/46501 (ethanol); El Marchal de Antón López, Almería, UTM: 30SWF3383, E. R.; 26 March 1998, B. A., MNCN 15.05/46502 (ethanol, SEM preparation and frozen material); Pool in Berchul, Félix, Almería, UTM: 30SWF298813, E. R., MNCN 15.05/46503 (ethanol and SEM preparation), 26 March 1998, B. A., MNCN 15.05/46504 (ethanol and frozen material); spring near the pool in Berchul, Félix, Almería, UTM: 30SWF298813, 26 March 1998, B. A., MNCN 15.05/46505 (ethanol); spring in Conchar, Granada, UTM.: 30SVF477912, 25 Sept. 1989, E. R., D. M., C. A., MNCN 15.05/46506 (dried); Faldés spring,



FIGS. 139–153. Shells of *Milesiana schuelei*. FIGS. 139–142: Holotype (SMF 253578/1); FIG. 143: Shell from Fuente del Mal Nombre, Padul (Granada); FIGS. 144, 148, 150, 153: Shells from Gaucín (Málaga); FIGS. 145, 151: Shells from Fuente Talama, Bayarcal (Almería); FIGS. 146, 149, 152: Shells from Fuente Los Minutos, Turrillas (Almería); FIG. 147: Shell from Benaoján (Málaga). Scale bar = 500 µm.

Sierra Harana Granada, UTM.: 30SVG592308, 23 April 1992, D. M., MNCN 15.05/46507 (ethanol), 12 Oct. 1992, E. R., D. M., MNCN 15.05/46508 (ethanol); 25 March 1998, B. A. MNCN 15.05/46509 (ethanol); Los Caños spring, Graena, Granada, UTM.: 30SVG810285, 27 Sept. 1989, E. R., D. M., C. A., MNCN 15.05/46510 (dried, ethanol); Pilar del Mono spring, Durcal, Granada, UTM.: 30SVF493951, 25 Sept. 1989, E. R., D. M., C. A., MNCN 15.05/46511 (dried, ethanol), 17 Oct. 1989, J. T., D. M., 27 March 1998, B. A., MNCN 15.05/46512 (ethanol); La Gitana spring, La Peza, Granada, UTM.: 30SVG703255, 25 March 1998, B. A., MNCN 15.05/46513 (ethanol); spring in Padul, Granada, UTM.: 30SVF4497, 25 Sept. 1989, E. R., D. M., C. A., MNCN 15.05/46514 (ethanol), 17 Oct. 1989, D. M.; 30 Sept. 1989, E. R., MNCN 15.05/46515 (ethanol, SEM preparation); Mal Nombre spring, Padul, Granada, UTM.: 30SUF445963, 27 March 1998, B. A., MNCN 15.05/46516 (ethanol and frozen material); spring in Gaucín, Málaga, UTM.: 30STF9244, 22 Nov. 1988; E. R., MNCN 15.05/46517 (ethanol, SEM preparation), 15 April 1998, B. A., MNCN 15.05/46518 (ethanol and frozen material); Matiaña spring, El Chorro, Málaga, UTM.: 30SUF468824, E. R., MNCN 15.05/46519 (ethanol), 14 April 1998, B. A., MNCN 15.05/46520 (ethanol and frozen material); Wet wall in El Chorro, Málaga, UTM.: 30SUF468824, E. R., MNCN 15.05/46521 (ethanol), 14 April 1998, B. A., MNCN 15.05/46522 (ethanol and frozen material); Cueva del Gato, Benaoján, Málaga, UTM.: 30SVF003673, 24 April 1992, D.M., MNCN 15.05/46523 (ethanol, SEM preparation); 15 April 1998, B. A., MNCN 15.05/46524 (ethanol and frozen material); Avellano River, La Cimada, Málaga, U.T.M.: 30SUF0976, E. R., MNCN 15.05/46525 (ethanol and SEM preparation).

TABLE 8. Shell measurements (in mm) of *Milesiana schuelei* from the following populations: 1 - Turrillas (El Nacimiento), Almería; 2 - Turrillas (Los Minutos spring), Almería; 3 - Padul, Granada; 4 - El Chorro, Málaga; 5 - Benaoján, Málaga.

	1 Mean ± SD; CV (Max-Min) (n = 15)	2 Mean ± SD; CV (Max-Min) (n = 29)	3 Mean ± SD; CV (Max-Min) (n = 10)	4 Mean ± SD; CV (Max-Min) (n = 17)	5 Mean ± SD; CV (Max-Min) (n = 27)
SL	0.75 ± 0.05; 0.07 (0.85-0.68)	0.80 ± 0.06; 0.07 (0.94-0.65)	0.68 ± 0.04; 0.07 (0.74-0.57)	0.68 ± 0.05; 0.08 (0.80-0.57)	0.92 ± 0.10; 0.11 (1.13-0.75)
SW	1.21 ± 0.08; 0.07 (1.35-1.10)	1.27 ± 0.08; 0.06 (1.42-1.01)	1.09 ± 0.07; 0.06 (1.18-0.97)	1.13 ± 0.08; 0.07 (1.27-1.04)	1.32 ± 0.09; 0.07 (1.54-1.17)
SL/SW	0.62 ± 0.06; 0.09 (0.73-0.51)	0.62 ± 0.05; 0.08 (0.73-0.53)	0.62 ± 0.03; 0.06 (0.70-0.57)	0.59 ± 0.04; 0.07 (0.68-0.52)	0.70 ± 0.07; 0.10 (0.87-0.58)
AH	0.65 ± 0.09; 0.14 (0.82-0.55)	0.62 ± 0.04; 0.07 (0.82-0.55)	0.52 ± 0.02; 0.04 (0.57-0.50)	0.53 ± 0.04; 0.07 (0.60-0.47)	0.67 ± 0.04; 0.06 (0.74-0.58)
LBW	0.65 ± 0.04; 0.07 (0.75-0.57)	0.71 ± 0.05; 0.07 (0.81-0.60)	0.43 ± 0.04; 0.10 (0.48-0.35)	0.60 ± 0.05; 0.09 (0.71-0.50)	0.79 ± 0.10; 0.13 (0.98-0.63)
WBW	0.81 ± 0.07; 0.08 (0.92-0.70)	0.82 ± 0.05; 0.06 (0.91-0.68)	0.69 ± 0.04; 0.06 (0.77-0.61)	0.72 ± 0.05; 0.08 (0.85-0.62)	0.88 ± 0.11; 0.12 (1.33-0.75)
AL	0.52 ± 0.03; 0.07 (0.60-0.46)	0.54 ± 0.03; 0.06 (0.91-0.68)	0.49 ± 0.03; 0.07 (0.55-0.42)	0.49 ± 0.04; 0.09 (0.61-0.44)	0.58 ± 0.05; 0.08 (0.67-0.52)
AW	0.50 ± 0.07; 0.15 (0.60-0.25)	0.53 ± 0.02; 0.05 (0.60-0.44)	0.48 ± 0.02; 0.04 (0.52-0.45)	0.49 ± 0.04; 0.09 (0.61-0.44)	0.58 ± 0.04; 0.07 (0.69-0.52)
WPW	0.33 ± 0.03; 0.10 (0.40-0.28)	0.33 ± 0.04; 0.13 (0.40-0.24)	0.27 ± 0.03; 0.12 (0.32-0.21)	0.27 ± 0.04; 0.17 (0.34-0.18)	0.38 ± 0.04; 0.10 (0.47-0.32)
WAW	0.12 ± 0.02; 0.19 (0.10-0.08)	0.14 ± 0.02; 0.18 (0.18-0.08)	0.12 ± 0.01; 0.15 (0.14-0.10)	0.09 ± 0.01; 0.15 (0.12-0.07)	0.15 ± 0.02; 0.15 (0.21-0.11)
NSW	3.22 ± 0.19; 0.06 (3.50-3.00)	3.13 ± 0.14; 0.04 (3.50-3.00)	3.00 ± 0.00; 0.00 (3.00-3.00)	3.02 ± 0.08; 0.02 (3.25-3.00)	3.28 ± 0.21; 0.06 (3.50-3.00)

TABLE 9. Operculum measurements (in mm) of *Milesiana schuelei* from Gaucín population (Málaga).

	Mean±SD; CV (Max-Min)		Mean±SD; CV (Max-Min)
OL	0.59 ± 0.12; 0.21 (0.78-0.46) (n = 5)	NL	0.24 ± 0.04; 0.16 (0.27-0.17) (n = 5)
OW	0.47 ± 0.07; 0.16 (0.60-0.40) (n = 5)	NW	0.31 ± 0.01; 0.05 (0.34-0.29) (n = 5)
OLWL	0.21 ± 0.10; 0.47 (0.36-0.11) (n = 5)	OL/OW	1.23 ± 0.10; 0.08 (1.38-1.11) (n = 5)
OLWW	0.15 ± 0.06; 0.42 (0.26-0.09) (n = 5)		

M. cf. *schuelei*: Robladillo de Gata, Cáceres, UTM: 29TQE0764, E. R., MNCN 15.05/46526 (ethanol, SEM preparation).

Material Examined for Morphometry and Histology

Shell measurements (Table 8) correspond to populations from Almería, Granada and Málaga. Operculum and radular measurements (Tables 9, 11) to Málaga and anatomical measurements (Tables 10, 12–14) to Almería, Granada, Málaga and Cáceres (more details in table captions). Male and females studied and measured were collected in the following months: March, May, Sept., Oct. and Nov. For histology, seven specimens preserved in ethanol were studied: four females from Benaoján, Málaga (April 1992) and two males and one female from Turrillas, Almería (Oct. 1990).

Diagnosis

Shell small, planispiral or valvatiform; operculum circular; ctenidium well developed; pleural-subesophageal connective absent; large pear-shaped prostate gland; penis slightly or completely unpigmented, with large, non-glandular penial lobe located in medial position; proximal seminal receptacle (SR2) small, sessile, rounded; distal seminal receptacle (SR1) always larger than SR2, pyriform, pedunculated; receptacles located very close to one another on opposite positions on renal oviduct.

Description

Shell: Planispiral or valvatiform (Figs. 139, 143–147, Table 8), 3–3.5 whorls (Figs. 142, 153); sutures deep; body whorl expanded near aperture; protoconch consisting of 1.5 whorls; protoconch width and width of nucleus are 315 µm and 110–126 µm, respectively; protoconch pitted (Figs. 154–156); aperture prosocline, rounded (Figs. 143–147); umbilicus wide, approximately 240 µm in diameter (Figs. 141, 150–152); outer peristome simple, thin, straight; inner peristome slightly reflected at columellar margin (Fig. 140, 148, 149).

Operculum: Circular with large, central nucleus (Fig. 157); muscle attachment area rounded (Fig. 158).

Body: Head (Fig. 170) with black pigmentation extending from around the eyes to middle of tentacles.

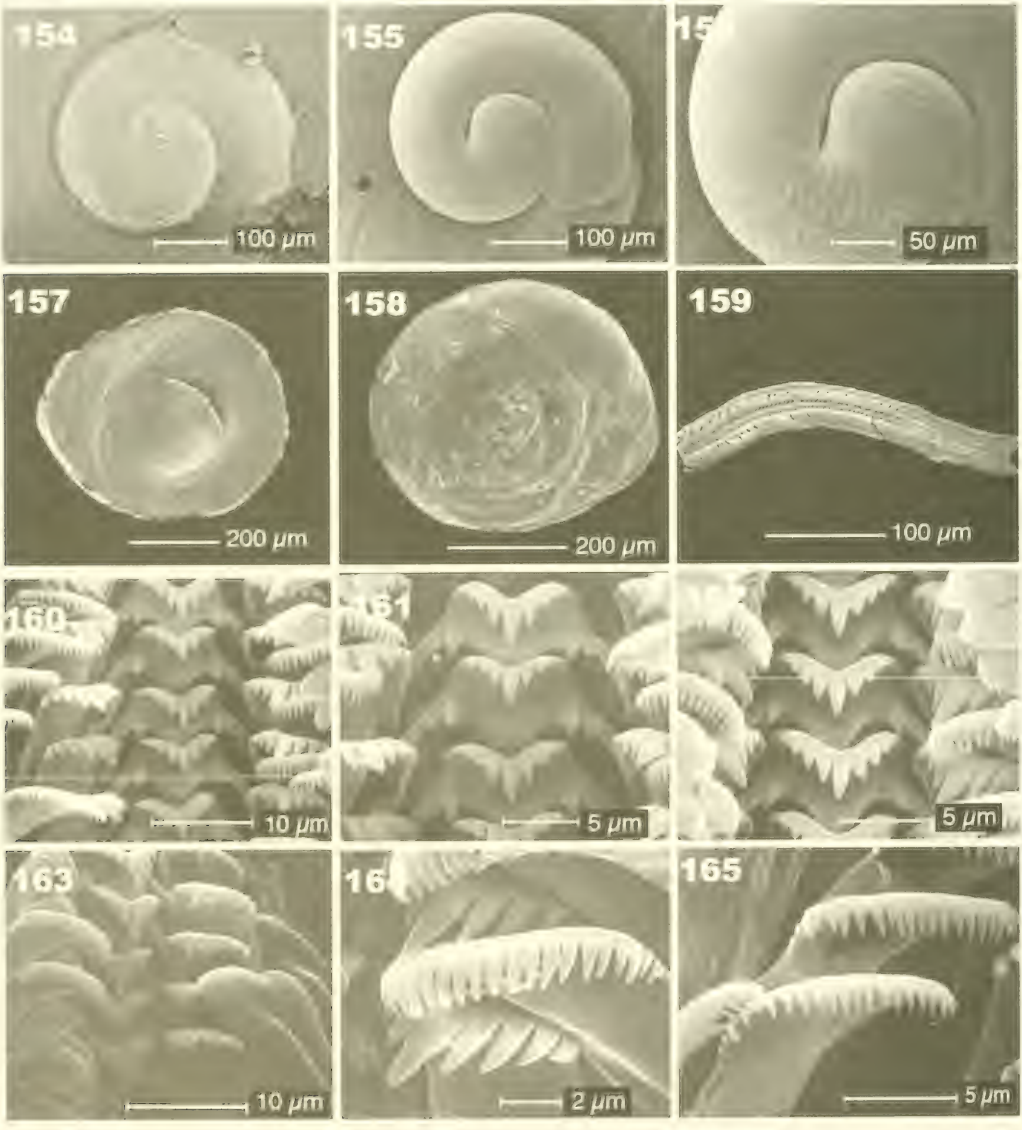
Nervous System: Pleuro-subesophageal connective absent, pleuro-supraesophageal connective middle-sized, RPG ratio 0.24 (concentrated). Esophagus runs straight underneath cerebral commissure (Fig. 166).

TABLE 10. Osphradium measurements (in mm) of *Milesiana schuelei* from the following populations: 1 - Turrillas (El Nacimiento), Almería; 2 - El Laujar de Andarax, Almería; 3 - La Cimada, Málaga; 4 - Padul, Granada; 5 - El Marchal, Almería; 6 - Lucainena de Las Torres, Almería; 7 - Gaucín, Málaga.

	1 Mean ± SD; CV (Max-Min) (n = 8)	2 Mean ± SD; CV (Max-Min) (n = 3)	3 Mean ± SD; CV (Max-Min) (n = 3)	4 Mean ± SD; CV (Max-Min) (n = 2)	5 Mean ± SD; CV (Max-Min) (n = 4)	6 (n = 1)	7 (n = 1)
Os L	0.17 ± 0.02; 0.13 (0.19-0.13)	0.23 ± 0.03; 0.11 (0.25-0.20)	0.24 ± 0.01; 0.05 (0.26-0.23)	0.16 ± 0.02; 0.14 (0.17-0.14)	0.22 ± 0.03; 0.14 (0.27-0.20)	0.21	0.16
Os W	0.08 ± 0.01; 0.09 (0.10-0.07)	0.10 ± 0.02; 0.16 (0.11-0.09)	0.11 ± 0.04; 0.38 (0.14-0.06)	0.08 ± 0.02; 0.28 (0.09-0.06)	0.10 ± 0.03; 0.30 (0.13-0.06)	0.08	0.09

Ctenidium – Osphradium: Ctenidium with 8–13 well-developed lamellae (Fig. 167). Osphradium oval, two to three times longer than it is wide (Table 10).

Stomach – Radula: Anterior and posterior stomach chambers are of approximately same size. Style sac protruding slightly anteriorly into intestinal loop (Fig. 168, Table 12). Rectum strongly U-shaped (Fig. 167). Radula long (40%) relative to maximum shell dimension (Fig. 159); central tooth with two basal cusps on each side (Table 11, Figs. 160–162), distance between internal cusps 7–8 µm approximately; central denticle long, tapered, followed on each side by four long, tapered denticles in decreasing order of size;



FIGS. 154–165. Protoconch, operculum and radula of *Milesiana schuelei*. FIG. 154: Holotype (SMF 253578/1). FIGS. 155, 156, 158, 161, 162: Protoconchs, operculum and radula from Gaucín (Málaga); FIGS. 159, 160, 163: Radula from Marchal de Antón López (Almería); FIG. 158: Inner side of the operculum; FIGS. 159, 160: Transverse rows; FIGS. 161, 162: Central teeth; FIGS. 163, 164: Central, lateral and inner marginal teeth; FIG. 165: Inner and outer marginal teeth.

TABLE 11. Radula formulae and measurements (in mm) of *Milesiana schuelei* from Benaoján (Málaga) population.

Radula characters	Formulae and measurements (in mm)
Central teeth	4-(3.5)+C+4(3)/2-2
Central teeth width	~ 8 µm
Left lateral teeth	4-5+C+3
Inner marginal teeth	~ 22 cusps
Outer marginal teeth	~ 10 cusps
Radula length	~ 351 µm
Radula width	~ 46 µm
Number of rows	~ 85

lateral teeth with 3–4 denticles on each side central one (Figs. 163, 164); denticles of inner marginal teeth larger than those of outer marginal teeth (Fig. 165).

Male Genitalia: With pear-shaped prostate gland (Fig. 169) almost two times longer than it is wide (Table 13), partially covered by rectum in pallial cavity; penis (Figs. 170, 171) generally unpigmented or with a slight dark pigmentation at base, with a blunt distal tip and one unpigmented, big, non-glandular lobe located in medial position; penial duct slightly undulating at the base, then running straight close to outer edge.

Female Genitalia: With renal oviduct making a narrow circle overlying the part between albumen and capsule glands (Fig. 172), oviduct glands (albumen + capsule glands) well developed, sometimes narrowing at outer edge between capsule and albumen glands; capsule gland larger than albumen gland; distal seminal receptacle (SR1) much larger than proximal (SR2); SR1 pyriform, pedunculated, SR2 rounded, sessile (Figs. 172, 173, Table 14), located rather close to one another; the renal oviduct widening distally with respect to SR2.

Discussion

Milesiana schuelei cannot be assigned to the genus *Islamia* because of differences in several diagnostic characters including some of the female genitalia and principally those related to the seminal receptacles. The numerous females studied and collected throughout different months of the year from populations of Almería, Granada, Málaga and Cáceres provinces had a remarkably large and pedunculated distal seminal receptacle (SR1), whereas the proximal one (SR2) was small and sessile. Moreover, illustrations in Boeters (1988: 218) depict a pedunculated distal seminal receptacle and a rounded and sessile proximal receptacle apparently protruding from the widened part of the renal oviduct, in a position corresponding to that of the proximal seminal receptacle. Both character states, a very large and pedunculated distal seminal receptacle (SR1) and a proximal one (SR2) small and sessile, are the opposite of those observed in *Islamia* (SR1 is always smaller or equal in size than SR2, and in addition SR1 is usually sessile while SR2 is always pedunculated).

Bernasconi (1975, 1977, 1984, 1985) described a larger distal seminal receptacle for

TABLE 12. Digestive system measurements (in mm) of *Milesiana schuelei* from the following populations: 1 - Turrillas (El Nacimiento), Almería; 2 - La Cimada, Málaga; 3 - Gaucín, Málaga.; 4 - El Laujar de Andarax, Almería.; 5 - Padul, Granada; 6 - El Marchal, Almería.

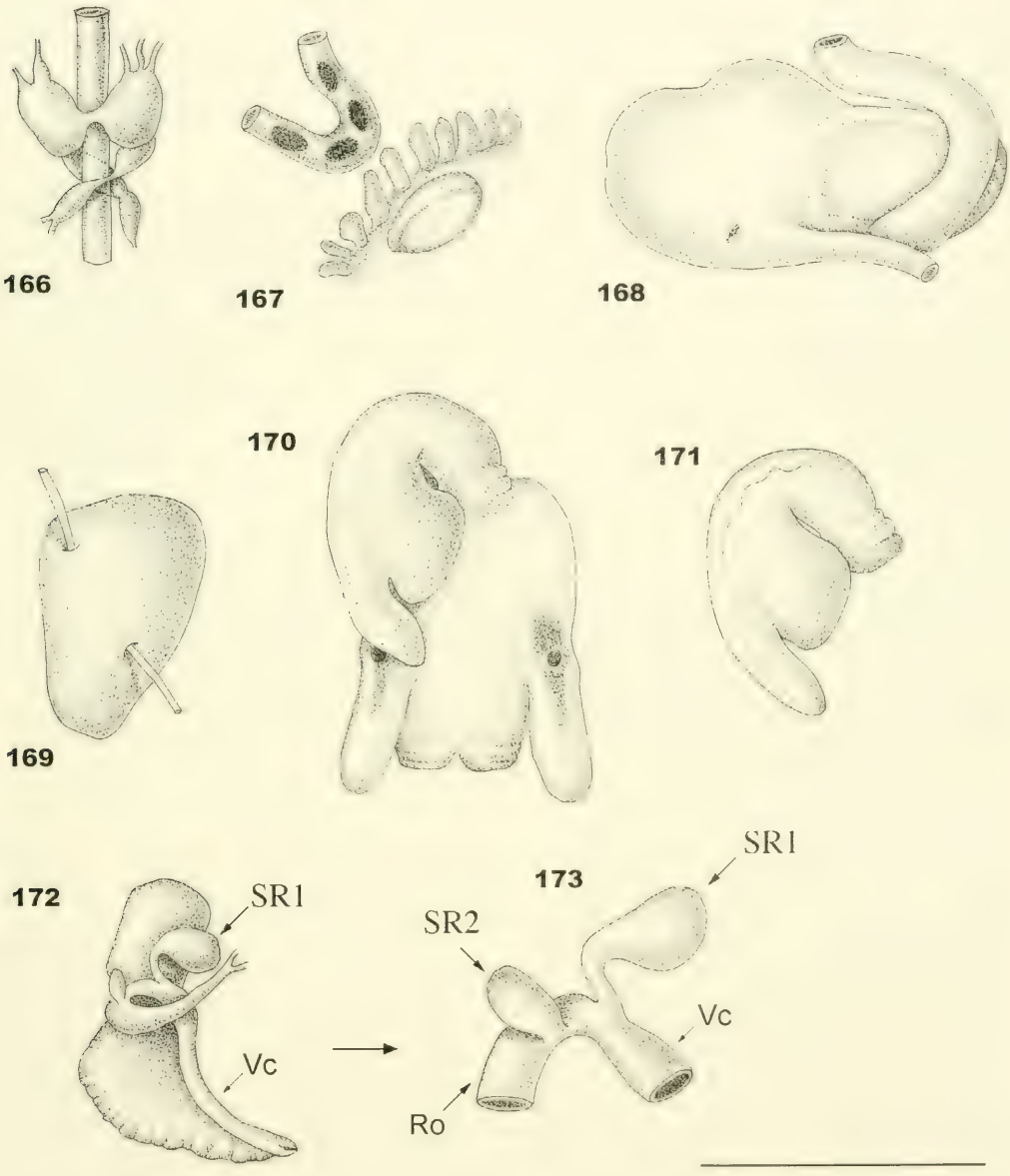
	1 Mean ± SD; CV (Max-Min) (n=3)	2 Mean ± SD; CV (Max-Min) (n=4)	3 Mean ± SD; CV (Max-Min) (n=2)	4 Mean ± SD; CV (Max-Min) (n=3)	5 Mean ± SD; CV (Max-Min) (n=2)	6 n = 1
Ss L	0.26 ± 0.02; 0.08 (0.28-0.23)	0.30 ± 0.02; 0.07 (0.33-0.29)	0.21 ± 0.02; 0.11 (0.22-0.19)	0.26 ± 0.01; 0.05 (0.28-0.25)	0.21 ± 0.06; 0.28 (0.26-0.17)	0.26
Ss W	0.19 ± 0.02; 0.14 (0.22-0.17)	0.24 ± 0.04; 0.15 (0.27-0.19)	0.17 ± 0.03; 0.18 (0.19-0.15)	0.21 ± 0.02; 0.08 (0.22-0.19)	0.17 ± 0.01; 0.08 (0.18-0.16)	0.20
St L	0.35 ± 0.02; 0.07 (0.37-0.32)	0.41 ± 0.03; 0.07 (0.45-0.38)	0.26 ± 0.02; 0.09 (0.28-0.24)	0.36 ± 0.04; 0.11 (0.39-0.32)	0.30 ± 0.04; 0.15 (0.33-0.27)	0.32
St W	0.26 ± 0.01; 0.04 (0.27-0.25)	0.39 ± 0.05; 0.12 (0.44-0.34)	0.29 ± 0.04; 0.13 (0.32-0.27)	0.33 ± 0.01; 0.04 (0.34-0.32)	0.25 ± 0.01; 0.02 (0.26-0.25)	0.33

TABLE 13. Male genitalia measurements (in mm) of *Milesiana schuelei* from the following localities: 1 - El Marchal, Almería; 2 - Turrillas (El Nacimiento), Almería; 3 - La Cimada, Málaga; 4 - Gaucín, Málaga; 5 - Benaoján, Málaga; 6 - Fuente Grande, S. Harana, Granada; 7 - Robladillo de Gata, Cáceres; 8 - Los Minutos spring, Turrillas, Almería; 9 - Lucainena de Las Torres, Almería; 10 - Padul, Granada.

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3 Mean ± SD; CV (Max-Min)	4 Mean ± SD; CV (Max-Min)	5 Mean ± SD; CV (Max-Min)	6	7	8	9	10
Pr L		0.43 ± 0.01; 0.02 (0.43-0.42) (n = 2)	0.68 ± 0.38; 0.56 (0.95-0.41) (n = 2)	0.29 (n = 1)		0.48 (n = 1)				0.33 (n = 1)
Pr W		0.20 ± 0.03; 0.16 (0.22-0.18) (n = 2)	0.29 ± 0.14; 0.49 (0.39-0.19) (n = 2)	0.16 (n = 1)		0.28 (n = 1)				0.16 (n = 1)
P L	0.45 ± 0.13; 0.29 (0.55-0.29) (n = 4)	0.48 ± 0.14; 0.30 (0.67-0.34) (n = 4)	0.63 ± 0.11; 0.17 (0.76-0.56) (n = 3)	0.52 ± 0.13; 0.25 (0.67-0.41) (n = 3)	0.8 1± 0.35; 0.44 (1.21-0.53) (n = 3)	0.60 (n = 1)	0.43 (n = 1)	0.53 (n = 1)	0.44 (n = 1)	
P W	0.14 ± 0.02; 0.17 (0.16-0.11) (n = 4)	0.15 ± 0.02; 0.14 (0.18-0.13) (n = 3)	0.15 ± 0.04; 0.28 (0.18-0.10) (n = 3)	0.16 ± 0.04; 0.28 (0.19-0.13) (n = 2)	0.20 ± 0.02; 0.12 (0.22-0.18) (n = 3)	0.11 (n = 1)	0.12 (n = 1)	0.15 (n = 1)	0.16 (n = 1)	
Pl. L	0.13 ± 0.02; 0.19 (0.15-0.10) (n = 4)		0.22 ± 0.09; 0.40 (0.27-0.12) (n = 3)	0.27 ± 0.00; 0.01 (0.27-0.27) (n = 3)	0.14 ± 0.05; 0.37 (0.20-0.11) (n = 3)	0.19 (n = 1)	0.23 (n = 1)			
Pl. W	0.17 ± 0.03; 0.19 (0.21-0.14) (n = 4)		0.11 ± 0.04; 0.32 (0.15-0.08) (n = 3)	0.14 ± 0.01; 0.09 (0.15-0.13) (n = 3)	0.11 ± 0.01; 0.08 (0.12-0.11) (n = 3)	0.16 (n = 1)	0.11 (n = 1)			
Head length	0.49 ± 0.09; 0.17 (0.57-0.39) (n = 4)	0.56 ± 0.05; 0.10 (0.62-0.53) (n = 3)	0.70 ± 0.16; 0.22 (0.87-0.57) (n = 3)	0.66 ± 0.27; 0.41 (0.98-0.50) (n = 3)	0.73 ± 0.15; 0.21 (0.88-0.57) (n = 3)	0.59 (n = 1)	0.42 (n = 1)	0.39 (n = 1)		
PL/Head length	0.94 ± 0.37; 0.39 (1.40-0.51) (n = 4)	0.74 ± 0.17; 0.23 (0.94-0.63) (n = 3)	0.94 ± 0.25; 0.27 (1.16-0.67) (n = 3)	0.81 ± 0.14; 0.17 (0.96-0.68) (n = 3)	1.11 ± 0.45; 0.40 (1.63-0.79) (n = 3)	1.01 (n = 1)	1.02 (n = 1)	1.35 (n = 1)		

French *Islamia* species, which later Bodon et al. (2001: 199) considered to be a misinterpretation.

Milesiana schuelei shows a wide range of inter-population variability in shell shape, body pigmentation, and narrowing between the oviduct glands. Even the size of SR1 varies although it is always much larger than SR2. In addition to the size and shape of the seminal receptacles, other characters that distinguish *M. schuelei* from other Iberian *Islamia* species include: a flatter shell, larger umbilicus, a well-



FIGS. 166–173. Anatomy of *Milesiana schuelei*. FIG. 166: Partial nervous system; FIG. 167: Osphradium and ctenidium; FIG. 168: Stomach; FIG. 169: Prostate; FIG. 170, 171: Head of a male and penis; FIG. 172: Anterior female genitalia; FIG. 173: Detail of the seminal receptacles; Abbreviations in text. Scale bar = 500 µm (FIGS. 166–172).

TABLE 14. Female genitalia measurements (in mm) of *Milesiana schuelei* from the following populations: 1 - El Marchal, Almería; 2 - Turrillas (El Nacimiento), Almería; 3 - La Cimada, Málaga; 4 - Gaucín, Málaga; 5 - El Laujar de Andarax, Almería.

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3 Mean ± SD; CV (Max-Min)	4 Mean ± SD; CV (Max-Min)	5 Mean ± SD; CV (Max-Min)
Op L	0.72 ± 0.15; 0.21 (0.87-0.56) (n = 3)	0.56 ± 0.13; 0.23 (0.78-0.45) (n = 6)	0.87 ± 0.02; 0.03 (0.88-0.85) (n = 2)	0.76 ± 0.14; 0.19 (0.89-0.61) (n = 3)	0.63 ± 0.11; 0.18 (0.80-0.56) (n = 4)
Op W	0.29 ± 0.04; 0.15 (0.33-0.24) (n = 3)	0.26 ± 0.04; 0.15 (0.31-0.21) (n = 6)	0.30 ± 0.02; 0.07 (0.32-0.29) (n = 2)	0.32 ± 0.08; 0.24 (0.38-0.23) (n = 3)	0.27 ± 0.02; 0.08 (0.30-0.24) (n = 4)
Ag. L	0.28 ± 0.07; 0.25 (0.35-0.21) (n = 3)	0.21 ± 0.06; 0.31 (0.28-0.16) (n = 3)	0.34 (n = 1)	0.28 ± 0.07; 0.25 (0.33-0.20) (n = 3)	0.31 ± 0.07; 0.22 (0.38-0.25) (n = 3)
Cg. L	0.43 ± 0.20; 0.46 (0.66-0.27) (n = 3)	0.40 ± 0.10; 0.26 (0.49-0.28) (n = 3)	0.55 (n = 1)	0.44 ± 0.13; 0.30 (0.58-0.31) (n = 3)	0.34 ± 0.07; 0.21 (0.41-0.28) (n = 3)
SR1 L	0.11 ± 0.02; 0.18 (0.12-0.09) (n = 3)	0.13 ± 0.02; 0.17 (0.16-0.12) (n = 6)	0.16 ± 0.01; 0.03 (0.16-0.15) (n = 3)	0.11 ± 0.00; 0.03 (0.11-0.11) (n = 2)	0.10 ± 0.02; 0.23 (0.13-0.09) (n = 3)
SR2 L	0.04 ± 0.01; 0.26 (0.04-0.03) (n = 2)	0.04 ± 0.02; 0.46 (0.06-0.01) (n = 6)	0.07 ± 0.01; 0.20 (0.09-0.06) (n = 3)	0.05 ± 0.02; 0.51 (0.07-0.03) (n = 2)	0.06 ± 0.01; 0.22 (0.07-0.05) (n = 3)

developed ctenidium with large lamellae, rectum U-shaped, central tooth with two basal cusps, and a penial lobe located in a medial position instead of close to the penial tip. The pleuro-subesophageal connective is absent in *Milesiana*, whereas it is present in all the other European genera for which information on this character is available (Radoman, 1983), except in the genus *Josefus* described herein.

Due to the peculiar structure of the female genitalia, *M. schuelei* can only be compared with *Pezzolia* Bodon & Giusti, 1986, another European valvatiform genus from Liguria (Italy), which has a distal seminal receptacle equal to or larger than the proximal receptacle. Nevertheless, the distal seminal receptacle in *Pezzolia* has no evident duct. This genus may at times have a very reduced bursa copulatrix. It has neither eyes nor ctenidium, and has only one basal cusp on the central tooth of the radula. This genus and its type species, *Pezzolia radapalladis* Bodon & Giusti, 1986, were described using extremely variable diagnostic genital characters (Bodon et al., 2001: 147–149, 158, 166, 167). According to these authors, *Pezzolia* may have a simple penis (with no glandular lobe) or there may be one or two glandular lobes, located in a medial position or one in a medial position and the other near the base of the penis. *Pezzolia* female genitalia can lack a bursa copulatrix (or if present, it is very small), and proximal seminal receptacle that can be equal to or smaller than the distal seminal receptacle. This unusual and extreme anatomical variability suggests that in order to clarify their taxonomic status, the morphological characters of all known populations of the genus *Pezzolia* and particularly those of the species *Pezzolia radapalladis*, *P.* sp. 1 and *P.* sp. 2 need to be carefully reviewed and studied.

The combination of two diagnostic characters (a large and pedunculated distal seminal receptacle and a short and sessile proximal receptacle), which is consistent in all studied populations of this widely distributed species, together with the absence of bursa copulatrix, the absence of pleuro-supraesophageal connective and other distinguishing shell and anatomical features, differentiates *M. schuelei* from all other known European Hydrobiidae valvatiform species. Therefore, we consider it justified creating distinct supraspecific taxa for this species, which we have called *Milesiana*.

Josefus Arconada & Ramos, n. gen.

Type species

Josefus aitanica, n. sp.

Etymology

In memoriam of our friend and colleague Jose Bedoya "Josefo", who, through his skills working with the SEM, helped us to discover the huge morphological diversity and complexity of this small fauna.

Diagnosis

Shell small valvatiform or depressed-trochiform; operculum without peg; central tooth with two basal cusps on each side; penis with a non-glandular lobe located in distal position; female genitalia with two seminal receptacles adjacent to one another, on the same side of unpigmented renal oviduct; bursa copulatrix absent.

Josefus aitanica Arconada & Ramos, n. sp.

Type Specimens

Holotype MNCN 15.05/46560 (SEM preparation) (Fig. 174), Paratypes MNCN 15.05/46560, 3 May 1994, E. R. (ethanol and SEM preparation – Figs. 177, 181, 182, 184 – and ethanol).

Type Locality

Torremanzanas, Alicante, UTM.: 30SYH2476.

Etymology

The name *aitanica* refers to Sierra de Aitana, a mountain chain in the distribution area of this species.

Populations Studied

This species was found in the provinces of Valencia and Alicante (Fig. 17). Lapica spring, Las Viñuelas, Valencia, UTM.: 30SXJ7155, 28 May 1998, B. A. & J. A., MNCN 15.05/46561 (dried and frozen material); La Granata, Tabernes de La Valldigna, Valencia, UTM.: 30SYJ358302, 21 March 1994, E. R., MNCN 15.05/46562 (ethanol), 27 May 1998, B. A. & J. A., MNCN 15.05/46563 (ethanol, SEM preparation and frozen material); Gamellons spring, Onteniente, Valencia, UTM.: 30SXH975942, 5 Oct. 1994, E. R., MNCN 15.05/46564 (ethanol), 29 May 1998, B. A., MNCN 15.05/46565 (ethanol and frozen material); Gaspar spring, Beniganim, Valencia, UTM.: 30SYJ2113, 5 April 1994, E. R., MNCN 15.05/46566 (ethanol), 29 May 1998, B. A. & J. A., MNCN 15.05/46567 (ethanol and frozen material), Pi spring, Beniganim, Valencia, UTM.: 30SYJ2113, 5 April 1994, E. R., MNCN 15.05/46568 (ethanol); Gamello spring, Cuatretonda, Valencia, UTM.: 30SYJ2514, 1 April 1994, G. T., MNCN 15.05/46569 (ethanol and SEM preparation); La Mina source, Jarafuel, Valencia, UTM.: 30SXJ645341, 28 May 1998, B. A. & J. A., MNCN.15.05/33290; Bella spring, Jarafuel, Valencia, Flores spring, Requena, Valencia, UTM: 30SXJ615725, 29 March 1992, G. T., MNCN 15.05/33263 (ethanol and SEM preparation), 27 May 1998, B. A. & J. A., MNCN 15.05/33289 (ethanol and frozen material); El Tollo spring, Requena, Valencia, UTM.: 30SXJ671513, 5 May 1994, E. R., MNCN 15.05/46570 (ethanol); El Moro spring, L'Algar springs, Callosa d'en Sarriá, Alicante, UTM.: 30SYH527831, 8 Dec. 1990, G. T., MNCN 15.05/46595 (ethanol); 30 May 1998, B. A. & J. A., MNCN 15.05/46571 (ethanol and frozen material); Reyinyosa spring, Bolulla, Alicante, UTM.: 30SYH5185, 30 April 1994, E. R., MNCN 15.05/46572 (ethanol), 30 May 1998, B. A. & J. A., MNCN 15.05/46573 (ethanol); Molí Montes spring, Agres, Alicante, UTM.: 30SYH1595, 3 May 1994, E. R., MNCN 15.05/46574 (ethanol); Azut spring, Alfafar, Alicante, UTM.: 30SYH12394, 4 May 1994, E. R., MNCN 15.05/46575 (ethanol), 29 May 1998, B. A. & J. A., MNCN 15.05/46576 (ethanol and frozen material).

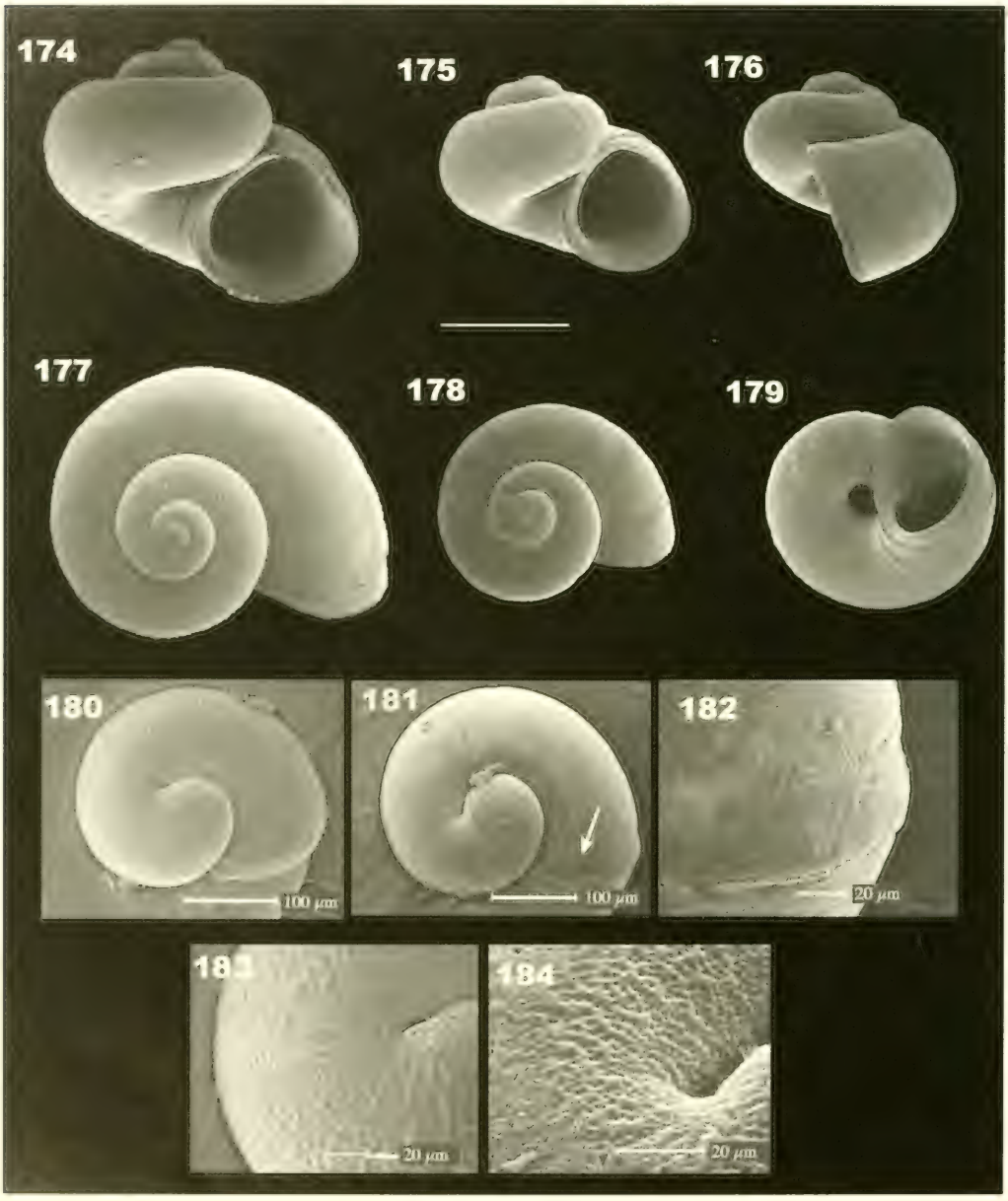
Specimens Examined for Morphometry and Histology

Shell and anatomical measurements (Tables 15, 17–19) correspond to populations from Alicante and Valencia. Operculum and radular measurements (Tables 15, 16) correspond to the population from type locality (more details in table captions). Male and females studied and measured were collected in the following months: March, April, May and Oct. For histology, one male and two females from type locality (May 1995) were studied.

Diagnosis

Operculum ovate; ctenidium absent; central tooth with two basal cusps on each side; eso-

phagus making a loop to the left posterior to cerebral ganglion complex; pleuro-subesophageal connective absent; rhomboid-shaped prostate gland; long pigmented penis with large non-glandular lobe located in distal position, never protruding from penis tip; two seminal receptacles small, sessile, rounded, equal in size, situated side by side on renal oviduct; all females with a nuchal node.



FIGS. 174–184. Shells of *Josefus aitanica*. FIGS. 174, 177, 181, 182, 184: Shells from Torremanzanas population (type locality); FIG. 174: Holotype (MNCN 15.05/46560); FIGS. 175, 176, 178–180, 183: Shells from Tabernes de la Valldigna population; FIGS. 181, 182: Varix separating protoconch and teleoconch. Scale bar = 500 µm (FIGS. 174–179).

Description

Shells: Valvatiform or depressed-trochiform (Table 15; Figs. 174, 175) with 3–3.5 whorls (Figs. 177, 178); about 1.5 spire whorls (Figs. 180, 181); highly developed body whorl (Figs. 177, 178); protoconch pitted (Figs. 183, 184), with 1.5 whorls; protoconch width 300 µm and width of nucleus approximately 105 µm; occasional varix observed at the end of protoconch seen in all populations (Figs. 181, 182); prosocline and rounded aperture; umbilicus of intermediate size, about 125 µm in diameter (Fig. 179); external lip (Figs. 176, 177) sometimes becoming thinner at its outer margin.

Operculum: Yellowish, oval, with rounded, big, central nucleus (Fig. 185); muscle attachment area rounded (Fig. 186).

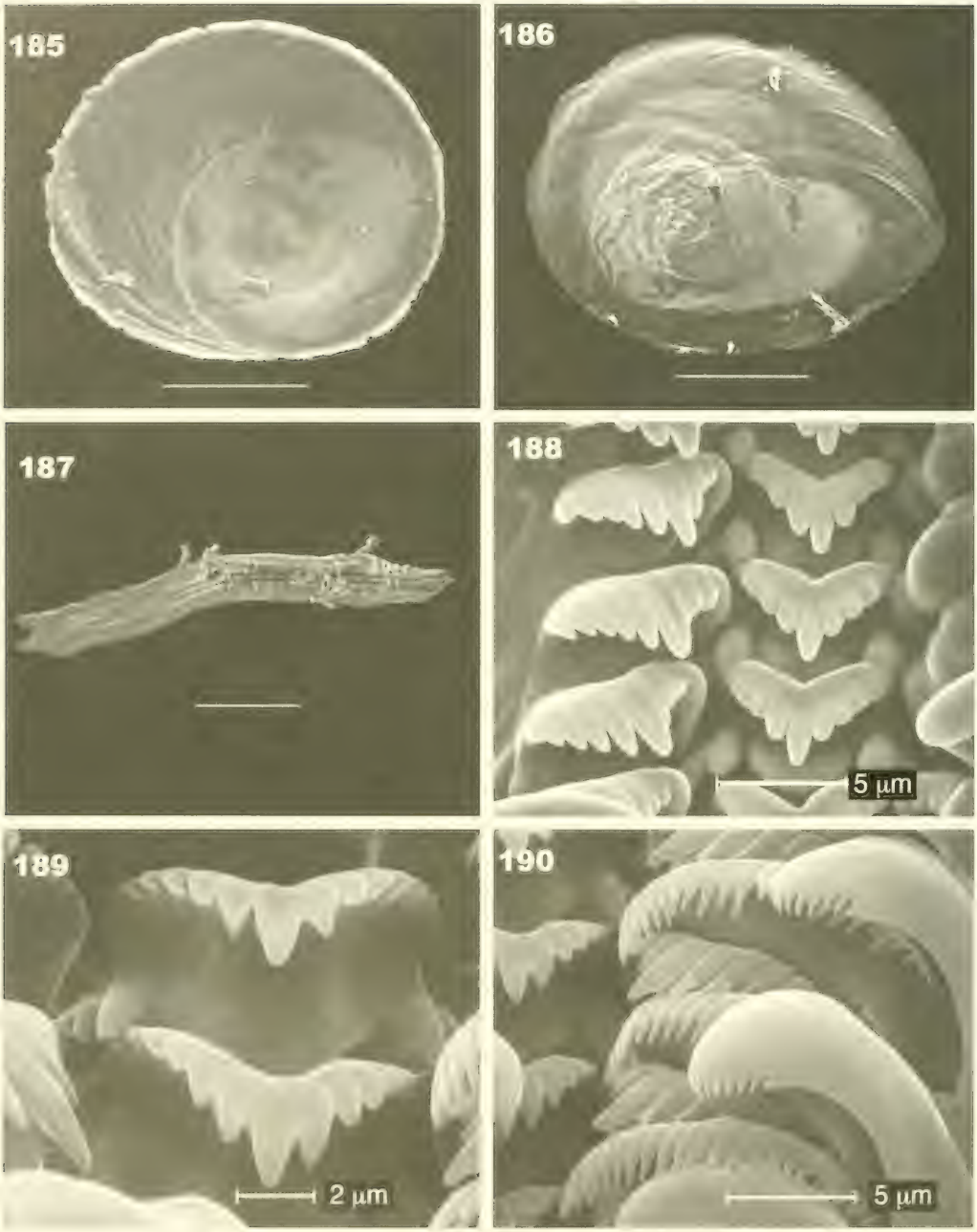
Body: Head with black-pigmented area from middle of tentacles to back of eye lobes (Figs. 191, 197); external body pigmentation dark.

Nervous System: Mid-sized pleuro-supraesophageal connective; pleuro-subesophageal connective absent (Fig. 192); supaesophageal ganglion small; RPG ratio 0.22 (concentrated). Esophagus making a marked loop posterior to left posterior to cerebral ganglia (Fig. 193).

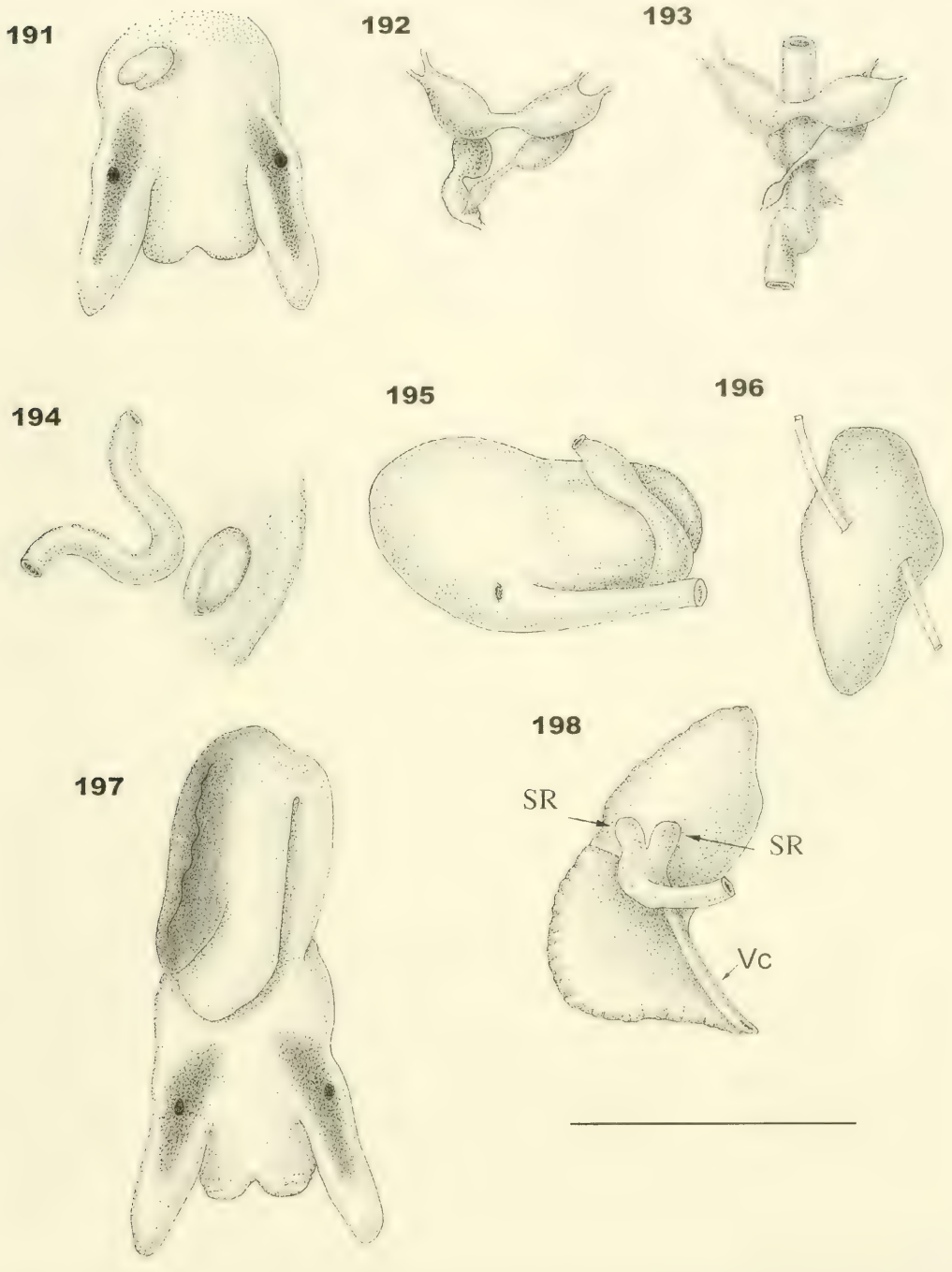
Ctenidium – Osphradium: Ctenidium absent (Fig. 194). Osphradium oval, two times longer than it is wide (Table 15).

TABLE 15. Shell. operculum and osphradium measurements (in mm) of *Josefus aitanica* from the following populations: 1 - Callosa d'en Sarriá, Alicante; 2 - Requena (Flores spring); 3 - type locality.

	1 Mean ± SD; CV (Max-Min) (n = 21)	2 Mean ± SD; CV (Max-Min) (n = 10)		3 Mean ± SD; CV (Max-Min)
SL	0.96 ± 0.06; 0.06 (1.07-0.83)	1.35 ± 0.17; 0.13 (1.53-0.97)	OL	0.60 ± 0.05; 0.08 (0.64-0.57) (n = 2)
SW	1.08 ± 0.08; 0.08 (1.24-0.83)	1.33 ± 0.14; 0.11 (1.58-1.06)	OW	0.47 ± 0.00; 0.01 (0.47-0.46) (n = 2)
SL/SW	0.89 ± 0.10; 0.11 (1.26-0.83)	1.01 ± 0.07; 0.07 (1.15-0.91)	OLWL	0.21 ± 0.04; 0.21 (0.25-0.18) (n = 2)
AH	0.62 ± 0.03; 0.05 (0.68-0.57)	0.84 ± 0.09; 0.11 (0.09-0.06)	OLWW	0.15 ± 0.01; 0.11 (0.16-0.13) (n = 2)
LBW	0.85 ± 0.05; 0.06 (0.94-0.70)	1.19 ± 0.14; 0.12 (1.34-0.89)	NL	0.27 ± 0.00; 0.00 (0.27-0.27) (n = 2)
WBW	0.75 ± 0.05; 0.07 (0.91-0.64)	1.07 ± 0.12; 0.11 (1.22-0.81)	NW	0.30 ± 0.00; 0.02 (0.30-0.29) (n = 2)
AL	0.60 ± 0.03; 0.05 (0.64-0.53)	0.79 ± 0.08; 0.10 (0.90-0.62)	OL/OW	1.29 ± 0.12; 0.09 (1.38-1.20) (n = 2)
AW	0.53 ± 0.03; 0.06 (0.60-0.48)	0.68 ± 0.07; 0.11 (0.78-0.54)	Os L	0.19 ± 0.08; 0.39 (0.30-0.10) (n = 5)
WPW	0.35 ± 0.04; 0.11 (0.41-0.24)		Os W	0.08 ± 0.03; 0.36(0.12-0.05) (n = 5)
WAW	0.14 ± 0.02; 0.17 (0.21-0.08)			
NSW	3.15 ± 0.18; 0.06 (3.50-3.00)	3.30 ± 0.16; 0.05 (3.50-3.00)		



FIGS. 185–190. Operculum and radula of *Josefus aitanica*. FIGS. 185, 186, 189, 190: Opercula and radula from Torremanzanas population (type locality); FIGS. 187, 188: Radula from Cuatretonda population: FIG. 185: Outer side of the operculum; FIG. 186: Inner side of the operculum; FIG. 187: Transverse rows: FIG. 188: Central and lateral teeth; FIG. 189: Central teeth; FIG. 190: Lateral, inner and outer marginal teeth. Scale bar = 200 µm (FIGS. 185, 186); 100 µm (FIG. 187).



FIGS. 191–198. Anatomy of *Josefus aitanica*. FIG. 191: Head of a female and nuchal node; FIGS. 192, 193: Partial nervous system; FIG. 194: Rectum and osphradium; FIG. 195: Stomach; FIG. 196: Prostate; FIG. 197: Head of a male and penis; FIG. 198: Anterior female genitalia; Abbreviations in text. Scale bar = 500 µm.

TABLE 16. Radula formulae and measurements (in mm) of *Josefus aitanica* from type locality.

Radula characters	Formulae and measurements (in mm)
Central teeth	5+C+5/2-2
Central teeth width	~ 6.3 µm
Left lateral teeth	5+C+3
Inner marginal teeth	~ 22 cusps
Outer marginal teeth	~ 24 cusps
Radula length	~ 400 µm
Radula width	~ 43 µm
Number of rows	~ 85

TABLE 17. Digestive system measurements (in mm) of *Josefus aitanica*. Populations from: (a) Torremanzanas, Alicante (type locality); (b) Callosa d'en Sarriá, Alicante; (c) Tabernes, Valencia; (d) Requena, Valencia.

	n = 1
Ss L	0.18(a); 0.27(b); 0.26(c); 0.24(d)
Ss W	0.18(a); 0.22(b); 0.14(c); 0.21(d)
St L	0.36(a); 0.30(b); 0.28(c); 0.36(d)
St W	0.33(a); 0.37(b); 0.28(c); 0.34(d)

Stomach – Radula: Length and width equal, stomach chambers same size; style sac protruding anteriorly into the intestinal loop (Table 17, Fig. 195). Rectum U-shaped (Fig. 194). Radula (Table 16, Fig. 187) long (41%) relative to maximum shell dimension; central trapezoidal tooth with two basal cusps on each side that points towards the lateral margins (Figs. 188, 189); cutting edge markedly concave, five denticles in decresing order of size at each side of central denticle,

TABLE 18. Male genitalia measurements (in mm) of *Josefus aitanica* from the following localities: 1 - Torremanzanas. Alicante (type locality); 2 - Beniganim. Valencia; 3 - Onteniente, Valencia; 4 - Tabernes, Valencia; 5 - Callosa d'en Sarriá, Alicante; 6 - Requena (El Tollo), Valencia; 7 - Agres, Alicante.

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3 Mean ± SD; CV (Max-Min)	4 Mean ± SD; CV (Max-Min)	5	6	7
Pr L	0.37 ± 0.03; 0.07 (0.39-0.36) (n = 2)						0.44 (n = 1)
Pr W	0.19 ± 0.03; 0.16 (0.21-0.17) (n = 2)						0.22 (n = 1)
P L	0.64 ± 0.24; 0.37 (0.94-0.34) (n = 5)	0.73 ± 0.12; 0.16 (0.81-0.65) (n = 2)	0.93 ± 0.11; 0.12 (1.03-0.81) (n = 3)	0.70 ± 0.04; 0.05 (0.72-0.66) (n = 3)	0.39 (n = 1)	0.63 (n = 1)	1.01 (n = 1)
P W	0.17 ± 0.06; 0.33 (0.24-0.10) (n = 5)	0.12 ± 0.00; 0.01 (0.12-0.12) (n = 2)	0.18 ± 0.02; 0.12 (0.21-0.16) (n = 3)	0.17 ± 0.02; 0.13 (0.19-0.15) (n = 2)		0.15 (n = 1)	0.22 (n = 1)
Pl.L	0.16 ± 0.05; 0.29 (0.23-0.10) (n = 5)	0.12 ± 0.03; 0.27 (0.14-0.09) (n = 2)	0.17 ± 0.02; 0.09 (0.19-0.16) (n = 3)	0.12 ± 0.02; 0.14 (0.10-0.14) (n = 3)	0.16 (n = 1)	0.14 (n = 1)	0.13 (n = 1)
Pl.W	0.11 ± 0.04; 0.38 (0.18-0.07) (n = 5)	0.08 ± 0.00; 0.04 (0.09-0.08) (n = 2)	0.14 ± 0.02; 0.12 (0.15-0.12) (n = 3)	0.08 ± 0.02; 0.22 (0.10-0.07) (n = 3)	0.09 (n = 1)	0.10 (n = 1)	0.12 (n = 1)
Head length	0.57 ± 0.13; 0.23 (0.75-0.46) (n = 5)	0.66 (n = 1)	0.74 ± 0.05; 0.07 (0.77-0.70) (n = 2)	0.59 ± 0.06; 0.10 (0.66-0.54) (n = 3)	0.57 (n = 1)	0.46 (n = 1)	0.81 (n = 1)
P L/ Head length	0.87 ± 0.52; 0.14 (1.57-0.14) (n = 5)	0.98 (n = 1)	1.3 5± 0.02; 0.01 (1.36-1.33) (n = 2)	1.19 ± 0.18; 0.15 (1.35-1.00) (n = 3)	0.69 (n = 1)	1.37 (n = 1)	1.25 (n = 1)

TABLE 19. Female genitalia measurements (in mm) of *Josefus aitanica* from the following populations: 1 - Torremanzanas, Alicante (type locality); 2 - Callosa d'en Sarriá, Alicante; 3 - Requena (Flores spring), Valencia; 4 - Tabernes, Valencia.

	1 Mean ± SD; CV (Max-Min)	2 Mean ± SD; CV (Max-Min)	3 Mean ± SD; CV (Max-Min)	4 Mean ± SD; CV (Max-Min)
Op L	0.58 ± 0.09; 0.15 (0.70-0.47) (n = 5)	0.64 (n = 1)	0.65 ± 0.10; 0.15 (0.72-0.58) (n = 2)	0.64 (n = 1)
Op W	0.20 ± 0.03; 0.15 (0.16-0.15) (n = 5)	0.26 (n = 1)	0.27 ± 0.04; 0.14 (0.30-0.24) (n = 2)	0.28 (n = 1)
Ag. L	0.26 (n = 1)	0.34 (n = 1)	0.24 ± 0.02; 0.09 (0.26-0.22) (n = 2)	0.37 (n = 1)
Cg. L	0.36 (n = 1)	0.30 (n = 1)	0.42 ± 0.12; 0.29 (0.50-0.33) (n = 2)	0.27 (n = 1)
SR1 L	0.08 ± 0.02; 0.19 (0.10-0.07) (n = 3)	0.05 ± 0.01; 0.16 (0.05-0.04) (n = 2)	0.08 ± 0.02; 0.18 (0.10-0.07) (n = 2)	0.07 ± 0.01; 0.08 (0.07-0.06) (n = 2)
SR2 L	0.05 (n = 1)	0.05 ± 0.01; 0.16 (0.05-0.04) (n = 2)	0.06 ± 0.01; 0.13 (0.06-0.05) (n = 2)	0.06 (n = 1)

lateral teeth with five denticles on each side a central one (Fig. 188); denticles of inner marginal teeth larger than those of outer marginal teeth (Fig. 190).

Male Genitalia: Prostate gland (Fig. 196; Table 18), almost rhomboidal, more slender anteriorly and located quite posterior to rectum loop; posterior vas efferens entering near middle prostate region and anterior vas efferens exits close to this point; penis large, dark pigmented (Fig. 197), with a well-developed, non-glandular, subterminal, unpigmented lobe that is longer than penis tip; penial duct undulating along penis length at right edge.

Female Genitalia: Two seminal receptacles, small, sessile, rounded, equal in size, arising side by side on the renal oviduct facing the albumen gland (usual position where SR2 arises from proximal oviduct) (Fig. 198); renal oviduct not widening posteriorly to SR2 and makes a tight circle over pallial oviduct; oviduct glands (albumen + capsule glands) do not usually narrow, although some females narrow slightly at outer edge, between capsule and albumen gland; albumen gland smaller than capsule gland, and occupying approximately 40% of total length of pallial oviduct; ovary overlying posterior chamber of stomach. Unpigmented nuchal node (Fig. 191) in an analogous position to that of penis, occupying $^1/_4$ of total head length, 0.14 µm approximately.

Discussion

Josefus aitanica shows little interpopulation variability in the size of the oviduct glands, the presence/absence of narrowing between capsule and albumen glands, and the size and colour of the penis. All females studied and collected in different months throughout the year – March, April, May, Oct. – had a nuchal node, similar to that described in females of the genus *Islamia*. No cases of parasitism were detected. The esophagus forms a tight pleat below the left posterior portion of the pleuro-oesophagal ganglionic complex, whereas it is only slightly curved in *I. henrici*, the only Hydrobiidae species in which this character has been described. The new species can be distinguished from all the other Hydrobiidae by the shape and position of the seminal receptacles, which are both sessile, equal in size

and emerge adjacent to each other on the same side of the renal oviduct. In the very few *Islamia* species where the two seminal receptacles have been observed close to one another (*I. valvataeformis* or *I. pallida*), they appear on opposite sides of the renal oviduct and, unlike in *J. aitanica*, are never equal in size and shape. The loop made by the renal oviduct is rather small and quite tight, and there is no widening of the oviduct before the loop.

DISCUSSION

Habitat Status and Conservation

The species described here live in apparently non-polluted springs, rich in aquatic vegetation. Specimens can be found on vegetation, stones, wet walls and in mud. *Milesiana schuelei* has the widest geographical distribution range of the species studied. In the last decade, *M. schuelei* has been severely threatened in Almería Province due to engineering projects aimed at optimising water resources in this extremely arid area, thus depleting groundwater resources essential for hydrobiid survival. In contrast, *Islamia globulus* populations are well conserved, since water resources are sufficient in its distribution area. *Islamia ateni* is only known from its type locality (Balneario de San Vicente), a thermal spring that was seriously affected by the construction of a motorway. Since then, no specimens have been found, suggesting they are probably now extinct. Specimens of *I. pallida*, *I. henrici henrici* and *I. h. giennensis* are rare in the springs where they were discovered. Both species have a very narrow distribution and are highly threatened by human activities. The populations of the last two subspecies have been declining since they were first found. Channelization has dessicated many of the natural habitats of *I. h. giennensis*. The species has disappeared from some of the springs that previously held many of the better-conserved populations.

The same is occurring with *Josefus aitanica*, although the majority of its populations are not yet threatened. *Islamia lagari* is restricted to a very small area (Sierra de Can Parés), although no live specimens have been collected for years. Following IUCN criteria we classify these species as follows: Extinct (EX) – *Islamia ateni*; Critically Endangered (CR) – *Islamia pallida*, *I. lagari* and both subspecies of *I. henrici* as; Lower Risk (LR) – *Islamia globulus*, *Josefus aitanica* and Conservation Dependent (cd) – *Milesiana schuelei*.

Genital Morphology and Functionality

Taxonomy at the rank of genus and family levels has been traditionally based on anatomical characters, especially those of the male and female genitalia. Among these, penis structure and number and position of the sac-like structures associated with the renal oviduct have usually received more taxonomic weight as they are generally constant in species and species groups.

The exact function of the sac-like structures on the renal oviduct of females of *Islamia* and *Neohoratia* has long been in question. It has been thought that these structures are either two seminal receptacles or a small bursa copulatrix and a seminal receptacle. In the past, authors described these structures in many species as a seminal receptacle and a pin-like or sessile bursa copulatrix (Bole, 1970; Bernasconi, 1975). Histological observations and other direct morphological evidence have clarified many previous doubts regarding these structures. Pearly-whitish refringence is undoubtedly related to the way spermatozoa are organized in the seminal receptacles or in other sperm storage areas of the renal oviduct (Davis & Kang, 1990; Davis et al. 1990; Ramos et al., 2001). The bursa copulatrix is almost translucent and its contents are never refringent. The location of the sac-like structures in relation to the ovary and the pallial glands (albumen + capsule glands) is also useful for identification. When the bursa copulatrix is absent and there are two seminal receptacles, the proximal seminal receptacle (SR2) emerges from the oviduct close to the end of the loop, and the distal seminal receptacle (SR1) originates at a point closer to where the oviduct enters the albumen gland, close to but more proximally located than the usual position of the bursa copulatrix (Bodon et al., 2001).

The epithelium differs between the bursa and the seminal receptacles, as does the physiological function of these organs and the way spermatozoa are dispersed within them. In the receptacles, the spermatozoa face the cilia of the inner epithelial cells, while they have no directional pattern in the bursa (see Genital Histology above). Bodon et al. (2001) stated that *Islamia ateni*, *I. globulus*, and *I. lagari* have two seminal receptacles. Histological evidence and morphological observation of the female genitalia of *Milesiana schuelei*, *Islamia globulus*, *I. h. henrici*, and *Josefus aitanica* indisputably confirm their assertion, and we apply it to all the species studied herein. Given

that the female genitalia of *Neohoratia subpiscinalis* (Kuscer, 1932) are currently described as having a poorly developed bursa copulatrix and a single seminal receptacle (Bole, 1993; Bodon et al., 2001), we redefine the taxonomic status of some Iberian taxa that were previously referred to and included in the genus *Neohoratia* (as *N. globulus globulus*, *N. g. lagari*, *N. ateni*) and ascribe them to *Islamia*, following previous papers (Bodon et al., 2001).

Without providing real histological evidence (serial sections), some authors have interpreted the refringent area, or "*banda traslucida*", in the penial lobe of *Islamia* species to be a mass of glandular cells (Giusti et al., 1981: 51, Bodon et al., 2001: 133). This area can also be observed in the penis when mounted on microscope slides. This interpretation led Bodon et al. (2001: 134) to conclude that *Islamia* had a "penis with one glandular (rarely non-glandular) lobe". This is the first study to investigate the penial lobe of *Islamia* species using histological serial sections. The males we observed show this refringency in the penial lobe (also seen in microscope slides), although it lacks glandular tissue. We conclude that morphological refringence in penial structures cannot be attributed to a mass of glandular cells.

Bodon et al. (2001) studied two males from the type locality of *I. valvataeformis* as well as *I. globulus* from two population of Huesca. He concluded that the refringence observed in the penial lobe of both species was made up of a mass of glandular cells. We were unable to study specimens of the type species of the genus, but the serial sections of the *I. globulus* we examined clearly demonstrated that the refringence observed in its penial lobe was of a non-glandular nature. In view of our findings, we suggest eliminating from the diagnosis of the genera any reference to the nature of the tissue observed in the refringent area of the penial lobe if the tissue has not been studied using serial sections. Further histological studies of this kind for the type species *I. valvataeformis* are particularly needed.

Character Variability in the Genus *Islamia*

Radoman (1973a) introduced the genus *Islamia* (type species: *Horatia servaini* Bourguignat, 1887, a junior synonym of *Hydrobia valvataeformis* Möllendorf, 1873, according to Radoman, 1983, from Vrelo Bosne, near Sarajevo), with two subgenera, *Islamia* and *Adriolitorea* (type species: *I.* (*Adriolitorea*) *zermanica* Radoman 1973, from the Zrmanja River, in the middle freshwater section). Each subgenus contained two species from the Balkans: *I.* (*Islamia*) *servaini* (Bourguignat, 1887), *I.* (*Islamia*) *bosniaca* Radoman, 1973; *I.* (*Adriolitorea*) *zermanica* Radoman, 1973; and *I.* (*Adriolitorea*) *latina* Radoman, 1973. Radoman (1973a) stated that the four species are anatomically identical except for a slight difference in penis structure, which justified their separation into two groups ("*Bien que l'anatomie de toutes ces espéces soit identique, il y a une légère difference dans la structure du pénis, ce qui les sépare en deux groupes*"): The penis is slightly split at the top in *Islamia*, whereas the penial branches are longer and slightly more slender in *Adriolitorea*. Based on this difference the author suggested that there were two ancestors for these two groups of species, one from central Bosnia (*Islamia* s.s.) and the other from the coastal area (*Adriolitorea*). Later on, Radoman (1973b) included the following species in *Islamia*: a new species from Greece (*I. graeca* Radoman 1973), two new species from Turkey (*I. pseudorientalica* Radoman 1973, and *I. anatolica* Radoman 1973), plus one previously described species *I. burnabasa* (syn. *Horatia burnabasa* Schütt, 1964). The last three live in sympatry (type locality: Kirkgöz, Anatolia, Turkey). Although these descriptions were based on conchological characters, Radoman (1973b) concluded that all the species were anatomically identical to other species of the genus *Islamia*. In his 1983 paper, he assigns all eight above-mentioned species from Bosnia-Herzegovina, Croatia, Greece and Turkey plus *I. trichoniana* Radoman, 1978, from Greece to *Islamia*. The subgenus *Adriolitorea* was, therefore, regarded as a synonym of *Islamia*. According to Radoman (1973a, 1983) *Islamia* is characterised by: "(1) shell valvatoid, with a roundish-ovoid aperture and wide umbilicus, (2) central tooth of the radula with two basal cusps (one on each side, according to drawings of Radoman, 1973a), (3) a long pleuro-supraintestinal and a short pleuro-subintestinal connective, and (4) two seminal receptacles present (rs1 and rs2), nearby at the same level, draining into the oviduct. A genital chamber absent." The penis is described as "very large, muscular, wide, split at the top, vas deferens draining at the point of the right branch. Near the penis point, on the ventral side, a muscular fold is present. Penis shape is to some extent variable in different species of this genus" (Radoman, 1983: 124, figs. 69, 70). In fact, while the size and shape of the two penial branches differ among

these species, all possess a muscular pleat at the centre of the ventral side of the penis. Radoman did not mention any glandular tissue inside the penis branches. Description of the female genital system was only provided for the type species (*I. valvataeformis*) (Radoman, 1973a, 1983), and according to Radoman's comments (1973a, b) female genitalia do not seem to vary among species. In other words, only conchological and penial characters differ among *Islamia* species.

The tenth species assigned to *Islamia* was *Valvata pusilla* Piersanti, 1952 (Giusti et al., 1981), from Italy (type locality: Grotta delle Fontanelle, Napoli). In the description of this species, the authors introduced for the first time the concept that the translucid band observed on the penial lobe corresponded to a mass of glandular cells. They also described three other groups of populations as "*Islamia* sp. forma A", "forma B", and "forma C" from three different areas of Italy without giving them a taxonomical category. These four groups of populations, as well, were differentiated only by penial and conchological characters.

According to Bodon et al (2001), *Islamia* includes 19 species to date, in addition to those of Spain. In this paper, the authors considered *Mienisiella* Schütt, 1991, to be a junior synonym of *Islamia*, thus expanding the distribution area of the genus to Lebanon – *I. gaillardoti* (Germain, 1911) – and to Israel – *I. mienisi* (Schütt, 1991), the type species of *Mienisiella*. Whereas the penial and conchological characters in these latter two species differ, they both have female genitalia that are similar to those previously described for *Islamia* species.

Considering all these species, Bodon et al. (1995) distinguished a group comprised of "oriental" species from the Balkan Peninsula (Croatia, Bosnia, Greece) and Turkey and an "occidental" species' group located in France, Spain, and Italy. The oriental taxa shared two penial characters: a very well-developed glandular penial lobe and a non-glandular (muscular) pleat on the ventral side of the penis. These two characters are also found in *Islamia pusilla* (Piersanti, 1952), the unique species inhabiting south central Italy (Giusti et al., 1981), and in *I. cianensis* Bodon et al., 1995, from Sicily, although the penial lobe is more reduced in the last species. The degree of development of the muscular pleat of the penis and the distance between seminal receptacles in the female genitalia have sometimes been considered to be "minor anatomical features" (Bodon et al., 2001: 199) and at times, if constant, "sufficient to support the existence of two groups of species representing two distinct branches in the radiation of *Islamia*" (Bodon et al., 2001: 201): The "oriental" species' group located in the Balkan Peninsula (including type species, *I. valvataeformis*), Turkey, Israel, and part of Italy (two species: *I. pusilla* and *I. cianensis*) have two seminal receptacles that are very close to each other and a penis with a well-developed muscular pleat.

The "occidental" species' group from France (*I. minuta*, *I. consolationis*, *I. globulina*, *I. spirata*) and Spain have two seminal receptacles that are generally substantially separated from each other and a penis with a less developed or completely absent muscular pleat. The Italian species, *I. gaiteri*, is an exception to this hypothesis, because it has two very closely adjacent seminal receptacles (as in most *Islamia* species), a penis with no muscular pleat, and a knob-like penial lobe that projects only slightly and without light microscope evidence of internal glandular tissue (Bodon et al., 1995: 51, figs. 20, 24–27). None of the Iberian species has a penis with muscular pleat. The degree of variation of this character throughout the distribution area of *Islamia* suggests that an East-West sort of cline exists in the development of the muscular pleat. It is prominent in oriental species, weakens westward and disappears completely in westernmost species. Variability observed in the female genitalia of Iberian species ranges from seminal receptacles that appear at the same point (*I. pallida*), are separated (*I. globulus*, *I. lagari* and *I. henrici*), or even at substantial distances from each other (*I. ateni*). The variability found in these two genital characters (distance between seminal receptacles and a penis with or without muscular pleat) among the supposedly "occidental" species' group suggests that neither of these features alone, nor a combination of these characters, are adequate enough to differentiate taxa at the genus or subgenus level. Therefore, it would be more appropriate to consider them as "species-specific anatomical features".

In hydrobioid taxa, the structures associated with the renal oviduct in the female genitalia are relatively more important taxonomically than those of the male genitalia (Davis & Carney, 1973). In a more recent study of Asian hydrobioids (Davis et al., 1992), involving 48 informative anatomical characters, 33% were derived from the female reproductive system, 23% from the male reproductive system, while only 19% were derived from the digestive sys-

tem and 4% from the nervous system.

Apart from the distance between seminal receptacles, other female genitalia characters of Iberian *Islamia* species also differ greatly, such as the size and shape of the two seminal receptacles. In general, the proximal seminal receptacle is larger than the distal receptacle (according to previously published diagnoses), but they can be almost equal in size in some, as they are in *I. pallida.* Another important character that has yet to be considered is the insertion point of the seminal receptacles. Both receptacles emerge on opposite sides of the renal oviduct in all known *Islamia* species. This character may have been overlooked due to the minute size of the female genitalia and to the fact that the renal oviduct is contorted. However, it is worth noting that while the seminal receptacles of all the *Islamia* species in the literature seem to have been correctly drawn, they have been incorrectly simplified in taxonomic schemes (e.g., in Bodon et al., 2001: figs. 180, 181).

Another female genital characteristic, the presence of a narrowing at the outer margin of the pallial oviduct between the capsule and albumen gland, described by Boeters (1988) as diagnostic for the Iberian "*Neohoratia*" species, does not always hold true in all species. It is sometimes present in *I. globulus*, *I. ateni*, and *I. h. gienensis* and absent in *I. pallida* and *I. h. henrici*. The same situation was reported for Italian species: while *I. cianensis* and *I. piristoma* Bodon & Cianfanelli, 2002, show a slight narrowing in the transition area between the two oviduct glands, *I. pusilla* and *I. gaiteri* lack this character (Giusti et al., 1981; Bodon et al., 1995; Bodon & Cianfanelli, 2002). Therefore, even though this feature could be useful at the species level, it is obviously irrelevant at the supraspecific level.

Other characters that are variable among *Islamia* species, although constant at the species level are: the number of basal cusps of the central tooth, the presence/absence of body or ocular pigmentation, and the presence/absence of a nuchal node or a reduced non-functional penis-shaped structure on the head of females. *Islamia h. henrici* and *I. pallida* are the only known *Islamia* taxa that have this last character. Despite this uniqueness, and because the influence of environmental parameters on the development of this structure is still a matter of discussion, and because water parameters have not been measured in all localities, we prefer to adopt a conservative position and not consider this character to be diagnostic. If in fact this character turns out to be diagnostic, a taxonomic re-arrangement of these species may be warranted. The absence/presence of ctenidium is also constant at the intraspecific level, except in the two *I. henrici* subspecies. The RPG ratio is also constant at the species level, except in the two *I. henrici* subspecies, but it is not quite useful at genus level, unless for the three genera here described. The nervous system is slightly elongated in *I. ateni* (although it has the smallest value in this category, 0.50), moderately concentrated in *I. globulus* (0.43), *I. pallida* (0.42) and *I. h. henrici* (0.30), and concentrated in *I. h. gienensis* (0.14), *M. schuelei* (0.24) and *J. aitanica* (0.22).

The shells of the *Islamia* species known to date (Radoman, 1973a, b, 1983; Giusti & Pezzoli, 1981; Schütt, 1991; Bodon et al., 1995) vary little in shape. They are mostly valvatiform, although some French species have the spire raised to different degrees (Bodon et al., 2001). *Islamia pallida* and *I. henrici* also have planispiral or valvatiform shells, whereas shells of *I. globulus*, *I. lagari* and *I. ateni* are ovate-conic (bythinelliform). It is well known that shell features are not sufficiently diagnostic at the genus level if they are not supported by anatomical differences. Therefore, the variability here described should be included in the diagnosis of *Islamia*, which reinforces the need to review a number of species described from different sites in Europe and Turkey and assigned to *Islamia* on the basis of shell characters (Bodon et al., 2001). This would probably lead to the conclusion that *Islamia* is a taxonomic mess and probably polyphyletic, as unpublished molecular genetic data suggests (Wilke, pers. comm.).

An interesting character is the shape of the esophagus posterior to the pleuro-esophageal ganglionic complex of the nervous system, a character never mentioned nor figured to date for any Hydrobiidae species. The esophagus runs straight in all species studied in this paper except in *I. henrici*, in which it shows a weak curvature to the right side of body (Figs. 17B, 18A), and in *J. aitanica*, in which it makes a marked loop to the left (Fig. 25C). As the shape of the esophagus is constant in all studied specimens of all the species, we rule out the possibility that curvatures are caused by manipulation or retraction of the animal during fixation. More research will reveal if this feature has potential taxonomic value or not.

Islamia has been related genetically to other European genera: *Alzoniella* Giusti & Bodon, 1984, *Fissuria* Boeters, 1981, and *Avenionia* Nicolas, 1882. These genera have been ten-

tatively assigned to the nominal subfamily Islamiinae Radoman, 1973 (Wilke et al., 2001). Neverthess, important differences in morphological character and character states clearly distinguish them from each other: *Alzoniella* has a conical or cylindrico-conical shell, a bursa copulatrix with a short to medium anterodorsal duct and two seminal receptacles, and a penis with one or more "glandular" penial lobes located in its concave side (Giusti & Bodon, 1984; Bodon, 1988; Boeters, 1999, 2000); *Fissuria* has a valvatiform shell, an oval bursa copulatrix of variable size, a short to long anterodorsal bursal duct, two equally-sized seminal receptacles, and a penis with 3–4 lobes containing "mass of glandular tissue" (Bodon et al., 2001); *Avenionia* has a cylindro-conical, bythinelloid shell, a rudimentary gastric caecum, a penis with a very large subapical lobe, with three "glandular" swellings on its apical border, a "glandular" lobe located on the dorsal side of the penis close to the base of the subapical lobe, and female genitalia with a wide bursa copulatrix, a short and anteroventral bursal duct, and two seminal receptacles (Bodon et al., 2000). *Islamia* is also distinguished from the two new Iberian genera, *Milesiana* and *Josefus*, by a set of character and character states that have been previously discussed.

Difficulties in defining synapomorphies between the so-called "hydrobioids" (Davis, 1979), together with the many conflicts that exist between morphological and molecular genetics (Wilke et al., 2001), call attention to the need for detailed anatomical studies designed to provide ways to accurately group species and to effectively distinguish closely related genera of this complex group.

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THE MICHIGAN PHYSIDAE REVISITED: A POPULATION GENETIC SURVEY

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ABSTRACT

We report an analysis of gene frequencies at 7 polymorphic allozyme-encoding loci in 16 populations of physid snails collected from Michigan, surveyed as a step toward integrating Te's (1978) influential classification of the Physidae with a more comprehensive system based on genetic interrelationships and breeding data. Analysis of a genetic distance matrix revealed three groups – two populations of *Aplexa hypnorum* together, five populations of *Physa acuta* together, and nine populations of *P. gyrina*, *P. sayii*, and *P. parkeri* combined. Allozyme divergence among the populations of this last cluster, referred to as the "*gyrina* group," was comparable to that seen among the five populations of the well-characterized *P. acuta* cluster, which breeding experiments have demonstrated biologically conspecific. These results suggest that Michigan populations assigned to *P. gyrina*, *P. sayii*, and *P. parkeri* may comprise a single biological species, the globose and often shouldered shell morphology of the latter resulting from local and perhaps phenotypically plastic responses to lacustrine environments. The 14 "taxonomic units" from Michigan that Te included in his analysis may represent as few as four biological species. A reduction in nominal higher levels of classification within the Physidae is called for.

Key words: Gastropoda, Pulmonata, *Physella*, allozyme polymorphism, protein electrophoresis.

INTRODUCTION

The freshwater pulmonate family Physidae includes some of the more common and widespread gastropod species on earth (Burch, 1989; Dillon, 2000; Dillon et al., 2002). In North America, the most influential classification of the family is currently that of George A. Te (1978, 1980). Te's analysis, based on 71 characters scored primarily from the shell and reproductive anatomy, suggested that the 85 taxonomic units he recognized might be divided into four genera: *Aplexa*, *Stenophysa*, *Physa* and *Physella*, the last genus with three subgenera (*Petrophysa*, *Costatella*, and *Physella s.s.*). This classification was adopted by Burch for his "North American Freshwater Snails" (Burch, 1989), and subsequently by Brown (1991), Turgeon et al. (1998), and many others.

A wealth of data regarding genetic relationships among the North American physids has accumulated in the 25 years since Te proposed his classification. Reports have been published detailing gene frequencies at allozyme-encoding loci among a variety of nominal species (Buth & Sulloway, 1983; Liu, 1993; Dillon & Wethington, 1995; Jarne et al., 2000). More recently, data have become available on DNA sequence divergence (Remigio et al., 2001; Wethington & Guralnick, 2004; Wethington et al., in prep.) and microsatellite polmorphisms (Bousset et al., 2004). Controlled breeding studies have uncovered little reproductive isolation among physid populations long assumed to represent different species, prompting calls for a reappraisal of systematic relationships within the family (Dillon et al., 2002, 2004; Dillon & Wethington, 2004; Dillon et al., in press 2). The classification system proposed by Wethington (2003; Wethington & Lydeard, in press) would return the number of genera to two – *Physa* and *Aplexa*.

Ideally, a new classification of the Physidae would integrate Te's morphological observations with more recent allozyme, DNA, and breeding data into a single unified system. Unfortunately, however, Te did not report collection localities or museum lot numbers for the 85 taxa upon which his 1978 classification was based, nor did he provide figures, keys, or any practical method by which the species

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he recognized might be distinguished. Since any effort to modernize or update Te's system would ideally begin with a resampling of his taxa to gather correlative genetic information, progress in physid systematics has been slowed.

Fortunately, Te (1975) did publish one preliminary paper, "Michigan Physidae, with systematic notes on *Physella* and *Physodon*". Although limited to just the six species and eight subspecies he recognized in the state, Te provided figures, a dichotomous key (based on shell characters), anatomical notes, synonymy, range data, and a "partial phylogenetic tree" for this subset. The purpose of the present paper is to report the results of a survey of genetic divergence at allozyme-encoding loci among a large sample of physid populations from Michigan, identified using the conchological key of Te (1975), as a step toward reconciling Te's 1978 classification with more recent classifications based on genetic data (Wethington, 2003; Wethington & Lydeard, in press).

The physid fauna of Michigan includes three nominal species sharing the "type B" penial morphology, *Physa gyrina*, *P. sayii*, and *P. parkeri*, all assigned by Te to the subgenus "*Physella*". He noted some minor differences among these three species in the length ratios of the glandular and non-glandular portions of their penial sheaths, as well as the transparency of the non-glandular region and terminal swelling in the glandular. But Te (1975) wrote, "*Physa gyrina*, *P. sayii* and *P. parkeri* are all related in one species complex. As such, there are intermediate forms that may be difficult to place; this is especially a problem between *P. gyrina* and *P. sayii*."

Burch & Jung (1992) also found the Michigan species of the subgenus *Physella* difficult to distinguish. They wrote, "Our approach has been to note morphological groups that correspond to named entities (nominal species) that seem distinct enough to possibly be good species." Burch & Jung recognized four "named entities" of *Physella* (s.s.) inhabiting northern Michigan: globose, strongly shouldered *P. parkeri*, elliptical or elongate-ovate *P. gyrina*, ovate thin *P. sayii*, and ovate thick *P. magnalacustris*, which Te considered a subspecies of *P. sayii*. As the systematic relationships within this group have continued to prove especially problematic, populations of physids from the subgenus *Physella* were the objects of particular attention in the investigation reported here.

METHODS

Our field survey was designed to sample the physid species reported by Te (1975), identified using the conchological key he provided, collected from their representative ranges across the state of Michigan. Ultimately, we sampled 16 populations, including two of *Aplexa hypnorum*, two of *Physa sayii*, three of *Physa parkeri*, four of *Physa gyrina*, and five of *Physa acuta*. The last-listed species was identified as "*P. integra*" by Te, a name that has subsequently been synonymized (Dillon et al., 2002). Sample sites are shown in Figure 1, with locality data and sample sizes listed in the Appendix. We were unable to collect the sixth species reported by Te, *Physa jennessi*, from any of the seven Michigan sites he listed.

Whole-snail homogenates were centrifuged and analyzed via horizontal starch gel electrophoresis using methods and apparatus as described by Dillon (1992). Multiple buffer systems were employed where possible to screen for hidden variation (Coyne & Felton, 1978). The AP6 buffer system of Clayton & Tretiak

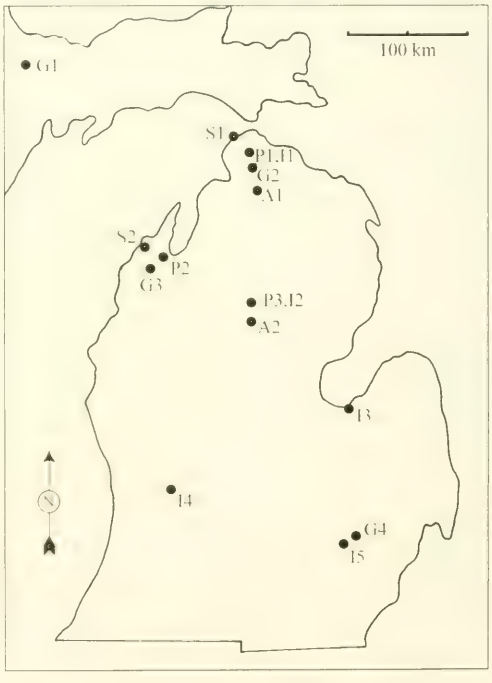


FIG. 1. Outline map of the state of Michigan, showing sample sites. A = *Aplexa hypnorum*, G = *Physa gyrina*, I = *Physa acuta*, P = *Physa parkeri*, S = *Physa sayii*. See Appendix for locality data.

(1972) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), leucine aminopeptidase (LAP), glucose phosphate isomerase (GPI), and isocitrate dehydrogenase (ISDH). We employed the TC6.8 buffer system of Mulvey & Vrijenhoek (1981) to resolve GPI, ISDH, phosphoglucomutase (PGM2), and mannose phosphate isomerase (MPI). The TEB8 system (buffer III of Shaw & Prasad, 1970) was used to analyze LAP, 6PGD, and the esterases (EST3).

Our initial runs included control samples of the well-characterized *P. acuta* population inhabiting the main pond at Charles Towne Landing State Park, Charleston, South Carolina (population C or CTL in Dillon & Wethington, 1995; Dillon et al., 2002; Wethington & Dillon, 1991). Putative alleles were named according to the electrophoretic mobility of their allozyme products in millimeters, setting the mobility of the most common allele in population C to 100. Mendelian interpretation has been confirmed for EST3 and LAP by Dillon & Wethington (1994), and for GPI, PGM, and 6PGD in planorbids by Mulvey & Vrijenhoek (1984) and Mulvey et al. (1988).

Data analysis was performed using Biosys version 1.7 (Swofford & Selander, 1981). Because large numbers of alleles were resolved at some loci, our sample sizes dictated that genotypes be pooled into three classes: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes before testing for Hardy-Weinberg equilibrium. Yates-corrected chi-square statistics were then employed for this purpose. We calculated matrices of Nei (1978) unbiased genetic identity and Cavalli-Sforza & Edwards (1967) chord distance. As distances of the latter type are Pythagorean in Euclidean space, they were used as the basis for an UPGMA cluster analysis (Wright, 1978) and a neighbor-joining tree (PAUP* 4.0b10; Swofford 1998).

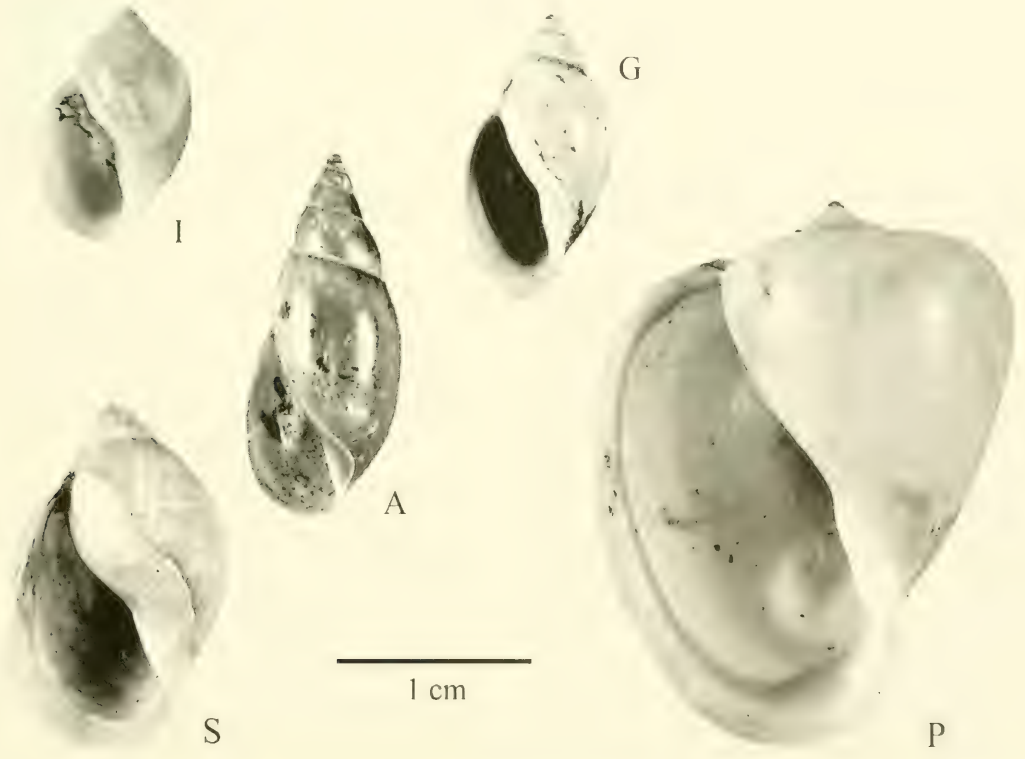
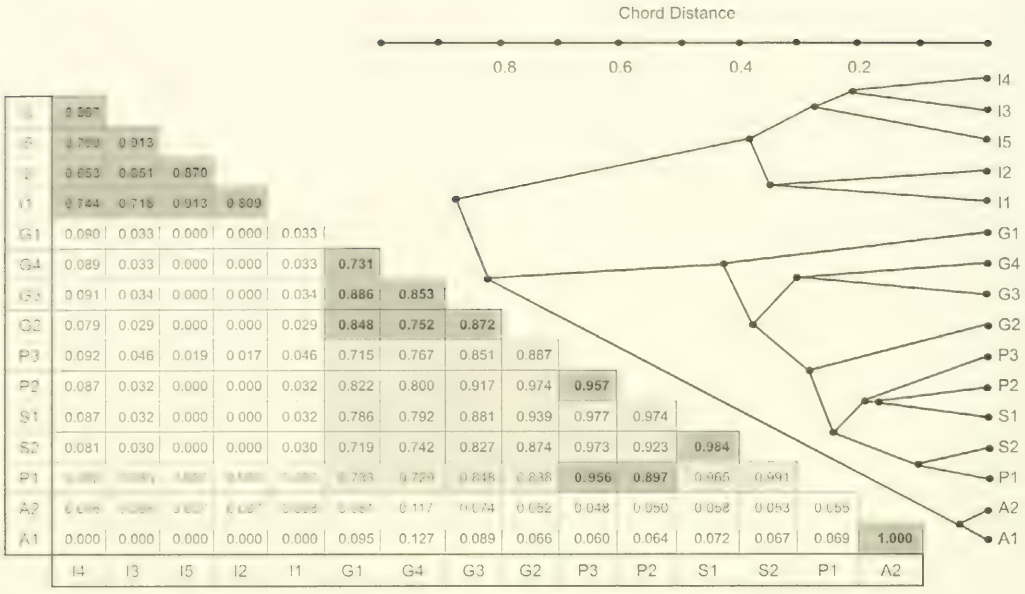


FIG. 2. Exemplar shells of the five physid species examined in this study. I – *Physa acuta* (population I1), S – *Physa sayii* (population S1), G – *Physa gyrina* (population G1), A – *Aplexa hypnorum* (population A2), P – *Physa parkeri* (population P1). See appendix for locality data.



	I4	I3	I5	I2	I1	G1	G4	G3	G2	P3	P2	S1	S2	P1	A2
I4															
I3															
I5															
I2															
I1															
G1	0.090	0.033	0.000	0.000	0.033										
G4	0.089	0.033	0.000	0.000	0.033	0.731									
G3	0.091	0.034	0.000	0.000	0.034	0.886	0.853								
G2	0.079	0.029	0.000	0.000	0.029	0.848	0.752	0.872							
P3	0.092	0.046	0.019	0.017	0.046	0.715	0.767	0.851	0.887						
P2	0.087	0.032	0.000	0.000	0.032	0.822	0.800	0.917	0.974	0.957					
S1	0.087	0.032	0.000	0.000	0.032	0.786	0.792	0.881	0.939	0.977	0.974				
S2	0.081	0.030	0.000	0.000	0.030	0.719	0.742	0.827	0.874	0.973	0.923	0.984			
P1						0.733	0.729	0.818	0.838	0.956	0.897	0.965	0.991		
A2							0.117	0.074	0.052	0.048	0.050	0.058	0.053	0.055	
A1	0.000	0.000	0.000	0.000	0.000	0.095	0.127	0.089	0.066	0.060	0.064	0.072	0.067	0.069	1.000

FIG. 3. Nei's (1978) unbiased genetic identities are shown below the diagonal, with nominally conspecific comparisons darkly shaded and other comparisons within the *gyrina* complex shaded lightly. Above the diagonal is the result of a UPGMA cluster analysis based on Cavalli-Sforza & Edwards (1967) chord distance.

RESULTS

We found Te's (1975) conchological key difficult to apply to natural populations collected from the wild, failing entirely in smaller individuals. Although *Aplexa* and (generally) *P. acuta* could be distinguished unambiguously, shell morphological variation within and among populations of *P. gyrina*, *P. sayii*, and *P. parkeri* often thwarted positive identification. Nor have any anatomical distinctions been subsequently described that might facilitate this process. We would have preferred to sample more populations of *P. sayii* in particular, but intergradation with both *P. gyrina* and *P. parkeri* made identification of this taxon especially problematic. The shells chosen for illustration in Figure 2 are exemplars. Voucher specimens have been deposited in the University of Michigan Museum of Zoology.

Allele frequencies at the seven enzyme-encoding loci are given in Table 1. Of the 16 x 7 = 112 loci examined, a total of 54 were polymorphic by the 95% criterion. Chi-square analysis revealed heterozygote deficits nominally significant at the 0.05 level in six of these cases – Est3 at population I4, Isdh in population I3, Est3 in population G3, and three polymorphic loci in population I5: Est3, Lap, and Isdh.

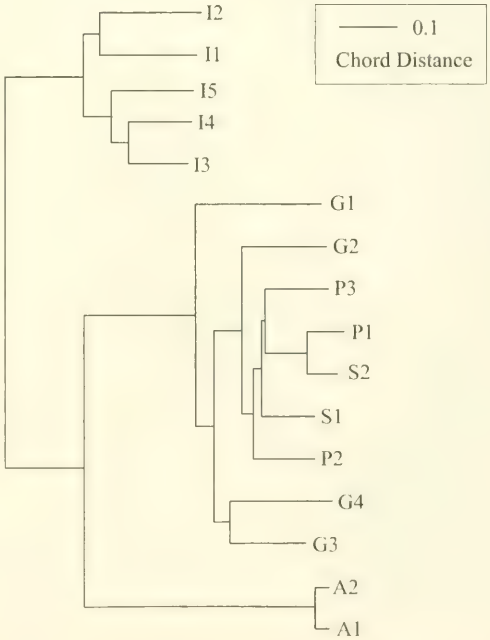


FIG. 4. Neighbor-joining tree (PAUP*; Swofford 1998) based on the matrix of Cavalli-Sforza & Edwards (1967) chord distance.

TABLE 1. Gene frequencies at seven polymorphic enzyme loci in 16 populations of physid snails from Michigan.

Allele	A1	A2	G1	G2	G3	G4	I1	I2	I3	I4	I5	P1	P2	P3	S1	S2
EST3																
104	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.411	0.136	0.379	0.000	0.000	0.000	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.563	0.864	0.345	0.000	0.000	0.000	0.000	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.839	0.933	0.027	0.000	0.276	0.000	0.000	0.000	0.000	0.000
91	0.000	0.000	0.000	0.018	0.152	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.000	0.000	0.081	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.097	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
89	0.000	0.000	0.000	0.982	0.217	0.741	0.000	0.000	0.000	0.000	0.000	0.611	0.857	0.859	0.849	0.871
87	0.000	0.000	0.379	0.000	0.630	0.259	0.000	0.000	0.000	0.000	0.000	0.343	0.125	0.141	0.023	0.129
84	0.000	0.000	0.621	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.047	0.000
LAP																
100	0.000	0.000	0.000	0.000	0.000	0.000	0.813	0.288	0.264	0.672	0.192	0.000	0.000	0.000	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.188	0.712	0.698	0.328	0.808	0.000	0.000	0.000	0.000	0.000
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.000	0.850	1.000	0.771	0.679	0.000	0.000	0.000	0.000	0.000	0.990	0.958	0.926	0.938	1.000
88	0.000	0.000	0.150	0.000	0.229	0.321	0.000	0.000	0.000	0.000	0.000	0.010	0.042	0.074	0.063	0.000
82	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PGD																
100	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.964	0.750	0.942	0.000	0.000	0.000	0.000	0.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.250	0.019	0.000	0.000	0.000	0.000	0.000
94	0.325	0.348	0.000	0.000	0.000	0.107	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.013
92	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.175	0.130	0.643	1.000	0.783	0.000	0.000	0.000	0.000	0.000	0.000	0.990	1.000	0.968	0.938	0.987
86	0.500	0.478	0.357	0.000	0.217	0.893	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.032	0.013	0.000

(*continues*)

(continued)

Allele	A1	A2	G1	G2	G3	G4	I1	I2	I3	I4	I5	P1	P2	P3	S1	S2
MPI																
110	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
104	0.000	0.000	1.000	1.000	1.000	1.000	0.017	0.000	0.170	0.446	0.000	1.000	1.000	0.904	1.000	1.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.967	0.984	0.802	0.527	1.000	0.000	0.000	0.096	0.000	0.000
96	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016	0.028	0.027	0.000	0.000	0.000	0.000	0.000	0.000
PGM2																
115	0.000	0.000	0.304	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
112	0.000	0.000	0.696	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.967	0.978	1.000
110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.022	0.000
103	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.296	0.197	0.371	0.794	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.704	0.803	0.629	0.206	0.000	0.000	0.000	0.000	0.000
98	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ISDH																
104	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.029	0.091	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.391	1.000	0.602	0.176	0.568	0.000	0.000	0.000	0.000	0.000
94	0.000	0.000	0.000	0.000	0.000	0.000	0.609	0.000	0.389	0.794	0.341	0.000	0.000	0.000	0.000	0.000
92	0.750	0.794	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.135	0.077	0.058	0.000
85	0.250	0.206	1.000	1.000	1.000	0.983	0.000	0.000	0.000	0.000	0.000	1.000	0.788	0.615	0.942	0.984
82	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.077	0.308	0.000	0.016
GPI																
102	0.000	0.000	0.000	0.018	0.022	0.106	0.000	0.000	0.000	0.000	0.000	1.000	0.149	0.628	0.571	0.936
100	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
98	0.000	0.000	1.000	0.982	0.478	0.227	0.000	0.000	0.000	0.000	0.000	0.000	0.541	0.117	0.296	0.064
94	0.000	0.000	0.000	0.000	0.500	0.667	0.000	0.000	0.000	0.000	0.000	0.000	0.311	0.255	0.133	0.000
95	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 3 shows the matrix of Nei's genetic identity among all pairs of populations and the results of an UPGMA cluster analysis based on Cavalli-Sforza and Edwards Chord distance. The cophenetic correlation (Sokal & Rohlf, 1962) for this analysis was very high, $r_{CS} = 0.993$ (Sneath & Sokal, 1973: 304), indicating a good fit between the branch length and the original distance matrix. The neighbor-joining tree is shown in Figure 4.

DISCUSSION

Fits to Hardy-Weinberg expectation were good in almost all populations, with scattered nominally significant values of chi square probably attributable to Type 1 statistical error. The exception was population I5, where significant heterozygote deficits were apparent at three of five polymorphic loci examined. Outcrossing is strongly preferred in laboratory populations of *Physa acuta*, self-fertilization resulting in a substantial fitness decrement (Wethington & Dillon, 1993, 1996, 1997). Evidence of inbreeding has nevertheless often been reported in natural populations of *Physa* (Dillon & Wethington, 1995; Jarne et al., 2000) and other pulmonates (Jarne 1995). Some low level of self-fertilization may be an unavoidable consequence of the pulmonate reproductive system (Dillon et al., in press 1). At the I5 site, low population densities may have increased the frequency of self-fertilization beyond the background levels that were more difficult to detect in other populations at our sample sizes.

Both the neighbor-joining tree and the UPGMA cluster analysis revealed three distinct groups – the two populations of *Aplexa* together, the five populations of *P. acuta* together, and the nine populations of *P. gyrina*, *P. sayii*, and *P. parkeri* combined (Figs. 3, 4). The five *P. acuta* populations, clustered at a chord distance of 0.37, showed a minimum genetic identity of 0.718. This is quite similar to the level of genetic divergence among the ten populations of *P. acuta* sampled from the Charleston area by Dillon & Wethington (1995). This level is also strikingly similar to that displayed within the nine populations of the *gyrina/sayii/parkeri* group, clustered at a chord distance of 0.43 with a minimum genetic identity of 0.715. The specific distinction between *P. gyrina*, *P. sayii*, and *P. parkeri*, hereafter referred to as the "*gyrina* group", is called into question.

Physa gyrina ranges broadly across North America, throughout Canada and the United States as far south as Virginia and Kentucky. In Michigan, Te reported populations from a wide variety of shallow habitats – creeks, brooks, pools, ponds, and ditches. The ranges of *Physa sayii* and *P. parkeri* are more restricted to the Great Lakes region and to deeper waters, Te giving the habitat of the former as "lakes and rivers" and the habitat of the latter as "large lakes".

Both Figures 3 and 4 depict the *sayii/parkeri* cluster as a subset within the larger *gyrina* group. This suggests to us that the generally larger, inflated, and globose shell that characterizes populations referred to these two nomena may be a regional (and possibly ecophenotypic) response to the colonization of lacustrine habitats by populations of the more typical *P. gyrina* morphology. We hypothesize that individuals inhabiting larger lakes and rivers may tend to live longer, and hence grow larger of body, than individuals inhabiting ponds and creeks. It also possible that the rotund, globose and often shouldered shell phenotype characterizing *P. parkeri* (and sometimes *P. sayii*) may be related to a deepwater habitat unaffected by current or wind.

The tendency for physid snails to develop rotund shells as a phenotypically plastic response to the threat of fish predation is well documented (DeWitt, 1998; DeWitt et al., 1999, 2000; Langerhans & DeWitt, 2002). More recently, Britton & McMahon (2004) have reported that physids respond to increased water temperature by developing wider shell spire angle, a variable positively correlated with shell globosity. It seems clear that the minor differences in shell morphology upon which rest the distinctions among the several nominal species of the *gyrina* group need not reflect any heritable variance whatsoever.

Breeding experiments would provide the ideal test to confirm that the three nominal species of the *gyrina* group inhabiting Michigan are in fact biologically conspecific. Dillon & Wethington (2004) reported the results of no-choice mating experiments between a line of *P. parkeri* from Douglas Lake and *P. gyrina* collected from its type locality near Council Bluffs, Iowa. Our control *P. parkeri* hatched and reared under laboratory conditions did not develop the shoulder on their shell characteristic of wild-collected animals, remaining superficially indistinguishable from control *P. gyrina*. Control *parkeri* hybridized readily with

P. gyrina, producing viable F1 offspring. The growth, survival rate, and fecundity of *P. parkeri* were, however, significantly below those posted by control *P. gyrina*, in both the control pairs and in the outcross *parkeri* x *gyrina* experiment. We were ultimately unable to carry either control *P. parkeri* or *parkeri* x *gyrina* hybrids to the F2 generation under our culture conditions, leaving the question of reproductive isolation an open one. Our experiments nevertheless confirmed that the life history adaptations evolved by *P. parkeri* have a heritable basis, although some key aspects of shell morphology, upon which the taxonomy is based, may not.

The overall form of the analyses shown in Figures 3 and 4 is consistent with the phylogeny suggested by Wethington (2003) and Wethington & Lydeard (in press). Mitochondrial COI and 16s sequence data, analyzed via parsimony, yielded a tree in which the genera *Aplexa* and *Physa* split first, followed by a split between the clade containing *P. acuta* and the clade containing the *gyrina* group. The analysis of Wethington & Lydeard also resolved two clades within the *gyrina* group: a "typical" subset and a "globose" subset that included *parkeri* and *sayii* (subspecies *magnalacustris*.) The authors attributed this distinction to geographical factors, however, not to reproductive isolation.

Our allozyme data, taken together with the partial results of the Dillon & Wethington (2004) breeding experiments, suggest that the nominal taxa *P. parkeri* and *P. sayii* may best be treated as junior synonyms of *P. gyrina*. Final confirmation of this hypothesis will await careful analysis of reproductive interactions between populations of these three nominal species in natural sympatry. Given the difficulty we and other workers have encountered distinguishing members of the *gyrina* group in the field, however, it may materialize that no practical site for such a study can be identified.

The 85 taxonomic units upon which Te (1978, 1980) based his classification included all 14 of the taxa he recognized from Michigan: *Aplexa hypnorum* (*tryoni* and *hypnorum s.s.*), *Physa jennessi* (subspecies *skinneri*), *Physa gyrina* (*elliptica*, *hildrethiana*, and *gyrina s.s.*), *Physa sayii* (*magnalacustris*, *vinosa*, and *sayii s.s.*), *Physa parkeri* (*latchfordii* and *parkeri s.s.*), and *Physa integra* (*brevispira*, *walkeri*, and *integra s.s.*). Including *P. jennessi*, the validity of which we have no reason to doubt, our allozyme data suggest that these 14 taxa may comprise just four biological species. It is clear that Te's analysis was based on a set of taxonomic units divided much more finely than biological species. This suggests to us that the revised classification of Wethington & Lydeard, returning the Physidae to a simpler two-genus system, has much to recommend it.

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APPENDIX

Locality data and sample sizes

A1 *Aplexa hypnorum*. Woodland pond at the Maple Bay access of Burt Lake, Cheboygan Co., Michigan. 45.4867°N, 84.7088°W. N = 21.

A2 *Aplexa hypnorum*. Houghton Lake at state campground, Roscommon Co., Michigan. 44.3388°N, 84.6648°W. N = 26.

G1 *Physa gyrina*. Little Lake at state campground, 1 km S of town of Little Lake, Marquette Co., Michigan. 46.2815°N, 87.3337°W. N = 31.

G2 *Physa gyrina*. Little Carp River at Hogsback Rd., 1 km N of Burt Lake, Cheyboygan Co., Michigan. 45.5520°N, 84.6854°W. N = 28.

G3 *Physa gyrina*. Turtle Lake at Miller Rd., 5 km W of Bendon, Benzie Co., Michigan. 44.6178°N, 85.9090°W. N = 24.

G4 *Physa gyrina*. Twin Sun Lakes at Highgate Beach, Wixom, Oakland Co., Michigan. 42.5466°N, 83.5085°W. N = 33.

I1 *Physa acuta*. Douglas Lake at the University of Michigan Biological Station, Cheboygan Co., Michigan. 45.5634°N, 84.6783°W. N = 32.

I2 *Physa acuta*. Higgins Lake near boat ramp at Sam O Set Blvd., Sharps Corners, Roscommon Co., Michigan. 44.4246°N, 84.6942°W. N = 31.

I3 *Physa acuta*. Saginaw Bay at Quanicassee Wildlife Area, Tuscola Co., Michigan. 43.5896°N, 83.6774°W. N = 57.

I4 *Physa acuta*. Pond near the junction of Mi 11 and Mi 37, Grand Rapids , Kent Co., Michigan. 42.9168°N, 85.5771°W. N = 44.

I5 *Physa acuta*. Kent Lake at Kensington MetroPark, Oakland Co., Michigan. 42.5336°N, 83.6462°W. N = 29.

P1 *Physa parkeri*. Douglas Lake at the University of Michigan Biological Station, Cheboygan Co., Michigan. 45.5634°N, 84.6783°W. N = 59.

P2 *Physa parkeri*. Long Lake at Long Lake Rd., 10 km SE of Traverse City, Grand Traverse Co., Michigan. 44.7140°N, 85.7316°W. N = 37.

P3 *Physa parkeri*. Higgins Lake near boat ramp at Sam O Set Blvd., Sharps Corners, Roscommon Co., Michigan. 44.4246°N, 84.6942°W. N = 47.

S1 *Physa sayii*. Lake Michigan at Wilderness State Park, Emmet Co., Michigan. 45.7474°N, 84.9045°W. N = 49.

S2 *Physa sayii*. Crystal Lake 3 km N of Frankfort, Benzie Co., Michigan. 44.6607°N, 86.2320°W. N = 39.

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EXTREME MITOCHONDRIAL SEQUENCE DIVERSITY IN THE INTERMEDIATE SCHISTOSOMIASIS HOST *ONCOMELANIA HUPENSIS ROBERTSONI*: ANOTHER CASE OF ANCESTRAL POLYMORPHISM?

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ABSTRACT

Today, the human blood fluke, *Schistosoma japonicum*, is transmitted in China by two subspecies of the rissooidean snail taxon *Oncomelania hupensis*: *O. h. hupensis* and *O. h. robertsoni*. Whereas the eastern Chinese subspecies *O. h. hupensis* has been studied extensively using mitochondrial DNA sequences, very little data existsfor the western subspecies *O. h. robertsoni*. Preliminary phylogeographic studies indicate that the latter shows a very high degree of genetic diversity with Kimura 2 parameter distances in the cytochrome oxidase I (COI) gene of up to 0.0932 (= 9.32%) among four sequences previously deposited in GenBank. Extreme degrees of intraspecific heterogeneity in gastropods have been reported before, and possible explanations include the presence of cryptic species complexes, isolation followed by secondary contact, heteroplasmy and duplications within the mitochondrial genome, the presence of "pseudogenes", and the retention of ancestral mitochondrial polymorphism.

Given the great significance of understanding phylogeographic patterns in the intermediate schistosomiasis host *Oncomelania h. robertsoni* for comprehending host/parasite relationships, DNA sequences of two mitochondrial genes (COI and LSU rRNA) from 66 *O. hupensis robertsoni* specimens are used to (1) assess the phylogenetic position, (2) study the degree of heterogeneity within and between "populations", (3) provide a preliminary overview of the geographic distribution of major genetic groups and (4) study the phylogenetic concordance of the two gene fragments.

Phylogenetic analyses, parametric bootstrapping and studies of sequence polymorphism show that: (1) all COI sequences are fully protein-coding with no insertions or deletions, (2) both individual and combined analyses of the COI and LSU rRNA genes show at least four distinct haplotype groups within *O. h. robertsoni*, (3) monophyly of the four clades cannot be confirmed, (4) there is high concordance in cluster patterns and arrangement of individual haplotypes of both gene fragments, (5) two of the genetic clades recovered appear to be localized, whereas the other two are widely distributed, and (6) sympatry of individuals belonging to different clades occurs. Moreover, based on preliminary AFLP analyses it could be shown that (7) there is no phylogenetic concordance between the mitochondrial and nuclear data presented here, and (8) the nuclear data from AFLP genotyping indicate a lack of clear population structure.

Given the results of the present study, it is cautiously suggested that retention of ancestral mitochondrial DNA polymorphism possibly in combination with some effects of secondary contact (introgression) is the most probable explanation for the occurrence of deviant lineages in *O. h. robertsoni*. On the basis of nuclear, morphological, and ecological data, it is also suggested that there is no evidence of organismal subdivision in *O. h. robertsoni*. It is strongly recommended that future studies incorporate more data from nuclear loci in order to better understand phylogeography, population genetics, and host-parasite coevolution in *O. h. robertsoni*.

Key words: schistosomiasis, *Oncomelania*, China, mitochondrial DNA, phylogeography, AFLP.

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INTRODUCTION

The human blood fluke, *Schistosoma japonicum*, responsible for one of the most serious disease problems in China, schistosomiasis, uses small dioecious rissooidean gastropods of the species *Oncomelania hupensis* as intermediate hosts. Molecular and morphological analyses, together with breeding experiments and biogeographic studies of *O. hupensis*, indicate that there are three subspecies on the mainland of China (Davis, 1992; Davis et al., 1995, 1999). *Oncomelania h. robertsoni* is restricted to high elevations on the plateaus and mountains of Yunnan and Sichuan above the Three Gorges. *Oncomelania h. hupensis* is found throughout the Yangtze River drainage below the Three Gorges; it has spread to Guangxi Province probably via the Grand Canal from Hunan. *Oncomelania h. tangi* is restricted to Fujian Province along the coast. The latter subspecies has been eradicated except for two known populations, and the parasite presumably is extinct.

Of the two wide-spread Chinese subspecies *O. h. hupensis* and *O. h. robertsoni*, the former has received considerable attention in genetic studies (allozymes and mitochondrial gene sequences) dealing with questions of population structure, phylogeography, infectivity and the nature of shell ribbing (e.g., Davis et al., 1995; Wilke et al., 2000a; Shi et al., 2002).

In contrast, very little is known about the genetics of the western Chinese subspecies *O. h. robertsoni*. In fact, whereas as of June 2005, 140 nucleotide sequences are available for *O. h. hupensis* from GenBank, only ten sequences (from a total of four specimens) exist for *O. h. robertsoni*. However, preliminary studies in a phylogeographic framework of other *O. hupensis* subspecies indicated a very high degree of genetic diversity within the few mitochondrial sequences available for *O. h. robertsoni*. In fact, of the four sequences published for the mitochondrial cytochrome c oxidase subunit I (COI) gene (GenBank accession numbers AF213339, AF253075, AF253076, AF531547), two sequences (AF253075 and AF213339) differ by K2P (Kimura 2 parameter) distances of 0.0932. To give a comparison, the highest pairwise K2P distance among more than 100 COI sequences for the eastern Chinese subspecies *O. h. hupensis* (which is regarded as genetically highly diverse) is with 0.0340 (GenBank accession numbers AF254484 and AF254509) only about 36% as high as in *O. h. robertsoni*. Moreover, in many phylogenetic studies of rissooidean gastropods, K2P distances in the COI gene compared to the amount found in *O. h. robertsoni* typically reflect species, if not genus level relationships (e.g., Wilke et al., 2000b; Wilke, 2003). To complicate matters, in further studies involving a single population of *O. h. robertsoni* from the lower Anning River Valley in Sichuan (site A8, see below), we even found pairwise K2P divergences of up to 0.1027 *within* the site.

Extreme degrees of intraspecific mitochondrial heterogeneity in gastropods have been reported before, and potential explanations involve, among others, the presence of cryptic species complexes, isolation followed by secondary contact, heteroplasmy and duplications within the mitochondrial genome, the presence of nuclear "pseudogenes", or the retention of ancestral mitochondrial polymorphism.

In order to shed light on the problem of heterogeneity within *Oncomelania h. robertsoni*, we here use mitochondrial DNA (mtDNA) sequences from a larger data set of 66 specimens from 13 sites. In addition to the protein-coding COI gene, we study the mitochondrial gene for large subunit ribosomal RNA (LSU rRNA) to test for potential conflicts between these gene fragments that could help to reveal methodological problems.

The specific goals of this paper are:

(1) to assess the phylogenetic position of *Oncomelania h. robertsoni* within the framework of other *O. hupensis* ssp.,
(2) to study the degree of mitochondrial heterogeneity within and between "populations" of *O. h. robertsoni,*
(3) to provide a preliminary overview of the geographic distribution of major mtDNA groups within *O. h. robertsoni,* and
(4) to study the phylogenetic concordance of different mitochondrial gene fragments.

We also use the results of preliminary AFLP (amplified fragment lengths polymorphism) genotyping of highly variable nuclear loci from a subset of 24 specimens to discuss the high degree of mtDNA diversity in the light of nuclear data (for a review of the performance of AFLP data in animal population genetics see Bensch & Åkesson, 2005).

MATERIALS AND METHODS

Specimens Studied

The current study includes 66 specimens of *Oncomelania hupensis robertsoni* Bartsch, 1946, from 13 sites in Yunnan and Sichuan provinces, China (Table 1, Appendix).

TABLE 1: Locality information for Chinese specimens of *Oncomelania hupensis robertsoni* studied (M = Meishan Area, A = Anning River Valley, Y = Yunnan).

Locality code	Original locality #	Latitude Longitude	Locality	No. specimens studied
M 1	D 98.16	29.99163 °N 103.41580 °E	Sichuan, Danling County, Ernming Township, Xiaoqiao Village	2
M 2	D 98.14	30.13993 °N 103.61167 °E	Sichuan, Dongpo County, Panao Township, Magau Group 2 Village	10
M 3	MG 96.18	30.0373 °N 103.9002 °E	Sichuan, Meishan County, Fusheng Township, Zhongfu Village	5
M 4	-	30.067 °N 104.138 °E	Sichuan, Mianzhu	1*
A 1	D 98.04	27.93525 °N 102.20540 °E	Sichuan, Xichang County, Xixiang Township, Gucheng Village	4
A 2	D 98.03	27.9318 °N 102.1962 °E	Sichuan, Xichang County, Xxixiang Township, Gucheng Village	4
A 3	D 98.12	27.87505 °N 102.30867 °E	Sichuan, Xichang County, Chaunxing Township, Minhe group 2 Village	4
A 4	D 98.09	27.8000 °N 102.204 °E	Sichuan, Xichang County, Jingjiu Township, Zhoutun Village	4
A 5	D 98.07	27.7995 °N 102.3087 °E	Sichuan, Xichang County, Hainan Township, Gucheng group 2 Village	4
A 6	D 98.05	27.7973 °N 102.3157 °E	Sichuan, Xichang County, Hainan Township, Gucheng group 5 Village	4
A 7	D 98.11	27.7468 °N 102.1903 °E	Sichuan, Xichang County, Jingjiu Township, Jingjiu Village	4
A 8	Xi Chang**	26.9637 °N 102.1328 °E	Sichuan, Miyi County, Panlian Township, Shuanggou Village	12
Y 1	Dali**	25.4510 °N 100.2007 °E	Yunnan, Dali City, Da Jin Ping, Zi Ran Village	8

* from GenBank (Attwood et al., 2003)
** previously studied using allozyme electrophoresis by Davis et al. (1995)

As primary outgroup taxon (which was used to root the mtDNA trees) served a yet undescribed representative of the genus *Tricula* (*Tricula* sp.; Davis et al., 1998) (GenBank AF213341, AF212895). Like *Oncomelania*, *Tricula* belongs to the family Pomatiopsidae. Additional outgroup taxa used in the current study are *Oncomelania minima* Bartsch, 1936 (GenBank DQ212795, DQ212858), as well as four other subspecies of *O. hupensis*: *O. h. hupensis* (Gredler, 1881) (GenBank AF254547, DQ212859), *O. h. tangi* (Bartsch, 1936) (GenBank DQ212796, DQ212860), *O. h. formosana* (Pilsbry & Hirasé, 1905) (GenBank DQ112283, DQ212861), and *O. h. quadrasi* (Moellendorff, 1895) (GenBank DQ112287, DQ212862).

DNA Isolation and Sequencing

The method used for isolating DNA from snails was modified from that of Spolsky et al. (1996). Individual alcohol-preserved specimens were first soaked for 10 min in 1 ml ice-cold exchange buffer (0.02 M Tris base, 0.1 M EDTA, pH 8.0). Then, either the soft body of a whole specimens or part of the foot (depending on the size of the specimen) was cut in pieces and incubated overnight in a water bath at 58°C in 200 µl Turner lysis buffer (0.02 M Tris base, 0.1 M EDTA, 0.5% Sarkosyl, pH 8.0) and 3 µl of 20 µg/µl Proteinase K. After digestion, 35 µl of 5 M NaCl and 35 µl of a 5% CTAB/0.5 M NaCl solution were added. Extraction was carried out with 270 µl chloroform. After centrifugation for 5 min at 9,000 rpm, the aqueous phase was transferred into a new tube and 270 µl of CTAB precipitation buffer (1% CTAB, 0.05 M Tris base, 0.01 M EDTA) was added, mixed and placed at room temperature for 45 min. After pelleting the CTAB-DNA for 10 min at 12,000 rpm, the supernatant was disposed and the pellet redissolved in 100 µl of NaCl/TE (0.01 Tris base,

0.001 M EDTA, 1 M NaCl, pH 8.0) and 1 µl of 10 mg/ml RNase. After incubation for 8 min at 65°C, the DNA was precipitated over night at –20°C by adding 250 µl of ice-cold 96% ethanol. After centrifugation for 15 min at 12,000 rpm, the pellet was washed twice with 300 µl of ice-cold 70% ethanol, air-dried for 5–10 min and finally redissolved in approximately 50 µl H_2O. Quality and quantity of the isolated genomic DNA were checked on a 1% agarose gel.

The primers used to amplify a fragment of the COI gene with a target length of 658 base pairs (excluding 51 bp primer sequence) were LCO1490 and HCO2198 as described by Folmer et al. (1994). The primers for amplification of a LSU rRNA fragment with a target length of 505–508 bp (excluding 42 bp primer sequence) were 16Sar-L and 16Sbr-H of Palumbi et al. (1991). Sequences (forward and reverse) were determined using the LI-COR (Lincoln, NE) DNA sequencer Long ReadIR 4200 and the Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ).

The COI sequences were aligned unambiguously by eye using BioEdit 5.0.9 (Hall, 1999). All sequences are fully protein-coding with no insertions or deletions. However, the first few base pairs (bp) behind the 3' end of each primer were difficult to read. We therefore uniformly cut off the first and last ten bp of each sequence, leaving a 638 bp-long completely overlapping fragment for the COI gene. Alignment of LSU rRNA sequences was done using ClustalX (version 1.81; Thompson et al., 1997). No manual refinement was necessary as the alignment yielded only five gaps: three single-nucleotide gaps as well as one gap of up to two nucleotides and one gap of up to three nucleotides within a stretch of thymine bases. The total length of the aligned LSU rRNA is 510 bp. All sequences are available from GenBank (for GenBank accession numbers and DNA voucher numbers see the Appendix).

AFLP Genotyping

Genomic DNA was digested with the frequent cutter restriction enzyme *Mse*I (New England Biolab, NEB) and the rare cutter *Eco*RI (NEB). Adaptors (Table 2) were ligated to the genomic DNA using T4 ligase (NEB). Both digestion and ligation were carried out in a single reaction running for 12h at 37°C.

The ligation product was used to perform a pre-selective PCR amplification with NEB Taq polymerase (for *Eco*RI and *Mse*I primers see Table 2). The quality of the ligation/pre-amplification was checked on a 1% agarose gel.

Selective amplification was performed from 1:40 diluted pre-amp DNA as duplex PCR (one unlabeled *Mse*I each with the two IRDye-labeled *Eco*RI primers; Table 2). A total of 12 primer combination was used for the PCR.

TABLE 2: AFLP primers.

Primer	Sequence
Adapters	
*Eco*RI	5'-CTC GTA GAC TGC GTA CC-CAT CTG ACG CAT GGT TAA-3'
*Mse*I	5'-GAC GAT GAG TCC TGA G-TA CTC AGG ACT CAT-3'
Pre-amplification primers	
E01 E-A (*Eco*RI)	5'-GAC TGC GTA CCA ATT CA-3'
M02 M-C (*Mse*I)	5'-GAT GAG TCC TGA GTA AA-3'
Selective amplification primers	
700 E-AAC	5'-*IRD700*-GAC TGC GTA CCA ATT CAA C-3'
800 E-AAG	5'-*IRD800*-GAC TGC GTA CCA ATT CAA G-3'
M-CGA	5'-GAT GAG TCC TGA GTA ACG A-3'
M-CTT	5'-GAT GAG TCC TGA GTA ACT T-3'
M-CTC	5'-GAT GAG TCC TGA GTA ACT C-3'
M-CAT	5'-GAT GAG TCC TGA GTA ACA T-3'
M-CTA	5'-GAT GAG TCC TGA GTA ACT A-3'
M-CTG	5'-GAT GAG TCC TGA GTA ACT G-3'

Labeled PCR products were separated on an 8% acrylamide gel using the DNA sequencer LI-COR Long ReadIR 4200 and digitally captured with the software package SAGA Generation 2 (MX module version 3.2.1.) from LI-COR. We manually selected the most informative and consistent bands for analysis. These 102 polymorphic loci were scored for 32 individuals of *O. hupensis*. Samples with > 10% ambiguities (more than ten ambiguous values for 102 loci scored) were removed from the data set and not included in any analyses, thus reducing the sample size from 32 to 24 individuals.

Preliminary Statistical Analyses (mtDNA)

Possible dissimilarities between the COI and LSU rRNA data sets are of primary interest for the current study. In order to test whether there were significant differences in incongruence-length between the COI and LSU rRNA data sets, the HOMPART command in PAUP* v. 4.0b10 (Swofford, 2002) was used to perform a partition-homogeneity test (Farris et al., 1995). As the test did not reveal a significant conflict ($P = 0.2580$; 10,000 replicates), the two data sets were used in a combined analysis.

Given the potential high degree of sequence diversity in *Oncomelania hupensis robertsoni*, as indicated by preliminary analyses, we used the test of Xia et al. (2003) implemented in the software package DAMBE 4.2.13 (Xia & Xie, 2001) to test for saturation prior to the phylogenetic analyses. The Xia et al. test did not reveal a significant degree of saturation ($I_{ss} = 0.301$, $I_{ss.c} = 0.801$, $P = 0.0000$).

Nucleotide diversities and divergences (corrected according to the K2P-parameter-model) were calculated using MEGA 2.1 (Kumar et al., 2000) with standard errors estimated by 1,000 bootstrap replications with pairwise deletion of gaps and missing data.

Phylogenetic Reconstruction (mtDNA)

The performance of different phylogenetic methods is highly controversial, and as numerous factors such as degree of heterogeneity and sample size may affect the quality of phylogenetic reconstruction (e.g., Huelsenbeck, 1995; Wiens & Servedio, 1998; Kolaczkowski & Thornton, 2004), we here use both maximum parsimony (MP) and Bayesian inference (BI) based methods.

Phylogenetic analyses based on the MP criterion were conducted in PAUP* 4.0b10 (Swofford, 2002) using the heuristic search option with tree bisection reconnection branch-swapping, 100 replications of random stepwise additions, and MAXTREES set to 10,000. Node support was evaluated with 10,000 bootstrapping replications.

Phylogenetic reconstruction based on BI was conducted using the software package MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001). First, we compared several independent runs using the default random tree option to monitor the convergence of the –ln likelihoods of the trees. The –log likelihoods started at around –8,100 and converged on a stable value of about –4,300 after approximately 60,000 generations. We then did a final run using the Metropolis-coupled Markov chain Monte Carlo variant with four chains (one cold, three heated) and 1,000,000 sampled generations with the current tree saved at intervals of 10 generations. A 50% majority rule tree was constructed from all sampled trees with the first 10,000 trees (100,000 generations) ignored as burn in.

MP and BI analyses were conducted with simple and optimal model of sequence evolution (the latter based on the Akaike Information Criterion implemented in Modeltest 3.6; Posada & Crandall, 1998), respectively.

Parametric Bootstrapping (mtDNA)

A parametric bootstrapping approach was used to specifically test the monophyly of *Oncomelania h. robertsoni* (for a review of the parametric bootstrap see Hillis et al., 1996). First we ran Modeltest to find the optimal model of sequence evolution for the aligned sequences of all *O. h. robertsoni* haplotypes. We then conducted maximum likelihood (ML) searches in PAUP* v. 4.0b10 under the constraint that *O. h. robertsoni* is NOT monophyletic (null hypothesis). The resulting tree was, together with the aligned sequences, imported into Seq-Gen 1.2.5. (Rambaut & Grassly, 1997) to generate 100 random data sets based on the model suggested by Modeltest. We then analyzed in PAUP the differences in tree lengths between the constrained and unconstrained trees for each of the 100 replicates. The frequency of differences in tree lengths was plotted and compared to the tree length difference (constrained vs. unconstrained) of the original unpermutated data set. Finally, we estimated how likely it was that this difference could have been observed randomly.

Intraspecific Genomic Polymorphism (AFLP)

AFLP genotyping is used here in a first attempt to study the degree of nuclear polymorphism in

O. h. robertsoni on the DNA fingerprint level. However, given the limited number of specimens used for AFLP genotyping, we restrict our analyses to estimating diversity indices and to computing a minimum spanning network (MSN) among genotypes in a preliminary assessment of genetic structure in our data set.

A matrix of corrected average pairwise differences between *Oncomelania h. hupensis* and *O. h. robertsoni* as well as within *O. h. robertsoni* was calculated in Arlequin 2.0 (Schneider et al., 2000). The matrix was also used to construct the MSN for the AFLP haplotypes via Arlequin 2.0.

RESULTS

MtDNA Sequence Polymorphism

Among the 66 specimens of *Oncomelania h. robertsoni* studied, a total of 40 haplotypes was found for the combined COI/LSU rRNA fragments. The average nucleotide diversity (corrected to the K2P-model) among all individuals of *O. h. robertsoni* is 0.046 ± 0.004 with a pairwise maximum of 0.117 between individuals A8d (Anning River Valley) and M2e as well as M2g (both from Meishan Area).

Within *Oncomelania h. robertsoni*, we detected four relatively distinct genetic groups (characterized by average genetic divergences of > 0.04 and numbered I, IIa, IIb, and IIc in Table 3 and Fig. 1). The divergences among these groups range from 0.042 ± 0.006 (between groups IIa and IIc) to 0.085 ± 0.010 (between groups I and IIc). In comparison, the overall level of genetic divergence among representatives of other *Oncomelania hupensis* subspecies ranges from 0.0097 ± 0.0029 (between *O. h. hupensis* and *O. h. formosana*) to 0.1024 ± 0.0103 (between *O. h. formosana* and *O. h. quadrasi*).

It should be noted that in phylogeographical studies, the evolutionary relationships above and below the species level are different in nature and their resolution requires a different set of methods (Posada & Crandall, 2001). Therefore, many workers use phylogeographical tools (e.g., network, population structure and gene flow analyses) to infer within-species relationships. However, preliminary tests show that the diversity in our data set is too high for these analyses. Therefore, we have to restrict the following mtDNA analyses to standard phylogenetic tests (MP and BI phylogenetic reconstruction as well as parametric bootstrapping).

MtDNA Phylogenetic Analyses

Given the great significance of data set congruence for addressing potential problems of heteroplasmy and NUMTs, we also performed and compared separate phylogenetic analyses with the individual COI and LSU rRNA data sets, despite the fact that the partition-homogeneity test did not reveal significant conflicts. Both MP and BI analyses revealed four distinct phylogenetic groups of *O. hupensis robertsoni* in the COI and LSU rRNA phylogenies. A manual comparison of the two trees showed a high congruence between the cluster patterns in the COI and LSU rRNA trees, that is, in both gene trees, the same specimens clustered in the same groups (individual trees not shown here). However, there were differences in the trees relative to the monophyly of the four groups within *O. hupensis robertsoni*. Whereas MP and BI analyses of the COI data set resulted in trees that showed the four major groups of *O. h. robertsoni* to be monophyletic, in the LSU rRNA data set the four groups were either paraphyletic (BI analysis: clade I clustered together with the other

TABLE 3: Average K2P nucleotide divergences between four major genetic groups of *Oncomelania h. robertsoni* (below diagonal line) and average nucleotide diversities within major groups (diagonal line). For a geographic distribution of these groups, see Fig. 1.

	I	IIa	IIb	IIc
I	0.018 ± 0.003			
IIa	0.083 ± 0.009	0.011 ± 0.002		
IIb	0.085 ± 0.009	0.043 ± 0.006	0.005 ± 0.001	
IIc	0.085 ± 0.010	0.042 ± 0.006	0.043 ± 0.007	0.010 ± 0.003

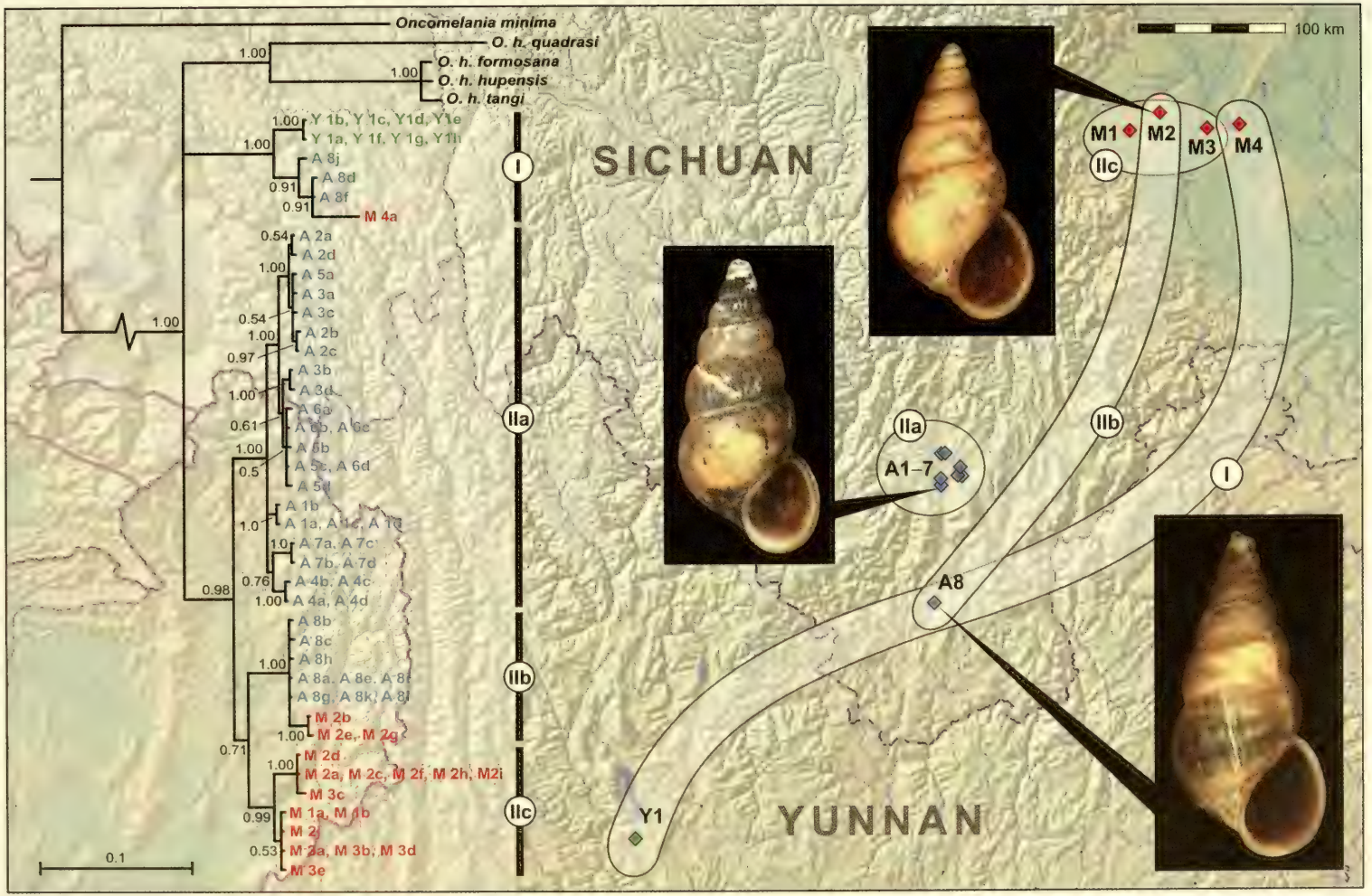

FIG. 1. Bayesian phylogram for *Oncomelania hupensis* ssp. based on 1,148 bp of combined fragments of the COI and LSU rRNA genes showing the 50% majority-rule consensus of topologies sampled. The scale bar represents the substitutions per site according to the model of sequence evolution applied. Posterior probabilities are provided for each clade. The four major clades in *O. h. robertsoni* are labeled I, IIa, IIb, IIc. The geographic distribution of these clades is shown on the map. Geographic areas and corresponding individual codes are color coded (green = Yunnan, blue = Anning River Valley, red = Meishan Area). The primary outgroup specimen, *Tricula* sp., was removed from the tree *a posterior*. For individual codes see the Appendix.

four subspecies of *O. hupensis*) or unresolved (MP analysis).

We then combined the two data sets and performed several phylogenetic analyses using MP and BI. In all combined analyses, we could recover the four distinct clades of *O. h. robertsoni*, generally with good support values. However, both MP and BI could not fully resolve the relationships among these clades (similar to the MP analysis of the LSU rRNA data set-see above): there is a trichotomy of (1) the clade comprising the four other subspecies of *O. hupensis* used in the present study, (2) clade I of *O. h. robertsoni*, and (3) a clade composed of sub-clades IIa, IIb, and IIc of *O. h. robertsoni* (Fig. 1).

The most basal clade in *O. h. robertsoni* (clade I) has a wide geographic distribution. Haplotypes belonging to this clade were found in all geographic areas sampled in the present study, that is, in Yunnan, in the southern Anning River Valley, and in eastern Meishan Area. In contrast, based on the limited data presented here, clade IIa appears to be a localized clade with haplotypes coming exclusively from localities in the northern Anning River Valley. Clade IIb has a wider distribution, ranging from the southern Anning River Valley to a single locality in Meishan Area. Finally, clade IIc is a localized clade restricted to Meishan Area.

Sympatric specimens belonging to different clades were found in two localities: at site A8 (southern Anning River Valley): of the 12 specimens studied, nine belong to clade I and three to clade IIb and at site M2 (central Meishan Area): from ten specimens studied, three belong to clade IIb and seven to clade IIc (Fig. 1).

Parametric Bootstrapping

Given the inability to solve the problem of *Oncomelania h. robertsoni* monophyly using the phylogenetic methods above, a parametric bootstrapping test was performed.

The alternate hypothesis of non-monophyly cannot be rejected ($P \geq 0.41$) as the observed difference in tree lengths between the constrained and unconstrained tree in the original data set is smaller than the observed difference in 59% of the simulated data sets (Fig. 2). In other words, a tree that has been forced to show *O. h. robertsoni* non-monophyletic is not significantly worse than an unconstrained tree.

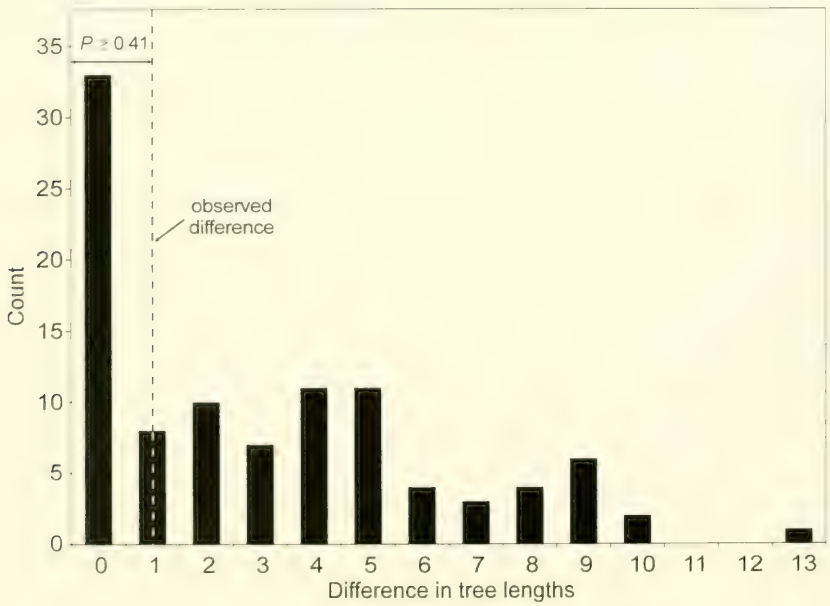

FIG. 2. Result of the parametric bootstrap analysis for the hypothesis of non-monophyly of *Oncomelania h. robertsoni*. The black bars show the number of simulated data sets and the corresponding differences in tree lengths between the constrained (non-monophyly criterion) and unconstrained trees. The dashed line shows the observed difference for the original data set. 41 of the 100 sampled data sets have tree length differences equal or smaller than in the original data set. Therefore, the null hypothesis of non-monophyly of *O. h. robertsoni* cannot be rejected.

AFLP Analysis

All 24 specimens analyzed had unique AFLP fingerprints. Estimates of diversity indices (average pairwise differences among all *O. h. robertsoni* genotypes divided by the total number of loci scored as well as the average pairwise differences between *O. h. hupensis* and *O. h. robertsoni* divided by the total number of loci) resulted in a within *O. h. robertsoni* diversity of 0.113 ± 0.045 and in a divergence between the two respective subspecies of 0.214 ± 0.034.

The MSN network (Fig. 3) shows most *O. h. robertsoni* genotypes clustering in a star-like pattern. The single *O. h. hupensis* genotype scored is distinct from the *O. h. robertsoni* genotypes. Within *O. h. robertsoni*, some genotypes (e.g., Y1f, M2g, A8g, M2b, and A2b) are relatively distinct as well. Given the star-like nature of the network, no clear population structure is recognizable and specimens from the same site do not cluster together in distinct groups. Also, the four major mtDNA clades found in the present study (Fig. 1) are not reflected in the AFLP data.

DISCUSSION

Given the great significance of phylogeographic patterns in the intermediate schistosomiasis host *Oncomelania h. robertsoni* for understanding host/parasite relationships, there are several interesting findings in our study that potentially can help to shed some light on our observation of high rates of mtDNA sequence divergences within *Oncomelania h. robertsoni*:

(1) all COI sequences are fully protein-coding with no insertions or deletions;
(2) both individual and combined analyses of the mtDNA COI and LSU rRNA genes show four distinct haplotype groups within the subspecies of interest (note that given our still preliminary sampling design, it is well possible that more haplotype groups will be recovered in future studies);
(3) neither the phylogenetic analyses nor the parametric bootstrapping test performed here are conclusive relative to the monophyly of the four *O. h. robertsoni* clades found;
(4) both the partition-homogeneity test and visual inspections of the individual COI and

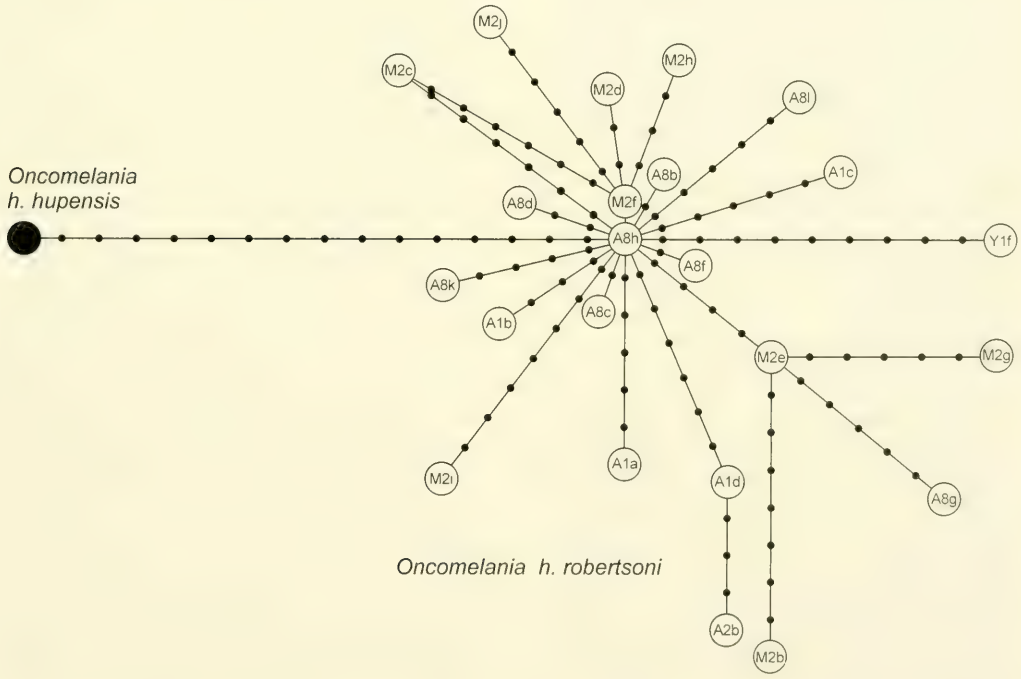

FIG. 3. Minimum spanning network for observed AFLP genotypes of *Oncomelania h. robertsoni* (large white circles) based on 102 polymorphic loci. For comparison, a specimen of *O. h. hupensis* (large black circle) was included. Small black circles indicate the scored differences between the haplotypes. For individual codes see the Appendix.

LSU rRNA trees revealed high concordance in cluster patters and arrangement of individual mtDNA haplotypes;
(5) two of the mtDNA clades recovered appear to be localized, whereas two are widely distributed;
(6) sympatry of individuals belonging to different mtDNA clades does occur;
(7) there is no phylogenetic concordance between the mitochondrial and preliminary nuclear data presented here; and
(8) the nuclear data from AFLP genotyping indicate a lack of clear population structure in *O. h. robertsoni*.

Based on these results, we will focus in our discussion on the high rates of intraspecific mtDNA variability and discuss some of the explanations found in the literature and their relevance for the *O. h. robertsoni* problem.

Presence of a Cryptic Species Complex

The presence of cryptic species radiations has been reported for many mollusc groups, though morphostasis seems to be particularly common in rissooidean gastropods (e.g., Ponder et al., 1995; Hershler et al., 1999; Wilke & Pfenninger, 2002). In fact, several studies on snail hosts in SE Asia revealed cryptic radiations in the family Pomatiopsidae (e.g., Davis, 1992; Attwood & Johnston, 2001). However, the taxon of concern in the present paper, *Oncomelania hupensis*, is one of the morphologically and ecologically best studied snail taxa in Southeast Asia. Particularly the three Chinese subspecies (*O. h. hupensis*, *O. h. robertsoni*, and *O. h. tangi*) were subject to extensive shell morphological and quantitative anatomical studies and comparative anatomical analyses did not reveal significant differences within these subspecies. In fact, a comparative anatomical study of *O. h. robertsoni* populations from Yunnan and Sichuan provinces showed that they are anatomically undistinguishable (George Davis, unpublished data). Moreover, an allozyme study (Davis et al., 1995) of the same three populations of *O. h. robertsoni* from Sichuan and Yunnan provinces showed low levels of heterogeneity within and between populations that are not indicative of a marked departure from the other subspecies. In fact, the allozyme heterogeneity within *O. h. robertsoni* was lower than in the eastern Chinese subspecies *O. h. hupensis*. This lack of population structure within *O. h. robertsoni* could be confirmed in our AFLP study. Given these findings, the presence of a cryptic taxon complex within *O. h. robertsoni* can very likely be ruled out as a cause for the high degree of mtDNA diversity within this subspecies.

Duplications within the Mitochondrial Genome

Duplications of genes or gene fragments within the mitogenome involving protein-coding genes are most often explained with the mechanism of tandem duplication of gene regions as a result of slipped strand mispairing, followed by the deletions of genes (Inoue et al., 2003, and references therein). Most duplications involve short fragments where control regions and tRNA genes seem to be particularly prone to mispairing but there are also reported cases of duplication portions > 8 kbp (e.g., Moritz & Brown, 1987; Inoue et al., 2003).

If duplications of mtDNA genes were responsible for the observed mtDNA patterns in *O. h. robertsoni*, then this would involve a large portion of the mtDNA genome containing both COI and LSU rRNA genes. While this does not seem to be impossible (see above), it is not very likely as this explanation requires a high number of assumptions.

Presence of Nuclear Mitochondrial DNA (NUMT or "Pseudogenes")

Nuclear copies of mitochondrial genes, so-called nuclear mitochondrial DNA (NUMT) or "pseudogenes" (e.g., Lopez et al., 1994; Bensasson et al., 2001) have been observed in many animal species and if unnoticed, can severely confound phylogenetic and population genetic studies (Zhang & Hewitt, 1996). According to Bensasson et al. (2001), symptoms of NUMT contamination of mtDNA can include: (A) PCR ghost bands, (B) sequence ambiguities (e.g., if encountered in forward and reverse strands), (C) frame shift mutations, and (D) stop codons. None of these symptoms were observed in the sequence data generated for the present study i.e., there were no ghost bands in the PCR products, there were no relevant alignment conflicts in forward and reverse strands, there were no insertions or deletions in the alignment of the protein-coding COI gene, and the gene portion studied was free of stop codons. Moreover, as the individual COI and LSU rRNA phylogenies are concordant, both genes would have had to move simultaneously into the nuclear genome. Given all these facts, we can rule out the presence of NUMTs in our data sets.

Heteroplasmy

Heteroplasmy, that is, the presence of more than one type of mtDNA in males/female or even in the same organism, has been reported from several invertebrate species (e.g. Zouros et al., 1992; Fujino et al., 1995; van Herwerden et al., 2000; Steel et al., 2000).

The mitochondrial genome is usually inherited maternally, but paternal 'leakage' and/or biparental inheritance patterns are common in some groups (e.g., *Mytilus*; Hoeh et al., 1991). In addition to biparental inheritance, animal mitochondrial heteroplasmy can also be caused by mutation of the genome within the individual or within the original oocyte (Steel et al., 2000). In most cases, heteroplasmy only involves variations in the number for repeats within the mitochondrial control region (e.g., Hoarau et al., 2002) but base substitutions in coding genes also have been found (e.g., van Herwerden et al., 2000; Steel et al., 2000). While paternal or biparental inheritance can not be completely dismissed as cause for the mitochondrial diversity observed in *O. h. robertsoni*, it is not likely as, for example, all 28 specimens studied from sites A1–7 belong to the same clade. Another possible scenario would be the existence of distinct populations of mitochondria due to non-concerted evolution. Fujino et al. (1995) and van Herwerden et al. (2000) found heteroplasmy in the COI and ND1 genes, respectively, of several digenetic trematode species. The workers suggested that structurally different forms of mitochondria are present in the tegumental and parenchymal cells of adults. Given the life style of the amphibious *Oncomelania h. robertsoni*, that is, the ability to closely shut the shell with its operculum to avoid dehydration during dry conditions and the fact that some freshwater snails (including snail hosts for schistosomiasis) have been shown to be capable of switching between aerobic and anaerobic respiration (Jurberg et al., 1997; van Hellemond et al., 1995, 2003), structurally different types of mitochondria associated with different metabolic respiratory processes could, at least in theory, exist in *Oncomelania h. robertsoni*. However, as the full phylogenetic concordance of COI and LSU rDNA haplotypes (based on the comparison of the individual COI and LSU rDNA trees) does not support the existence of more than one type of mitochondrion in a single individual, non-concerted evolution appears to be extremely unlikely as well.

Temporal Isolation Followed by Secondary Contact

An increasing number of studies shows that temporal isolation followed by secondary contact has deeply influenced the phylogeography of many Palearctic species (e.g. Taberlet et al., 1998; Hewitt, 2000). Particularly, processes resulting from fragmentation into glacial refuges followed by range expansions via postglacial colonization routes may lead to secondary contact zones among formerly disjointed lineages (e.g., Pfenninger & Posada, 2002). Pleistocene glaciations and climate changes certainly must have affected the rivers and streams of the area that is currently populated by *O. h. robertsoni*. However, we doubt that these phylogeographic processes alone are responsible for the extant mtDNA patterns seen today. The divergence between the major clades of *O. h. robertsoni* with K2P differences of up to 8.5% for the combined COI/LSR rDNA data set are indicative of much older divergence times than late Pleistocene or Holocene. Wilke (2003) suggested an average COI local clock rate of 1.83 ± 0.21% uncorrected distance/my for Protostomia lineages that are not affected by saturation. Given an uncorrected average pairwise COI distance of 8.7% between clades I and II in our analysis (Fig. 1), the oldest split in *O. h. robertsoni* is potentially some 4 my old (i.e., early Pliocene) and predates the split of all other *O. hupensis* subspecies. Secondary contact of formerly isolated population may therefore not fully explain the patterns observed here, particularly as there is no compelling supporting evidence from our AFLP data or previous allozyme studies conducted by Davis et al. (1995).

Retention of Ancestral mtDNA Polymorphism

The conflict between our mtDNA und nuclear data sets combined with the potentially long age of the *O. h. robertsoni* clades, as discussed above, may be indicative of a problem in some mtDNA analyses: retained ancestral polymorphism.

A mtDNA phylogeny represents a gene tree that may not be congruent with the species tree (i.e., no reciprocal monophyly in the descendant taxa) because of the retention of ancestral lineages due to stochastic processes (e.g., Avise, 2000; Moore, 1995). This is particularly true for species with ancient divergences

(Avise, 2000) and the problem cannot be solved using multiple mtDNA genes, as the animal mitochondrial genome is inherited as a single unit. Therefore, phylogenies derived from multiple mtDNA genes are not independent estimates of a species' phylogeny (Moore, 1995; Page, 2000).

Long-term substantial isolation among populations of *O. h. robertsoni* could have disrupted gene flow and therefore allowed the retention of anciently separated matrilines. As pointed out by Avise (2000), the evolutionary continuance of isolated populations may buffer against the extinction of lineages within a species. However, this would not explain the occurrence of different matrilines in sympatry as seen in sites M2 and A8 (Fig. 1). Perhaps there is secondary contact among these lines after all (i.e., introgression), either due to post-Pleistocene range expansions or human impact (like transport of snails or their eggs with rice plants). However, as our AFLP data (and previous allozyme and morphological and ecological data) do not support the mtDNA matrilines, we suggest that there is no evidence of organismal subdivision in *O. h. robertsoni* (for a very similar case involving *Drosphila simulans*: Ballard et al., 2002).

It is beyond the scope of this paper to discuss the distinct selective forces acting on the mitochondrial and nuclear genomes. However, tests for deviation from a strictly neutral model of evolution in our mtDNA data sets based on Fu and Li's D^* and F^* (Fu & Li, 1993) as implemented in DnaSP 3.53 (J. Rozas & R. Rozas, 1999) showed that the COI data set deviates significantly from expectations under neutrality both in Fu and Li's D^* (1.78, $P < 0.02$) and in Fu and Li's F^* (1.80, $P < 0.05$). Neutrality was not rejected in the (smaller) LSU rDNA data set with values of 0.56 ($P > 0.10$) and 0.57 ($P > 0.10$) for Fu and Li's D^* and F^*, respectively. At least the results for the COI data set suggest that selection and/or population level processes like expansion, contraction, or subdivision (Ballard & Whitlock, 2004) are acting upon the mtDNA in *O. h. robertsoni*.

Interestingly, one of the extrinsic forces that has been shown to influence mtDNA evolution in natural populations are parasites (e.g., Turelli & Hoffmann, 1995; Ballard et al., 2002). Whether, the parasite of *O. h. robertsoni*, *Schistosoma* sp., has a similar effect on the mtDNA evolution of its host would need to be tested in future studies.

In the present paper, we offer DNA data from two mitochondrial gene fragments as well as preliminary data from AFLP genotyping as a first step to assess the problem of deviant lineages in *O. h. robertsoni*. We suggest that the presence of a cryptic species complex or the occurrence of NUMTs are unlikely to explain the phylogeographic patterns observed. Though, we cannot completely dismiss the occurrence of heteroplasmy or duplications within the mitochondrial genome, which have been observed in molluscs before, these explanations are unlikely as well. The most probable scenario is the retention of ancestral mtDNA polymorphism possibly in combination with some effects of secondary contact. Based on our preliminary AFLP data, we also suggest that there is no evidence of organismal subdivision in *O. h. robertsoni*. However, these hypotheses need to be tested thoroughly in future study.

Nevertheless, we find it important to present our preliminary findings in order to draw attention to the problem observed. As intermediate host for schistosomiasis in western China, *Oncomelania h. robertsoni* is receiving growing attention in ecological and parasitological studies. It is strongly suggested that future studies incorporate more data from nuclear loci in order to better understand phylogeography, population genetics and host-parasite co-evolution in *O. h. robertsoni*.

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APPENDIX

Individual codes (M = Meishan Area, A = Anning River Valley, Y = Yunnan), DNA voucher numbers and GenBank accession numbers for Chinese specimens of *Oncomelania hupensis robertsoni* studied.

Individual code	DNA voucher #**	GenBank accession # COI/LSU rRNA	Individual code	DNA voucher #**	GenBank accession # COI/LSU rRNA
M1a	0963	DQ212797/-	A4d	0958	DQ212828/DQ212883
M1b	0965	DQ212798/-	A5a	0951	DQ212829/DQ212884
M2a	0941	DQ212799/DQ212863	A5b	0952	DQ212830/DQ212885
M2b	0942	DQ212800/DQ212864	A5c	0953	DQ212831/DQ212886
M2c	0943	DQ212801/DQ212865	A5d	0954	DQ212832/-
M2d	1022	DQ212802/-	A6a	0947	DQ212833/DQ212887
M2e	1388	DQ212803/DQ212866	A6b	0948	DQ212834/-
M2f	1389	DQ212804/DQ212867	A6c	0949	DQ212835/-
M2g	1390	DQ212805/DQ212868	A6d	0950	DQ212836/DQ212888
M2h	1391	DQ212806/DQ212869	A7a	0937	DQ212837/DQ212889
M2i	1392	DQ212807/DQ212870	A7b	0938	DQ212838/-
M2j	1393	DQ212808/DQ212871	A7c	0939	DQ212839/DQ212890
M3a	MG14	DQ212809/-	A7d	1021	DQ212840/-
M3b	MG15	DQ212810/-	A8a	0019	DQ212841/-
M3c	MG16	DQ212811/-	A8b	0020	DQ212842/DQ212891
M3d	MG30	DQ212812/-	A8c	0021	DQ212843/DQ212892
M3e	MG33	DQ212813/-	A8d	0022	DQ212844/DQ212893
M4a	-*	AF531547/AF531545*	A8e	0023	DQ212845/-
A1a	0932	DQ212814/-	A8f	0026	DQ212846/DQ212894
A1b	0934	AF213339/AF212893	A8g	0028	DQ212847/DQ212895
A1c	0935	DQ212815/-	A8h	0029	DQ212848/DQ212896
A1d	1018	DQ212816/-	A8i	0030	DQ212849/-
A2a	0928	DQ212817/DQ212872	A8j	0050	DQ112252/-
A2b	0929	DQ212818/DQ212873	A8k	0051	DQ212850/DQ212897
A2c	0930	DQ212819/DQ212874	A8l	0057	DQ212851/DQ212898
A2d	0931	DQ212820/DQ212875	Y1a	0045	AF253074/DQ212899
A3a	0959	DQ212821/DQ212876	Y1b	0046	DQ212852/-
A3b	0960	DQ212822/DQ212877	Y1c	0048	AF253075/-
A3c	0961	DQ212823/DQ212878	Y1d	0055	DQ212853/-
A3d	0962	DQ212824/DQ212879	Y1e	0066	DQ212854/-
A4a	0955	DQ212825/DQ212880	Y1f	1505	DQ212855/DQ212900
A4b	0956	DQ212826/DQ212881	Y1g	1506	DQ212856/DQ212901
A4c	0957	DQ212827/DQ212882	Y1h	1508	DQ212857/DQ212902

* from Attwood et al. (2003)
** deposited at the DNA voucher collection of the Justus Liebig University, Giessen

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A SYSTEMATIC REVISION OF THE SOUTHEAST ASIAN FRESHWATER GASTROPOD *BROTIA* (CERITHIOIDEA: PACHYCHILIDAE)

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ABSTRACT

We here present morphological and molecular genetic data on species of the Southeast Asian freshwater pachychilid *Brotia*, based on examination of material originating from various museum collections world wide, including type material, as well as material from field collections in Thailand and Indonesia. We show that a number of previous systematic assumptions about *Brotia* are in need of correction. Based on our analyses, we suggest a revised and more specific characterisation of this genus and outline the taxonomic and systematic implications of our findings. Accordingly, *Brotia* is restricted herein to viviparous pachychilids possessing as morphological characteristics a subhaemocoelic brood pouch, a pallial oviduct with only a simple, deep, and papillated spermatophore bursa, as well as embryonic shells with a wrinkled apical whorl. This typical embryonic shell structure results from a peculiar mode of ontogeny that includes a yolk sac protruding from the apical whorl during most stages of embryonic development, which are retained in the maternal brood pouch. A molecular phylogeny based on two mitochondrial gene fragments (646 bp of COI and 826 bp of 16S) shows that *Brotia* as encompassed here forms a monophyletic group. The application of the revised concept results in a significantly reduced number of species assigned to *Brotia* with implications also for a considerable reduction of the distributional area covered by members of the genus. In total, 35 species are recognized; the systematic affinities of eight of them remain unclear, however. Data on the morphology, distribution and if known on the biology of these species is presented.

Key words: taxonomy, systematics, Cerithioidea, Pachychilidae, *Brotia*, *Melania*, morphology, viviparity.

INTRODUCTION

It is a major challenge of modern biosystematic research to provide classifications that correctly reflect phylogenetic relationships among organisms. The complexity of work required to achieve this goal has inspired the allegory of an Herculean task, as so aptly formulated by Graf (2001) for his catalogue of North American Pleuroceridae. However, while in the Greek myth the job was finished by unusual ways and means, in systematics accuracy is needed. In addition to a well-founded hypothesis on the natural relationships between taxa, a sound systematics also necessitates thorough revision of the taxonomy, which alone is a challenge as exemplified for various freshwater gastropods, such as Pleuroceridae (Graf, 2001), Pachychilidae (Köhler & Glaubrecht, 2002a), or Neotropic Ampullariidae (Cowie & Thiengo, 2003). In the current work, we attempt to combine both a taxonomic revision and a phylogenetic study, in order to improve our understanding of Asian freshwater snails of the genus *Brotia* H. Adams, 1866, which still are poorly known.

Brotia is a member of the Pachychilidae, a family that was earlier incorporated within the so-called "melanians" or "Melaniidae", which represent a polyphyletic assemblage of freshwater Cerithioidea (reviewed by Glaubrecht, 1996, 1999; molecular phylogeny in Lydeard et al., 2002). Views on the correct familiar assignment of *Brotia* have changed in recent decades due to our steadily improving knowledge. According to earlier systematic opinions, the genus was affiliated either with Thiaridae (e.g., Morrison, 1954; Brandt, 1968, 1974;

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Davis, 1971) or Pleuroceridae (e.g., Vaught, 1989); this was reviewed by Köhler & Glaubrecht (2002a). Molecular phylogenetic studies, however, show that *Brotia* is a pachychilid (Köhler et al., 2004), which in turn represent one of six cerithioidean freshwater clades next to, for example, the Thiaridae, Pleuroceridae, Melanopsidae, to name a few only here (for a molecular phylogeny of the Cerithioidea, see Lydeard et al., 2002).

Freed from systematic misconceptions, Pachychilidae were recently highlighted as an ideal focal group to address biological aspects of more general importance connected, for example, to processes of speciation and morphological adaptation (Rintelen et al., 2004), the evolution of different modes of reproduction (Köhler et al., 2004), as well as biogeographical problems (Glaubrecht, 2000; Glaubrecht & Rintelen, 2003).

Taxonomy and systematics especially of the Asian Pachychilidae have remained confusing for a long time, as outlined by Köhler & Glaubrecht (2001, 2002a). Most Asian pachychilid species sooner or later were attributed to *Brotia* by one or the other author (e.g., Martens, 1900; Thiele, 1928, 1929; Rensch, 1934; Abbott, 1948; Solem, 1966; Brandt, 1968, 1974), which rendered *Brotia* a taxon frequently referred to in systematic literature but in turn most vaguely defined by means of its morphology, distribution, and species composition.

In an initial study, it was shown that *Brotia* as perceived up to then was an assemblage composed of four species groups characterized by the possession of different reproductive and embryonic shell morphologies (Köhler & Glaubrecht, 2001). In the meantime, it has been substantiated that each of these groups indeed represents a distinct evolutionary lineage. The degree of morphological distinctiveness of these lineages has been considered large enough to justify the treatment as separate genera. Accordingly, in addition to *Brotia*, the following Asian pachychilid genera are currently recognized: *Sulcospira* Troschel, 1858 (Köhler & Glaubrecht, 2005), *Pseudopotamis* Martens, 1900 (Glaubrecht & Rintelen, 2003), *Tylomelania* S. Sarasin & F. Sarasin, 1897 (Rintelen et al., 2004; Rintelen & Glaubrecht, 2005), *Adamietta* Brandt, 1974, *Paracrostoma* Cossmann, 1900, and *Jagora* Köhler & Glaubrecht, 2003. Therefore, the status of various supraspecific pachychilid taxa was clarified in the last few years. Although we now know much better which species do not belong to *Brotia*, this has not necessarily improved our knowledge of *Brotia* itself.

For this reason, the current work aims at a taxonomic and systematic revision of the genus by comparative analysis of morphological and mitochondrial sequence data. This shall contribute to a stable and unequivocal taxonomy and systematics of *Brotia* as a group frequently referred to in accounts on Asian freshwater gastropods and at the same time provide the fundament for future studies on the phylogeny, evolution, and biogeography of these promising model organisms.

MATERIAL AND METHODS

Nomenclatural Remarks

The treatment of some species group names introduced by Troschel (1857) is subject to dispute. Bouchet & Rocroi (2005) argue that the non-hierarchical usage of the names "Thiarae" and "Pachychili" as well as "Bithyniae", "Lithoglyphi", "Hydrobiae", and "Ancyloti" by Troschel (1857) stands in contrast with the procedure in the rest of his work, in which the ranks assigned to the formed names are indicated by formal endings, such as "-idea", "-ina", or "-acea". In case of the above-cited group names, Troschel (1857) explicitly refrained from such an assignment of family ranks for the somewhat ambiguous data he was faced with. For this reason, it was suggested by Bouchet & Rocroi (2005) to ignore these names. However, it has also been pointed out that some of these names, such as Bithyniidae, Thiaridae, or Hydrobiidae, are commonly published with Troschel as author. In contrast to the suggestion of Bouchet & Rocroi (2005) and unless it might otherwise be stipulated by an official decision of the ICZN, we prefer to further employ the names introduced by Troschel (1857), not only because we regard them as available and valid irrespective of the circumstance that the author refrained from the assignment of a specific rank, but also in order to keep continuity in the use of zoological names.

Material Examined

This study is based on examination of material from various museum collections world wide (see under repositories). Most samples investigated comprise dry shells only; some others were fixed in 70–96% ethanol or in formalin. In many cases, preserved museum material was not suitable for a more detailed

examination of gross anatomy, for example, by histology, because the bodies were in a bad condition due to partial decay. It has furthermore proven impossible to extract DNA from museum material. In order to achieve a broader basis for our examinations, new collections were undertaken in Thailand and Indonesia. This voucher material is preserved in 75–96% ethanol, and is deposited with the Malacological Collection of the ZMB. To allow proper and quick fixation of the soft bodies, some shells were cracked prior to ethanol preservation. Consequently, the basis of available samples varies considerably between the different species in respect both to quality and quantity of available material. From some species hardly more than some dry shells were accessible, rendering it impossible to sufficiently assess the morphological and geographical range, whereas from others material was available suitable for various kinds of examinations.

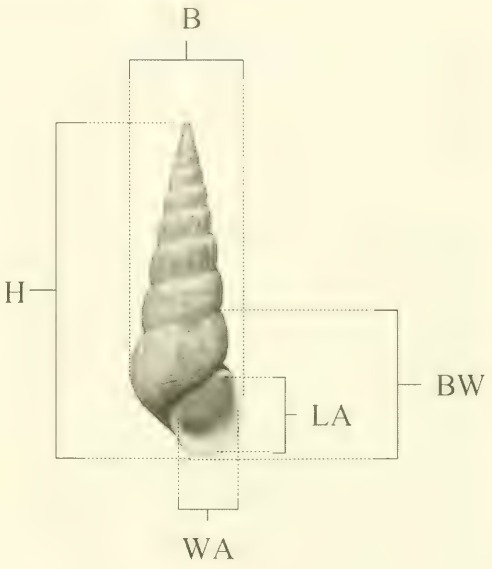

FIG. 1. Shell parameters used for morphometrical analyses.

Morphological Examination

Dimensions of adult and embryonic shells were measured with callipers to 0.1 mm using standard parameters (Figs. 1, 2). These parameters were analysed using statistic software SPSS (vs 9.0). Anatomy was studied using a stereo microscope. Extracted radulae were cleaned as described by Holznagel (1998) and mounted on stubs and coated with Gold-Palladium for SEM examination with a Jeol FSM 6300 scanning electron microscope. Embryonic shells extracted from ethanol preserved specimens or from dried shells were cleaned mechanically and by sonication and prepared for SEM as given for the radulae. Since in viviparous freshwater Cerithioidea a distinct transition from the larval or primary shell (= protoconch) to the adult or secondary shell (= teleoconch) is lacking for the loss of free larval stages, we apply the more general term "embryonic shell" for all shelled stages retained in the brood pouch. Embryonic shell parameters were measured as shown in Figure 2. Soft tissues were treated with hexamethyl-

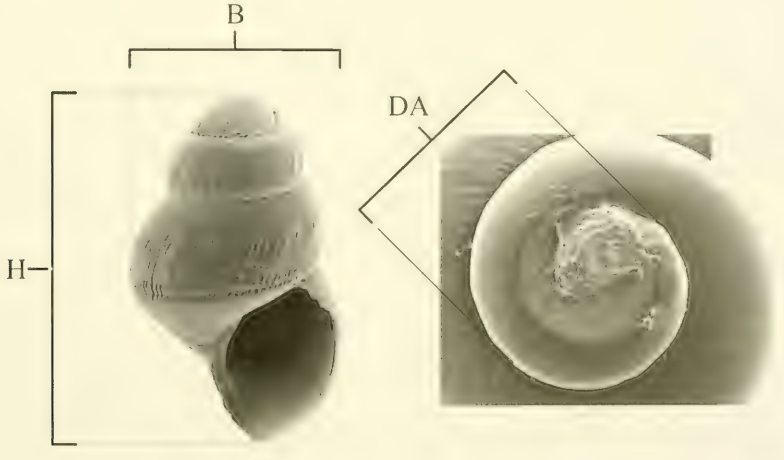

FIG. 2. Embryonic shell parameters used for morphometrical analyses.

disilazane prior to SEM as described by Nation (1983). Stomach morphology was examined using the methodology and terminology described by Strong (2003).

Molecular Genetics

Two fragments of the mitochondrial genes of the Cytochrome Oxidase I ("COI", 646 bp) and the 16S rRNA ("16S", 826 bp) were sequenced. The data set contains 40 sequence pairs belonging to 16 species of *Brotia,* five sequence pairs belonging to four species of *Adamietta*, and a sequence pair each of two species of *Paracrostoma*. Two additional sequence pairs belonging to *Jagora* were included as outgroup representatives. DNA was purified from about 1–2 mm^3 of foot tissue from specimens preserved in ethanol by CTAB extraction (Winnepenninckx et al., 1993). PCR amplification of the fragments were performed in 25 µl volumes containing 1x Taq buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 1 U Taq polymerase, approximately 100 nM DNA and ddH_2O up to volume on a Perkin Elmer GeneAmp 9600 or 2400 thermocycler. After an initial denaturation step of 3 min at 95°C, cycling conditions were 35 cycles of 1 min each at 95°C, 45–53°C, and 72°C, with a final elongation step of 5 min. Primers used were LCO 1490 5'-GCTCAA CAAATCATAAAGATATT-3' and HCO2198 var. 5'-TAWACTTCTGGGTGKCCAAARAAAT-3' (Folmer et al., 1994, modification of HCO2198 by A. B. Wilson) for COI, and 16SF 5'-CCGCACTTAGTGATAGCTAGTTTC-3' (Wilson et al., 2004) and H3059-Inv 5'-CGGTYTG AACTCAGATCATGT-3' (Palumbi et al., 1991) for 16S, respectively. PCR products were purified with QiaQuick PCR purification kits (Qiagen) following the standard QiaQuick PCR purification protocol. Both strands of the two genes were cycle sequenced with the original primers using ABI Prism BigDye™ terminator chemistry and visualized on an ABI Prism 377 automated DNA sequencer. The resulting sequence electropherograms of both strands were corrected manually for misreads and merged into one sequence file using BioEdit Version 5.0.1 (Hall, 1999). Sequences are accessible via GenBank (accession numbers in Table 6).

Sequence Analysis

COI sequences were aligned manually and checked by translating the DNA sequences into amino acids in DAMBE 4.1.19 (Xia & Xie, 2001) using the genetic code for invertebrate mitochondrial DNA. 16S sequences were aligned using the online version of ClustalW provided by the hompeage of the Europaen Bioinformatics Institute (www.ebi.ac.uk/clustalw/) (Thompson et al., 1994) using default settings. A combined data set was constructed by concatenating the sequences. Pair-wise genetic distances were calculated with PAUP* (Swofford, 1999). Phylogenetic trees were reconstructed using Neighbor Joining (NJ) (Saitou & Nei, 1987) and Maximum Parsimony (MP) as implemented in PAUP*. In addition, a Bayesian method of inference (BI) was employed to estimate phylogenetic relationships (e.g., Huelsenbeck et al., 2002; Holder & Lewis, 2003) using MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). NJ analyses were conducted using the random initial seed option to break ties and under a general time reversible model of sequence evolution (GTR+I+Γ; Gu et al., 1995) to correct for multiple substitutions. In the MP analyses, the heuristic search algorithm was employed with ten random additions of taxa and tree bisection-reconstruction (TBR) branch swapping. Gaps were treated as fifth base. Other settings were left on default. Prior to BI analyses, it was explored which substitution model fits best the sequence data set by running a hierarchical likelihood ration test implemented in MrModeltest (Nylander, 2002). For BI analysis a Metropolis-coupled Markov chain Monte Carlo (4 chains, chain temperature = 0.2) was run for 750,000 generations. A 50% majority-rule consensus tree was constructed for the last 2,500 trees in order to assess the posterior clade probabilities for each node (bpp).

Repositories and their Abbreviations

AMS	Australian Museum, Sydney, Australia
ANSP	Academy of Natural Sciences, Philadelphia, Pennsylvania, U.S.A.
BMNH	Natural History Museum, London, United Kingdom
CAS	California Academy of Sciences, San Francisco, California, U.S.A.
IMC	Indian Museum, Calcutta, India
MCZ	Museum of Comparative Zoology, Cambridge, Massachusetts, U.S.A.
MHNG	Muséum d'Histoire Naturelle, Genève, Switzerland
MNHN	Muséum National d'Histoire Naturelle, Paris, France
MZB	Zoological Museum, Bogor, Indonesia

NMB Naturhistorisches Museum, Basel, Switzerland
RMNH Natural History Museum Naturalis, Leiden, The Netherlands
SMF Senckenbergmuseum, Frankfurt/Main, Germany
ÜMB Überseemuseum, Bremen, Germany
USNM National Museum of Natural History, Smithsonian Institution, Washington D.C., U.S.A.
ZMA Zoölogisch Museum, Amsterdam, The Netherlands
ZMB Museum für Naturkunde, Humboldt-Universität Berlin [formerly Zoologisches Museum], Germany
ZMH Zoologisches Museum und Institut, Universität Hamburg, Germany
ZSI Zoological Survey of India, Calcutta, India
ZSM Zoologische Staatssammlung, München, Germany
ZMZ Zoologisches Museum, Zürich, Switzerland

Abbreviations

B breadth of shell
BW height of the body whorl
bp brood pouch
bpp brood pouch pore
c cerebral ganglion
cg capsule gland
cr crescent fold
crt septate crescent thickening
ct ctenidium
DA diameter of apical whorl of embryonic shell
dg digestive gland
dgd digestive gland duct
eg egg capsule
ey eye
ft foot
gg genital groove
gp gastric pad
gs gastric shield
H height of shell
hd head
int intestine
kd kidney
LA length of aperture
lf lateral fold
ll lateral lamina
m median
mc mantle cavity
me mantle edge
mf marginal fold
ml medial lamina
mr mantle roof
N number of whorls
oes oesophagus
og oviductal groove
op operculum
ovd oviduct
ovr ovary
p pedal ganglion
pl pleural ganglion
rad radula
s statocyst
sa sorting area
sb spermatophore bursa
sbg sub-oesophageal ganglion
sd standard deviation
sg sperm gutter
sn snout
snn snout nerve
spg supra-oesophageal ganglion
ss style sac
st stomach
t_1 major typhlosole
t_2 minor typhlosole
tn tentacle
tnn tentacular nerve
ts testis
WA width of aperture

SYSTEMATIC ACCOUNT

Pachychilidae Troschel, 1857

Brotia H. Adams, 1866

Brotia H. Adams, 1866. Type species, by monotypy: *Melania pagodula* Gould, 1847.
Antimelania Fischer & Crosse, 1892. Type species, by subsequent designation in Pilsbry & Bequaert (1927): *Melania variabilis* Benson, 1836.
Wanga Chen, 1943. Type species, by original designation: *Melania henriettae* Griffith & Pidgeon, 1834.

Taxonomy and Systematics

Brotia was originally established for the round and multispiral operculum of the type species, which however is a characteristic exhibited by a number of pachychilid taxa and not peculiar for *Brotia* (Köhler & Glaubrecht, 2001, 2002a, 2003). In the 19th and 20th century, a vast number of species were affiliated with *Brotia* by a number of authors without sufficient knowledge of their gross morphology (e.g., Brot, 1874–1879; Martens, 1897,

1900; Martens & Thiele, 1908; Abbott, 1948). This procedure has caused considerable systematic confusion as to the taxonomy of *Brotia* and other described supraspecific taxa from Asia (see overview in Davis, 1971: 68, 69; Köhler & Glaubrecht, 2002a). A first, more comprehensive treatment of *Brotia* species based also on features of the soft body was presented by Brandt (1974). This author also suggested a subdivision of *Brotia* into three subgenera: (1) *Brotia* s. str., (2) *Senckenbergia* Yen, 1939, and (3) *Paracrostoma* Cossmann, 1900. This suggestion was refuted, however, by Köhler & Glaubrecht (2001), who argued that radular and opercular features alone are insufficient to differentiate supraspecific taxa among the Pachychilidae. Instead, it was shown that characters of the reproductive tract and embryonic shells are more informative at this level. Using these morphological structures, a preliminary subdivision of *Brotia* into four species groups was suggested by Köhler & Glaubrecht (2001). Two of these groupings have since been established as genera independent of *Brotia*: *Tylomelania* endemic to Sulawesi (Rintelen et al., 2004; Rintelen & Glaubrecht, 2005) and *Jagora* endemic to the Philippines (Köhler & Glaubrecht, 2003). The status of the two remaining groupings, so-called "*Brotia pagodula* group" and "*Brotia testudinaria* group", have remained unresolved thus far. Only recently it has been suggested on basis of molecular genetic data that both species groups indeed form distinct monophyletic lineages (Köhler et al., 2004). According to this mitochondrial phylogeny, it was suggested to transfer all species of the "*Brotia testudinaria* group" designated by Köhler & Glaubrecht (2001) to *Adamietta* Brandt, 1974 (Köhler et al., 2004: 2221). In regard to this suggestion, in the current study *Brotia* is restricted to the members of the "*Brotia pagodula* group" as delineated by Köhler & Glaubrecht (2001). Accordingly, morphological features characteristic for *Brotia* are (1) an irregularly wrinkled apical whorl of the embryonic shell and (2) a pallial oviduct possessing a simple, deep, and ciliated spermatophore bursa.

Morphology and Differential Diagnosis

Shell: Relatively large, often up to 4 or 5 cm. Moderately thick, broadly to elongate conical, turreted spire, apex eroded or truncated. Sculpture variable comprising axial ribs, sometimes with nodules, and spiral ridges or lines. Body whorl comparatively large; aperture ovate, well rounded or angled below, pointed above. No features peculiar to *Brotia*.

Embryonic Shell: Relatively large among viviparous pachychilids; average height 1 to 6 mm, up to four whorls. Apical whorl asymmetrical, irregularly wrinkled; initial shell sharply delimited from subsequent whorls with more or less smooth sculpture (for peculiar ontogeny of *Brotia* causing wrinkles see below).

Operculum (Fig. 3C): Either round, up to eight whorls, central nucleus or slightly oval for last whorl increasing in diameter with up to six whorls.

External morphology and mantle cavity (Figs. 3A, B): Animals light to dark brown, dark grey or black, often with light patches; broad, furrowed snout. Cephalic tentacles moderately long, each with tiny eye on side of base. Females with subhaemocoelic brood pouch; "egg transfer" or "genital groove" on right side of head connects pallial oviduct with brood pouch pore near base of right tentacle; present also in males. Mantle margin smooth; mantle cavity occupying approximately two thirds of first whorl. Osphradium delicate, slightly undulating, forming narrow ridge embedded in shallow trench, lying adjacent to anterior part of ctenidium. Ctenidium large, broad tapering posteriorly; beginning shortly behind mantle edge, extending posteriorly almost to end of cavity, on average twice as long as osphradium. Hypobranchial gland inconspicuous, adjacent to rectum.

Radula: Taenioglossate, relatively large, robust. Up to 30 mm long corresponding to half of shell height. Posteriorly embedded in connective tissue, coiled behind buccal mass in radular sac. Rachidian squarish, with one pronounced, more or less pointed central cusp flanked by up to three accessory denticles that taper in size; glabella well developed. Anterior margin of rachidian concave or straight, lower rim concave by posteriorly extending glabella. Lateral teeth with rounded glabella; major cusp flanked by up to three smaller denticles on each side. Inner marginal teeth with two, outer marginal with up to three denticles; hooked; simple flange or ledge at outer margin; more pronounced in outer marginal teeth.

Nervous System (Fig. 3E): Cerebral commissure long, cerebro-pleural connectives short. Sub-oesophageal ganglion fused with left pleural ganglion. Pedal ganglia deeply embedded in propodium, connected to pleural

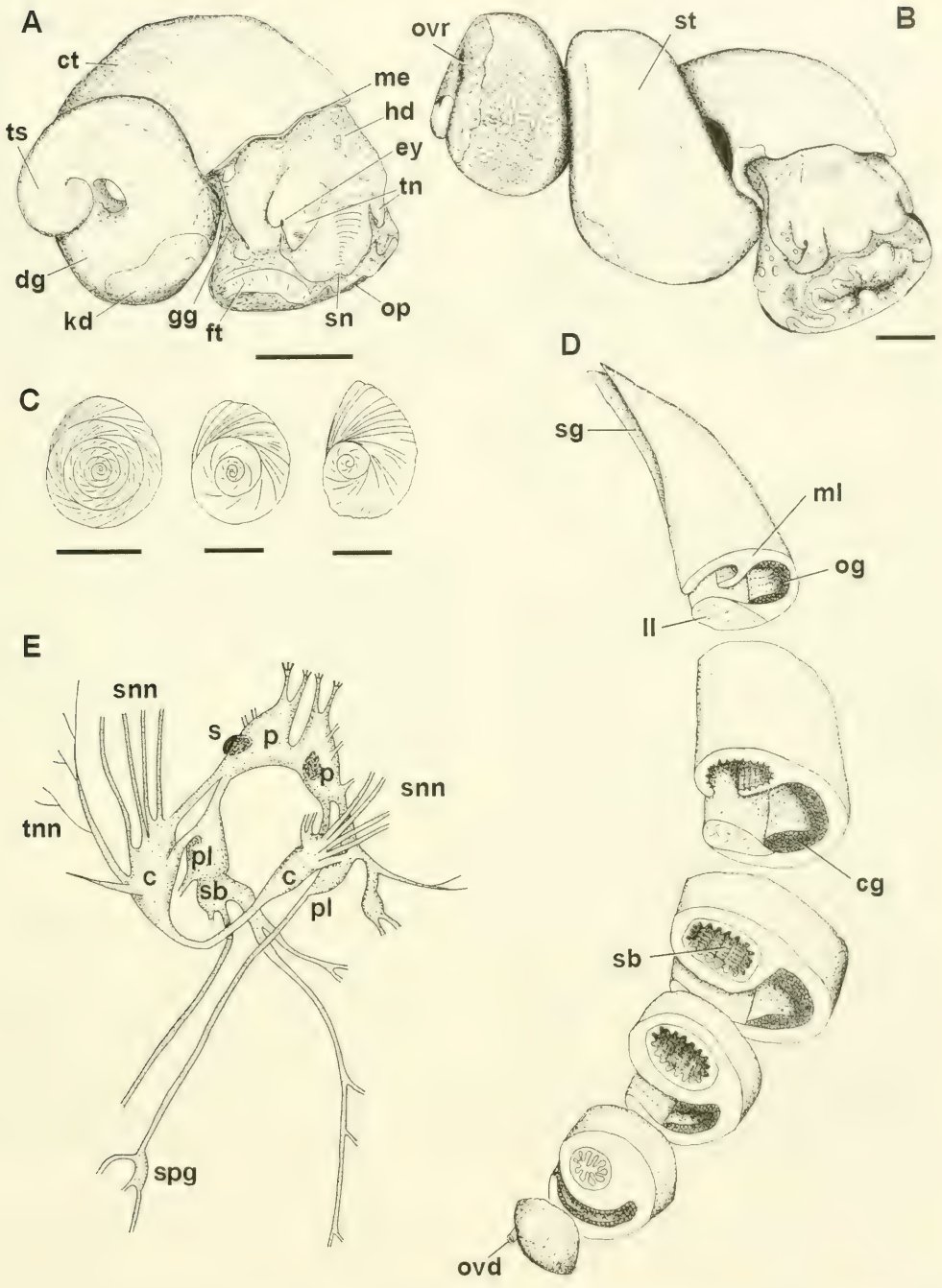

FIG. 3. Soft anatomy of *Brotia*. A: External anatomy of *B. pagodula* (Thailand, male); B: External anatomy of *B. episcopalis* (Sumatra, female); C: Opercula (from left to right: *B. pagodula*, *B. episcopalis*, *B. costula*; D: Pallial oviduct of *B. pagodula*; schematic reconstruction showing various cross-sections from anterior to posterior; E: Schematic reconstruction of nervous system of *B. pagodula*. Scale bars = 10 mm.

and cerebral ganglia by relatively long connectives. Pedal ganglia closely joined, statocysts located basally.

Alimentary System: Oesophagus longitudinally folded, transverse septae not present. Stomach typical pachychilid (Strong & Glaubrecht, 1999), including presence of sorting area, single digestive gland duct, narrow glandular pad, cuticular gastric shield, crescent ridge and groove (e.g., Fig. 4, *B. citrina*). Major and minor typhlosole may be fused. Epithelium of style sac heavily ciliated with golden gloss. Crystalline style cylindrical or club-like.

Reproductive System

Gonochoristic with balanced sex ratio. Subhaemocoelic brood pouch occupying almost entire visceral cavity, compartmentalized with lamellae of thin adventitious tissue embedding embryos (Figs. 5A–E for histological sections). Juveniles within pouch of same ontogenetic stage. Gonads comparatively large, comprising last two to three visceral whorls, adjacent to and dorsal of digestive gland. Ovary orange to light brown consisting of broad lobes (Fig. 6E). Testis light yellow consisting of highly branched thin tubes. Pallial gonoduct open in both sexes. Pallial oviduct comprising deep oviductal groove bounded by parallel laminae (Fig. 6A); ciliated sperm gutter forming along free edge of medial lamina, opening to papillated spermatophore bursa approximately at two thirds of oviductal length (Fig. 6B); large capsule gland comprises almost entire length of pallial oviduct; capacious, ciliated spermatophore bursa formed by median lamina (Figs. 6C, D); Fig. 3D, schematic reconstruction of pallial oviduct).

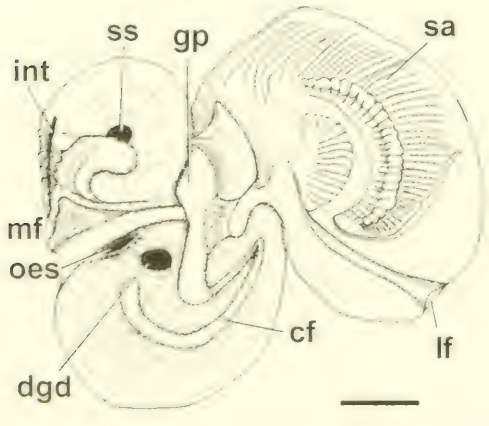

FIG. 4. Stomach anatomy of *B. citrina* (Thailand, Mae Sot; ZMB 200.212). Scale bar = 5 mm.

Habitat

Most species inhabit small, clear mountain streams; some occur also in lakes. Often confined to specific habitats, such as upper course of rivers, and restricted to single rivers or river systems. Rarely more than two species cooccur with notable exception of endemic species flock in Kaek River, central Thailand (Glaubrecht & Köhler, 2004).

Distribution

Southeast Asia, from foot hills of Himalayas in northeast India and Bangladesh to Myanmar, Thailand, Malaysian Peninsula, Sumatra, Java, and Borneo. Reports from Java and Borneo are scarce, date back to 19th century. *Brotia* as here defined does not occur in most parts of Indochina, in Sulawesi, in the Philippines, or on the Smaller Sunda Islands.

Fossil Record

Fossil record in continental Southeast Asia extends back to middle Miocene. Gurung et al. (1997) report on *Brotia* species (e.g., *B. palaeocostula* and other undetermined species) from middle Miocene to Pliocene deposits of Nepal (Churia group). Annandale (1919) mentions fossil *Brotia* from Miocene and Pleistocene sediments of Lower Burma, for example, "*B. variabilis*" from Miocene of Pegu, "*B. baccata*" from Lake Inlé (Shan States) of presumably post-Pleistocene age. From latter deposits, Bequaert (1943) noted three forms of *Brotia* and *Sulcospira*, respectively that persist to the Recent. His reference to *Sulcospira* is here attributed to *Brotia*, *Sulcospira* being endemic to Java (Köhler & Glaubrecht, 2005).

Affinities of Miocene and Pliocene fossils of Java reported by Martin (1914) and Oostingh (1935) remain doubtful, not only since the dating of these sediments was questioned (Oostingh, 1935: 2). Judging from figures in both publications, we consider the species in question, for example, "*Brotia oppenoorthi*", not congeneric with Recent *Brotia*. Instead, at least some species represent thiarids.

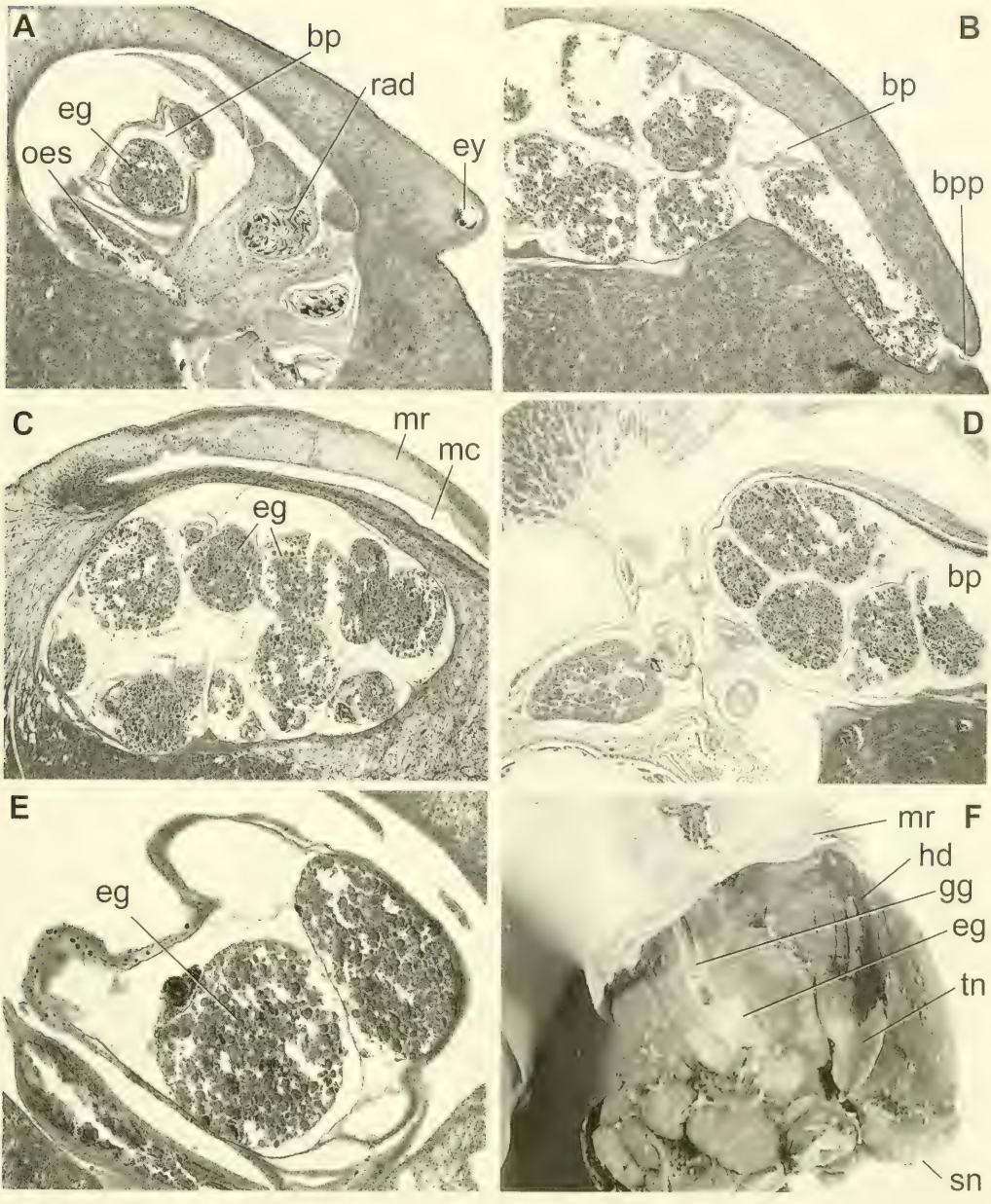

FIG. 5. Brood pouch morphology. A–E: Histological sections of the head-foot of *Brotia episcopalis* (ZMH, Trang); A: Longitudinal section of head, showing the visceral cavity with radula, buccal mass, oesophagus, and anterior part of brood pouch situated just behind buccal mass; B: Cross-section at about mid head; brood pouch occupies most of visceral cavity, brood pouch pore visible; C: Cross-section some mm posterior to B; brood pouch filled with numerous egg capsules each embedded in thin membrane; D: Cross-section at posterior end of brood pouch; E: Detail of A; egg capsules in higher magnification; F: Macro-anatomical photograph of *B. pseudosulcospira* (ZMB 200.196); head of female with egg capsule sitting in genital groove just in front of brood pouch pore.

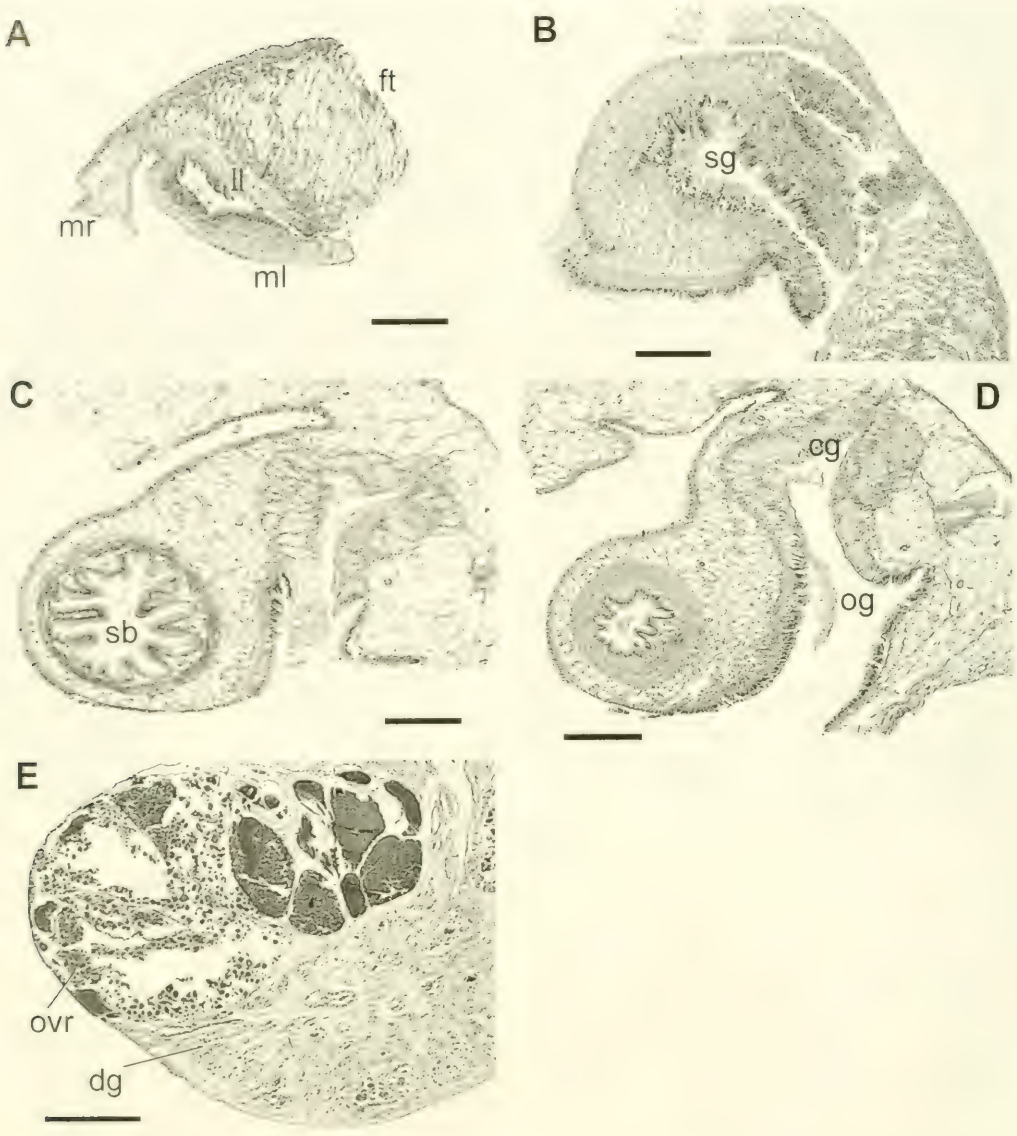

FIG. 6. Female reproductive anatomy. A–D: Histological sections of pallial oviduct of *B. pagodula* (ZMH: Myanmar): A: Cross-section at anterior end of pallial gonoduct; lateral lamina fused with mantle, simple medial lamina free; B: Cross-section at about one third of oviduct length; heavily ciliated sperm gutter formed by medial lamina; C: Cross-section at about half of oviduct length; spermatophore bursa formed by medial lamina, capsule gland comprising base of oviductal groove; D: Cross-section at about two thirds of oviduct length; ciliated spermatophore bursa; E: Cross-section of visceral whorl of *B. episcopalis* (ZMH. Trang); ovary filled with egg capsules, adjacent and posterior to digestive gland. Scale bars = 1 mm.

Also fossil shells from Europe were attributed to *Brotia* (Papp, 1953). The fossil taxon *Tinnyea* was treated as a subgenus of *Brotia* (Papp, 1953) and various species have been affiliated with this Southeast Asian taxon, such as "*Brotia escheri*" (Brongniart, 1822) and "*B. vasarhelyii*" (Hantken, 1887) from Pannonian deposits near Budapest, Hungary – Upper Miocene (Lörenthey, 1902), Burgenland, Austria – Upper Miocene (Fischer, 1994), and

Mainz Basin, Germany – Upper Oligocene to Lower Miocene (Kadolsky, 1995). Placement of these and other fossil species in *Brotia* does refer only to (rather superficial) shell similarity and ignores the uncertain freshwater origin of the deposits. Assignment of European fossils to *Brotia* is rejected here; whether some fossils might be included in the Pachychilidae awaits critical evaluation of the fossil material, which we have not examined yet.

ACCOUNT OF RECENT SPECIES IN ALPHABETICAL ORDER

Brotia armata (Brandt, 1968) (Figs. 7, 8, 12A, B)

Brotia (*Paracrostoma*) *pseudosulcospira armata* Brandt, 1968: 275, pl. 10, fig. 62 ("Maenam Kaek in Phitsanulok Prov. at Gaeng Song rapids, 45 km E Pitsanulok" = Thailand, Prov. Phitsanulok, Kaek River at Kaeng Song rapids, approximately 60 km E of Phitsanulok), holotype SMF 197380, 35 paratypes ZMH; types seen.

Paracrostoma pseudosulcospira armata – Brandt, 1974: 186, pl. 13, fig. 43; Köhler & Glaubrecht, 2002a: 144.

Brotia armata – Glaubrecht & Köhler, 2004: 283–287.

Paracrostoma morrisoni Brandt, 1974: 188, 189, pl. 14, fig. 47 ("Maenam Kaek at Sopa Falls, 71 km E of Pitsanulok" = Thailand, Prov. Phitsanulok, Kaek River at Sopha Falls, 71 km E of Phitsanulok), holotype SMF 215966, six paratypes SMF 215967, 12 paratypes SMF 271191, 38 paratypes SMF 193587, 11 paratypes BMNH 1976119, 14 paratypes RMNH 55135/14; types seen; Köhler & Glaubrecht, 2002a: 141, 142.

Paracrostoma paludiformis dubiosa Brandt, 1974: 188, pl. 14, fig. 46 ("Kaek River, 80 km E of Pitsanulok" = Thailand, Prov. Phitsanulok, Kaek River, 80 km E of Phitsanulok), holotype SMF 215964, six paratypes SMF 215964, five paratypes RMNH 55284/5; types seen; Köhler & Glaubrecht, 2002a: 142.

Taxonomy and Systematics

Originally described as subspecies of *B. pseudosulcospira*, it was transferred to *Brotia* and is treated as distinct species in the Kaek River species flock by Glaubrecht & Köhler (2004). According to these authors, *P. morrisoni* and *P. paludiformis dubiosa* are considered as synonyms.

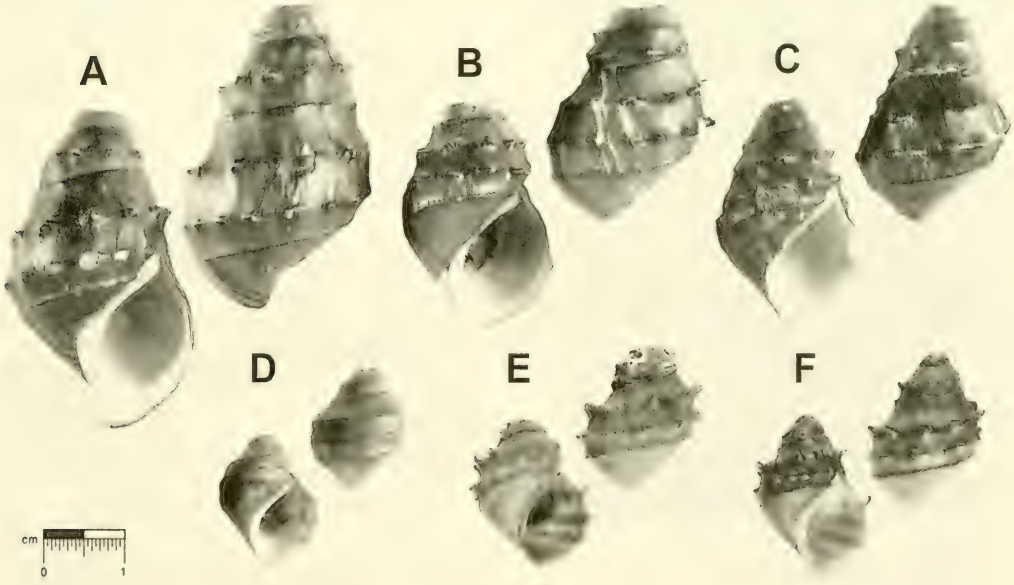

FIG. 7. Shell morphology of *B. armata*. A–D: Paratypes of *P. pseudosulcospira armata* SMF 193587; E–F: Paratypes of *P. morrisoni* SMF 215967. Scale bar = 10 mm.

Material Examined

Thailand: Prov. Phitsanulok, Kaek River: Sakunothayan Falls, 33 km E of Phitsanulok (ZMB 200.265; ZMH); Kaeng Song rapids, 45 km E of Phitsanulok (SMF 193587; ZMB 200.193); resort, 53 km E of Phitsanulok (ZMB 200.254); Poi Falls, 60 km E of Phitsanulok, 16°50.75'N, 100°45.06'E (ZMB 200.268); Thung Salaeng Luang National Park, 90 km E of Phitsanulok, 16°52'N, 100°38"E (USNM 794081; ZMB 200.252, 200.265).

Differential Diganosis

Shell relatively small, conical to oval, not more than three rather flattened whorls; one to three spiral cords supporting a spiral row of sometimes spiny nodules.

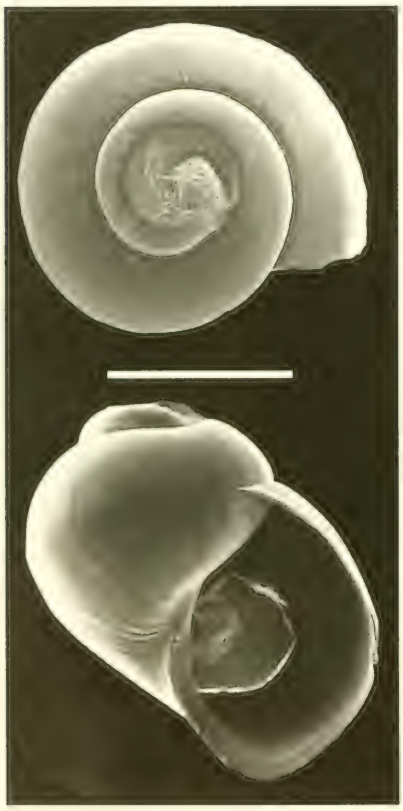

FIG. 8. Embryonic shell morphology of *B. armata*. SEM images of embryonic shell removed from brood pouch (paratype BMNH 1976111); apical and front view. Scale bar = 1 mm.

Description

Shell (Fig. 7): Relatively small, oval to conical, up to three flattened to slightly convex whorls, tip eroded. One to three spiral cords, especially upper ones supporting spiral rows of spiny nodules; on body whorl often additional cord visible. Some shells almost smooth. Aperture broadly ovate, large compared to shell, basal margin produced. Size: H = 26–38 mm, B = 18–24 mm.

Embryonic Shell (Fig. 8): Smooth except for axial growth lines, sharp transition between apical area and penultimate whorl after about half of first whorl. Size of juveniles kept in brood pouch: 2.0–2.5 mm, 2.5 whorls.

Operculum: Oval, up to four whorls that increase in diameter, sub-central nucleus.

Radula (Figs. 12A, B): Length of ribbon: m = 18.4 mm (sd = 4.4 mm; n = 15), up to 180 rows of teeth. Central tooth with elongated main cusp and two or three much smaller accessory denticles on each side that taper in size; glabella narrow with straight lateral margins, rounded posterior rim that does not reach the basal rim of central tooth. Laterals with broad main cusp flanked by one to two accessory denticles on each side. Inner and outer marginals with large, broad outer cusp and spiny inner denticle.

Stomach: Typical, as in *B. binodosa* (Fig. 13).

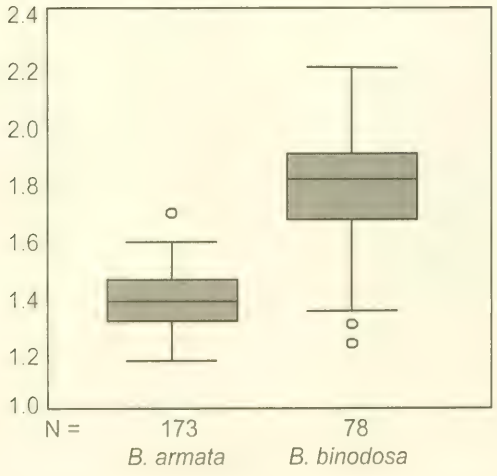

FIG. 9. Comparison of *B. armata* and *B. binodosa* by means of shell parameter H/B. Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

TABLE 1. Result of disriminant analysis of shell parameters of *B. armata* and *B. binodosa*.

	Predicted group membership	
	B. armata	*B. binodosa*
B. armata	134 (97.8%)	3 (2.2%)
B. binodosa	11 (14.1%)	67 (85.9%)

Distribution

Thailand: Prov. Phitsanulok: Endemic to Kaek River; only in middle course between Sakunothayan Falls (33 km E Phitsanulok) and Thung Salaeng Luang NP (90 km E Phitsanulok).

Remarks

Brotia binodosa with similar sculpture is more turreted and slender possessing more whorls. Both species deviate mainly in proportion of shell height to width (H/B, Fig. 9), although not statistically significant (Table 1). *Brotia pseudosulcospira* lacks spines, is larger with a darker, thicker, smoother shell.

Brotia binodosa (Blanford, 1903)
(Figs. 10, 11, 12C, 13)

Melania binodosa Blanford, 1903: 282, 283, pl. 8, fig. 2 ("Siam, in fluminibus majoribus" = in large rivers, Thailand; restricted to Sopha Falls, at the Kaek River near Phitsanulok by Brandt 1974: 175), holotype BMNH 1903.2.28.2, paratype BMNH 1903.2.28.3 (Figs. 8A, B); types seen.

Brotia binodosa – Solem, 1966: 15, figs. 1a, b; Glaubrecht & Köhler, 2004: 287–289.

Brotia (*Brotia*) *binodosa binodosa* – Brandt, 1974: 174, 175, pl. 12, fig. 26.

Brotia spinata – Köhler & Glaubrecht, 2002a: 148 [partim].

Brotia (*Brotia*) *binodosa spiralis* Brandt, 1974: 176, pl. 12, fig. 27 ("Thailand: Kaek River, 38.5 km E Pitsanulok" = Thailand, Prov. Phitsanulok, Kaek River 38.5 km E of Phitsanulok), holotype SMF 220340; type seen.

Brotia spinata spiralis – Köhler & Glaubrecht, 2002a: 130.

Taxonomy and Systematics

Revised by Glaubrecht & Köhler (2004), who suggested *B. binodosa spiralis* to represent a junior synonym. Member of the Kaek River species flock in Central Thailand.

Material Examined

Thailand: Prov. Phitsanulok: Chattrakan Fall, Kwae Noi River in the Chattrakan NP, N of Nakhon Thai (ZMB 200.202); Kaek River (SMF 193577; RMNH 55288): Kaeng Song Falls (SMF 193874); resort, 53 km E of Phitsanulok

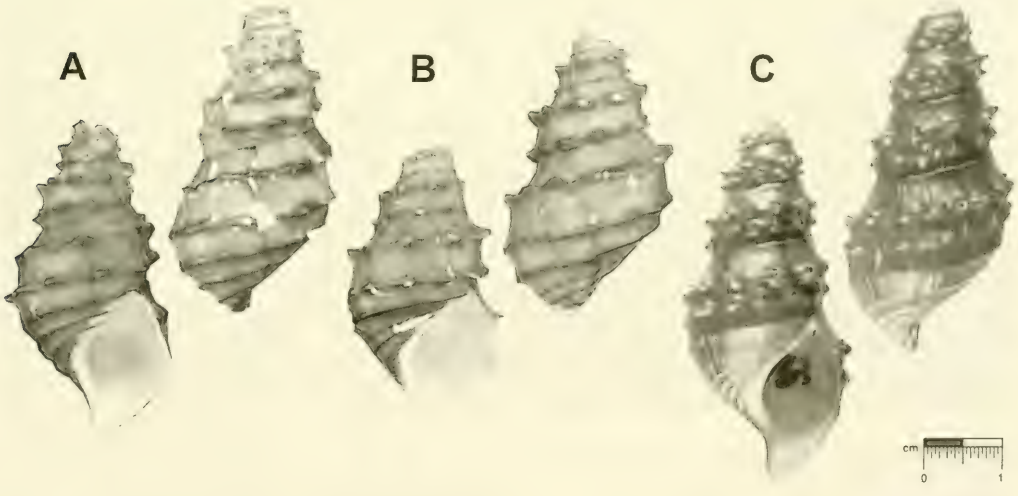

FIG. 10. Shell morphology of *B. binodosa*. A: Holotype of *M. binodosa* BMNH 1903.2.28.2; B: Paratype BMNH 1903.2.28.3; C: Thailand, Kaek River, Sopha Falls (ZSM 19983219). Scale bar = 10 mm.

FIG. 11. Embryonic shell morphology of *B. binodosa*. SEM images of embryonic shell removed from brood pouch (Thailand, Kaek River; ZSM 19983219); apical and front view. Scale bar = 0.3 mm.

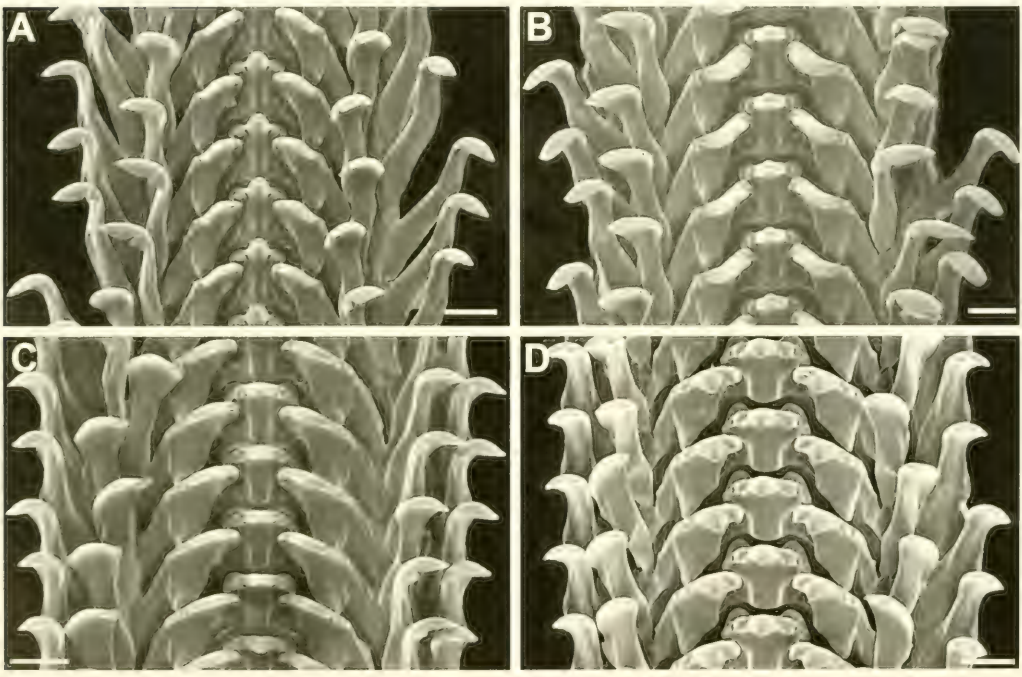

FIG. 12. Radular morphology of *B. armata*, *B. binodosa*, and *B. citrina*. SEM images of radula segments viewed from above. A: *B. armata* (Thailand, Kaek River; ZMB 200.252); B: *B. armata* (Thailand, Kaek River; ZMB 200.254); C: *B. binodosa* (Thailand, Kaek River; ZMB 200.192); D: *B. citrina* (Thailand, Pa Charoen; ZMB 200.207). Scale bars = 100 µm.

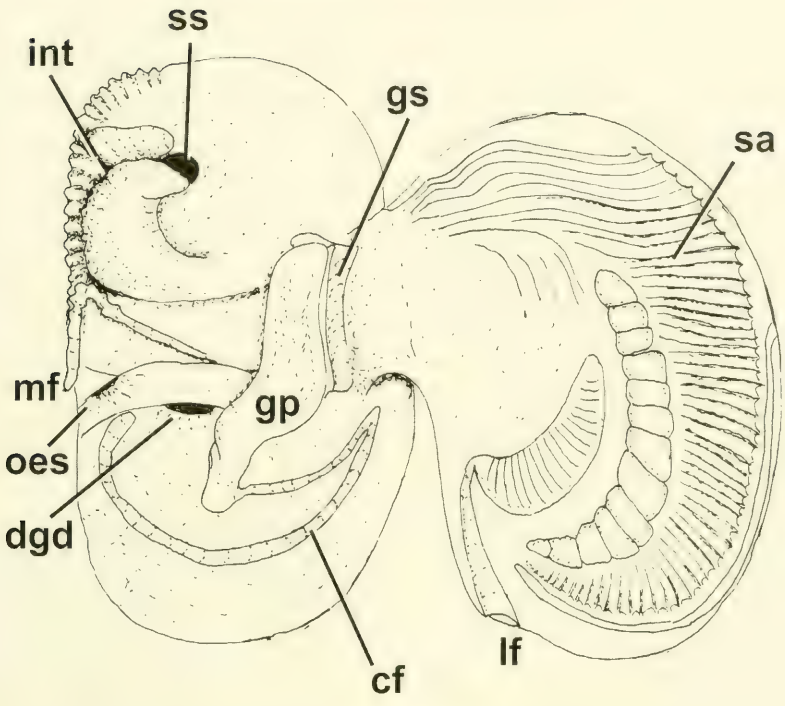

FIG. 13. Stomach anatomy of *B. binodosa* (ZMB 200.269; Thailand, Kaek River).

(ZMB 200.267); Poi Falls (ZMB 200.269; SMF 205137); Sopha Falls (ZSM 19983214, 6, 8; RMNH 55288/6; SMF 193575, 220339; AMS 146761); Thung Salaeng Luang NP (ZMB 200.192; ZSM 19983217; SMF 193578; BMNH; AMS 146760); Tap Tami Falls (ZSM 19983215; SMF 193576; ZHM).

Differential Diganosis

Shell elongately turreted, sculptured by two spiral rows of pointed nodules or tiny spines, each supported by a spiral cord.

Description

Shell (Fig. 10): Medium sized, spire elongately turreted with three to four whorls, eroded tip. Whorls convex with subsutural depression, separated by narrow, inconspicuous suture. Sculptured by more or less developed spiral ridges, most prominent at the base, and two spiral rows of pointed nodules or tiny spines, each supported by a spiral cord. Shell thin but solid; colour brown to red-brown, glossy surface. Basal whorl relatively large. Aperture oval, angled, produced below, inside white. Shell size: H = 25–35 mm. B = 14–18 mm.

Embryonic Shell (Fig. 11): Conical, comprising up to $3^1/_2$ whorls. Sculpture smooth, faint growth lines. Spiral keel at about the centre of the whorl from third whorl on. In some specimens, this keel supports two spiral rows of smooth knobs.

Operculum: Oval, with up to five whorls gradually increasing in diameter; nearly central nucleus.

*Radula (*Fig. 12C): Length of ribbon: m = 20 mm (sd = 1; n = 3), up to 190 rows of teeth. Very similar to *B. armata*, rachis tends to be more squarish in size.

Stomach (Fig. 13): Typical, as in *B. citrina* (Fig. 4).

Reproductive System

Three dried shells contained between 131 and 145 shelled juveniles varying in height between 1 and 3 mm, respectively (ZSM 19983217).

Distribution

Thailand: Prov. Phitsanulok: Only known from Kaek River and adjacent Kwae Noi River.

Remarks

Very similar to *B. spinata* (Godwin-Austen, 1872). *B. binodosa* is more slender, columella more curved (Blanford, 1903). Shell of *B. armata* is more conical possessing fewer whorls. Discriminant analysis of shell parameters: Figure 9, Table 1.

Brotia citrina (Brot, 1868)
(Figs. 4, 12D, 14, 15)

Melania citrina Brot, 1868: 11, 12, pl. 3, fig. 13 ("Siam" = Thailand), lectotype and three paralectotypes MHNG, coll. Brot (designated by Köhler and Glaubrecht, 2002a) (Figs. 14A–C); types seen; Brot, 1875: 106, 107, pl. 13, fig. 5.

Melania citrinoides Brot, 1886: 101, 102, pl. 5, fig. 4 ("Siam" = Thailand), lectotype and four paralectotypes MHNG, coll. Brot (designated by Köhler & Glaubrecht, 2002a) (Figs. 14D–H).

Brotia citrina – Köhler & Glaubrecht, 2002a: 131, fig. 1I (non Brandt, 1974).

Taxonomy and Systematics

Brandt (1974) based his diagnosis on material that we re-determined as *B. dautzenbergiana.* Material of *B. citrina* was apparently not available to him, except for the types. Consequently, Brandt's (1974) description of *B. citrina* and his conclusions in respect to its systematics are refuted and attributed to *B. dautzenbergiana*, which is considered distinct (see below). Types of *M. citrina* and *M. citrinoides* do only differ in average shell height but not in respect to other morphological or morphometrical characteristics. This feature is not considered sufficient to indicate separate

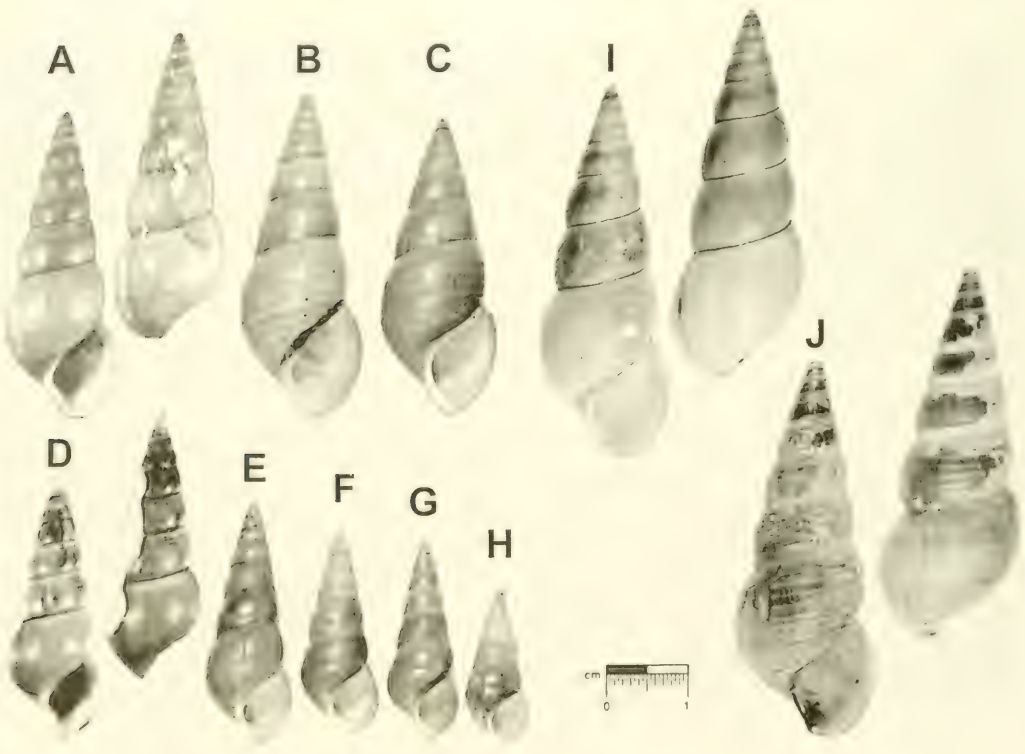

FIG. 14. Shell morphology of *B. citrina*. A: Lectotype of *M. citrina* MHNG, front and rear; B–C: Paralectotypes MHNG; D: Lectotype of *M. citrinoides* MHNG, front and rear; E–H: Paralectotypes MHNG; I: Thailand (ZMB 26.874); J: Thailand, Pa Charoen (ZMB 200.207). Scale bar = 10 mm.

status. For this reason, we agree with Brandt (1974) treating both taxa as synonyms.

Material Examined

Thailand: Prov. Kamphaeng Phet: Pa Charoen waterfall, S of Mae Sot, 16°30.51'N, 98°44.89'E (ZMB 200.207); Nang Khruan waterfall near Mae Sot, 16°24.59'N, 98°39.27'E (ZMB 200.212).

Differential Diganosis

Highly turreted shell, thin but solid, smooth except for growth lines and fine, closely spaced spiral lirae; aperture wide, produced below; colour yellowish to olive-brown. Rachidian cusp relatively broad, upper rim well rounded.

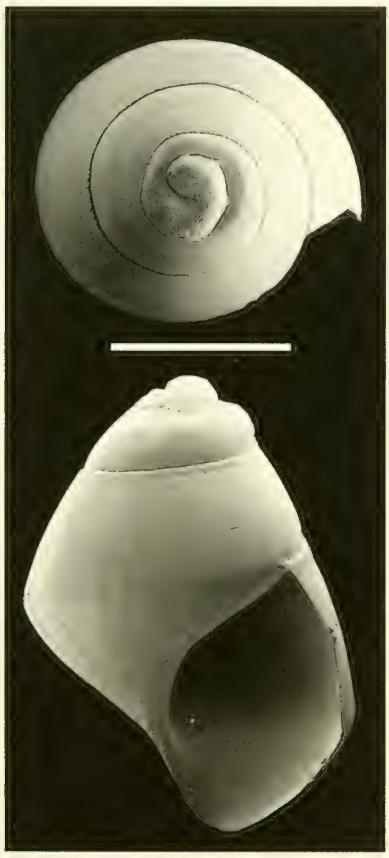

FIG. 15. Embryonic shell morphology of *B. citrina*. SEM images of embryonic shell removed from brood pouch (Thailand, Pa Charoen; ZMB 200.207); apical and front view. Scale bar = 1 mm.

Description

Shell (Fig. 14): Elongately turreted, thin but solid, six to ten convex and regularly rounded whorls; narrow suture. Sculpture of regularly spaced spiral ridges becoming more prominent at the base, fine axial growth lines; some shells completely smooth. Colour yellowish to light olive brown, glossy surface. Aperture wide, oval, angled, produced below, pointed above, sharp to thin margin. Shell size: H = 21–63 mm, B = 9–22 mm.

Embryonic Shell (Fig. 15): Smooth with faint growth and spiral lines; conspicuous subsutural depression; colour light greenish brown with broad chestnut brown spiral band.

Operculum: Round, up to eight regular whorls, almost central nucleus.

Radula (Fig. 12D): Central tooth relatively broad, basal margin well rounded. Central cusp flanked by three smaller denticles on each side. Inner marginals with two cusps, the outer one being broader. Outer marginals with mostly two, sometimes three cusps, outer one being broader.

External Anatomy: Animal dark grey with light grey patches. Columellar muscle well developed, relatively short and broad.

Stomach (Fig. 4): Inner septate crescent pad of the sorting area weakly developed, outer one well developed, laminated part of sorting area with fine, densely arranged laminae; typhlosoles fused at $^{4}/_{5}$ of style sac length.

Reproductive System

Females contained between 18 and 56 juveniles that varied in height between 2.2 and 5.5 mm, up to 3.5 whorls (n = 3; ZMB 200.207). Large embryos lay anteriorly in the pouch.

Distribution (Fig. 36)

Thailand: Prov. Kamphaeng Phet: Two localities in vicinity of Mae Sot as only known records. Not recorded by Brandt (1974) otherwise giving an excellent overview of the gastropod fauna of Thailand.

Habitat

Relatively cold, fast flowing, clear streams, well oxygenated, on limestone substratum. Buried in sand or mud, under rotten leaves or sunken wood presumably feeding on detritus.

TABLE 2. Result of disriminant analysis of shell parameters of *B. citrina* and *B. dautzenbergiana*.

	Predicted group membership	
	B. citrina	*B. dautzenbergiana*
B. citrina	19 (95.0%)	1 (5.0%)
B. dautzenbergiana	2 (4.7%)	41 (95.3%)

Remarks

From *B. dautzenbergiana* to be distinguished by its more conical shell, uneroded spire, in average fewer whorls, and lack of dark brown spiral band; or by statistical analysis of shell parameters (Table 2, Fig. 16).

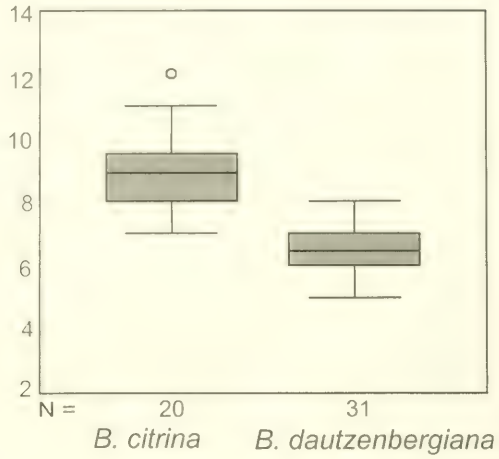

FIG. 16. Comparison of *B. citrina* and *B. dautzenbergiana* by means of number of whorls (N). Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

Brotia costula (Rafinesque, 1833)
(Figs. 17–19)

Melania costula Rafinesque, 1833: 166 ("Ganges"); types not traced.
Antimelania costula – Morrison, 1954: 15 [partim].
Brotia costula – Benthem Jutting, 1956: 374–378, fig. 76 [partim]; 1959: 92–95 [partim]; Brandt, 1974: 175, pl. 13, figs. 37–39 [partim]; Köhler & Glaubrecht, 2001: 295–299 [partim]; Köhler & Glaubrecht, 2002a: 132 [partim].
Brotia costula episcopalis – Subba Rao & Dey, 1986: 26 [partim].
Brotia (*Antimelania*) *costula* – Subba Rao, 1989: 108, 109 [partim].
Melania carolinae Griffith & Pidgeon, 1834: 598, pl. 13, fig. 3 ("India"), *ex* Gray ms, lectotype and paralectotype BMNH 1874.10.12.11 (designated by Köhler & Glaubrecht, 2002a) (Figs. 17B, C); types seen.
Melania plicata I. Lea, 1835: 20, pl. 23, fig. 95 (*non M. plicata* Menke, 1830) ("Bengal, Calcutta").
Melania variabilis Benson, 1836: 746, 747 (*non M. variabilis* Defrance, 1823) ("The river Gumti at Jonpur, and tolly's nullah near Calcutta" = Gomati River, Jaunpur, Uttar Pradesh, 25°44'N, 82°41'E), lectotype BMNH 1872.12.2.2 (designated by Köhler & Glaubrecht, 2002a) (Fig. 17A); types seen; Souleyet, 1852: 545; Reeve, 1860: species 204; Brot, 1870: 281 [partim]; Brot, 1875: 85–87, pl. 10, figs. 1, 1a–d [partim].
Melania (*Melanoides*) *variabilis* – Nevill, 1885: 251, 252 [partim].
Melanoides (*Tiara*) *variabilis* – Preston, 1915: 23, 24.
Acrostoma variabilis – Annandale, 1920: 110; Annandale et al., 1921: 560–562, pl. 6, figs. 3–6; Prashad, 1921: 485–488 [partim].
Brotia variabilis – Rensch, 1934: 239 [partim]; Bequaert, 1943: 433, 434, pl. 33, figs. 11–16; Solem, 1966: 15 [partim].
Brotia (*Antimelania*) *variabilis* – Adam & Leloup, 1938: 85, 86 [partim].
Melania varicosa Troschel, 1837: 174 ("Bengalien, Ganges" = Bengal, Ganges), lectotype ZMB 2.226a (here designated for the stabilisation of the name) (Fig. 17F) and 13 paralectotypes ZMB 2.226b; types seen; Philippi, 1844: 15, 16, pl. 3, fig. 2.
Melanoides varicosa – H. Adams & A. Adams, 1854: 297.
Melania indica Souleyet, 1842: pl. 31, figs. 12–15 ("India, Ganges"), five syntypes MNHN; types seen; Souleyet, 1852: 545.
Melanoides indica – H. Adams & A. Adams, 1853: pl. 31, figs. 5, 5a, b.
Melania menkiana [sic !] I. Lea, 1842: 242 (replacement name for *M. plicata* I. Lea, 1835, *non M. plicata* Menke, 1830; misspelled for intended "*M. menkeana*"); Brot, 1860: 280; Hanley & Theobald, 1874: 110.
Melania menkeana Brot, 1875: 91, 92, pl. 11, fig. 1, 1a, b (replacement name for *M. menkiana* Lea, 1842).
Melania (*Melanoides*) *variabilis menkeana* – Nevill, 1885: 260.

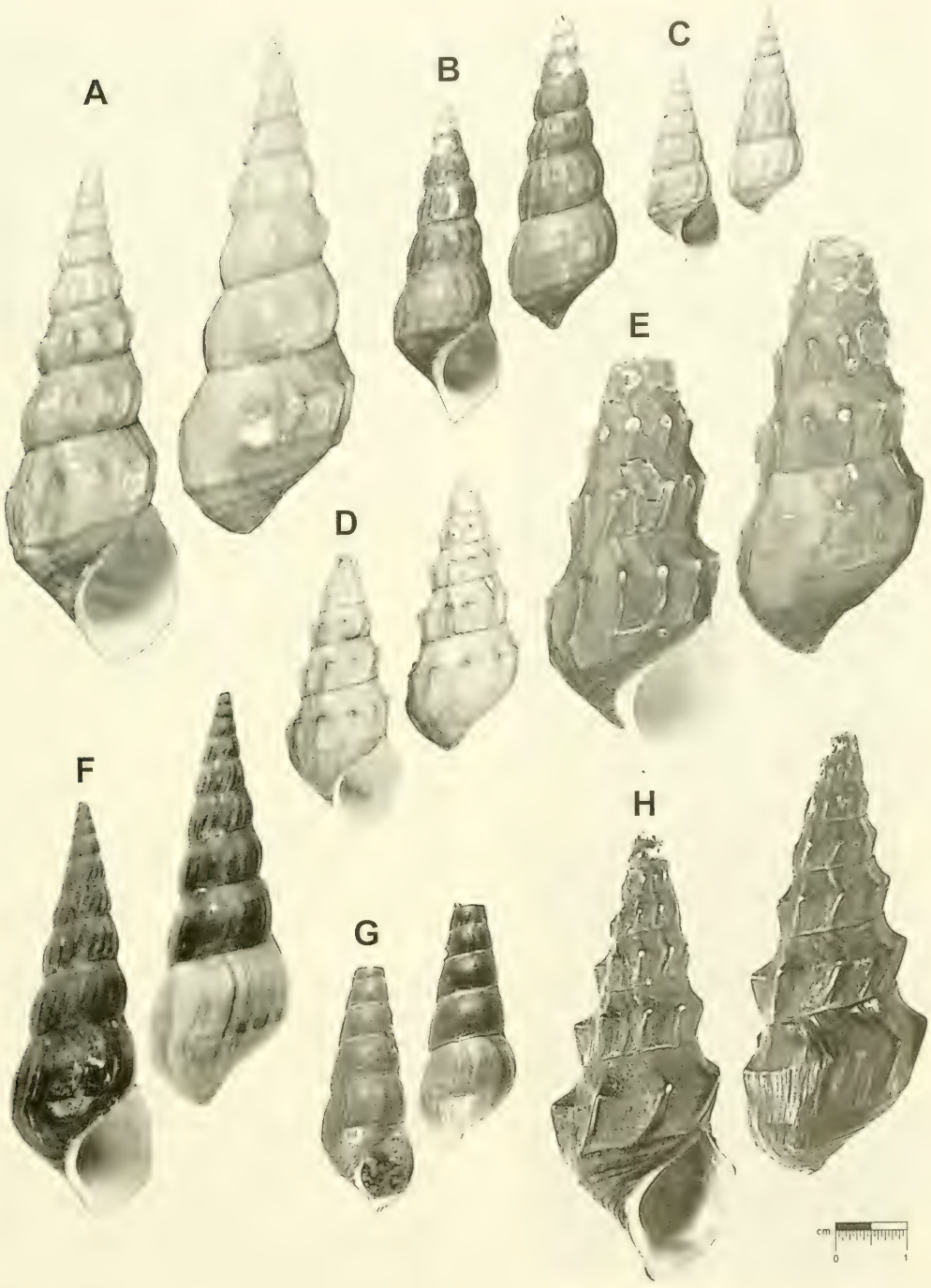

FIG. 17. Shell morphology of *B. costula*. A: Lectotype of *M. variabilis* BMNH 1872.12.2.2; B: Lectotype of *M. carolinae* BMNH 1874.10.12.11/A; C: Paralectotype BMNH 1874.10.12.11/B; D: Lectotype of *M. spinosa* BMNH 1907.10.28.79; E: Paralectotype BMNH 1907.10.28.80; F: Lectotype of *M. varicosa* (ZMB 2.226a); G: Syntype of *M. hainesiana* USNM 119741; H: Bangladesh, Chittagong (RMNH 76332). Scale bar = 10 mm.

Brotia menkeana – Yen, 1939: 59, pl. 5, fig. 13.
Melania spinosa Hanley, 1854: pl. 1, fig. 7 ("River Jumna, Sylhet, British India" = River Jamuna, Sylhet, Prov. Chittagong, Bangladesh, 24°53'N, 91°52'E) (*non M. spinosa* Gray, 1824), lectotype BMNH 1907.10.28.79 and paralectotype BMNH 1907.10.28.80 (Figs. 17D, E) (designated by Köhler & Glaubrecht, 2002a); types seen; Brot, 1875: 92, 93, pl. 12, fig. 2.

FIG. 18. Embryonic shell morphology of *B. costula*. SEM images of embryonic shell removed from dried material (ZMB 35.811); apical and front view. Scale bar = 1 mm.

Melania variabilis var. *spinosa* – Hanley & Theobald, 1873: pl. 75, fig. 6.
Melania hainesiana I. Lea, 1856: 144 ("India"), nine syntypes USNM 119741 (Fig. 17G); types seen; I. Lea, 1864: 78, pl. 22, fig. 18; Brot, 1875: 109, 110, pl. 14, fig. 4.
Melania (*Melanoides*) *variabilis* var. *hainesiana* – Nevill, 1885: 255.
Melania corrugata Reeve, 1859: pl. 3, fig. 10 ("India, Java") (*non M. corrugata* Lamarck, 1822).
Melania spinata – Brot, 1875: 89, 90, pl. 10, fig. 2a (*non M. spinata* Godwin-Austen, 1872).
Melania episcopalis – Hanley & Theobald, 1873: 31, 32, pl. 72, fig. 7, pl. 75, figs. 5, 7 (*non M. episcopalis* H. Lea & I. Lea, 1850).
Melania (*Melanoides*) *variabilis episcopalis* – Nevill, 1885: 256 [partim].
Melania (*Melanoides*) *variabilis* subvar. *aspera* Hanley & Theobald, 1874: pl. 109, fig. 6; Nevill, 1885: 252.
Melania (*Melanoides*) *variabilis* subvar. *cincta* Hanley & Theobald, 1874: pl. 109, fig. 5; Nevill, 1885: 252.
Melania (*Melanoides*) *variabilis* subvar. *microstoma* Nevill, 1885: 261 ("Sylhet").
Melania (*Melanoides*) *variabilis* var. *pseudospinosa* Nevill, 1885: 258.
Melania (*Melanoides*) *variabilis* var. *semilaevigata* Nevill, 1885: 254 ("Cachar and Sylhet").
Melania (*Melanoides*) *variabilis* subvar. *subtuberculata* Nevill, 1885: 252 ("Calcutta").
Melania (*Melanoides*) *variabilis* subvar. *subspinosa* Nevill, 1885: 252 ("Calcutta").

Taxonomy and Systematics

This species was delineated in various ways by previous authors, the plethora of synonyms witnessing serious difficulties in species recognition especially by 19th century authors. Presupposing that *B. costula* is highly variable, 20th century authors frequently subsumed similar taxa from across Southeast Asia under this name (e.g., Rensch, 1934; Benthem Jutting, 1956; Brandt, 1974; Köhler & Glaubrecht, 2001). Brandt (1974) hypothesised that *B. costula* forms a "rassenkreis" of three geographical subspecies: (1) the nominate form ranging from NE India to Indochina, (2) *B. c. varicosa* (Troschel, 1837), with suggested occurrence on Sumatra, Java and Borneo, and (3) *B. c. peninsularis* Brandt, 1974, restricted to the Malay Peninsula. This suggestion was refuted using comparative morphological and

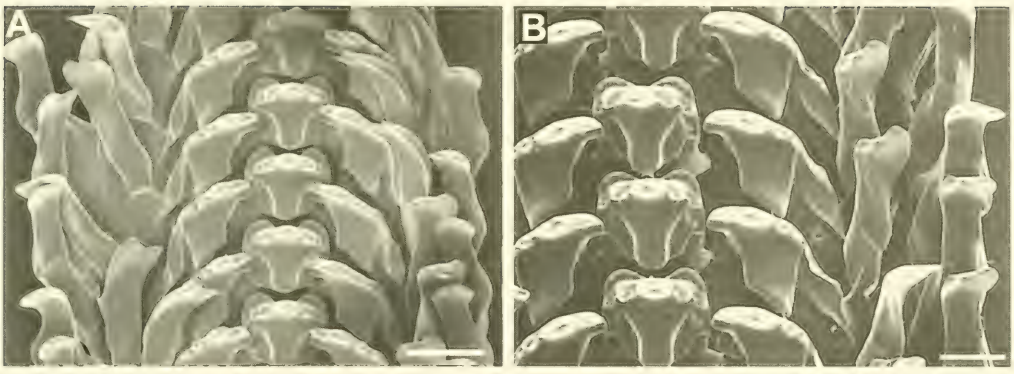

FIG. 19. Radula morphology of *B. costula*. Radula segments viewed from above. A: India, Sikkim (ZMB 2.227); B: India, Manipur (BMNH). Scale bars = 100 µm.

molecular genetic data (Köhler & Glaubrecht, 2001). Köhler & Glaubrecht (2001) demonstrate that taxa from Borneo and Java, which were assumed to constitute the *varicosa* subspecies, among other features possess a different embryonic shell morphology and, thus, are clearly distinct from *B. costula*. In addition, the molecular phylogeny shows that taxa from Sumatra, such as *B. torquata*, Malay Peninsula, such as *B. episcopalis* and *B. peninsularis*, and Myanmar, such as *B. herculea*, are also distinct (Figs. 78, 79). *Brotia costula* is encompassed here in a much more restricted way by means both of its distribution and its morphology. Accordingly, under *B. costula* we subsume only those taxa described from northern India, especially from the Ganges plain and Bengal, that exhibit corresponding shells, opercula and radular patterns (if available). Forms possessing spiny axial ribs, such as *M. menkiana*, are tentatively considered conspecific unless data on soft body morphology or molecular genetics may show otherwise. Here we follow Benson (1936: 747) who stated for *M. variablis* that "... several of these varieties [i.e., with or without spiny nodules] would, if viewed apart, be easily mistaken for distinct species, but they melt into each other so gradually, occasionally showing characters of more than one variety combined in the same shell, that no doubt remains of their blending in one species".

Material Examined

India (ZMB 200.044, 200.061, 200.064; CAS 6199): Ganges (ZMB 200.058, 200.062); Sikkim (ZMB 2.227, 200.078); Assam (ZMB 200.042, 200.052; BMNH 1935.10.9.5-17, 1888.12.4.1492-3), Guwahati (ZMZ 522377); Brahmaputra (ZMB 200.302-3); Durang (BMNH); Himalayas (BMNH 1841.7.23.9); Meghalaya: Jaintia-Khâsi hills (BMNH); Manipur (BMNH); Keladyne River (BMNH 1899.12.4.1761-2); Kolkata (ZMB 20.738; 200.063; BMNH; CAS 25326); Bengal (ZMB 45.849; BMNH 1888.12.4.1480-2; ZMZ 522371); Bengal, River Toolsi Ganga (BMNH); Bengal, River Atrai (BMNH); Settlepore (ZMZ 522372); Madhya Pradesh: Jonapura (ZMZ 522370); Bhutan: Duars, West Bhutan (BMNH); Bangladesh: Chittagong (BMNH; RMNH 71332; ZMB 35.811); Rajshahi: Basudebpur (BMNH); Malaudi (BMNH); River Jamuna (BMNH 1907.12.30.207); Sylhet (ZMB 200.071); Nepal: Prov. Narayani, Chitwan Distr., Bis Hajaar Lakes, 27°36.44'N, 84°26.34'E (ZMB 112.783), Prov. Koshi, Sunsari Distr., Haripur, tributary of the Sapta Koshi, 26°33.28'N, 86°59.6'E (ZMB 112.660).

Differential Diganosis

Shell highly turreted, large, up to 12 whorls, sculptured by regularly spaced axial ribs throughout, only exceptionally these ribs may lack completely; in some specimens, ribs support a spiral row of spiny nodules.

Description

Shell (Fig. 17): Medium sized to large, solid but not very thick, 6 to 12 whorls, pyramidal spire, frequently eroded tip; colour uniform light to olive-brown; whorls well rounded in diameter, separated by well-defined, thin

suture; sculpture of basal spiral ridges and regularly spaced axial ribs that occasionally support small, spiny nodules arranged in a spiral band at centre of whorl; some specimens smooth; aperture wide, well rounded at base, comprising about $^1/_5$ of shell height. Size: H = 20–87 mm, B = 8–36 mm.

Embryonic Shell (Fig. 18): Smooth except for fine growth lines. Maximum height 4 mm, 3.5 whorls. Average proportions: H = 2.3 mm, B = 1.1 mm, HA = 0.27 mm, BA = 0.48 mm, DA = 0.63 mm (for n = 6).

Operculum: Slightly oval, four to six whorls, central nucleus; almost fits aperture.

External Morphology: Uniformly coloured, dark grey to black; grey foot sole with scattered light spots.

Radula (Fig. 19): Ribbon length of up to 30 mm, corresponding to about half of the shell height, about 180 rows of teeth. Rachidian with single main cusp, three smaller denticles on each side tapering in size; upper margin concave by inflated, rounded corners; lower rim rounded; glabella narrow, well rounded at its base, lateral margins slightly concave. Laterals with main cusp flanked by three smaller denticles. Inner and outer marginals with two to three denticles, somewhat pointed, of about same size and shape.

Distribution (Fig. 20)

Northeast India (Bihar, Uttar Pradesh, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Sikkim, Assam, West-Bengal), Bangladesh, Bhutan, and Nepal. Namely, Ganges-Meghna-Brahmaputra River system with affluent rivers.

Habitats

Clear creeks with sandy bottoms, large rivers, and even ponds (Subba Rao, 1989).

Remarks

Reports from Sri Lanka (Annandale, 1920), Hainan and China (Yen, 1939), Sumatra and Java (Rensch, 1934; Benthem Jutting, 1956), Thailand, the Mekong, Borneo (Brandt, 1974), Melanesia (Abbott, 1948), and the Philippines (Bandel & Riedel, 1998) refer to other species.

Conchologically similar are *B. episcopalis* from the Malay Peninsula, *B. sumatrensis* from Sumatra, *B. herculea* from Myanmar, and *B. jullieni* from Cambodia; all were repeatedly synonymized with *B. costula*. *Brotia episcopalis* and *B. sumatrensis* tend to be smaller and more conical in shape. In *B. episcopalis*, the upper whorls are smooth and

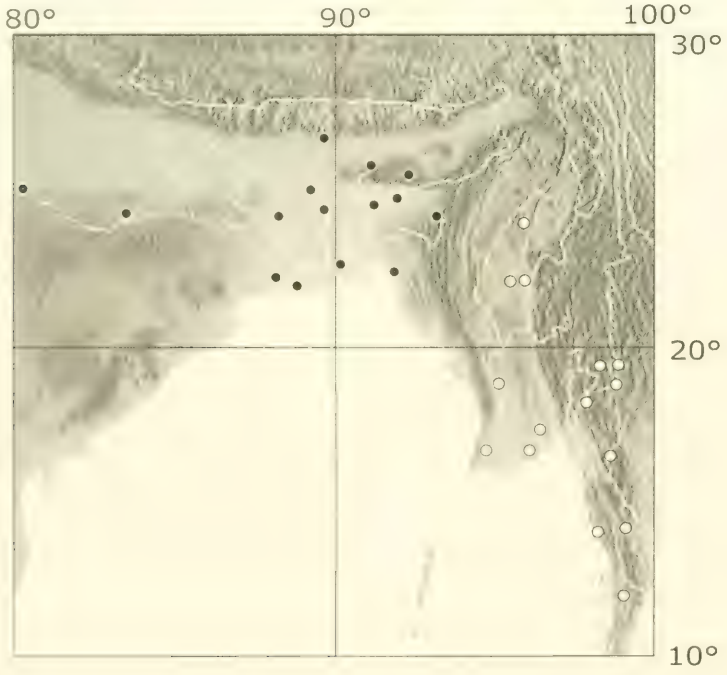

FIG. 20. Distribution of *B. costula* (closed circles) and *B. herculea* (open circles).

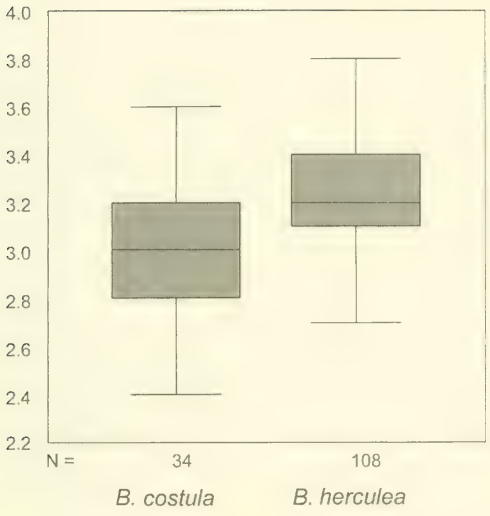

FIG. 21. Comparison of *B. costula* and *B. herculea* by means of shell parameter H/LA. Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

axial ribs are more conspicuous and not as regularly spaced as in *B. costula*, in which closely spaced axial ribs are always present. *B. jullieni* exhibits a larger, broader, and more conical shell, with a more pronounced spiral sculpture (e.g., Figs. 21, 27, 41 for comparison of shell parameters).

Adamietta species formerly assigned to *B. costula*, such as *A. infracostata* (Mousson, 1849), differ in embryonic shell morphology (Köhler & Glaubrecht, 2001, for the "*Brotia testudinaria*-group").

Brotia dautzenbergiana (Morlet, 1884)
(Figs. 22–24)

Melania dautzenbergiana Morlet, 1884: 399, 400, pl. 8, fig. 1a–c ("Les ruisseaux se jetant dans le Prec-Thenot, sur sa rive droite dans les environs de Kompong Tull" = streams discharging into the Prec-Thenot on its right bank near Kompong Tull, Cambodia), lectotype and three paralectotypes MNHN (designated by Köhler & Glaubrecht, 2002a) (Fig. 22A); types seen; Fischer-Piette, 1950: 154.

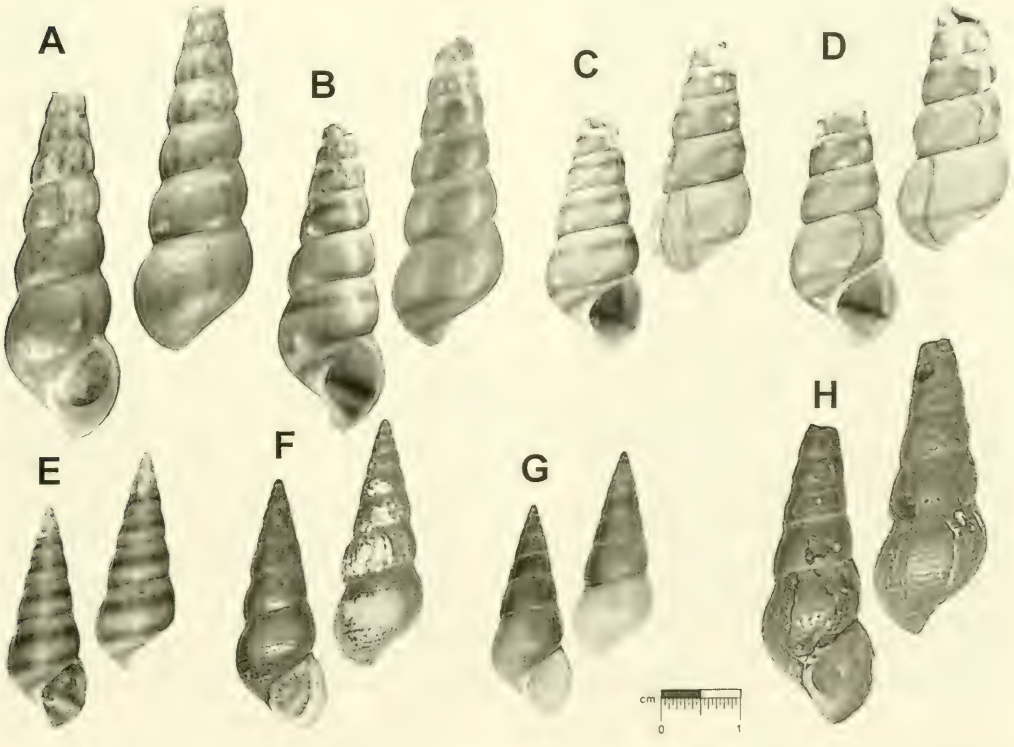

FIG. 22. Shell morphology of *B. dautzenbergiana*. A: Lectotype of *M. dautzenbergiana* MNHN; B: Lectotype of *M. dugasti* MNHN; C–D: Paralectotypes of *M. dugasti* BMNH; E: Myanmar (ZMB 49.626); F–G: Thailand, Lampang (ZMB 200.229); H: Thailand, Thoern (ZMB 200.213). Scale = 10 mm.

Stenomelania dautzenbergiana – Habe, 1964: 55, pl. 1, fig. 19.

Brotia dautzenbergiana – Köhler & Glaubrecht, 2002a: 133, fig. 1O.

Melania dugasti Morlet, 1893: 153, 154, pl. 6, fig. 1 ("Laos, Nam-Si, affluent du Nam Moun" = Laos. River Nam Si. affluent of the Nam Moun), lectotype MNHN, four paralectotypes MNHN, three paralectotypes BMNH 1893.12.8.117-119, three paralectotypes MHNG (designated by Köhler & Glaubrecht, 2002a) (Figs. 22B–D); types seen; Fischer-Piette. 1950: 160.

Brotia citrina – Brandt, 1974: 179, pl. 13, figs. 33, 34 (*non M. citrina* Brot, 1868).

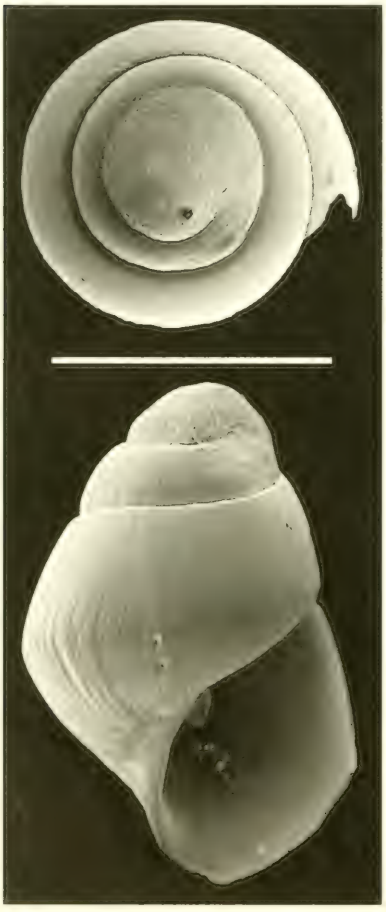

FIG. 23. Embryonic shell morphology of *B. dautzenbergiana*. SEM images of embryonic shell removed from brood pouch (Thailand, Lampang; ZMB 200.229); apical and front view. Scale bar = 1 mm.

Taxonomy and Systematics

Melania dugasti is considered as a junior synonym of *M. dautzenbergiana* for the most similar shell. Brandt (1974) assumed that both taxa are synonyms of *B. citrina*. However, the species can be distinguished by several morphological features. Treatment as distinct species is corroborated by molecular phylogenetic data (Figs. 78, 79).

Material Examined

Myanmar: North Shan, affluent to the Salween, Meungyaw (ZMB 46.626, 200.293), Nampai river, Lashio (ZMB 49.627); Mandalay (ZMB 200.264); Thailand: Prov. Chiang Mai, Lampang River in Lampang (ZMB 200.225-6), bridge 20 km from Lampang, highway to Uttaradit, 18°7.89'N, 99°97.33'E (ZMB 200.229), bridge at highway 106 near Thoern, 17°39.31'N, 98°7.91'E (ZMB 200.213); Prov. Kamphaeng Phet, Huai Hin Fon near Mae Sot (ZMH); Prov. Nan, Thung Thing (RMNH 71320); Huai Mae Lau (ZMH).

Differential Diganosis

Shell highly elongated, thin, slender; whorls well rounded; suture narrow, deeply incised. Sculpture smooth, only with growth lines and spiral lines, surface not glossy. Light brown, with dark brown patches or spiral band.

Description

Shell (Fig. 22): Medium sized, solid but not thick; elongately turreted, cylindrical, mostly truncated with five to ten remaining regular, convex whorls. Suture narrow, accompanied by subsutural depression. Upper whorls smooth except for growth lines, last whorls sculptured by numerous fine spiral lines forming regular pattern with crossing growth lines. Surface not glossy; colour of periderm yellowish to brownish green or olive, often with broad, dark brown spiral band, occasionally with dark axial flames at upper whorls. Shells often grey or black due to layer of mineral deposits. Aperture ovate with protracted base. Size: H = 23–44 mm, B = 10–16 mm.

Embryonic Shell (Fig. 23): Conical, smooth with faint growth lines; up to 2.5 mm high, 2.0–2.5 whorls; average proportions: H = 1.8 mm, B = 1.1 mm, HA = 0.21, BA = 0.41, DA = 0.66 (for n = 15).

Operculum: Slightly ovate, up to five fast in diameter increasing whorls, nucleus slightly eccentric.

Radula (Fig. 24): Upper rim of rachidian slightly concave, lateral corners not excavated, lower rim rather straight, slightly convex; main cusp flanked by two or three smaller denticles on each side, glabella well rounded at the base, v-shaped, its lateral margins slightly concave. Lateral teeth with main cusp, flanked by two accessory cusps on each side tapering in size. Inner and outer marginal teeth with two cusps, outer cusp broad, rounded; inner cusp pointed, considerably smaller. Outer marginals with conspicuous hooked outer flange.

Stomach: Corresponds to *B. citrina* (Fig. 4).

Reproductive System

Females (n = 9) contained 11 to 275 juveniles, height 1.0 to 2.5 mm.

Distribution (Fig. 36)

Myanmar, central, northern to eastern Thailand, Laos, Cambodia, Vietnam. Widespread and fairly common in most parts of the Indochinese Peninsula. Few more precise localities available, though. River systems of the Salween, and the Chao Praya, as well as some affluents of the Mekong, but not Mekong itself.

Remarks

Similar to *B. citrina* from which *B. dautzenbergiana* is distinguished by its more elongated shell, eroded tip, dark brown spiral band. Both species can be discriminated by shell parameters, although not statistically significant (Table 2).

Brotia episcopalis (H. Lea & I. Lea, 1851)
(Figs. 25, 26, 67A)

Melania episcopalis H. Lea & I. Lea, 1851: 184 ("sluggish river, Malakka" = Melaka, Prov. Negeri Melaka; 2°12'N, 102°15'E), lectotype and paralectotype MCZ 221841 (designated by Köhler & Glaubrecht, 2002a) (Figs. 25A, B); types seen; Hanley, 1854: pl. 3, fig. 27; Brot, 1875: 97, 98, pl. 12, figs. 1, 1a.

Melanoides episcopalis – H. Adams & A. Adams, 1854: 297.

Melania (*Melanoides*) *episcopalis* – Chenu, 1859: 288, fig. 1952.

Melania (*Melanoides*) *variabilis episcopalis* – Nevill, 1885: 256 [partim].

Brotia costula episcopalis – Davis, 1971: 53–86.

Brotia episcopalis – Köhler & Glaubrecht, 2002a: 134, fig. 1P.

Melania heros Brot, 1875: 339, 400, pl. 34, fig. 8 (unknown locality), holotype MHNG (Fig. 25D); type seen.

Sermyla perakensis Morgan, 1885: 421, pl. 8, figs. 14a–f ("Perak"), lectotype and paralectotype MNHN (designated by Köhler & Glaubrecht, 2002a) (Fig. 25C); types seen.

Brotia costula – Brandt, 1974: 175, pl. 13, fig. 37–39 [partim]; Köhler & Glaubrecht, 2001: 296–299, figs. 1D, 10A–C, G, H [partim] (*non M. costula* Rafinesque, 1833).

Brotia (*Antimelania*) *costula* – Subba Rao, 1989: 108, 109 [partim] (*non M. costula* Rafinesque, 1833).

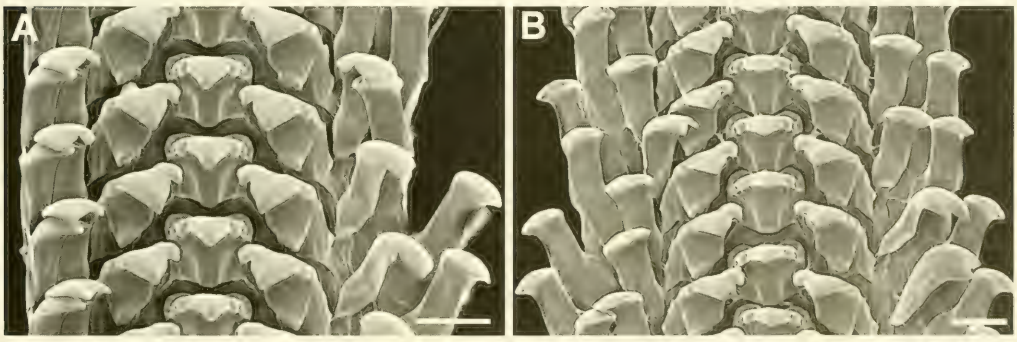

FIG. 24. Radular morphology of *B. dautzenbergiana*. SEM images of radula segments viewed from above. A: Thailand, Lampang (ZMB 200.229); B: Thailand, Thoern (ZMB 200.213).

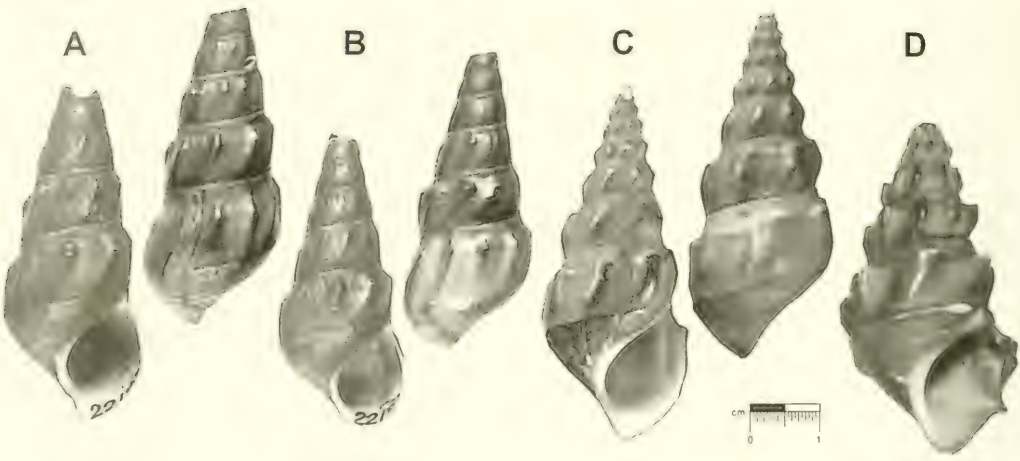

FIG. 25. Shell morphology of *B. episcopalis*. A: Lectotype of *M. episcopalis* MCZ 221841; B: Paralectotype MCZ 221841; C: Lectotype of *Sermyla perakensis* MNHN; D: Holotype of *Melania heros* MHNG. Scale bar = 10 mm.

Brotia variabilis – Bequaert, 1943: 433, 434, pl. 33, figs. 11–16 [partim]; Solem, 1966: 15 (*non M. variablis* Benson, 1836).

Taxonomy and Systematics

Frequently subsumed under *B. costula* by 20th century authors (Benthem Jutting, 1949, 1956; Brandt, 1968, 1974; Köhler & Glaubrecht, 2001). However, molecular genetic data shows that *B. episcopalis* is distinct (Figs. 78, 79). *Melania heros* and *Sermyla perakensis* are considered as synonyms.

Material Examined

Thailand: Prov. Trang: Trang (ZSM 19983228). Prov. Nakhon Si Thammarat: Khlong Nga, Chawang (ZMH); Malaysia: Prov. Kedah: Baling, River to east coast (ZMA). Prov. Pahang, Taman Negara National Park (ZMA; ZMB 200.041, 200.047); Sungei Kenong (ZMB 200.139); Sungei Mantine, affluent to Sungei Serau (ANSP A8907). Prov. Selangor: Sungei Buaya – NW Rawang (ZMA); Sungei Kelang, 17 mi. S Kuala Lumpur (ZMA); rapidly flowing river, 16 mi. N of Kuala Lumpur (CAS 30197). Prov. Negeri Perak: Tong Temple near Ipoh (ZMA; ZMB 200.046); Perak River (ZMB 200.054). Prov. Negeri Melaka: Melaka (ZMB 52.656, 200.047, 200.050, 200.306-7; MHNG).

Differential Diganosis

Shell large, solid; conic, up to 11 whorls, with strong axial ribs. Upper rim of the rachidian flanked by heavily excavated lateral corners.

Description

Shell (Fig. 25): Large, solid, pyramidal, frequently eroded, 6 to 11 convex, rounded whorls; strong axial ribs, and basal spiral ridges; colour light brown to olive-brown. Aperture wide, oval, well rounded below. Size: H = 34–57 mm, B = 15–24 mm.

Embryonic Shell: No own data, but described and depicted by Davis (1971: 60, figs. 2h, i, 11): up to 3.5, perhaps even 4.0 whorls, 2 mm height, rather smooth.

Operculum: Oval, multispiral, up to six whorls, sub-central nucleus.

Radula (Fig. 67A): Up to 180 rows of teeth, length up to 20 mm, corresponding to about half of shell height. Upper margin of rachidian conspicuously concave, formed by two inflated, well rounded corners. Glabella slightly v-shaped, well rounded at its base, concave lateral margins. Main cusp flanked by mostly two smaller denticles on each side, sometimes only one. Laterals with short lateral extensions, pronounced inner flange, two main cusps flanked by two smaller denticles. Inner and outer marginal teeth with

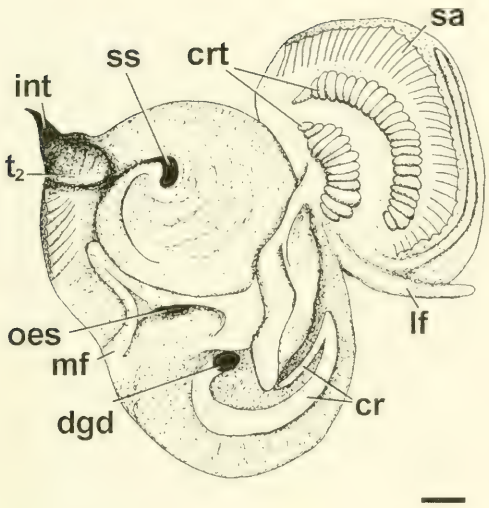

FIG. 26. Stomach anatomy of *B. episcopalis* (Thailand, Nakhon Si Thammarat; ZMH). Scale bar = 1 mm.

two pointed cusps of about same size and shape.

Stomach (Fig. 26): Typhlosoles fused at almost entire length of style sac; marginal fold narrowly angled posterior and underneath opening of intestine; flap-like posterior end of major typhlosole flat, partially covering opening of style sac.

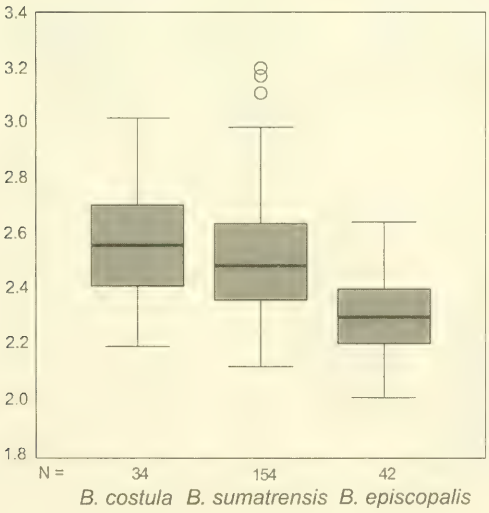

FIG. 27. Comparison of *B. costula*, *B. sumatrensis*, and *B. episcopalis* by means of shell parameter H/B. Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

Distribution (Fig. 68)

Southern Thailand and Malaysia: Malay Peninsula, S of Isthmus of Kra. Occurrence in Sumatra unclear.

Habitat

Streams and rivers, only rarely still waters (Davis, 1971; Kruatrachue et al., 1990). In great abundance in the Pahang River system in quiet, marginal waters together with *B. kelantanensis*, which lives among rocks in rapids (Davis, 1982: 392, referring to *B. costula* and "a second spiny species").

Remarks

Frequently confused with *B. costula* and *B. sumatrensis*. *Brotia costula* tends to have a more elongated shell with more closely spaced, regular ribs also on upper whorls. *Brotia sumatrensis* lacks marked transition from smooth upper whorls to strongly sculptured lower whorls and exhibits lesser pronounced axial ribs. Employing statistical analyses, *B. costula*, *B. episcopalis*, and *B. sumatrensis* cannot be discriminated by their morphometry (Fig. 27). A detailed description of morphology, reproductive biology, growth rates, and relevance as intermediate host of the lung fluke *Paragonimus westermanni* is given by Davis (1971). Our observations fit well to the comprehensive data reported in this paper.

Brotia godwini (Brot, 1875)
(Figs. 28, 31A)

Melania (*Melanoides*) *hanleyi* Godwin-Austen, 1872: 514, 515, pl. 30, fig. 2 (*non M. hanleyi* Brot, 1860) ("Diyung River, North Cachar hills" = Diyung River, Jaintia-Khâsi hills N of Silchar, Meghalaya, India, 24°48'N, 92°46'E), lectotype BMNH 19991561/A and paralectotype BMNH 19991561/B (designated by Köhler & Glaubrecht 2002a) (Figs. 28A, B); types seen.

Melania godwini Brot, 1875: 90, pl. 10, fig. 3 (replacement name for *M. hanleyi* Godwin-Austen, 1872).

Melania (*Melanoides*) *variabilis* var. *binodulifera* Nevill, 1885: 259 ("Khasi hills").

Brotia godwini – Köhler & Glaubrecht, 2002a: 136.

Taxonomy and Systematics

Melania godwini Brot, 1875, was employed as replacement name for *M. hanleyi* Godwin-Austen, 1872, being preoccupied by *M. hanleyi* Brot, 1860.

Material Examined

India: Assam, Lamin (ZMB 94.722); Cachar (ZMB 20.737).

Differential Diganosis

Stepped whorls, deeply incised suture, two spiral ridges, lower one at about a third of whorls diameter, upper one at about two thirds, more pronounced. Upper ridge supports spiral row of spiny tubercles; some specimens with axial ribs; aperture very wide.

Description

Shell (Fig. 28): Small to medium sized, conical to turreted, four to five convex, stepped whorls, eroded; colour chestnut brown. Spiral row of spiny tubercles and spiral lines, most prominent at base of shell; last whorl large, inflated; aperture wide, ovate, produced below, comprising up to $^1/_3$ of shell height.

Radula (Fig. 31A): Ribbon with 120 rows of teeth. Central tooth squarish, anterior rim slightly concave, very large main cusp flanked by two smaller denticles on each side, glabella v-shaped, basely rounded; lateral teeth with main cusp and one accessory denticle on each side; inner and outer marginals with large, broad, spatula-shaped outer cusp and much smaller, pointed inner denticle; inner marginals broader than outer ones.

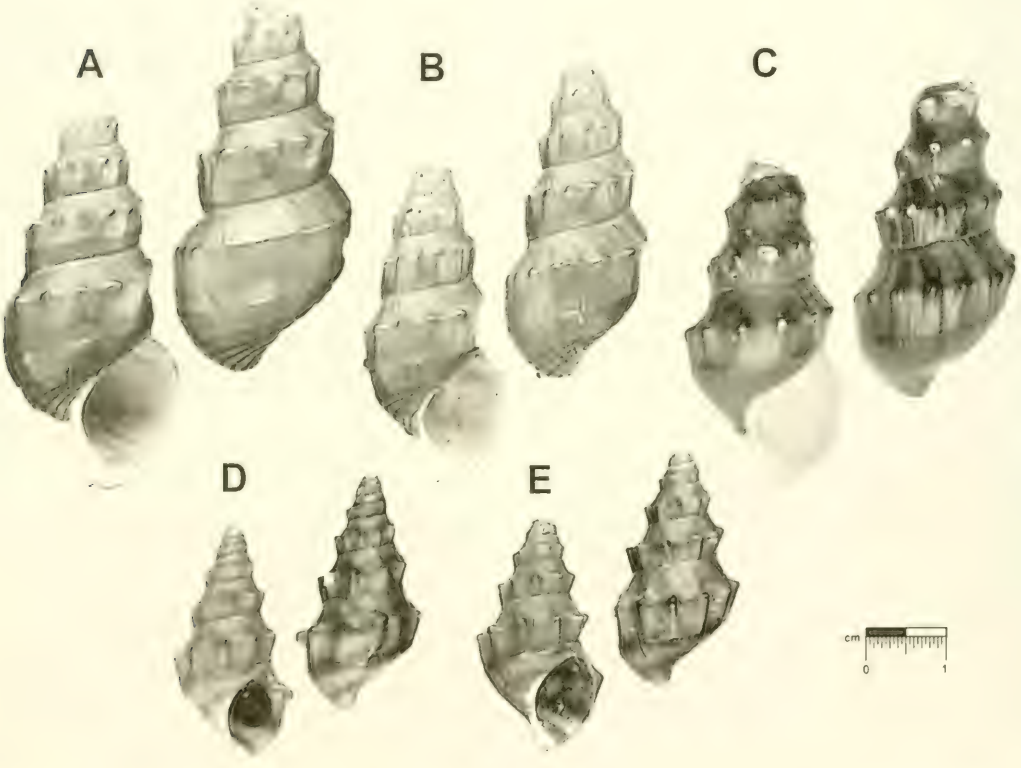

FIG. 28. Shell morphology of *B. godwini*. A: Lectotype of *M. hanleyi* Godwin-Austen BMNH 19991561/A; B: Paralectotype BMNH 19991561/B; C: Assam, Cachar (ZMB 20.737); D–E: Assam (ZMB 97.422). Scale bar = 10 mm.

Embryonic shell morphology, Soft body anatomy, Operculum: Unknown.

Distribution

India: Meghalaya, Assam, Manipur: tributaries of Brahmaputra (possibly also neighbouring regions of Myanmar and Bangladesh). Seemingly restricted to mountainous regions.

Remarks

Similar to spiny morphs of *B. costula*, but shell not as highly turreted, whorls much more stepped; radula differs in shape of glabella.

Brotia henriettae (Griffith & Pidgeon, 1834) (Figs. 29, 30, 31B, C)

Melania henriettae Griffith & Pidgeon, 1834: 598, pl. 13, fig. 2 ("China"), *ex* Gray ms; lectotype BMNH 19990495/A and paralectotype BMNH 19990495/B (designated by Köhler & Glaubrecht, 2002a) (Figs. 29A, B); types seen; Reeve, 1859: 1.
Semisulcospira henriettae – Yen, 1942: 204: pl. 15, fig. 66.
Brotia henriettae – Köhler & Glaubrecht, 2002a: 137, 138, fig. 2D.
Melania baccata Gould, 1847: 219 ("Thoungyin River, branch of the Salween, Burma"), Lectotype MCZ 169052 and paralectotype USNM 611239 (designated by Johnson, 1964) (Fig. 29E); types seen; Brot, 1875: 81, 82, pl. 9, fig. 6; Hanley & Theobald, 1873: 32, pl. 75, figs. 1, 2, 4; Annandale, 1918: 115, pl. 7, fig. 9; Johnson, 1964: 45.
Melania (*Melanoides*) *baccata* – Nevill, 1885: 262.
Melania (*Brotia*) *baccata* – Martens, 1899: 35, 36.
Melanoides (*Tiara*) *baccata* – Preston, 1915: 26.
Melania baccata subsp. *elongata* Annandale, 1918: 115, 116, pl. 7, figs. 3, 3a, 4–7 ("He-Ho Plain and Yawnghwe River" = He ho, N of Lake Inle, 20°44'N, 96°49'E), two syntypes ZSI 11155/2, according to Annandale (1918); types not seen.
Acrostoma elongatum – Annandale & Rao, 1925: 117.
Melania persculpta Ehrmann, 1922: 18–23, fig. 8 ("Loikaw-Fluß, Süd-Schan-Staaten" = Loikaw River, Southern Shan States, Myanmar), lectotype SMF 221813, 20 paralectotypes SMF 221814-5 (designated by Köhler & Glaubrecht, 2002a) (Fig. 29D); types seen.
Acrostoma baccata – Rao, 1928: 442–445, figs. 17, 18.
Brotia baccata – Bequaert, 1943: 431; Morrison, 1954: 384; Johnson, 1964: 45.
Brotia (*Brotia*) *baccata* – Brandt, 1974: 178, pl. 13, fig. 32.
Melania reticulata I. & H.C. Lea, 1851: 193 ("China"), holotype USNM 119663 (Fig. 29C); type seen.
Melanoides reticulata – H. Adams & A. Adams, 1854: 297.
Melania baccata var. *pyramidalis* Martens, 1899: 36.
Melania variabilis var. *pyramidalis* – Theobald, 1865: 274, fig. 7
Melania variabilis var. *glabra* Theobald, 1865: 273.
Melania variabilis var. *vittata* Theobald, 1865: 273, fig. 4; Nevill, 1885: 263.
Melania variabilis var. *turrita* Theobald, 1865: 273, 274, fig. 5.
Melania variabilis var. *baccifera* Theobald, 1865: 274, fig. 6.
Melania (*Melanoides*) *baccata* subvar. *recta* Nevill, 1885: 262 ("Upper Salween").
Melania (*Melanoides*) *subasperata* Nevill, 1885: 262 ("Shan States").
Melania (*Melanoides*) *subasperata* var. *sublaevigata* Nevill, 1885: 263 ("Shan States").

Taxonomy and Systematics

Noticing the confusing variety of different shell forms that also lead 19th century authors to introduce a plethora of names, Annandale (1918) wondered whether these forms should be regarded as representing one highly variable species or a flock of morphologically similar species. Indeed, the diversity of shell forms attributed to this species might be indicative for the existence of more then a single species. However, the question whether and, if so, how many different species are currently subsumed under the concept of *B. henriettae* cannot be answered satisfactorily since only dry shell material is available from Myanmar. Unless more detailed morphological and molecular genetic data will show otherwise, we follow Brot (1875) considering these forms as conspecific. It remains unclear, however, why Brot (1875) referred to *M. baccata* but not to the older name *M. henriettae*. This treatment was followed by later authors, rendering *M. baccata*

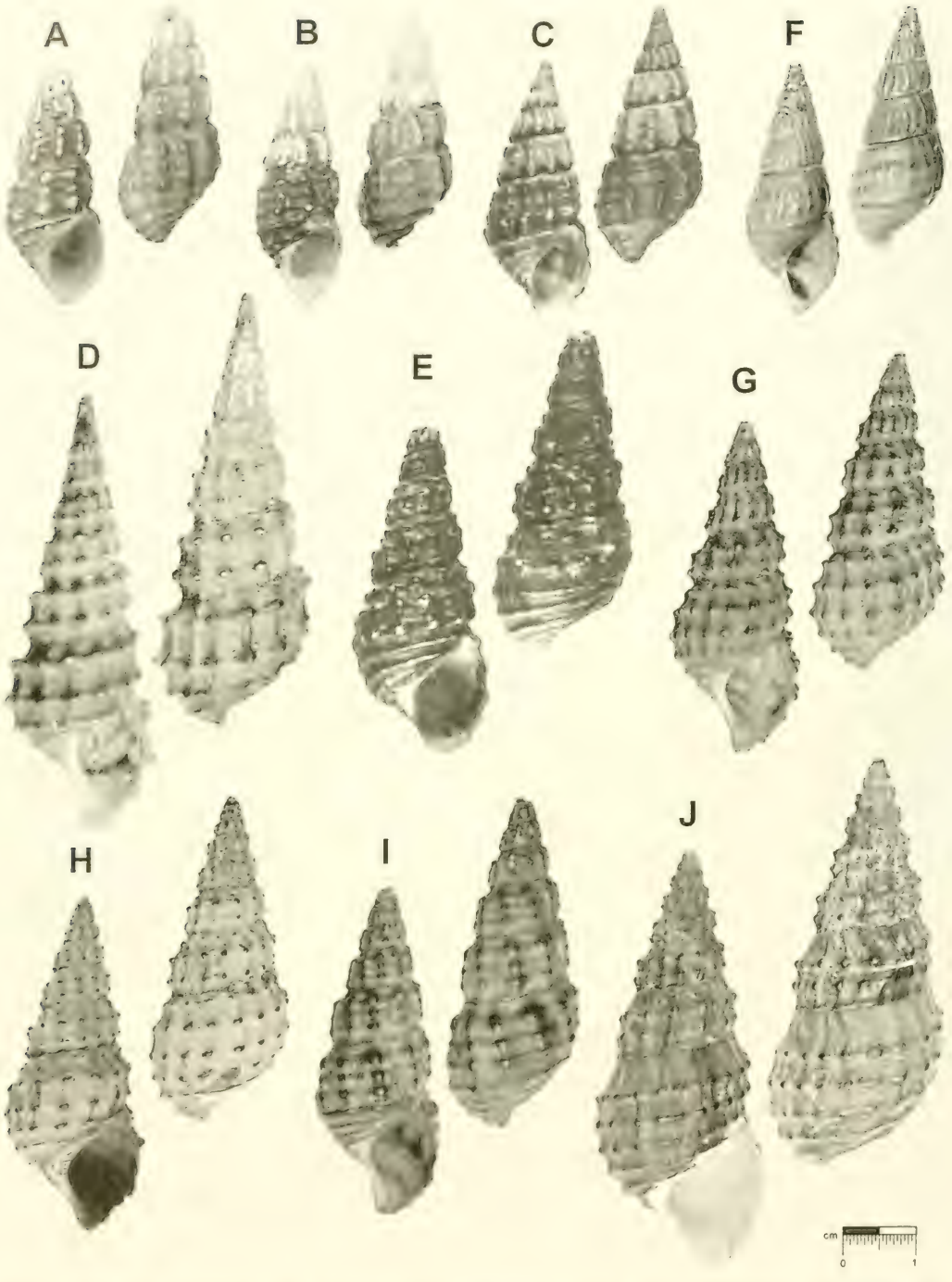

FIG. 29. Shell morphology of *B. henriettae*. A: Lectotype of *M. henriettae* BMNH 1999095/A; B: Paralectotype BMNH 1999095/B; C: Holotype of *M. reticulata* USNM 119663; D: Lectotype of *M. persculpta* SMF 221813; E: Lectotype of *M. baccata* MCZ 169052; F: Thailand, Pai (ZMB 200.221); G: Myanmar. Lashio River (ZMB 49.612); H: Myanmar (ZMB 200.006); I: China, Hienshow (ZMB 62.665); J: Myanmar, Myitnge (ZMB 49.613). Scale bar = 10 mm.

a name most commonly employed. Nonetheless, the name *M. henriettae* Griffith & Pidgeon, 1834, being available has priority over *M. baccata*.

Brotia henriettae is type species of *Wanga* Chen, 1943, by original designation. This genus is considered a junior synonym of *Brotia*.

Material Examined

China: Yunnan, Yaylayman (ZMB 27.511); Hienshow River (ZMB 52.665). Myanmar: Shan states (BMNH 1907.12.30.210; ZMB 200006-7); North Shan States: Lashio River at Myitnge (ZMB 49.616), Lashio River (ZMB 49.612, 200.076, BMNH 1899.6.21.72-5), Nampai River near Lashio (ZMB 49.611, BMNH 1899.6.21.90-91), tributary of the Nampai near Lashio (ZMB 49.615), small stream at Meungyaw (ZMB 49.618), Myitnge (ZMB 49.613, 200.004, 200.140), tributary of the Myitnge at Bagwyo near Thibaw (ZMB 49.614), small stream near Bangwyo (ZMB 200.002); ZMB 200.005); Chindwin, tributary of the Irawaddy at Matu (ZMB 49.620); affluent of the Salween near Lashio (ZMB 49.619, BMNH 1899.6.21.76-79); tributary of the Salween (ZMB 200.000), Gotheik cave (ZMB 200.001), Thoungyin River (BMNH 1888.12.4.1767-8); Thailand: Prov. Chiang Mai, Pai River in Pai, 19°21.57'N, 98°26.62'E (ZMB 200.221); Prov. Mae Hongsong, Som River at Ban Som (ZSM 19983220); Prov. Kamphaeng Phet, Moei River, 30 km S of Mae Sot, boarder to Myanmar, 16°26.96'N, 98°39.27'E (ZMB 200.210).

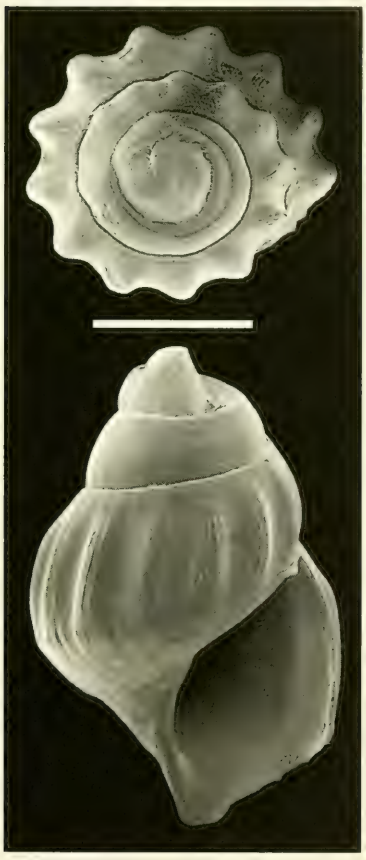

FIG. 30. Embryonic shell morphology of *B. henriettae*. SEM images of embryonic shell removed from dried shell (ZMB 49.613); apical and front view. Scale bar = 1 mm.

Diagnostic Characteristics

Pyramidal turreted, solid, flattened whorls, narrow suture; spiral lines support two or three spiral rows of closely spaced tubercles; in some specimens tubercles replaced by axial ribs; aperture well produced with sharp peristome; body whorl relatively large; operculum round with up to eight whorls, considerably smaller than aperture; embryonic shells with axial ribs from second whorl on.

Description

Shell (Fig. 29): Medium sized, solid; spire oval to cylindrical or highly turreted; six to eight flattened whorls, suture deeply incised; strong spiral cords support more or less distinct nodules. Two to three nodules frequently arranged in vertical rows, sometimes forming axial ribs. Aperture rather narrow, peristome thin, sharp. Colour light to chestnut brown. Size: H = 30–64 mm, B = 13–25 mm.

Embryonic Shell (Fig. 30): Conic to turreted, penultimate whorl with smooth sculpture, following whorls with strong axial ribs. Average proportions: H = 3.0 mm, B = 1.9 mm, HA = 0.24 mm, BA = 0.40 mm, DA = 0.90 mm (for n = 6) up to 3.5 whorls.

Operculum: Round, up to eight regular whorls, almost central nucleus; much smaller than aperture.

External Anatomy: Animal black with yellowish to light brown patches.

Radula (Figs. 31B, C): Up to 150 rows of teeth; radulae from different localities vary in breadth

and shape of main cusp. Generally, central tooth with concave upper rim, relatively broad central cusp flanked by two accessory denticles tapering in size, glabella with concave to angled lateral margins, basely well rounded. Lateral tooth with broad main denticle flanked by two inner and one or two outer accessory denticles. Inner and outer marginals with two cusps, outer one broad, spatula-shaped, inner one small, pointed. Inner marginals broader.

Stomach: Typical, as in *B. citrina* (Fig. 4); typhlosoles unfused.

Distribution (Fig. 36)

China (southern China), particularly Yunnan; Myanmar (Northern and Southern Shan states); Thailand (northern and western Thailand); river system of the Irawaddy and Salween.

Habitat

Clear mountain rivers and streams with strong current, attached to stones and rocks. In the Maenam Moei (= Thoungyin River) co-occurring with *B. pagodula* and *B. herculea*.

Fossil Record

In Tertiary and Pleistocene cave deposits of Myanmar (Bequaert, 1943); sub-fossil shells reported by Annandale (1918) from Myanmar.

Remarks

Similar sculpture in *B. iravadica*, frequently being smaller and more conical in shape, with fewer whorls.

Brotia herculea (Gould, 1846)
(Figs. 32–34)

Melania herculea Gould, 1846: 100 ("Tavoy River, British Burma" = Tavoy, Myanmar, 14°05'N, 98°12'E), lectotype MCZ 169436, two paralectotypes MCZ 87933, 17 paralectotypes MCZ 169437, two paralectotypes USNM 611234 (designated by Johnson, 1964) (Fig. 32A); types seen; Reeve, 1859:

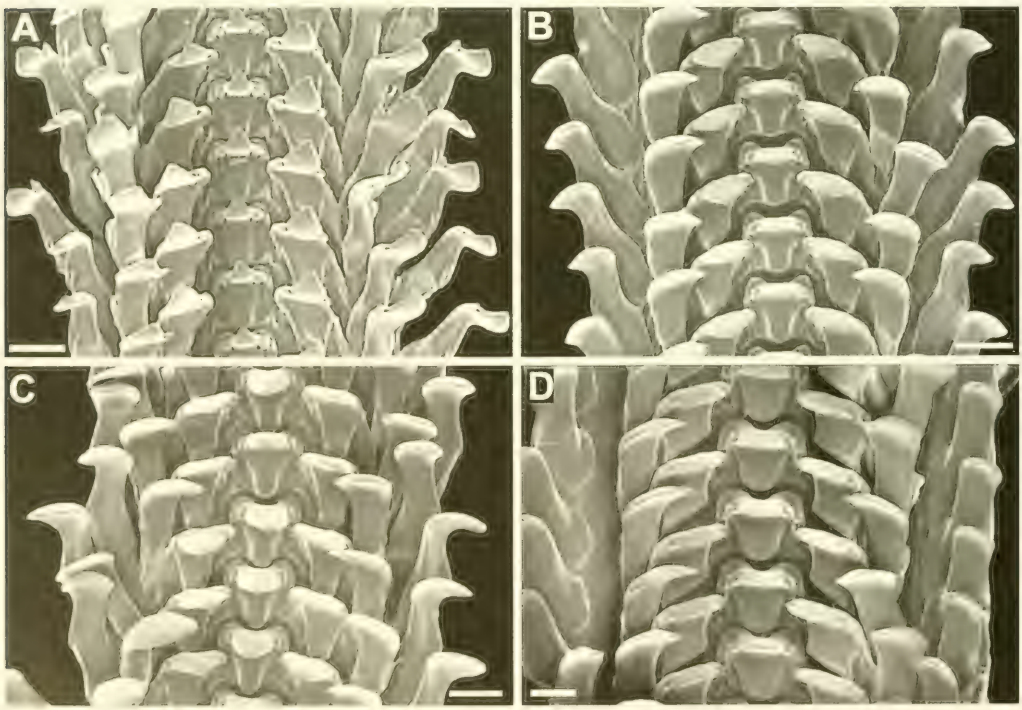

FIG. 31. Radular morphology of *B. godwini*, *B. henriettae*, and *B. jullieni*. A: *B. godwini* (Assam; ZMB 97 422); B. *B. henriettae* (Thailand, Pai; ZMB 200.221); C: *B. henriettae* (Thailand, Mae Sot; ZMB 200.210); D: *B. jullieni* (Cambodia; ZMH). Scale bars = 0.1 mm.

pl. 2, fig. 4; Hanley & Theobald, 1873: 31, pl. 72, fig. 5; Johnson, 1964: 87, pl. 35, fig. 10.
Melanoides herculea – H. Adams & A. Adams, 1854: 297.
Melania (Melanoides) herculea – Nevill, 1885: 251.
Melania balteata Reeve, 1860: pl. 20, species 144 (*non M. balteata* Philippi, 1858) (no locality given), lectotype ÜMB TK 304/1 and paralectotype ÜMB 308/1 (designated by Knipper, 1958, referring to *M. reevei)* (Figs. 32B, C); types seen.

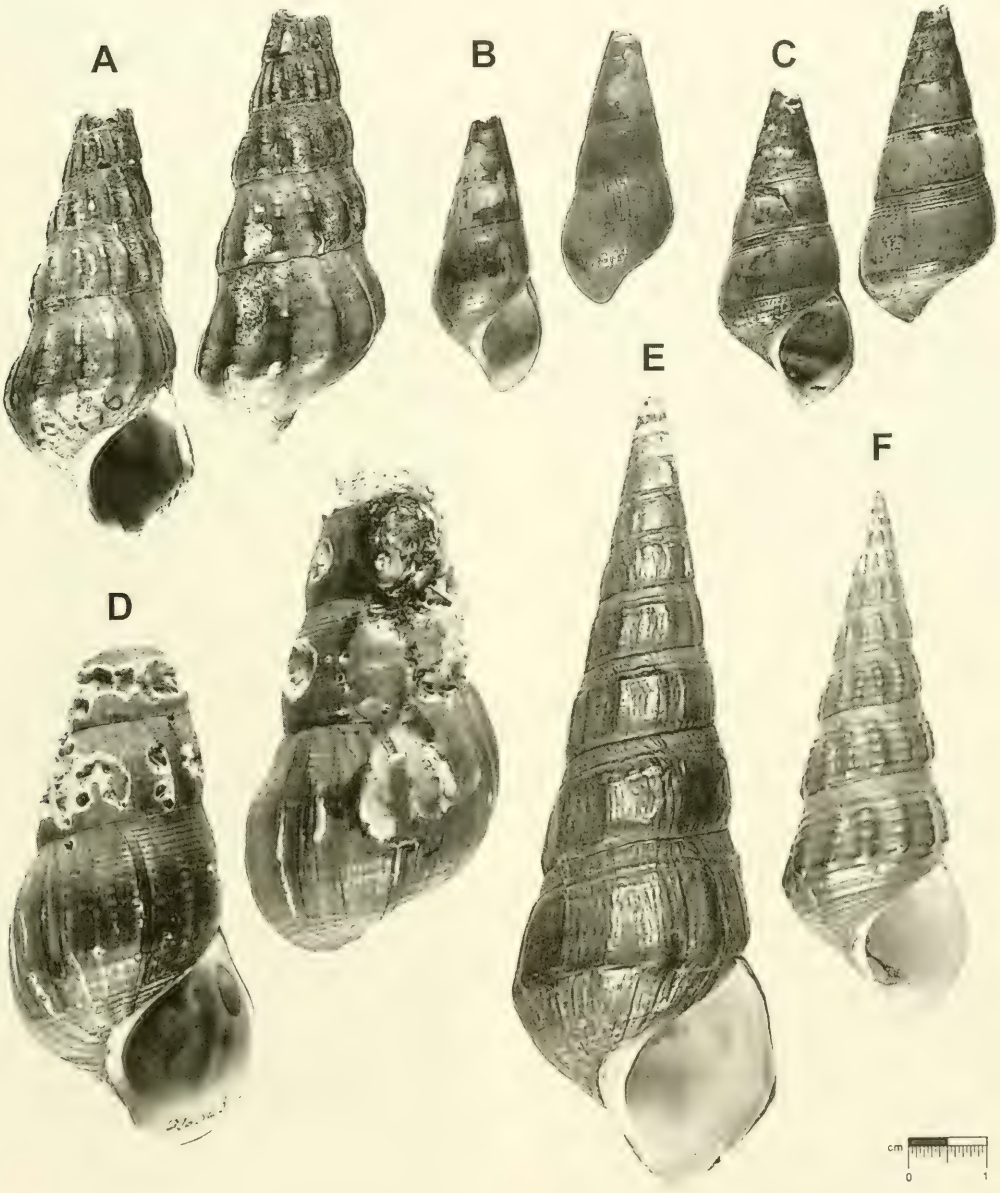

FIG. 32. Shell morphology of *B. herculea*. A: Lectotype of *M. herculea* MCZ 169436; B: Lectotype of *M. balteata* Reeve ÜMB TK 304/1; C: Paralectotype ÜMB TK 308/1; D: Lectotype of *M. gloriosa* ANSP 26363; E: Thailand, Raheng (MHNG); F: Thailand, Sai Yok (ZMB 200.235). Scale bar = 10 mm.

Melania reevei Brot, 1862: 46 (replacement name for *M. balteata* Reeve); Brot, 1875: 95, 96, pl. 11, figs. 4, 4a, pl. 13, fig. 6; Hanley & Theobald, 1876: 61, pl. 153, fig. 1.
Melania (Melanoides) reevei – Nevill, 1885: 248.
Melania (Melanoides) reevei var. *lanceolata* Nevill, 1885: 248, 249 ("Mandalay; Hezada, Pegu; Thyet Myo").
Melania (Melanoides) reevei var. *imbricata* Hanley & Theobald, 1876: pl. 153, fig. 4 (without locality); Nevill, 1885: 249.
Melania (Melanoides) reevei var. *soliduscula* Nevill, 1885: 249, 250 ("Pegu, Noung-ben-Ziek").
Melania (*Brotia* ?) *reevei* – Martens, 1899: 36.
Melania gloriosa Anthony, 1865: 207, pl. 18, fig. 3 ("Pegu" = Pegu, Myanmar), lectotype ANSP 26363, paralectotype MCZ 74106, three paralectotypes MCZ 74107, potential paralectotype MCZ 315666 (designated by Köhler & Glaubrecht, 2002a) (Fig. 32D); types seen; Brot, 1875: 94, 95, pl. 11, figs. 3, 3a, b; Hanley & Theobald, 1873: 31, pl. 72, figs. 1, 2; Baker, 1964: 190.
Melania (Melanoides) tourannensis var. *gloriosa* – Nevill, 1885: 250.
Melania variabilis – Brot, 1875: 85–87, pl. 10, fig. 1, 1a–d [partim].
Melania peguensis Hanley & Theobald, 1873: 31, pl. 72, fig. 6 [*nomen nudum*].
Melania (Melanoides) tourannensis var. *peguensis* – Nevill, 1885: 250.
Melania (Melanoides) tourannensis var. *compacta* Nevill, 1885: 250, 251 ("Henzada, Pegu").
Melania (Melanoides) tourannensis var. *beddomeana* Nevill, 1885: 251 ("near Moulmein").
Melania (*Melanoides*) *variabilis* subvar. *subvaricosa* Nevill, 1885: 252, 253 "Arakan, Pegu").
Melania (*Melanoides*) *variabilis* subvar. *semilaevigata* Nevill, 1885: 252.
Brotia costula – Benthem Jutting, 1956: 374–378, fig. 76 [partim]; 1959: 92–95 [partim]; Brandt, 1974: 175, pl. 13, figs. 37–39 [partim]; Köhler & Glaubrecht, 2001: 296–299, figs. 10D–F [partim]; Köhler & Glaubrecht, 2002a: 132 [partim].

Taxonomy and Systematics

Treated as synonym of *Brotia costula* by, for example, Benthem Jutting (1956, 1959) and Brandt (1974), this taxon is considered herein as a distinct species since *B. costula* and *B. herculea* occupy different positions in the phylogenetic trees (Figs. 78, 79). Together with its distinct shell morphology, this is reason enough to not treat *B. herculea* conspecific with the former.

A second taxon, *Melania reevei* Brot, has also frequently been considered a synonym of *B. costula* by 20th century authors. This name was employed as a replacement for *M. balteata* Reeve, being preoccupied by *M. balteata* Philippi. Most certainly identical with the latter are *Melania gloriosa* Anthony and *M. peguensis* Hanley & Theobald. The latter was introduced in error by Hanley & Theobald (1873), who intended to refer to Anthony's original figure but mixed up the legends of figures 2 and 3 of pl. 18 of Anthony's work. So, they employed the name "*M. peguensis*", which however refered to a bivalve species of *Monocondylaea*, instead of "*M. gloriosa*", which would have been the correct reference for the species of *Melania*.

Both *M. reevei* and *M. gloriosa* are tentatively subsumed under *B. herculea* for their somewhat similar shell and since both originate from the same area, Pegu. The type lots of *M. herculea* on one hand and the types of *M. reevei* and *M. gloriosa*, respectively, on the other hand mainly differ in the presence or absence of axial ribs. Examination of further series of dry shells from Pegu, though, reveals that the presence of ribs seems to be rather a variable feature, not sufficient to indicate the existence of two individual species. A more reliable decision on this aspect awaits the study of new alcohol preserved material, however.

Material Examined

Myanmar (BMNH; ZMB 49.621, 200.059-60): Pegu (ZMB 41.199, 200.051, 200.060, 200.065-6, 200.305; BMNH 1838.12.4.1757); Bassein District, Pegu (BMNH); Prome (MHNG); Mandalay (ZMB 47.125, 49.623; MHNG); Myadung (ZMB 27.512, 49.623); Yangon (ZMB 200.055-6, 200.067); Tenasserim (ZMB 200.304; BMNH); Chindwin near Matai (ZMB 49.624); Yu River, tributary of the Chindwin (ZMB 49.622, 49.625); Mu, tributary of the Irawaddy (ZMB 49.621); Thailand: Prov. Mae Hong Song, Nam Mae Yuam near Mae Sariang (ZSM 19983228, 19983247); Prov. Chiang Mai, Pai River approximately 20 km E Pai, 19°17.83'N, 98°27.93'E (ZMB 200.219), Pai River in Pai, 19°21.57'N, 98°26.62'E (ZMB 200.220); Mae

Ping, 60 km N Chiang Mai (MNHN; AMS 146766); bridge at the street from Samoeng to Chiang Mai, 18°44.23'N, 98°55.87'E (ZMB 200.253); Prov. Kanchanaburi, Sai Yok Falls 1 at Nam Tok, 14°14.16'N, 99°3.24'E (ZMB 200.235-7); Prov. Kamphaeng Phet, Maenam Moei, about 30 km S Mae Sot, boarder to Myanmar, 16°26.96'N, 98°39.27'E (ZMB 200.209); Prov. Tak, Mae Dao River, Mae Sot (AMS 146762), Maenam Moei, 8 km N Mae Ramat (AMS 146765).

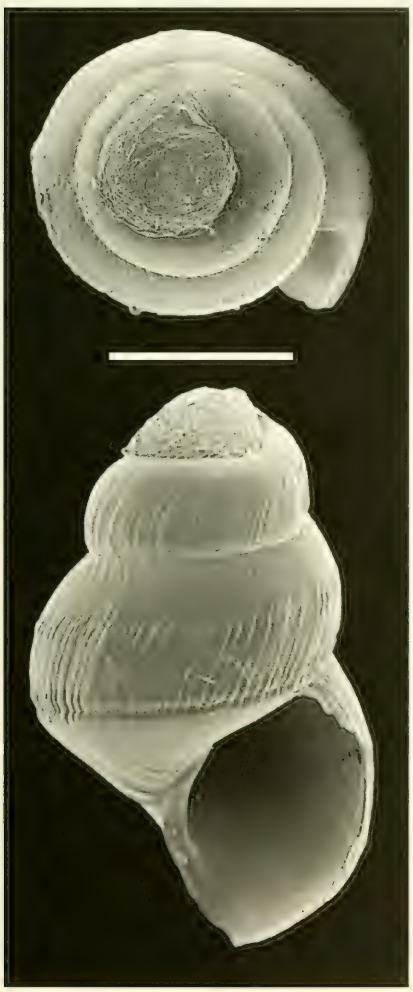

FIG. 33. Embryonic shell morphology of *B. herculea*. SEM images of embryonic shell removed from dried shell (ZMB 49.623); apical and front view. Scale bar = 1 mm.

Differential Diganosis

Shell robust, highly turreted, up to 12 flattened whorls, the basal ones convex, more or less rounded in diameter; aperture wide with protracted base. Strong axial ribs, that may also lack completely; spiral lines.

Description

Shell (Fig. 32): Large to very large, shell solid to thick, spire pyramidal turreted, up to 12 whorls, eroded tip; colour hazelnut to dark brown; spiral ridges most prominent at the base, in some specimens very conspicuous, in others almost completely absent; strong axial ribs may be present. Whorls flattened in diameter, with subsutural depression. Size: H= 28–98 mm, B = 10–34 mm.

Embryonic Shell (Fig. 33): Smooth, covered with axial wrinkles. Average proportions: H = 1.7 mm, B = 1.0 mm, HA = 0.25 mm, BA = 0.40 mm, DA = 0.61 mm (for n = 15), up to 3.5 whorls.

Operculum: Slightly oval, four to six whorls, central nucleus; almost fits aperture.

External Morphology: Uniformly coloured, dark grey to black; grey foot sole with scattered light spots.

Radula (Fig. 34): Ribbon length of up to 30 mm, corresponding to about half of the shell height, about 180 rows of teeth. Rachidian with single main cusp, three smaller denticles on each side tapering in size; upper margin concave by inflated, rounded corners; lower rim rounded; glabella narrow, well rounded at its base, lateral margins slightly concave. Laterals with main cusp flanked by three smaller denticles. Inner and outer marginals with two to three denticles, somewhat pointed, of about same size and shape.

Stomach (Fig. 35): Typhlosoles fused at almost entire length of style sac; opening to style sac partly covered by fleshy, flap-like proximal end of major typhlosole; proximal end of minor typhlosole thickened; crescent ridges below opening of digestive gland duct undulated; crescent pads adjacent to sorting area well developed, heavily undulated or ribbed.

Distribution (Fig. 20)

Myanmar and northwest Thailand: river systems of the Irawaddy, Chindwin, and Salween (with Moei River), and Chao Praya (with Ping and Nan Rivers).

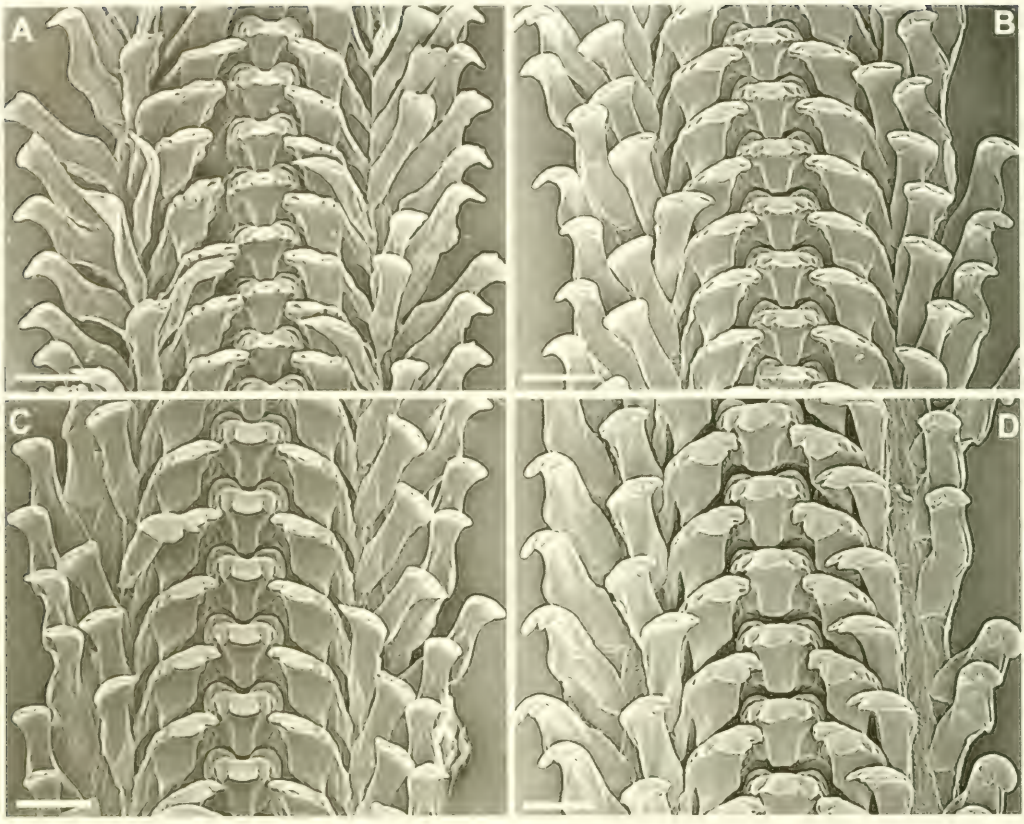

FIG. 34. Radula morphology of *B. herculea*. Radula segments viewed from above. A: Myanmar, Pegu (ZMB 41.199); B: Thailand, Pai (ZMB 200.220); C: Thailand, Sai Yok Falls, Nam Tok (ZMB 200.237); D: Thailand, Pai (ZMB 200.219). Scale bars = 100 µm.

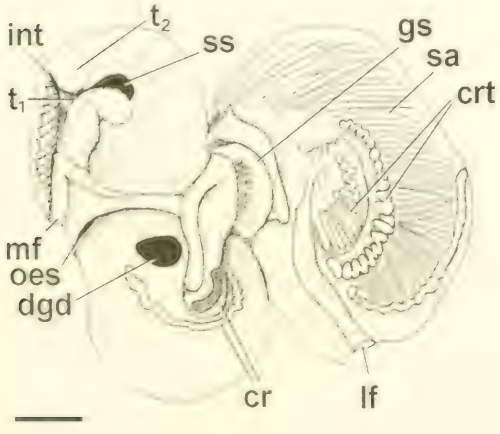

FIG. 35. Stomach anatomy of *B. herculea* (ZMB 200.209; Thailand). Scale bar = 5 mm.

Habitat and Ecology

Clear creeks and rivers on rock, mud, sand, roots, under and among piles of leaf litter in the water (Davis, 1982, referring to *B. costula*), what can be confirmed from own observations in Thailand. May be infested by drilling sabellids (Nematoda).

Remarks

Largest species of the genus. *B. costula* differs statistically significant in shell parameters H/B, H/LA, N (e.g., Fig. 21).

Brotia indragirica (Martens, 1900)
(Fig. 37)

Melania indragirica Martens, 1900: 10, 11 ("Indragiri-Fluß, Sumatra" = Indragiri River,

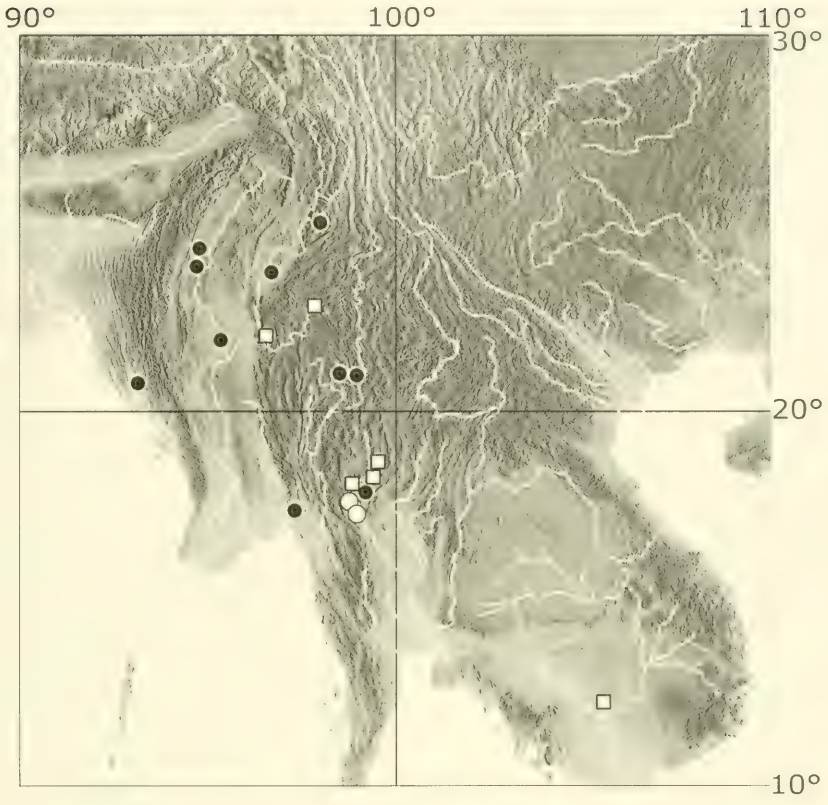

FIG. 36. Distribution of *B. citrina* (open circles), *B. dautzenbergiana* (open squares) and *B. henriettae* (close circles).

Sumatra (Indonesia), lectotype ZMB 51.777a, three paralectotypes ZMB 51.777b, five paralectotypes NMB 1202q (designated by Köhler & Glaubrecht, 2002a) (Fig. 37); types seen; Bullen, 1906: 14 (including an unnamed variety).

Brotia indragirica – Köhler & Glaubrecht, 2002a: 139, fig. 2L.

Taxonomy and Systematics

Only known from the types. For this reason, soft body, radula, and embryonic shells unknown. Shell clearly pachychilid being reason for affiliation with *Brotia* as the only pachychilid taxon known from Sumatra.

Differential Diganosis

Highly turreted, convex whorls flattened in diameter, keeled or angled; prominent, wavy spiral bands or ridges, along keel of the whorl spiral row of spiny nodules; aperture wide, well rounded.

Description

Shell (Fig. 37): Small, not thick but solid; spire turreted, eroded tip, four to five convex whorls,

FIG. 37. Shell morphology of *B. indragirica*. Lectotype of *M. indragirica* ZMB 51.777a.

upper half of whorls flattened; conspicuous, wavy spiral ridges, weak axial ribs; spiral row of spiny nodules at centre of whorls where spiral ridge meets axial ribs. Aperture wide, ovate, produced below. Colour yellowish brown. Size: H = 23–36 mm, B = 10–15 mm.
Embryonic Shell, Operculum, Radula, Soft Body: Anatomy unknown.

Distribution

Sumatra (provinces of West-Sumatra and Riau): Indragiri River and its affluent Kwantan, discharging into South China Sea (approximate centre of river at 0°33'S, 102°03'E).

Brotia insolita (Brot, 1868)
(Fig. 38)

Melania insolita Brot, 1868: 11, pl. 3, fig. 4 ("Inde?"), lectotype and seven paralectotypes MHNG, Brot collection, "Siam" (designated by Köhler & Glaubrecht, 2002a) (Fig. 38); types seen; Brot, 1875: 107, 108, pl. 13, fig. 7.
Brotia (*Brotia*) *insolita* – Brandt, 1974: 176, 177, pl. 13, figs. 29, 30.
Brotia insolita – Köhler & Glaubrecht, 2002a: 139, 140, fig. 2J.

Taxonomy and Systematics

Brot (1868) stated that the species originated from India, which was later corrected to Thailand (Brot, 1875). This corresponds with labelling of the types. Because the type locality could not further be specified, the So Pa Falls, Kaek River (Prov. Phitsanulok, central Thailand) were subsequently designated as the type locality by Brandt (1974: 177).

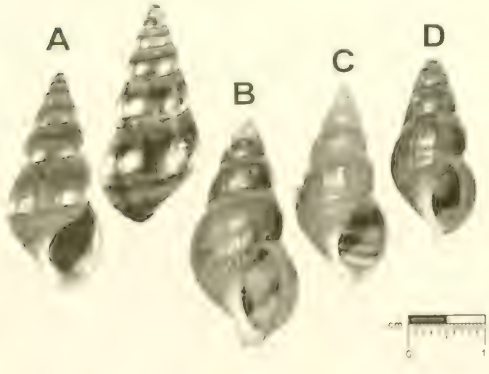

FIG. 38. Shell morphology of *B. insolita*. A: Lectotype of *M. insolita* MHNG; B–D: Three paralectotypes MHNG. Scale bar = 10 mm.

Material Examined

Thailand (ZMB 31.172); Cambodia (ZMB 26.870). Without locality (MHNG, Brot collection; labelled "*M. gloriosa*").

Differential Diganosis

Relatively small, conical, thin but solid; whorls well rounded; yellowish to greenish brown, dark brown spiral band may be present.

Description

Shell (Fig. 38): Small, relatively thin but solid. Shell conical in shape, the body whorl is comparatively large and inflated, while subsequent whorls taper considerably in size. Spire with up to six whorls, eroded. Sculpture consisting of faint growth lines and delicate regularly spaced spiral ridges, in some specimens these ridges become stronger at the base, inconspicuous axial ribs may be present too. Surface glossy, colour yellowish brown a spiral band of darker coloration may be present at the mid of the whorls. Aperture wide, oval and well rounded and produced at the base.
Embryonic Shell, Operculum, *Radula*, *Soft Body*: Anatomy unknown.

Distribution

Central Thailand to Cambodia, only vague.

Remarks

We were neither able to trace voucher material of Brandt from the Kaek River nor to find this species during our own field work. For this reason, we cannot confirm the occurrence in the Kaek River. Brandt (1974) described *B. manningi*, the shells of which are at best hard to distinguish from *B. insolita*. To complicate matters, *B. insolita* closely resembles some, but not all specimens of the type series of *B. siamensis*, among them the lectotype. The difficulties to reliably discriminate all these taxa will likely persist unless material suitable for studies on soft body morphology and molecular genetic is available. For the time being, we follow the treatment of Brandt (1974).

Brotia siamensis tends to be more elongate, often exhibiting axial ribs at the upper whorls. Whorls of *B. manningi* are flattened in diameter.

Brotia iravadica (Blanford, 1869)
(Fig. 39)

Melania iravadica Blanford, 1869: 445 ("Burma, Upper Irawaddy at Malé and Bhamo"), three syntypes BMNH 1888.12.4.1808-10; types not seen; Hanley & Theobald, 1873: 30, pl. 71, fig. 1.
Melania irawadica [sic !] – Brot, 1872: 34; Brot, 1875: 111, 112, pl. 14, figs. 7, 7a.
Melania (*Melanoides*) *baccata* var. *iravadica* – Nevill, 1885: 262.
Melania (*Melanoides*) *iravadica* – Nevill, 1885: 33.
Melania (*Brotia*) *baccata* var. *iravadica* – Martens, 1899: 35, 36.
Tiara (*Melanoides*) *baccata* var. *irawadica* [sic !] – Preston, 1915: 27.
Acrostoma iravadica – Rao, 1928: 446, 447.

Taxonomy and Systematics

Mostly treated as subspecies or variety of morphologically relatively plastic *B. henriettae*. We suggest this taxon represents a distinct species because of its deviant shell. Exceptionally known from the Irawaddy, but not from its tributaries where *B. henriettae* occurs. Whether both species occur in sympatry remains unclear.

FIG. 39. Shell morphology of *B. iravadica* (Myanmar, Irawaddy; ZMB 49.617).

Material Examined

Myanmar: Irawaddy (BMNH 1899.21.6.76-79, ZMB 200.010), Irawaddy near Yenyangyoung (ZMB 49.617, 200.005); Pegu (BMNH 1871.9.23.49). Shan States (BMNH 1888.12.4.1440).

Differential Diganosis

Relatively small, broadly conical, truncated, with two to four remaining whorls; two spiral bands of closely spaced nodules.

Description

Shell (Fig. 39): Relatively small, conical, truncated with two to four remaining whorls. Body whorl comparatively large compared to shell. Two spiral cords support rows of more or less developed nodules as well as some conspicuous spiral cords at base of shell. Aperture wide, produced below, columellar margin thin. Shell size: H = 18–33 mm, B = 9–19 mm.
Operculum: Round, central nucleus, considerably smaller than aperture.
Embryonic shell morphology, Radula, Soft body anatomy: Unknown.

Remarks

Can be distinguished from *B. henriettae* by its smaller and more conical shell and less pronounced sculpture.

Brotia jullieni (Deshayes, 1874)
(Figs. 34D, 40)

Melania jullieni Deshayes, in Deshayes & Jullien, 1874: 115, pl. 7, figs. 7–9 ("Thio-Compih, Cambodge" = Thio Compih, Sâmbok at the Mekong, Cambodia, 12°34'N, 106°01'E), lectotype and three paralectotypes MNHN (designated by Köhler & Glaubrecht, 2002a) (Fig. 40A); types seen; Morlet, 1889: 145.
Melania julieni [sic] – Brot, 1875: 93, 94, pl. 11, figs. 2, 2a.

Taxonomy and Systematics

Commonly treated as synonym of *B. costula* (e.g., Brandt, 1968, 1974; Davis, 1982), but herein considered distinct for its peculiar shell and radula.

Material Examined

Laos: Muong-Bet sur le Song-Ma (MNHN); Mekong (MNHN). Cambodia: Mekong near Pakse (ZMH); Vietnam: Environs de Gang, Tonkin (MNHN); Song Ya near Yuong-Het, Tonkin (MNHN).

Differential Diganosis

Extraordinarily large and robust; aperture wide, basely produced; strong axial ribs, fine spiral lines. Radular teeth each with a very broad, rounded main denticle.

Description

Shell (Fig. 40): Large, broadly pyramidal, eroded tip; aperture wide, comprising about $^1/_4$ of shell height; whorls rounded, suture thin; strong axial ribs and thin spiral ridges, at least at base of shell; colour yellowish to chestnut brown. Size: H = 55–65 mm, B = 24–30 mm.

Radula (Fig. 31D): Lateral corners of rachidian conspicuously enlarged; very broad, spatula shaped main cusp flanked by two much smaller, pointed accessory denticles; glabella almost squarish, well rounded at its base, concave lateral edges. Lateral teeth with very broad main cusp flanked by two smaller denticles on each side, short lateral extensions. Inner and outer marginals with broadly rounded outer cusp and tiny pointed inner cusp. Inner marginals broader than outer ones.

Embryonic shell morphology, Soft body anatomy: Unknown.

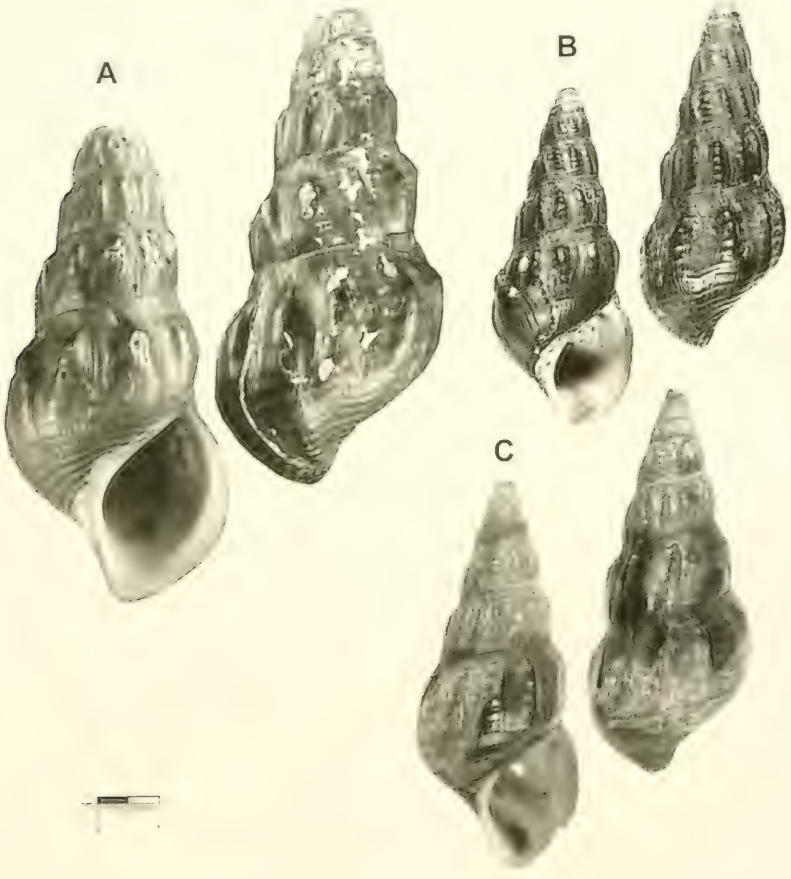

FIG. 40. Shell morphology of *B. jullieni*. A: Lectotype of *M. jullieni* MHNH; B: Cambodia (ZMZ 522392); C: Laos, Pakse (ZMH). Scale = 10 mm.

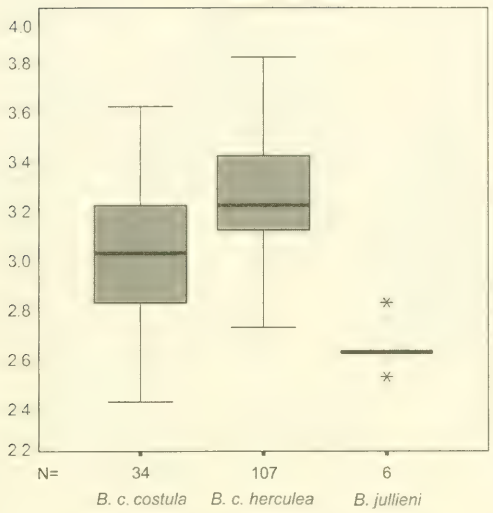

FIG. 41. Comparison of *B. costula* and *B. jullieni* by means of shell parameter H/LA. Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

Distribution

Laos, Cambodia, Vietnam, perhaps also northeast Thailand; Mekong River system.

Ecology

Frequently infested by drilling sabellids (*Caobangia* spec., Nematoda).

Remarks

Only superficially similar with more elongated *B. herculea* (statistical analyses of shell parameters: Fig. 41). Main cusps of the radular teeth and glabella of *B. jullieni* much broader, possessing only one accessory cusp instead of two in *B. herculea*.

A report from the Ping River near Tak, Thailand, by Morlet (1891: "Riviere de Menam-Pinh, de Raheng à Xieng-Moi") refers to *B. herculea*. Brandt (1974) and Davis (1982), referring to *B. costula*, stated that this species is the only cerithioidean in the Mekong.

Brotia kelantanensis (Preston, 1907)
(Figs. 42, 43A)

Melania kelantanensis Preston, 1907: 267, text-fig. ("Kelantan, Malay Peninsula"), types not seen.

Taxonomy and Systematics

Ignored by later authors, this species was reported only once by Davis (1982) mentioning an unidentified, spiny species in the Pahang River system. Herein assigned to *Brotia* for it's characteristic morphology.

Material Examined

Malaysia: Pahang, Taman Negara National Park (ZMA).

Differential Diganosis

Shell comparatively small, broadly conical, no more than four whorls; prominent spiral cord at the centre of the whorls supporting spiral row of strong, pointed spines or nodules.

Description

Shell (Fig. 42): Medium sized, pyramidal, conical, decollated, four remaining whorls; prominent spiral cord at centre of whorls supports spiral row of strong, pointed nodules, additional, weak spiral ridge on upper sector. Colour chestnut brown. Aperture round, relatively small compared to body whorl, slightly produced below. Shell size: H = 31 mm, B = 18 mm (n = 2).

Operculum: Oval, four whorls, central nucleus.

Radula (Fig. 43A): Ribbon 16 mm long, corresponding to about half of shell height, 100 rows of teeth (n = 1). Rachidian with two conspicuously excavated upper corners, concave upper rim; main cusp flanked by two smaller, accessory denticles; glabella narrow, well rounded below with concave lateral margins. Inner and outer marginals with two cusps, outer one broadly spatulate.

Embryonic Shell: Unknown.

Distribution

Malaysia (Malay Peninsula): Federal State of Pahang; Pahang River system.

Habitat

On rocks in rapids (Davis, 1982: 392).

Remarks

Hardly to be mistaken for any other species. Occurs in sympatry with *B. episcopalis*, which is more elongated and differs in average number of whorls and sculpture.

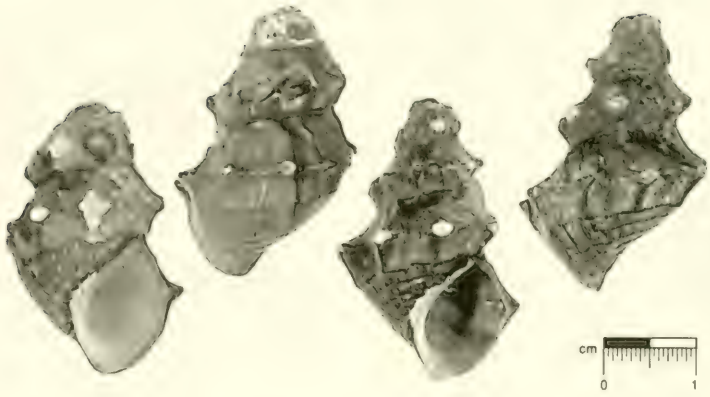

FIG. 42. Shell morphology of *B. kelantanensis* (Malaysia, Pahang; ZMA).

Brotia manningi Brandt, 1968
(Figs. 43B, 44)

Brotia (*Brotia*) *manningi* Brandt, 1968: 272, pl. 10, fig. 58 ("Thailand: Huai Lan at Ban Dam Pon, Lom Sak District, Phetchabun Province"), holotype SMF 197376, 22 paratypes MCZ 288652, 22 paratypes ZSM 19983239, 20 paratypes RMNH 55289/20 (Fig. 44); types seen; Brandt, 1974: 179, 180, pl. 13, fig. 35.
Brotia manningi – Köhler & Glaubrecht, 2002a: 141.

Taxonomy and Systematics

In absence of additional information and material, we follow the statement of Brandt (1968, 1974).

Differential Diganosis

Shell elongate conic with flattened, slightly convex whorls; aperture produced; almost smooth, only with faint spiral lines and growth lines.

Description

Shell (Fig. 44): Medium sized, spire conic with up to seven flattened whorls; suture narrow; smooth, faint growth lines; colour brown to olive, dark brown spiral band may be present. Aperture oval, well rounded to produced below. Size: H = 24–38 mm, B = 11–15 mm.
Operculum: Oval, up to four fast in diameter increasing whorls, sub-central nucleus.
Radula (Fig. 43B): Ribbon about 12 mm long with 80 rows of teeth (n = 1). Rachidian elon-

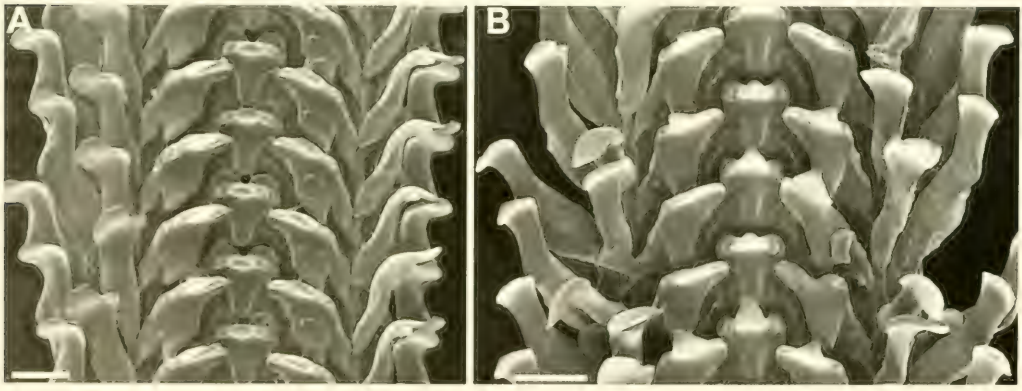

FIG. 43. Radula morphology of *B. kelantanensis* and *B. manningi*. A: *B. kelantanensis* (Malaysia, Pahang; ZMA); B: *B. manningi*, paratype ZSM 19983239). Scale bars = 100 µm.

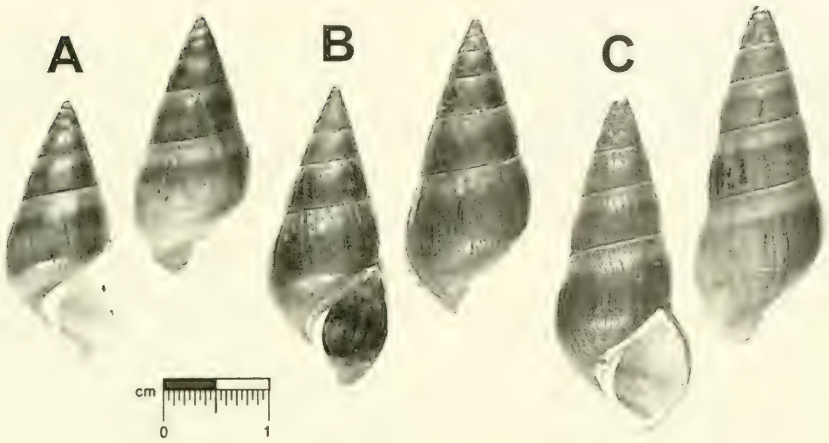

FIG. 44. Shell morphology of *B. manningi*. A–C: Paratypes ZSM 19983239. Scale = 10 mm.

gate, anterior rim slightly concave, inconspicuously excavated upper lateral corners; cutting edge with pronounced main denticle flanked by two, much smaller accessory denticles on each side; glabella narrow, rounded below, not reaching basal margin of rachidian. Laterals with very large main denticle. Inner and outer marginals relatively long, slender, broad outer cusp, smaller, spiny inner denticle.

Embryonic Shell: Unknown.

Distribution

Thailand: Central Thailand, Provinces of Nan, Loei, and Phetchabun (Brandt, 1974).

Remarks

Belongs to a group of taxa from central Thailand with similar shells. To be distinguished from *B. insolita* and *B. siamensis* only by subtle morphological differences. Distinct status requires confirmation by examination of further material suitable for morphological and molecular genetic studies. We were not able to trace material from the Kaek River, central Thailand, a locality reported by Brandt (1974).

Brotia microsculpta Brandt, 1968
(Figs. 45, 46A)

Brotia microsculpta Brandt, 1968: 272, pl. 10, fig. 59 ("Thailand: Maenam Kaek, in Thung Salaeng Luang Botanical Garden, 80 km E of Pitsanulok" = Kaek River, Thung Salaeng Luang NP, Prov. Phitsanulok), holotype SMF 197378/1, 10 paratypes SMF 205356/10 (Fig. 45); types seen; Köhler & Glaubrecht, 2002a: 141; Glaubrecht & Köhler, 2004: 289–291.

Brotia (*Brotia*) *microsculpta* – Brandt, 1974: 180, pl. 13, fig. 36.

Taxonomy and Systematics

Revised by Glaubrecht & Köhler (2004) based on morphological and molecular genetic data. Accordingly, *B. microsculpta* belongs to the Kaek River species flock in Central Thailand.

Material Examined

Thailand: Prov. Phitsanulok, Kaek River: Resort 53 km E Phitsanulok (ZMB 200.266); Poi Falls (ZMB 200.200); Sopha Falls, 71 km E of Phitsanulok (ZSM 19983240); Thung Salaeng Luang NP (ZMB 200.191).

Differential Diganosis

Shell small, conical to elongated, mostly three remaining, slightly rounded whorls; smooth sculpture. Aperture round, not produced. Operculum round, not oval as other Kaek River species. Radula relatively short, closely spaced rows of teeth, marginal teeth prolonged.

Description

Shell (Fig. 45): Relatively small, conic to elongate conic, not thick but solid; truncated,

FIG. 45. Shell morphology of *B. microsculpta*. Holotype SMF 197378/1. Scale = 10 mm.

mostly three remaining, convex whorls; smooth, fine axial growth lines, faint spiral lines. Aperture almost round, relatively small compared to shell, basely rounded but not produced. Size: H = 10–25 mm. B = 8–15 mm.

Operculum: Round to only slightly oval, 5–6 regular whorls, central nucleus.

Radula (Fig. 46A): Length of ribbon m = 11.8 mm (sd = 1.7 mm; n = 3), about 190 closely spaced rows of teeth. Radular teeth comparatively small. Rachidian relatively broad, main cusp flanked by three accessory denticles on each side, glabella narrow, with straight lateral margin, cut basal rim, not reaching base of rachidian. Inner and outer marginals very long, narrow, curved, large, broad outer cusp, one to three tiny inner accessory denticles.

Stomach: Typical, as in *B. citrina* (Fig. 4).

Embryonic Shell: Morphology unknown.

Habitat

Buried into sandy substrata in quiet parts of the swift river.

Distribution

Thailand: Prov. Phitsanulok: Endemic to Kaek River and its northern tributary Huai Chieng Nam (Brandt, 1974).

Remarks

Recognizable by its smaller shell, round operculum, and typical radula. *Brotia pseudosulcospira* is more conical, thicker, whorls more flattened. Only Kaek River species occurring on soft substrata.

Brotia pagodula (Gould, 1847) (Figs. 46B, 47, 48)

Melania pagodula Gould, 1847: 219 (*non M. pagodulus* Reeve, 1860) ("Thoungyin-River, tributary of the Salween River, Burma"), lectotype MCZ 169276 and paralectotype USNM 611238 (designated by Johnson, 1964) (Fig. 47A); types seen; Brot, 1875: 102, 103, pl. 13, fig. 2, Hanley & Theobald, 1876: 61, pl. 153, fig. 3.

Io pagodula – H. Adams & A. Adams, 1854: 300; Reeve, 1859: pl. 3, fig. 10.

Tiara (*Acrostoma*) *pagodula* – Preston, 1915: 32.

Brotia pagodula – Morrison, 1954: 382; Johnson, 1964: 121, pl. 44, fig. 2; Köhler & Glaubrecht, 2001: 292–295, figs. 1A, 9A–F; Köhler & Glaubrecht, 2002a: 142; Glaubrecht & Köhler, 2004: 283.

Brotia (*Brotia*) *pagodula* – Brandt, 1974: 173, 174, pl. 12, fig. 25.

Taxonomy and Systematics

Type species of *Brotia*.

Material Examined

Myanmar: (ZMB 26.708); Salween River, Tavoy (BMNH); Thailand: Prov. Kamphaeng Phet: Maenam Moei approximately 20 km E Mae Sot, 16°45.82'N, 98°45.14'E (ZMB 200.205), Maenam Moei approximately 30 km S Mae Sot, boarder to Myanmar, 16°26.96'N, 98°39.27'E (ZMB 200.208), Maenam Moei (USNM 776062), Maenam Moei, 8 km W of Mae Ramat (ZSM 19983241; ZMH; RMNH 71319); soft bodies already removed from the shells, without location (ZMH).

Differential Diganosis

Conical shell sculptured by spiral row of conspicuous spines; aperture wide, rhomboid, well produced below; radular teeth with very broad, enlarged main cusp; comparatively large juveniles in brood pouch.

Description

Shell (Fig. 47): Medium sized, spire broadly conical, decollated, up to five flattened whorls, narrow suture, spiral row of long, pointed

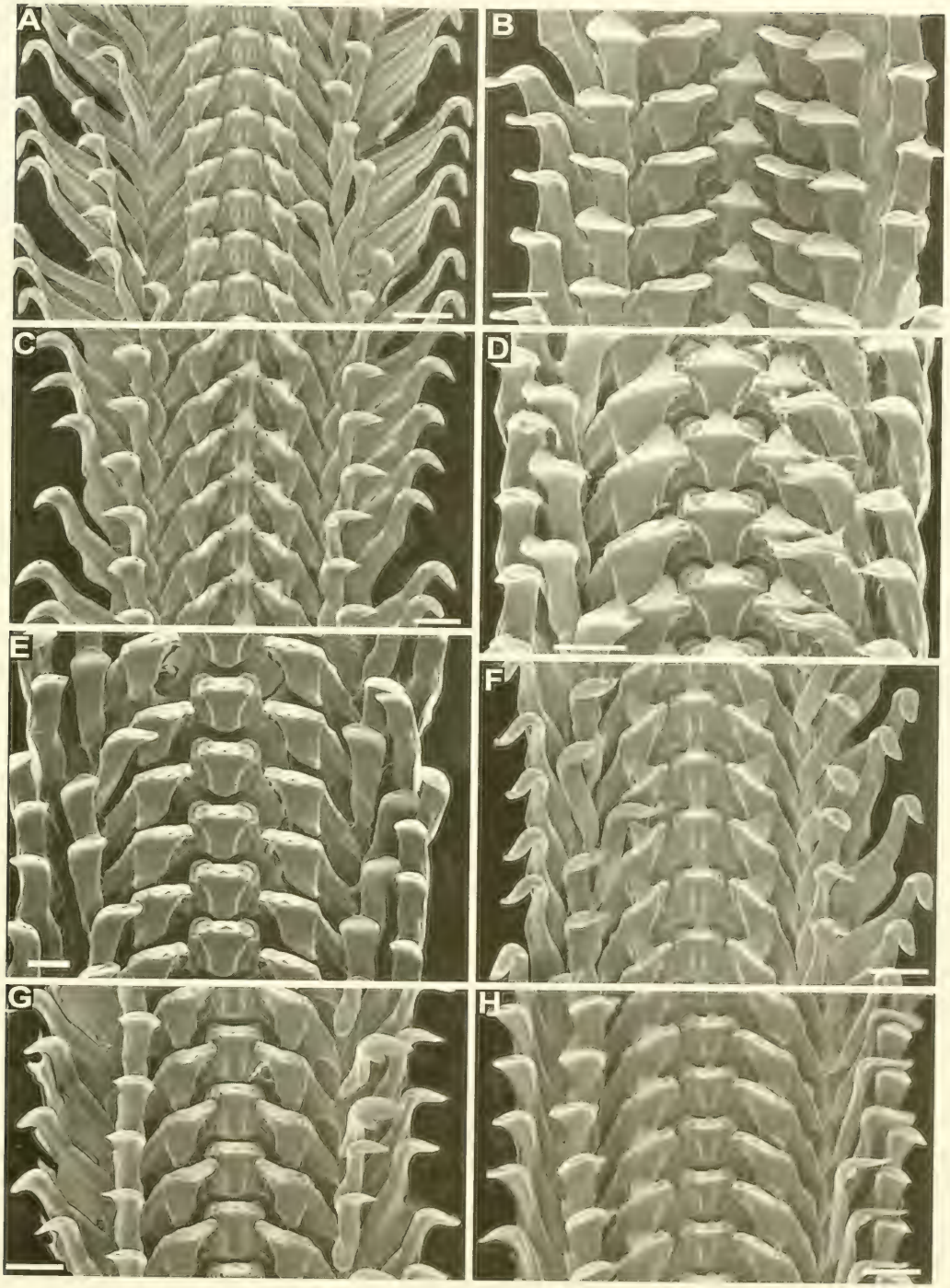

FIG. 46. Radular morphology of several *Brotia* species. A: *B. microsculpta* (Thailand, Kaek River; ZMB 200.200); B: *B. pagodula* (Thailand, Moei River; ZMH); C: *B. paludiformis* (Thailand, Kaek River; SMF 215963); D: *B. peninsularis* (Thailand, Surat Thani; ZMB 200.242); E: *B. praetermissa*, Paratype BMNH 20010482/B; F: *B. pseudosulcospira* (Thailand, Kaek River; ZMH); G: *B. solemiana* (Thailand, Pong River; SMF 193585); H: *B. subgloriosa* Paratype ZSM 19983219. Scale bars = 0.1 mm.

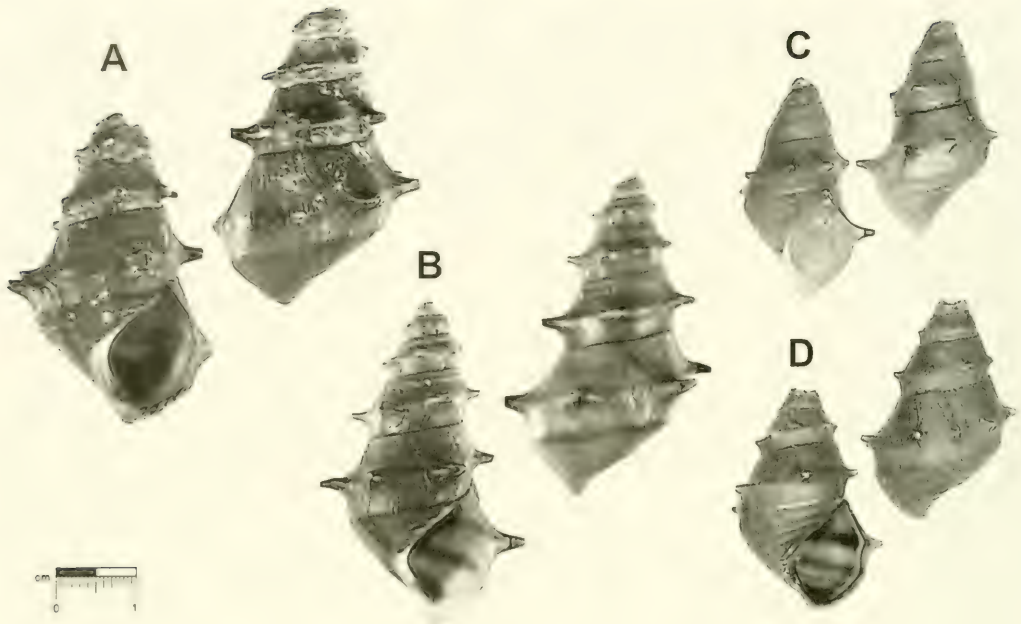

FIG. 47. Shell morphology of *B. pagodula*. A: Lectotype of *M. pagodula* (MCZ 169276; Thougyin); B: Thailand (ZMB 26.708); C: Thailand, Moei River (ZMB 200.205); D: Thailand, Moei River (ZMB 200.208). Scale = 10 mm.

spines; fine spiral lines at base of shell; light to chestnut brown colour, dark brown spiral band may be present. Aperture ovate with angular margin below, inside greyish white with brown bands. Size: H = 18–44 mm, B = 13–26 mm.

Embryonic Shell (Fig. 48): Smooth; up to four rapidly increasing whorls, comparatively large compared to adult as well as to other species.

Operculum: Round, 6 to 8 regularly increasing whorls; central nucleus; clearly smaller than aperture.

Radula (Fig. 46B): 125 to 170 rows of teeth, length of up to 20 mm, corresponding to half of shell height. Rachidian with straight upper rim, base convex by basally extending, broad glabella with more or less straight lateral margins and cut lower rim; very large main denticle flanked by two smaller denticles on each side. Laterals with large, broadly triangular main cusp flanked by two or three minute denticles on inner side and one or two at outer side. Inner and outer marginals broadly spatulate, with large main cusp and tiny inner denticle.

Stomach: Stomach as in *B. citrina* (Fig. 4), except for typhlosoles fused at almost entire length of style sac.

Reproductive System

Females contain between 1 and 50 juveniles (n = 6) varying in height between 3.5 and 6 mm.

Habitat

Attached to rocks in sectors with swift current.

Ecology

Specimens collected during a field trip in 2001 frequently infested with drilling sabellids (*Caobangia* spec., Nematoda).

Distribution (Fig. 49)

Myanmar, Thailand: Restricted to Salween and its tributary Thoungyin (= Maenam Moei), forming the border between Thailand and Myanmar.

Remarks

Can hardly be confused with any other species for its spiny shell. Spines of other species are considerably smaller (e.g., *B. binodosa*, *B. costula*, *B. spinata*).

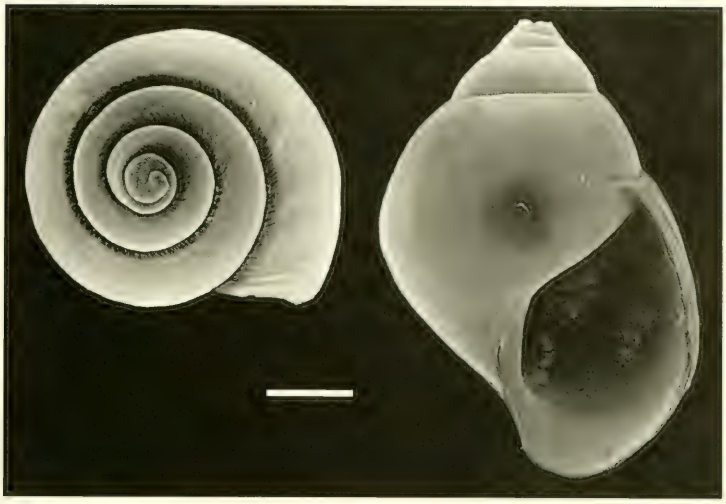

FIG. 48. Embryonic shell morphology of *B. pagodula*. SEM images of embryonic shell removed from brood pouch (ZMH); apical and front view. Scale bar = 1 mm.

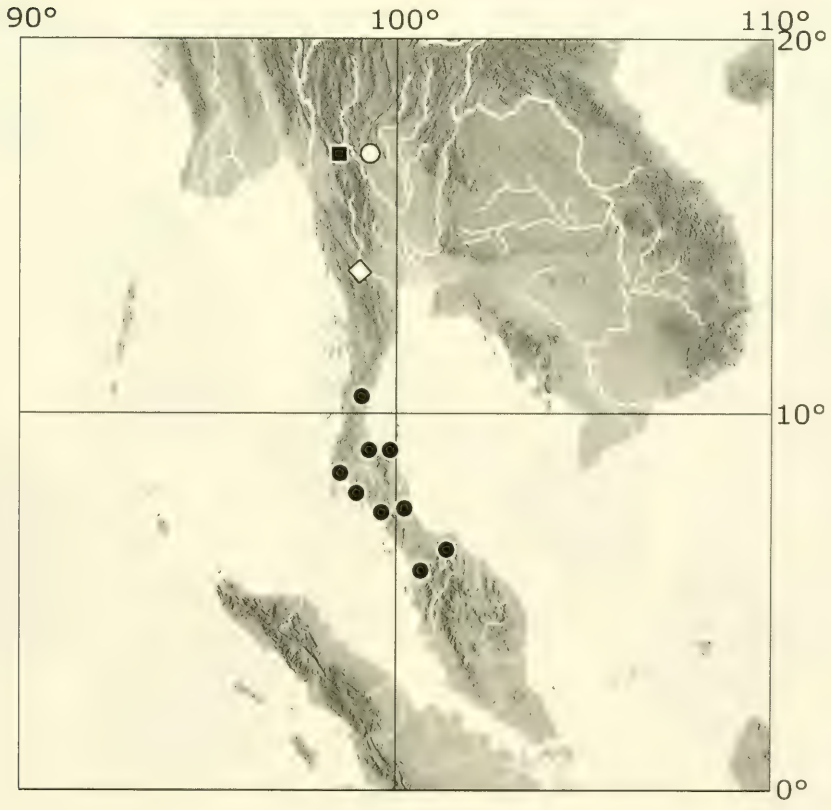

FIG. 49. Distribution of *B. peninsularis* (close circles), *B. siamensis* (open circle), *B. pagodula* (close rectangle) and *B. wykoffi* (open rectangle).

Brotia paludiformis (Solem, 1966)
(Figs. 46C, 50)

Paracrostoma paludiformis Solem, 1966: 17, pl. 1, figs. H–J, text-fig. 2 (*non Semisulcospira paludiformis* Yen, 1939) ("Thailand, Provinz Phitsanulok: Kaek River at the Thung Salaeng Luang Falls"); types not seen.
Paracrostoma paludiformis paludiformis – Brandt, 1974: 187, pl. 14, fig. 45.
Paracrostoma paludiformis – Köhler & Glaubrecht, 2002a: 121–156.
Brotia paludiformis – Glaubrecht & Köhler, 2004: 291, 292.

Taxonomy and Systematics

For specimens from the Kaek River, the name "*Paracrostoma paludiformis*" was first employed by Solem (1966) in reference to a presumably pleurocerid species from Hainan described by Yen (1939). Although, Solem (1966) erred in assuming that both taxa are conspecific, the name introduced by him is available as the species epitheton has been used in context with a changed generic affiliation.

This species belongs to the Kaek River species flock in Central Thailand and was revised and transferred to *Brotia* by Glaubrecht & Köhler (2004).

Material Examined

Thailand: Prov. Phitsanulok: Kaek River: Sopha Falls, 71 km E of Phitsanulok (ZMH; BMNH; SMF 215963).

Differential Diganosis

Shell conical, thick, very robust; two or three convexly rounded whorls; body whorl conspicuously inflated; entirely smooth except for growth lines; aperture broadly oval.

Description

Shell (Fig. 50): Medium sized to large, broadly ovate, two or three well rounded, convex whorls; spire eroded; body whorl large, inflated; smooth sculpture consisting of faint growth lines, only rarely with spiral row of small, rounded nodules; colour chestnut brown; aperture wide, oval, well rounded below. Shell size: H = 24–30 mm, B = 18–22 mm.
Operculum: Oval to slightly elongated, up to three whorls fast increasing in diameter, subcentral nucleus.
Radula (Fig. 46C): Length of ribbon: m = 23.4 mm (sd = 1.3 mm; n = 3), about 190 rows of teeth. Denticle morphology corresponding to *B. armata*.

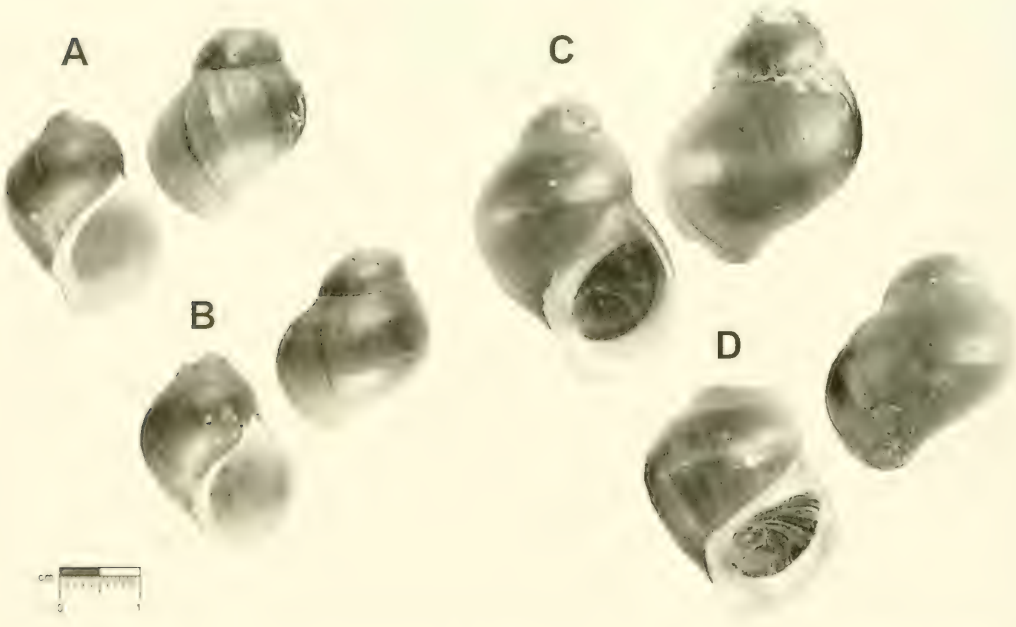

FIG. 50. Shell morphology of *B. paludiformis*. A–B: Thailand, Kaek River, Sopha Falls (SMF 215963); C–D: Thailand, Kaek River, Sopha Falls (ZMH). Scale = 10 mm.

Embryonic shell morphology, Soft body anatomy: Unknown.

Distribution

Thailand: Prov. Phitsanulok: Endemic to Kaek River; exclusively known from Sopha waterfalls.

Remarks

Very distinct species in it's globular shape and inflated body whorl. Somewhat similar is *B. pseudosulcospira*, which differs most conspicuously by its more flattened whorls.

Brotia peninsularis (Brandt, 1974)
(Figs. 46D, 51, 52)

Brotia (*Brotia*) *costula peninsularis* Brandt, 1974: 183, pl. 1, fig. 17 ("Thailand: Maenam Lampa, Province of Pattalung" = River Lampa, Prov. Phattalung), holotype SMF 220570, 17 paratypes SMF 220571, six paratypes SMF 220572, paratypes ZSM 19983232, paratypes ZMH; types seen.

Taxonomy and Systematics

Brandt (1974) mentioned a series of 50 paratypes (Brandt collection 496). Thus, additional type material may exist that was not traced. This taxon has been described as a subspecies of *B. costula*. However, it is considered here as distinct based on morphological and molecular genetic data.

Material Examined

Thailand: Prov. Surat Thani, Wiphawadi waterfalls, bridge at highway 401 to Nakhon Si Thammarat, 20 km off Surat Thani, 9°5.88'N, 99°46.33'E (ZMB 200.041-2), Pum Pin near Takuha, km 63.5 (ZMH; ZSM 19983231); Prov. Phang Nga, Khlong Ipan, bridge at street 4035 between Ao Luk and Phrasaeng (ZMB 200.043), Bok Ka Ra Ni falls near Phang Nga (ZMH; ZSM 19983233); Prov. Krabi, street 4 at Ao Luk, 8°91.44'N, 98°34.90'E (ZMB 200.046), creek between Krabi and Baling (ZSM 19982334), Klong Nga opposite Krabi (ZSM 19983230); Klong Sag, Ban Nai Sra (MCZ 288636; marked as paratypes); Yala, creek at new mine, NW Na Pupo (ZMH; ZSM 19983229).

Differential Diganosis

Shell rather small, thin but solid, conical; body whorl relatively large; regular spiral lines, rarely axial ribs.

Description

Shell (Fig. 51): Small, spire oval to conical turreted, moderately thick, up to eight flattened to rounded whorls, narrow suture; regular spiral ridges crossed by growth lines predominant sculpture; rarely, small spiny nodules formed on spiral ridges; colour lightly brown to olive-brown. Aperture oval, well rounded below, pointed above.

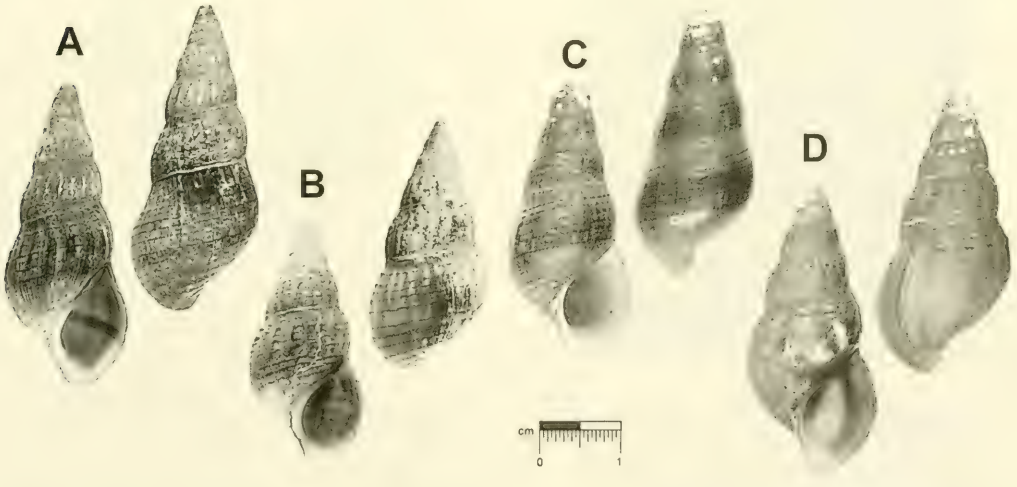

FIG. 51. Shell morphology of *B. peninsularis*. A–B: Paratypes ZMH; C: Paratype ZSM 19983232; D: Thailand, Surat Thani (ZMB 200.242). Scale = 10 mm.

Embryonic Shell (Fig. 52): Subsequent whorls smooth, sculptured only by growth lines. Average proportions: H = 3.2 mm, B = 0.4 mm, HA = 0.18 mm, BA = 0.33 mm, DA = 0.65 mm (for n = 10).

Operculum: Round to slightly oval, five to six whorls gradually increasing in diameter.

Radula (Fig. 46D): Rachidian with slightly concave upper rim, glabella well developed, rounded below, concave lateral margins; main cusp flanked by three smaller denticles on each side. Lateral cusp formula 2–13. Inner and outer marginals with two cusps, the outer one being broader; inner marginal teeth generally broader than outer ones.

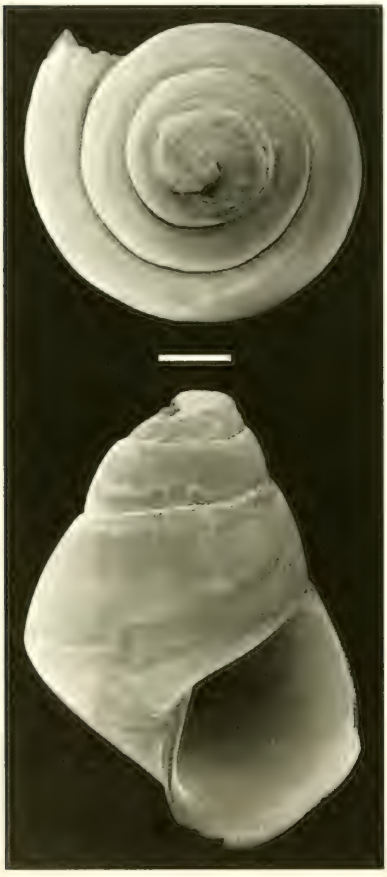

FIG. 52. Embryonic shell morphology of *B. peninsularis*. SEM images of embryonic shell removed from brood pouch (paratype ZMH); apical and front view. Scale bar = 0.3 mm.

Stomach: Typical (as in *B. citrina*; Fig. 4); except for both typhlosoles unfused at entire length of style sac.

Reproductive System

One female contained 23 juveniles (ZMB 200.242).

Habitat

Rather small, swift streams on limestone; attached to rocks and boulders, sitting directly in the water current.

Distribution (Fig. 49)

Thailand, Malaysia: Malay Peninsula S of Isthmus of Kra (Thai provinces Chumphon, Surat Thani, Krabi, Phang Nga, and Nakhon Si Thammarat as well as province of Pahang, Malaysia; Brandt, 1974).

Remarks

Type specimens of *B. siamensis* are very similar but can be discriminated statistically significant by parameters N and H/B (Table 3, Fig. 53).

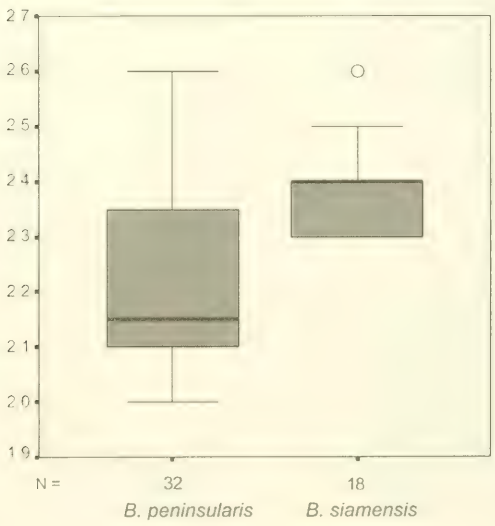

FIG. 53. Comparison of *B. peninsularis* and *B. siamensis* by means of shell parameter H/B. Box plot diagram showing median, the 25%- and 75%-percentile and largest non-extremes (less than 1.5 times of box height).

Brotia praetermissa Köhler & Glaubrecht, 2002
(Figs. 46E, 54–56)

Brotia praetermissa Köhler & Glaubrecht, 2002b: 353–355 ("Borneo"), holotype BMNH 20010482/A; three paratypes BMNH 20010482/B (Fig. 54); types seen.

Taxonomy and Systematics

This species was described from material in the BMNH and is one of two *Brotia* species recorded from Borneo, even though the locality data is vague.

Differential Diganosis

Shell highly turreted, with stepped whorls, conspicuous spiral ridges, one or two spiral rows of spiny nodules; operculum round, relatively small; inner and outer marginal teeth with very broad, oval main tooth, only some outer marginals with accessory cusp at inner side.

Description

Shell (Fig. 54): Highly turreted, about eight stepped whorls, covered by thick calcareous deposit; tip eroded; relatively deep suture; sculpture of six strong spiral ridges, most prominent at the base, one or two spiral rows of spiny nodules most prominent at second whorl; early whorls smooth or sculptured by inconspicuous axial ribs only. Colour hazelnut brown to yellowish brown (probably leached due to conservation). Average shell dimensions: H = 58.2 mm, B = 22.3 mm.

*Embryonic Shell (*Fig. 55): Turreted, flattened whorls, smooth texture, faint spiral lines, regular growth lines; about 4 mm in height.

Operculum: Round, up to 10 whorls, central nucleus; considerably smaller than aperture.

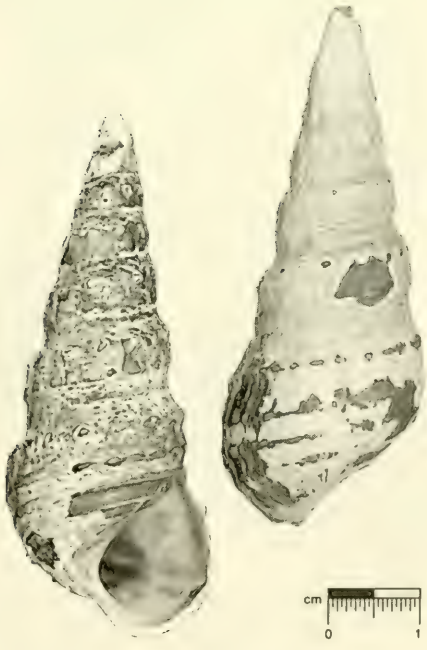

FIG. 54. Shell morphology of *B. praetermissa*. Holotype BMNH 20010482/A. Scale bar = 10 mm.

FIG. 55. Embryonic shell morphology of *B. praetermissa*. SEM images of embryonic shell removed from brood pouch (paratype, BMNH 20010482/B); apical and front view. Scale bar = 1 mm.

Radula (Fig. 46E): Ribbon about 20 mm long with 120 rows of teeth; rachidian with one main cusp flanked by two smaller denticles on each side that taper in size, glabella well developed with rounded basal margin; anterior rim of rachidian slightly concave by slightly excavated lateral corners, basal rim rounded. Main cusp of laterals flanked by two accessory denticles on each side, glabella well developed comparatively long lateral extensions. Inner marginal tooth with one very broad, spatula-shaped cusp; some of outer marginals in addition possess accessory cusp at inner side. Both, inner and outer marginals, curved or knee-shaped, outer ones with lateral flange at exterior side.

Stomach (Fig. 56): Major and minor typhlosole unfused, gastric pad large, sorting area with two well developed crescent septate thickenings.

Reproductive System

One female contained 18 shelled juveniles.

Distribution

Borneo (locality data vague).

Remarks

Somewhat similar is *Jagora asperata* from the Philippines, which can be distinguished by its different soft body, embryonic shell, and radular morphology (Köhler & Glaubrecht, 2003).

Brotia pseudoasperata Brandt, 1968
(Figs. 57–59)

Brotia (*Brotia*) *pseudoasperata* Brandt, 1968: 270, 271, pl. 10, fig. 57, text-fig. 39 ("Maenam San and its tributary Huai Kao Man", Prov. Loei, Thailand), holotype SMF 197375 ("Huai Kao Man, Phung Song, Loei"), 18 paratypes SMF 19381, 12 paratypes ZSM 19983244, nine paratypes ZSM 19983245, five paratypes RMNH 5240/5, 14 paratypes BMNH 1976072 (Fig. 57); types seen; Brandt, 1974: 177, 178, pl. 13, fig. 31.

Brotia pseudoasperata – Köhler & Glaubrecht, 2002a: 144.

Taxonomy and Systematics

Brandt (1968) stated that shells from Annam (China) and Laos erroneously attributed to "*Melania asperata*" belong to his species. Another lot of similar shells is known from Mt. Carin (Pegu, Myanmar; ZMB 47.129). However, it is still questionable whether all these references can really be attributed to this species. We rather suspect that *B. pseudoasperata* is restricted to the Heung River system. Species limits by means both of morphology and geographical distribution remain dubious unless material suitable for morphological and molecular genetic analyses will be available.

Differential Diganosis

Shell elongate turreted; closely spaced axial ribs that support one to three spiral rows of

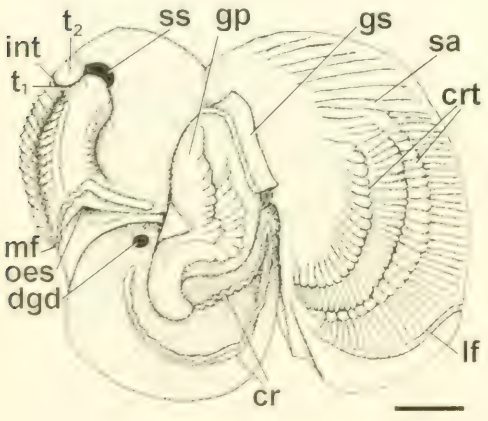

FIG. 56. Stomach morphology of *B. praetermissa*, paratype BMNH 20010482/B.

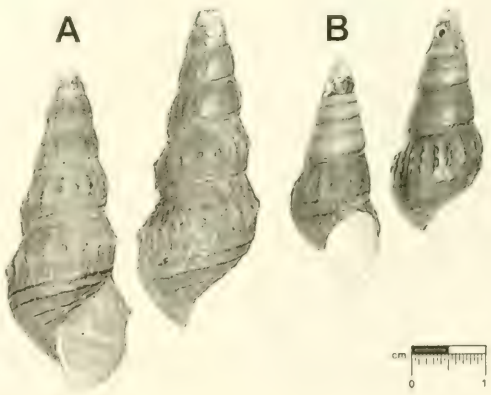

FIG. 57. Shell morphology of *B. pseudoasperata*. A–B: Paratypes ZSM 19983244. Scale bar = 10 mm.

spiny nodules; operculum round with up to eight whorls.

Description

Shell (Fig. 57): Medium sized, thin but solid, elongate turreted, tip eroded, up to seven convex whorls, narrow suture; thin, regularly spaced axial ribs that support one to three spiral rows of spiny nodules, the first approximately at mid of whorls, the second, if present, at upper half of whorls; upper whorls may be smooth; spiral ridges at base. Colour hazelnut brown, a dark brown spiral band may be present. Aperture broad, wide, well rounded, produced below. Size: H = 20–27 mm, B = 8–12 mm.

Embryonic Shell (Fig. 58): Ovate, smooth except for faint growth lines, comprising 2.0–2.5 whorls. Average proportions: H = 2 mm, B = 1.6 mm, HA = 0.22 mm, BA = 0.33 mm, DA = 0.81 mm (for n = 3).

Operculum: Round, up to eight gradually increasing whorls, central nucleus; clearly smaller than aperture.

Radula (Fig. 59): Ribbon with 90 to 120 rows of teeth. Upper rim of rachidian concave by inflated lateral corners, lower rim almost straight; cutting edge with one main denticle flanked by two smaller ones; glabella narrow, well rounded at base not exceeding lower rim of rachidian, v-shaped with concave lateral margins. Main cusp of laterals flanked by two accessory denticles on each side. Inner and outer marginal teeth with two cusps, outer one broad, rounded, inner one small, pointed.

Reproductive System

One female contained 19 shelled juveniles (ZSM 19983244).

Distribution

Thailand: With certainty known only from type locality (San River, affluent of Heung River, collecting area of the Mekong), and its tributary Huai Kao Man (Brandt, 1974). Reports from Laos, Vietnam, Myanmar should be treated with caution.

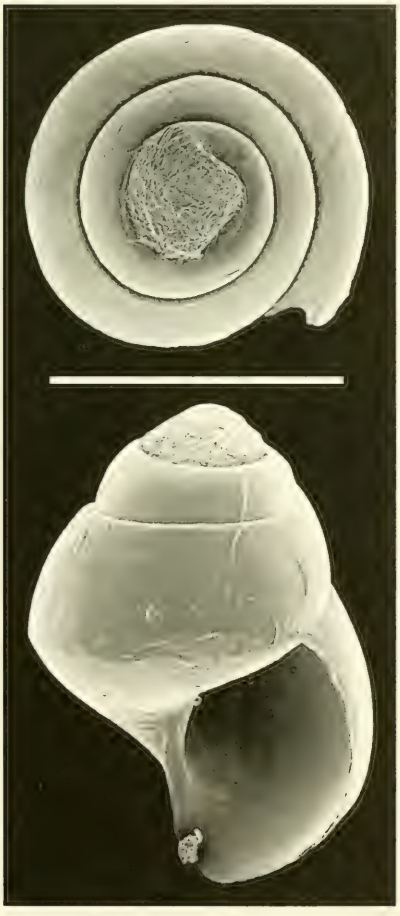

FIG. 58. Embryonic shell morphology of *B. pseudoasperata*. SEM images of embryonic shell removed from dried shell (Thailand, Huai Kao Man, ZSM 19983244); apical and front view. Scale bar = 1 mm.

FIG. 59. Radular morphology of *B. pseudoasperata*, paratype ZMH. Scale bar = 100 µm.

Brotia pseudosulcospira (Brandt, 1968)
(Figs. 46 F, 60, 61)

Brotia (*Paracrostoma*) *pseudosulcospira* Brandt, 1968: 274, 275, pl.10, fig. 61, text-fig. 40 ("Maenam Kaek in Pitsanulok Prov., at Wang Nok Nang Aen, Wang Tong District, Thailand" = Thailand, Provinz Phitsanulok, Wang Tong District, Kaek River at Wang Nok Nang Aen), holotype SMF 197379; 23 paratypes SMF 193586; five paratypes SMF 194061; 11 paratypes BMNH 1976120; 12 paratypes ZMH; 11 paratypes ZMH (alc.); types seen.

Paracrostoma pseudosulcospira pseudosulcospira – Brandt 1974: 185, pl. 13, fig. 42.

Paracrostoma pseudosulcospira – Köhler & Glaubrecht, 2002a: 144.

Brotia pseudosulcospira – Glaubrecht & Köhler, 2004: 292.

Taxonomy and Systematics

Brandt (1968) described a second subspecies, *P. p. armata*, which is considered distinct. A systematic revision based on morphological and molecular genetic data was presented by Glaubrecht & Köhler (2004).

Material Examined

Thailand: Prov. Phitsanulok, Kaek River: Sakunothayan Falls, 33 km E of Phitsanulok (ZMB 200.196, 200.299).

Differential Diganosis

Shell conical, up to three flattened whorls, rather smooth with growth lines, occasionally spiral cords at the base. Aperture widely ovate well rounded.

Description

Shell (Fig. 60): Medium sized, conical, robust, frequently with eroded spire, only two remaining, flattened whorls; smooth sculpture except for growth lines, occasionally more or less developed, regularly spaced spiral cords, but not at base of shell. Aperture widely ovate well rounded, slightly produced below. Size: H = 26–40 mm, B = 18–24 mm.

Embryonic Shell (Fig. 61): Smooth, with faint growth lines only; size of 2.0–2.5 mm, 2.5 whorls.

Operculum: Oval, up to four whorls fast increasing in diameter, sub-central nucleus.

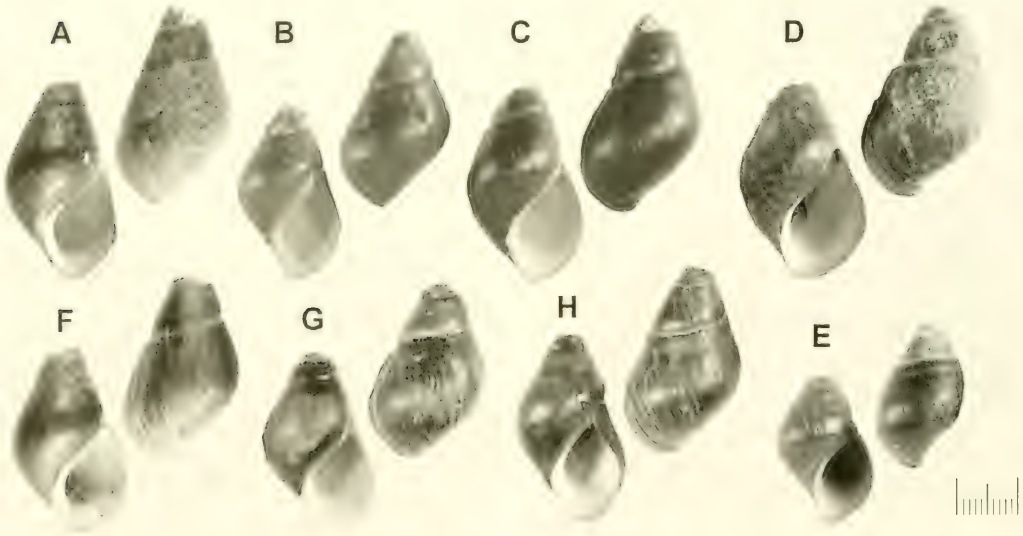

FIG. 60. Shell morphology of *B. pseudosulcospira*. A: Paratype SMF 193586; B–C: Paratypes ZMH; D–E: Paratypes ZMH (alc.); F: Paratype SMF 194061; G–H: Kaek River, Sakunothayan Falls (ZMB 200.299). Scale bar = 10 mm.

Radula (Fig. 46F): Length of ribbon: m = 25 mm (sd = 2.5 mm; n = 3), up to 180 rows of teeth. Central tooth comparatively broad, glabella very narrow; otherwise similar to *B. armata*.

Distribution

Thailand: Prov. Phitsanulok: Endemic to Kaek River, restricted to its westernmost portion (Wang Nok Nang Aen, E of Wang Tong and Sakunothayan Falls close by).

Remarks

The shell of *B. pseudosulcospira* is very characteristic. *Brotia paludiformis*, also being smooth, exhibits convexly rounded whorls and an inflated body whorl. It latter lacks spiral lirae as observed at least in some specimens of *B. pseudosulcospira*. *Brotia armata* has spiny nodules.

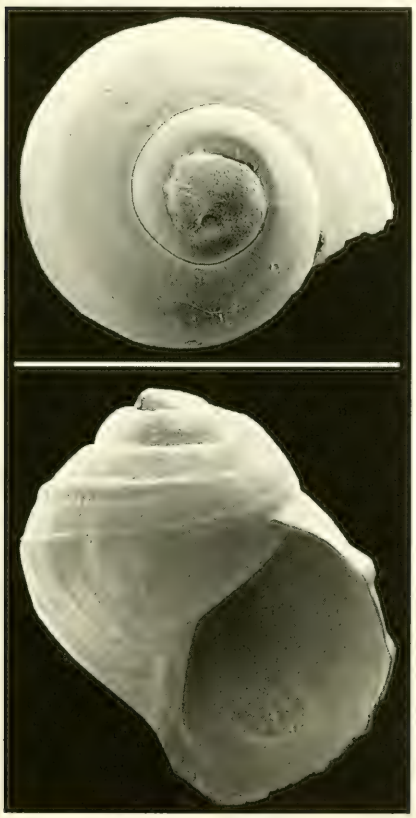

FIG. 61. Embryonic shell morphology of *B. pseudosulcospira*. SEM images of embryonic shell removed from dried shell (paratype ZMH); apical and front view. Scale bar = 1 mm.

Brotia siamensis (Brot, 1886)
(Fig. 62)

Melania siamensis Brot, 1886: 90, 91, pl. 7, figs. 3–3b ("Raheng, Siam" = Tak, Prov. Tak, Thailand), lectotype and 18 paralectotypes MHNG, coll. Brot (designated by Köhler & Glaubrecht, 2002a) (Fig. 62); types seen.
Brotia siamensis – Köhler & Glaubrecht, 2002a: 147, fig. 3H.

Taxonomy and Systematics

Treated in various ways by previous authors, this taxon was considered conspecific with *M. hamonvillei* Brot, 1887, by Bavay & Dautzenberg (1910) for the similar shell. This assumption was also followed by Köhler & Glaubrecht (2002a). In fact, both taxon names are used interchangeably for material in various museum collections (own observations). However, in spite of their conchological similarity, the taxa are not conspecific as is revealed by a different embryonic shell morphology (unpubl. data). *Melania hamonvillei* possesses a protoconch typical for species of *Adamietta* and certainly is not member of *Brotia*.

Melania siamensis was further been stated to be identical with *M. jullieni* by Morlet (1891) and *B. costula* by Brandt (1968, 1971). Also Köhler & Glaubrecht (2002a) noticed that some type specimens of *M. siamensis* are similar to *B. costula*, whereas some others are not (Fig. 57). However, this superficial similarity is no reason to assume that both taxa are conspecific, since their distributional areas are separated by a considerable geographic distance. Re-examination of Brandt's voucher material reveals that the author was also not sure how to distinguish between *B. siamensis* and *B. peninsularis*. The latter taxon was treated by him as a subspecies of *B. costula*. Some lots of this species were labelled by him with *B. siamensis*, however. Both taxa are indeed similar. *B. peninsularis* as considered here is restricted to the Malay Peninsula south of the Isthmus of Kra. The only confirmed record of *B. siamensis* is the type locality, Tak, about 700 km N of this isthmus. A reliable decision on the relationships of *B. siamensis* and *B. peninsularis* awaits the examination of well-preserved material from the area of Tak. For

TABLE 3. Result of disriminant analysis of shell parameters of *B. peninsularis* and *B. siamensis*.

	Predicted group membership	
	B. peninsularis	*B. siamensis*
B. peninsularis	29 (93.5%)	2 (6.5%)
B. siamensis	0 (0%)	18 (100%)

the time being, we consider both as distinct species, because they can be discriminated by statistical analyses of shell parameters with significance (Table 3).

Differential Diganosis

Shell variable, rather small, elongate turreted; apex frequently truncated; regularly spaced spiral ridges, sometimes axial ribs, mostly only on upper whorls; greenish to olive brown or dark brown to almost black, brown spiral band may be visible.

Description

Shell (Fig. 62): Medium sized, conical to elongate turreted, up to six convex whorls, apex frequently truncated; regularly spaced spiral ridges, most prominent at the base, axial ribs, mostly on upper whorls, may be lacking. Colour greenish to olive brown or dark brown to almost black; dark brown spiral band may be visible. Size: H = 2639 mm, B = 11–16 mm.

Embryonic Shell, *Radula*, *Operculum*, *Soft Body*: Unknown.

Remarks

Similar *B. peninsularis* tends to have larger body whorl compared to the shell height, whorls more rounded in diameter. *Brotia jullieni* has a much larger shell, larger body whorl, wider aperture, protracted basal lip. *Brotia costula* is larger, not truncated, pyramidal turreted, more elongated in shape, different sculpture. "*Melania hamonvillei*" has distinct embryonic shell structure, resembling, for example, *B. testudinaria* (Köhler & Glaubrecht, 2001).

Distribution (Fig. 49)

Thailand: Type locality only known reference: Tak (Prov. Tak, north-central Thailand) at banks of Ping River.

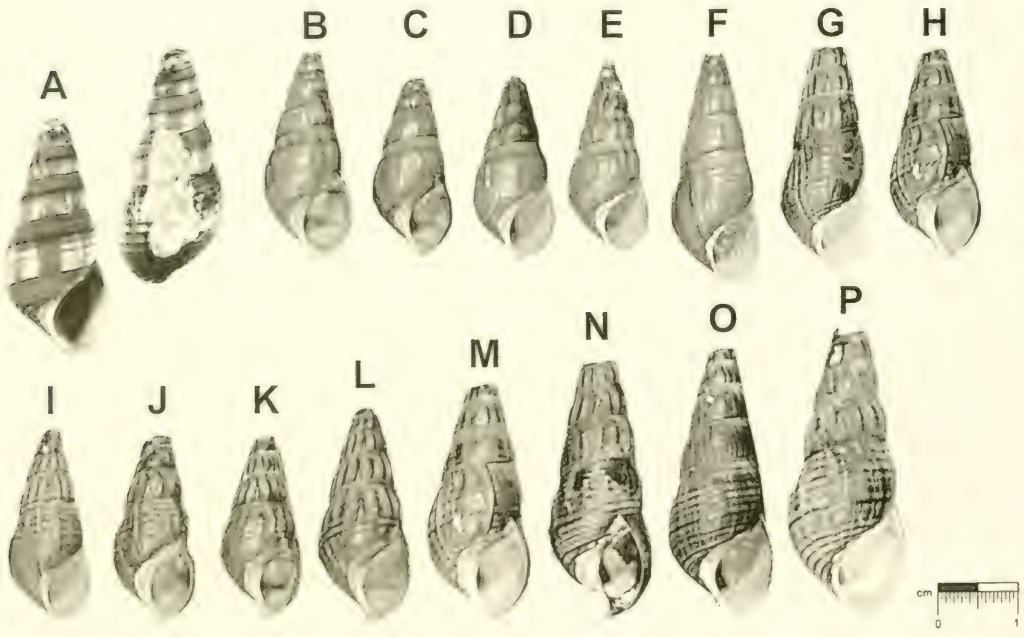

FIG. 62. Shell morphology of *B. siamensis*. A: Lectotype MHNG (front and rear); B–P: Paralectotypes MHNG. Scale bar = 10 mm.

Brotia solemiana (Brandt, 1968)
(Figs. 46G, 63)

Brotia (*Paracrostoma*) *solemiana* Brandt, 1968: 273, pl.10, fig. 60 ("Maenam Pong at Ban Pa Nok Kao, Loei Prov." = Thailand, Prov. Loei, Pong River bei Ban Nok Kao), holotype SMF 197377, seven paratypes SMF 193583, six paratypes SMF 193585, two paratypes RMNH 55233/2 (Fig. 63); types seen.
Paracrostoma solemiana – Brandt 1974: 186, pl. 13, fig. 44; Köhler & Glaubrecht, 2002a: 147.
Brotia solemiana – Glaubrecht & Köhler, 2004: 292, 293.

Taxonomy and Systematics

Brandt (1968, 1974) stated a slender shell, flattened whorls, an elongated aperture to be characteristic for this species. Furthermore, he assumed that it is endemic to the Pong River, between the provinces of Loei and Kon Kaen, central to western Thailand. Glaubrecht & Köhler (2004) attributed specimens also from the Kaek drainage to this species mainly due to a corresponding shell morphology. The description of soft body features is mainly based on these specimens.

Material Examined

Thailand: Prov. Loei, Loei River: Tat Kok Falls at the road 2216 near Wang Saphung (ZMB 200.174); Prov. Phitsanulok, upper course of the Kaek River at Sri Dit Falls (ZMB 200.203).

Differential Diganosis

Shell conical, two or three flattened whorls, smooth sculpture except for growth lines and occasionally fine spiral ridges, spiral lirae lack at base of shell; aperture widely ovate, acute or produced below.

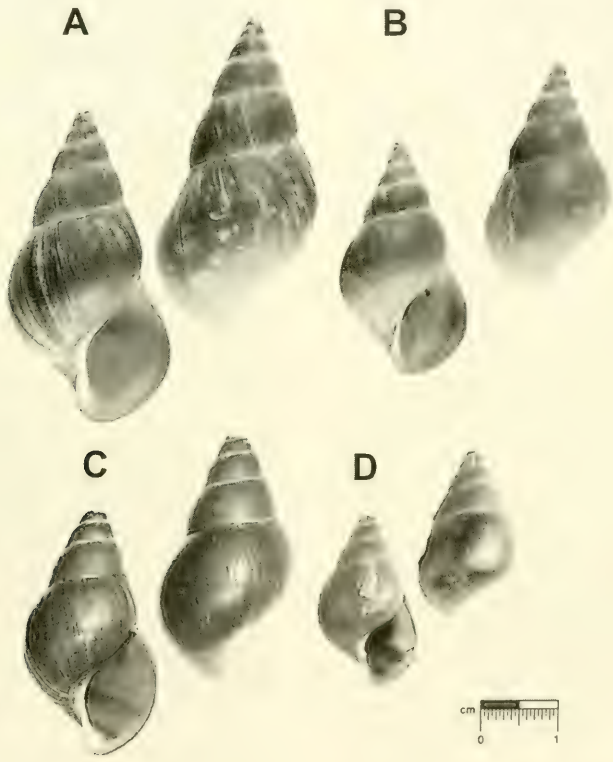

FIG. 63. Shell morphology of *B. solemiana*. A–B: Paratypes SMF 193585; C: Prov. Loei, Tat Kok Falls (ZMB 200.174); D: Kaek River, Sri Dit falls (ZMB 200.203). Scale bar = 10 mm.

Description

Shell (Fig. 63): Medium sized, conical, robust, with two or three flattened whorls, tip eroded; smooth sculpture except for growth lines, in some specimens inconspicuous spiral ridges, spiral lirae lack at base of shell; aperture widely ovate, acute or produced below. Colour yellowish to greenish brown. Size: H = 26–40 mm, B = 18–24 mm.
Operculum: Oval, up to four whorls, sub-central nucleus.
Radula (Fig. 46G): Length of the ribbon: m = 16.0 mm (sd = 3.4 mm; n = 4), 150–160 rows of teeth. Rachidian relatively narrow, otherwise widely corresponding to *B. armata*.
Stomach: Typical, as described for *B. citrina* (Fig. 4).
Embryonic Shell: Unknown.

Distribution

Thailand: Loei Prov.: Pong River, Prov. Phitsanulok: Kaek River at Sri Dit Falls in western most headwater.

Remarks

Brotia pseudosulcospira with more flattened whorls, more conical shell; *B. subgloriosa* generally larger, more turreted; *B. microsculpta* with smaller body whorl, rounded aperture, circular operculum. Radula of *B. solemiana* shorter as in other Kaek River species.

Brotia subgloriosa (Brandt, 1968)
(Figs. 46H, 64, 65)

Brotia binodosa subgloriosa Brandt, 1968: 269, pl. 10, fig. 56, text-fig. 38 ("Thailand: Huai Chieng Nam, tributary of the Kaek River, about 92 km E of Pitsanulok at the bridge of the Friendship Highway"), holotype SMF 19737, 20 paratypes SMF 193572, paratype ZSM 19983213, six paratypes ZSM 19983219, 11 paratypes ZMH (Fig. 64); types seen.
Brotia (*Brotia*) *binodosa subgloriosa* – Brandt, 1974: 175, 176, pl. 13, fig. 28.
Brotia spinata subgloriosa – Köhler & Glaubrecht 2002a: 129.
Brotia subgloriosa – Glaubrecht & Köhler, 2004: 293.

Taxonomy and Systematics

Described as a subspecies of *B. binodosa*, it was stated that both taxa are connected by intermediate morphs (Brandt, 1968). Such intermediates were not found by us among the voucher material examined; their existence is thus contended herein. According to Brandt (1968, 1974), *B. subgloriosa* and *B. binodosa* occur sympatrically in parts of the Kaek River, which conflicts a relation as geographical subspecies. For this reason, *B. subgloriosa* is considered as distinct species, perhaps closely related to *B. binodosa*. According to Glaubrecht & Köhler (2004) this species likely is member of the Kaek River species flock.

Differential Diganosis

Shell elongate turreted, entirely smooth, aperture elongate produced and relatively narrow.

Description

Shell (Fig. 64): Medium sized, solid, elongate turreted; up to five convex, rounded whorls, truncated tip; smooth except for thin growth lines. Colour olive-brown, often covered with dark mineral deposits. Basal whorl relatively large. Aperture wide, elongate, produced below. Size: H = 25–45 mm, B = 16–24 mm.
Embryonic Shell (Fig. 65): Conical, up to 3.5 whorls; smooth sculpture with faint growth lines.

FIG. 64. Shell morphology of *B. subgloriosa*. Paratype ZSM 19983213. Scale bar = 10 mm.

Operculum: Oval, up to five whorls gradually increasing in diameter, nearly central nucleus.

Radula (Fig. 46H): Length of ribbon: 18 mm (n = 1), 220 rows of teeth. Central tooth comparatively broad, glabella very narrow; otherwise corresponding to the radula of *B. armata*.

Reproductive System

Two dried shells (ZSM 19983219) contained 130 and 156 shelled juveniles, respectively that varied in height between 0.5 and 1.5 mm.

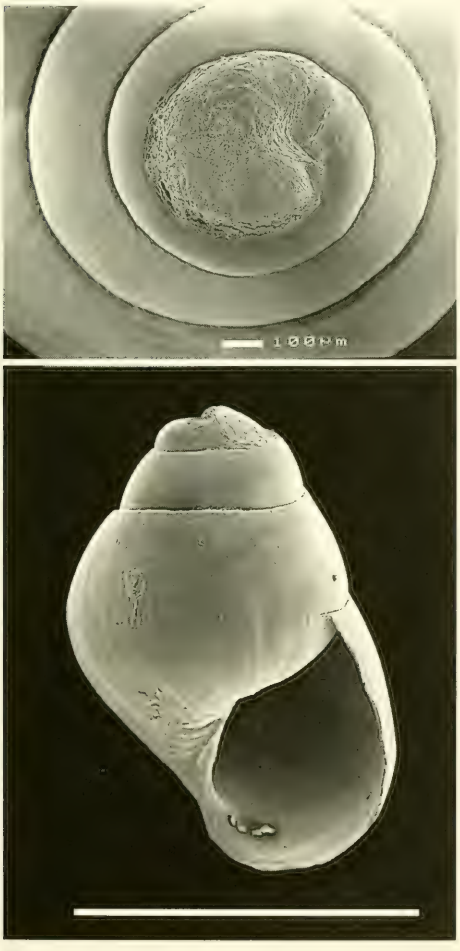

FIG. 65. Embryonic shell morphology of *B. subgloriosa*. SEM images of embryonic shell removed from dried shell (paratype ZSM 19983213); apical and front view. Scale bars = 0.1 mm (above), and 1 mm (below).

Distribution

Thailand: Endemic to Kaek River, between 65 km (at Sopha Falls) and 92 km E of Phitsanulok, and tributary Huai Chieng Nam (Brandt, 1968: 270).

Remarks

Superficially similar to other Thai species with smooth shells. *Brotia microsculpta* much smaller, with comparatively smaller, rounded aperture and operculum; *B. pseudosulcospira* more conical in shape with flattened whorls; *B. solemiana* more compact with comparatively broader but shorter shell.

Brotia sumatrensis (Brot, 1875)
(Figs. 66, 67B–E)

Melania (*Melanoides*) *sumatrensis* Brot, 1875: 87, pl. 10, fig. 2b, pl. 13, figs. 1a, b ("Sumatra: Palembang"), three syntypes MHNG, Brot collection, one syntype MCZ 112689 (Figs. 66A–C); types seen.

Melania sumatrensis – Schepman, 1886: 13.

Melania boeana Brot, 1881: 154, 155, pl. 6, fig. 1 ("Boea, Sumatra" = Bua, Sumatra), lectotype and four paralectotypes MHNG, Brot collection (designated by Köhler & Glaubrecht, 2002a) (Figs. 66F–J); types seen.

Melania (*Brotia*) *episcopalis* – Martens, 1900: 10.

Melania (*Melanoides*) *palembangensis* Strubell, 1897: 12 ("Südsumatra" = South Sumatra); types not seen.

Brotia costula – Benthem Jutting, 1956: 374–378, fig. 76 [partim]; Benthem Jutting, 1959: 92–95 [partim]; Brandt, 1974: 175, pl. 13, figs. 37–39 [partim]; Köhler & Glaubrecht, 2001: 296–299, figs. 1D, 10A–C, G, H [partim] (*non M. costula* Rafinesque, 1833).

Brotia (*Antimelania*) *costula* – Subba Rao, 1989: 108, 109 [partim] (*non M. costula* Rafinesque, 1833).

Brotia variabilis – Rensch, 1934: 239 [partim]; Bequaert, 1943: 433, 434, pl. 33, figs. 11–16 [partim]; Solem, 1966: 15 (*non M. variabilis* Benson, 1836).

Taxonomy and Systematics

Brotia sumatrensis has been subsumed under *B. costula* by most previous authors (see also under that species), but molecular genetic

data shows that the Sumatran species is distinct. Problems of earlier authors to satisfactorily diagnose this species persist to the present due to lack of well-preserved soft body material. Shells examined from various museum collections are remarkably plastic, which may indicate the existence of yet undiscovered, morphologically similar species on Sumatra. This renders a correct characterisation and delineation of *B. sumatrensis* problematic and provisional. For the time being, we assign similar shells to *B. sumatrensis*, as representing the oldest available name. Future studies may reveal a higher diversity of similar *Brotia* species on Sumatra. Brot (1875) struggled with the diagnosis of *B. sumatrensis* and was unsure whether this species should instead be considered a synonym of *M. infracostata* from Java. Schepman (1886: 13, 14, pl.1, figs. 3a, b, 4a, b) described and depicted a new var. *mitescens* for material with smooth shells, using a manuscript name of Martens. This variety is considered a synonym of *M. torquata* for it's rather round, small operculum and fragile shell. *Mela-*

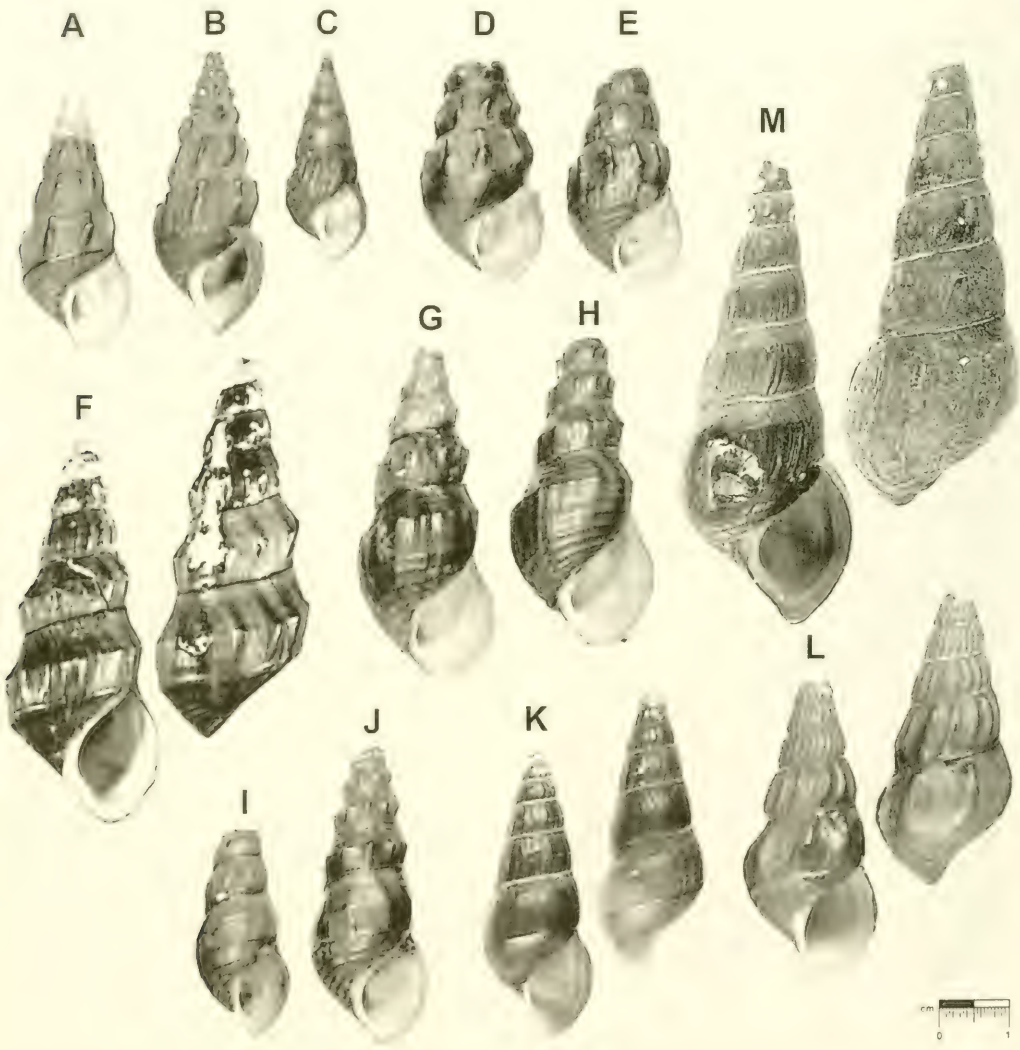

FIG. 66. Shell morphology of *B. sumatrensis*. A–C: Syntypes of *M. sumatrensis* MHNG; D–E: Sumatra (MHNG, coll. Brot); F: Lectotype of *M. boeana* MHNG, front and rear view; G–J: Paralectotypes MHNG; K: Sumatra, Lake Ranau (MZB); L: Sumatra, Jambi (MZB 9013); M: Sumatra, Lake Toba, Parapat (ZMB 200.119).

nia boeana Brot, 1881, is considered a synonym of *B. sumatrensis*, because we are not able to establish a significant distinction. The original series in the MHNG comprises in total seven specimens. Two of them originally are assigned to a var. b and, thus, not qualified as types (ICZN, Art. 72.4.1.). The type locality "Bua" is a common local village name that occurs several times in Sumatra. Consequently, the type locality of this taxon cannot be specified more accurately.

Material Examined

Indonesia: Sumatra (ZMB 200.045; BMNH 1890.2.21.1-4): Tandjung djatti (ZMA); Suengei Ketil, Kampung (ZMA); Sungei Mentjirin near Kampung (ZMA); Kepahiang (ZMB 26.715, 200.039; MHNG); Bengkajang (ZMB 200.040); Sungei Kalau (ZMA); Sungei Minahol (ZMA); Tibitinggi (ZMB 26.717, ZMB 27.680); Demarguri (ZMB 35.819). Prov. Aceh (ZMB 76671; ZMA; MZB 8786): Tributary of Alas River, Ketombe, SE Aceh (MZB 8624); Lake Takengon (ZMB 76.673, 200.136). Prov. Sumatera Utara: Trans-Sumatra highway, bridge 150 km N Bukittingi, 1°28.28'N, 99°19.41'E (ZMB 200.116); Lake Toba, harbour of Parapat, 2°49.17'N, 98°56.22'E (ZMB 200.119); Trans-Sumatra highway, 1°40.04'N, 99°10.05'E (ZMB 200.120); Sungei Belawan (ZMB 51.776); Sungei Kopas, Kisaran, east coast (ZMA); Tandjung Langkat (ZMA); Laut Tawar, N Sibangun (ZMA; ZMB 87.409, 200.124); Bukit Lawang, at the Wisma Cottage (ZMB 200.125); Bukit Lawang (MZB 7058); Bohorok river (MZB; ZMA); Berastagi, Mt. Sinabung, Gunung Leuser NP (ZMB 200124); Medan (NMB); Sungei Rambai near Langkat (ZMA), Sungei Deli near Medan (ZMA). Prov. Sumatera Barat: small stream at Pajakumbuh, N Bukittingi, 0°27.31'S, 100°36.2'E (ZMB 200.122); Danau di Atas (ZMB 200.069, 200.154; RMNH; ZMA); Sumpur (MZB); Ambulutu (MZB 4361); river in Pajakumbuh, N Bukittingi, 0°27.31'S, 100°36.2'E (ZMB 200.122); Pajakumbuh (RMNH); Lake Manindjau (MZB 8632); Lake Singkarah (ZMA). Prov. Riau: Arau River (MZB 9009); Kampar River, Pulau Jadang (MZB 9010). Prov. Jambi (NMB): Lake Kerinci (RMNH; MZB 4901, 9022); Sungei Merangiu, Gunung Raya (MZB 9013). Prov. Sumatera Selatan, Pagaralam (ZMA), Palembang (RMNH; NMB); Lake Ranau (ZMB 76.288-9; MZB); Sungei Lepan, Langkat (ZMA); Simpang (ZMB 76.296); Sumani (ZMB 200.070); Sungei Musi, Muara Klingi (ZMB 76.295), Air Putih, Tjurup (ZMB 76.298). Prov. Lampung (MZB 7028).

Differential Diganosis

Shell elongate turreted, thin but solid, slender, up to nine whorls; sculpture variable, from smooth to ribbed; no marked transition from smooth to ribbed whorls.

Description

Shell (Fig. 66): Relatively large, elongate turreted, slender in shape, up to nine whorls, rather thin. Sculpture variable; whorls either smooth or with axial ribs; no transition from smooth upper to ribbed lower whorls. One colour, chestnut brown. Shell size: H = 24–75 mm, B = 10–27 mm.

Operculum: Oval, four to six whorls, central nucleus.

Radula (Figs. 67B–E): Up to 200 rows of teeth; ribbon length up to 30 mm, corresponding to more than half of shell height. Upper margin of rachidian concave by two inflated, well rounded corners; lower corners slightly angled; glabella slightly v-shaped, narrow, well rounded at base, its lateral margins concave. Cutting edge of rachidian with single main cusp and two or three smaller denticles on each side of it; some specimens with single flanking denticle. Laterals with short lateral extensions, pronounced inner flange, two main cusps flanked by two smaller denticles. Inner and outer marginals with two cusps, pointed, of about same size and shape.

Stomach: Corresponds to *B. episcopalis* (Fig. 26).

Embryonic Shell: Unknown.

Distribution (Fig. 68)

Indonesia: Sumatra.

Habitat

From fast running, clear forest streams with sandy or stony bottom to muddy irrigation channels in rice fields; even in lakes with polluted waters (e.g., harbour of Parapat, Lake Toba).

Remarks

Brotia costula tends to be larger and more elongate, axial ribs are regular; *B. episcopalis* differs mainly by a marked transition from smooth upper whorls to strongly sculptured lower whorls, with lesser pronounced axial ribs.

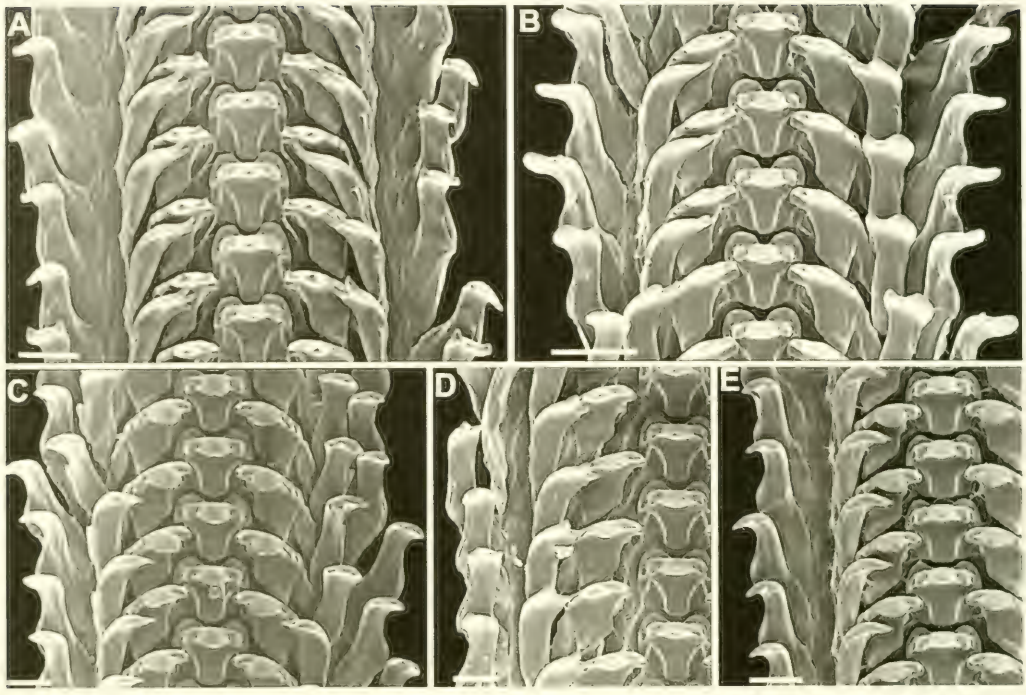

FIG. 67. Radular morphology of *B. episcopalis* and *B. sumatrensis*. A: *B. episcopalis*, Thailand, Nakhon Si Thammarat (ZMH); B: *B. sumatrensis* (Sumatra, Lampung; MZB 7028); C: South Sumatra (ZMB 200.116); D: Sumara, Lake Toba (ZMB 200.119); E: Trans-Sumatra highway (ZMB 200.120).

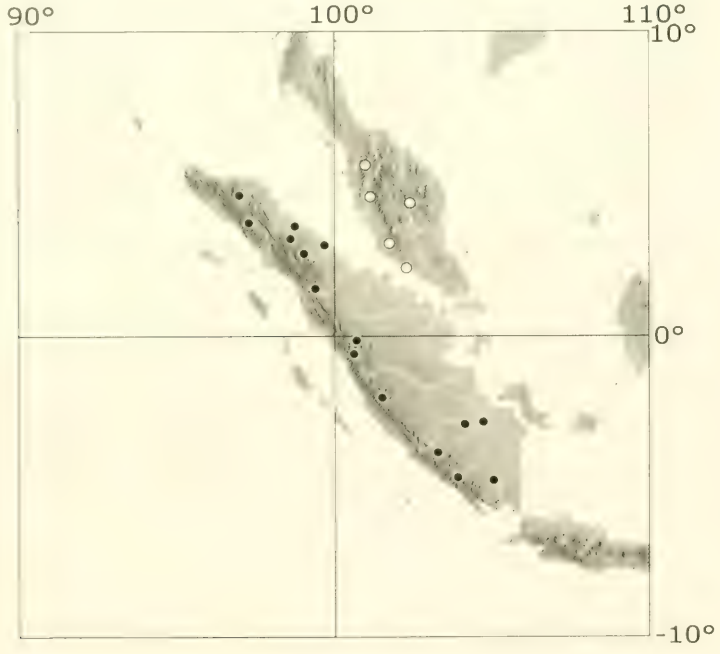

FIG. 68. Distribution of *B. sumatrensis* (close circles) and *B. episcopalis* (open circles).

Brotia torquata (Busch, 1842)
(Figs. 69, 70, 71A)

Melania torquata Busch, 1842 – In: Philippi, 1842: 3, pl. 1, fig. 18 ("Java"), lectotype ÜMB TK 291/1 (designated by Knipper, 1958) (Fig. 69A); type seen; Mousson, 1849: 70; Brot, 1870: 281; Brot, 1875: 110, 111, pl. 14, fig. 5, 5a [partim].

Melanoides torquata – H. Adams & A. Adams, 1854: 297.

Brotia torquata – Köhler & Glaubrecht, 2002a: 150.

Melania zollingeri Brot, 1868: 42, pl. 2, fig. 4 ("Java"), holotype MHNG, coll. Brot (Fig. 69 B); type seen; Brot, 1875: 111, pl. 14, fig. 6; Schepman, 1886: 14; Leschke, 1914: 252; Degner, 1928: 374; Benthem Jutting, 1959: 93.

Brotia zollingeri – Köhler & Glaubrecht, 2002a: 152, fig. 3Q.

Melania subplicata Schepman, 1886: 14, pl. 1, fig. 6 ("Bedar Alam" = Sumatra, SW part of Riau, Bedar Alam, 0°45'S, 102°15'E), lectotype ZMA and four paralectotypes RMNH 71330 (designated by Köhler & Glaubrecht, 2002a) (Figs. 69E, F); types seen; Martens, 1897: 37, pl. 2, fig. 15, pl. 4, fig. 26; Bullen, 1906: 15; Leschke, 1914: 218, 252; Degner, 1928: 374; Benthem Jutting, 1959: 93.

Melania sumatrensis var. *mitescens* Schepman, 1886: 13, 14 ("Soepajang en nabij

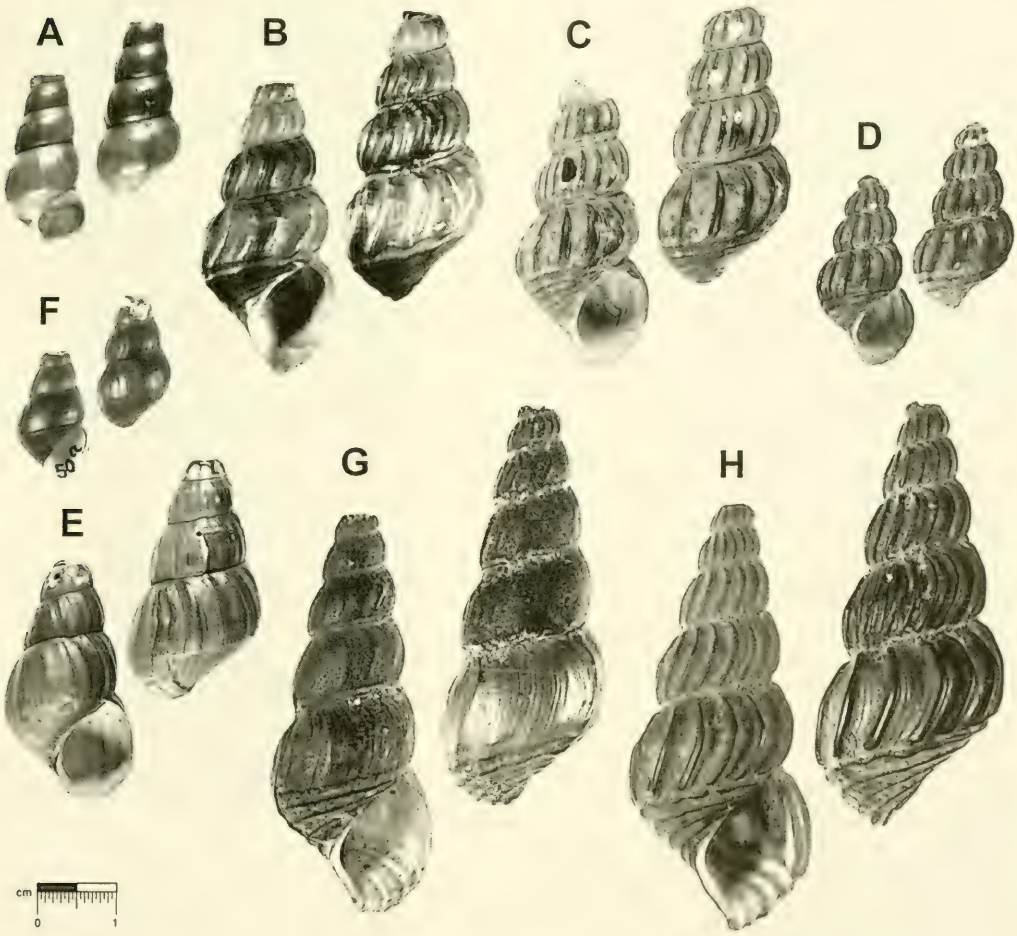

FIG. 69. Shell morphology of *B. torquata*. A: Lectotype of *M. torquata* ÜMB TK 291/1; B: Holotype of *M. zollingeri* MHNG; C: Lectotype of *M. curvicosta* ZMA; D: Paralectotype of *M. curvicosta* ZMA; E: Lectotype of *M. subplicata* ZMA; F: Paralectotype of *M. subplicata* ZMA; G–H: West Sumatra, Fort Kok (RMNH 71331). Scale bar = 10 mm.

Alahan pandjang" = Supajang and Alahan pandjang, nearby); types not seen.

Melania curvicosta Martens, 1897: 36, pl. 2, fig. 14, pl. 4, fig. 27 ("See von Manindjau, Sumatra = Laker Manindjau, Sumatra), lectotype, paralectotype ZMA, 12 paralectotypes ZMA (alc.), three paralectotypes ZMB 54.364 (designated by Köhler & Glaubrecht, 2002a) (Figs. 69C, D); types seen; Bullen, 1906: 15; Degner, 1928: 374.

Melania curvicosta var. *prestoniana* Bullen, 1906: 15, pl. 2, fig. 8; types not seen; Degner, 1928: 374.

Brotia costula – Knipper, 1958 [partim].

Taxonomy and Systematics

Busch (1842) stated type locality to be Java. However, the type is not accompanied by an original label. A newer label states "Bengal", which is not believed to represent the type locality. Likely due to this confusion, Brot (1875) stated that *M. torquata* is conspecific with *M. terebra* Benson, 1836, from Bengal. This is rejected herein, since the latter is a thiarid, neither sympatric with nor even similar to *B. torquata*. Studies of shell series show that several described taxa fall into a joint morphospace and that ribbed and smooth specimens occur syntopically, connected by intermediates. For this reason, these taxa are synonymized herein. Benthem Jutting (1956, 1959), Knipper (1958), and Brandt (1974) treated *M. torquata*, *M. zollingeri*, *M. subplicata*, and *M. curvicosta* as synonyms of *B. costula*. However, *B. torquata* can be distinguished from the latter by its different morphology; additional support is gained from molecular genetics. Rensch (1934: 233) affiliated "*M. zollingeri*" with "*Tiaropsis*". Re-examination of his voucher material in the ZMB shows that Rensch dealt with a thiarid, likely *Melania subcancellata* Boettger, 1890. Consequently, his systematic conclusions are obsolete.

Material Examined

Indonesia: Sumatra (ZMB 200.156). Prov. Sumatera Barat: Rao at the Trans-Sumatra highway, 0°26.7'N, 100°2.4'E (ZMB 200.121); Lake Manindjau (ZMB 54.360, 200.123, 200.147-50, ZMB 200.053; ZMA); Lake Manindjau near Banjur (ZMB 200.131); Lake Manindjau at Manindjau, 0°19'S, 100°22'E (ZMB 200.117); Fort Kok (RMNH 71331).

Differential Diganosis

Mostly small, delicate to thin, smooth or sculptured by convex, closely spaced axial ribs; operculum round; embryonic shells with more or less developed axial ribs from second whorl on; cutting edge of inner marginal teeth with two accessory cusps at inner side.

Description

Shell (Fig. 69): Small, thin, often even fragile, highly turreted, spire mostly eroded; three to five whorls; strong, closely spaced axial ribs, spiral striae at base, or entirely smooth.

FIG. 70. Embryonic shell morphology of *B. torquata*. SEM images of embryonic shell removed from brood pouch (Sumatra, Lake Manindjau; ZMB 200.117); apical and front view. Scale bar = 1 mm.

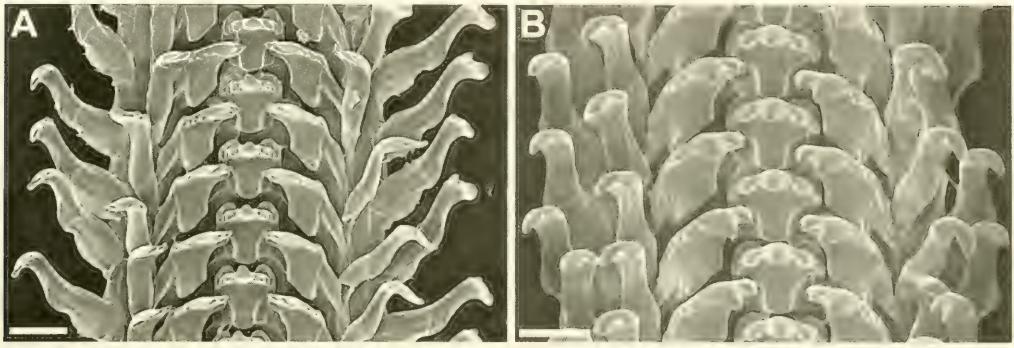

FIG. 71. Radular morphology of *B. torquata* and *B. verbecki*. A: *B. torquata* (Sumatra, Lake Manindjau; ZMB 200.117); B: *B. verbecki* (Sumatra, Lake Singkarah; ZMB 200.118). Scale bars = 10 µm.

When present, axial ribs curved across entire whorl from one suture to the other. Colour chestnut to dark brown. Aperture wide, basely rounded, pointed above. Size: H = 25–48 mm, B = 6–23 mm.

Embryonic Shell (Fig. 70): Conical turreted, three to four whorls; fine vertical growth lines, in some specimens distant axial ribs from the second whorl on. Average proportions: H = 2.4 mm, B = 1.7 mm, HA = 0.22 mm, BA = 0.34 mm, DA = 0.68 mm (for n = 9).

Operculum: Round, with about six regularly increasing whorls, central nucleus, flat, clearly smaller than aperture.

External Morphology: Animal small, up to four whorls; one colour dark grey to black; egg transfer groove beneath right tentacle inconspicuous and short; mantle cavity short occupying about $^2/_3$ of first whorl; osphradium relatively short corresponding to $^1/_3$ to $^1/_2$ of length of ctenidium.

Radula (Fig. 71A): Ribbon with about 100 rows of teeth. Upper margin of rachidian concave by two inflated, rounded corners; lower corners of basal plate rounded; glabella well rounded at base, its lateral margins concave; cutting edge of rachidian with single main cusp flanked by two accessory cups on each side that taper in size. Laterals with main cusp flanked by two inner and three outer denticles. Outer marginals with two pointed denticles, almost equal; inner marginals with pointed outer cusp and one or two inner accessory denticles.

Stomach: Typical, as in *B. citrina* (Fig. 4); except for unfused typhlosoles at entire length of style sac.

Distribution

Indonesia: Java, West-Sumatra. The only known reports from Java refer to types of *M. torquata* and *M. zollingeri*. Either these reports are in error or the species have become extinct or extremely rare. All other material from West-Sumatra.

Remarks

Somewhat similar to *B. verbecki* when considering similar shape and size of shell. Some specimens of *B. verbecki* even exhibit marked axial sculpture otherwise typical for most specimens of *B. torquata*. Shells of *B. torquata* are thinner and generally lack more pronounced spiral elements except for basal lirae. The two species can significantly be discriminated by shell morphometry (Table 4). The report of Leschke (1914) on *M. subplicata* from Bogor is incorrect; the voucher material in the ZMB is re-determined as *Adamietta testudinaria*.

TABLE 4. Result of disriminant analysis of shell parameters of *B. torquata* and *B. verbecki*.

	Predicted group membership	
	B. torquata	*B. verbecki*
B. torquata	10 (90.9%)	1 (9.1%)
B. verbecki	1 (2.6%)	37 (97.4%)

Brotia verbecki (Brot, 1886)
(Figs. 71 B, 72, 73)

Melania verbecki Brot, 1886: 90, pl. 6, figs. 9–9b ("Lac de Singkarah, gouvernement de Padang, Sumatra occid." = Lake Singkarah, Padang distr., West Sumatra), lectotype, 11 paralectotypes MHNG, coll. Brot, paralectotype MCZ 112682 (designated by Köhler & Glaubrecht, 2002a) (Figs. 72A–D); types seen; Martens, 1897: 38.

Melania verbecki var. *laevis* Martens, 1897: 38 (Lake Singkarah), 32 syntypes ZMB 200.152.

Brotia verbecki – Köhler & Glaubrecht, 2002a: 151, 152, fig. 3P.

Melania papillosa Martens, 1897: 38, 39, pl. 2, fig. 21 ("See Singkarah, Sumatra" = Lake Singkarah), lectotype ZMA, 18 paralectotypes ZMA, 15 paralectotypes ZMB 200.025 (designated by Köhler & Glaubrecht, 2002a), (Figs. 72F–K); types seen.

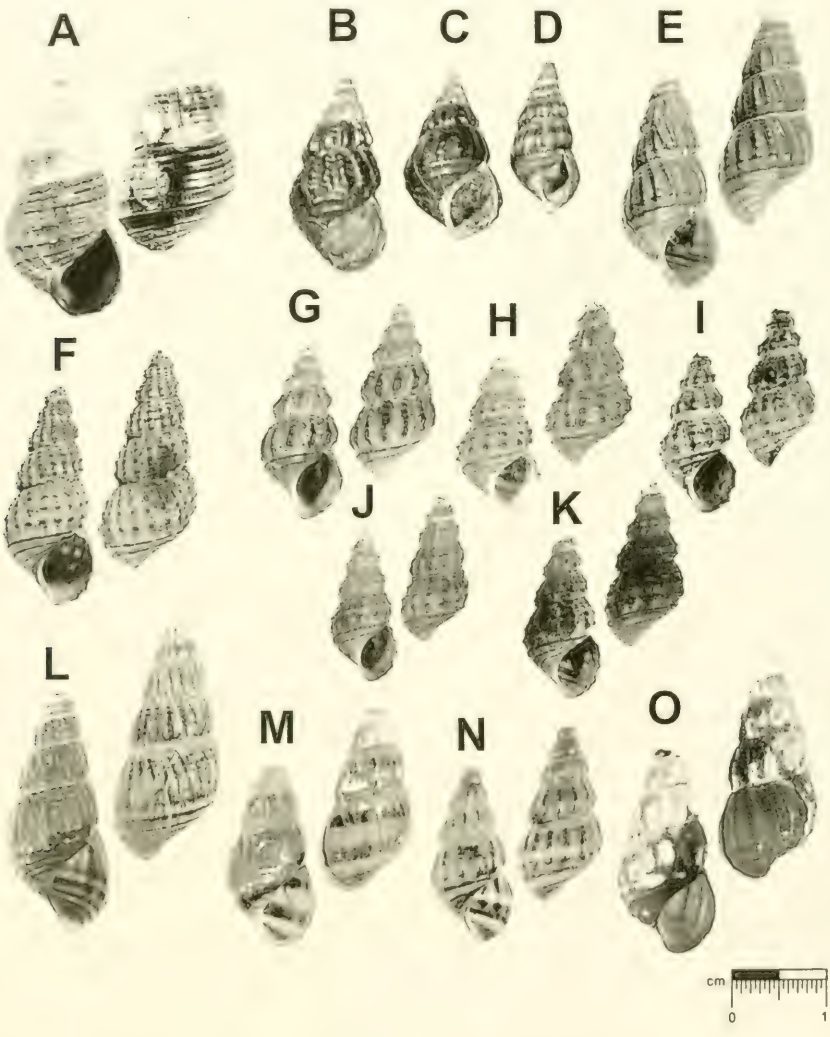

FIG. 72. Shell morphology *B. verbecki*. A: Lectotype of *M. verbecki* MHNG; B–D: Paralectotypes MHNG; E: Lectotype of *M. stricticosta* ZMB 200.102; F: Lectotype of *M. papillosa* ZMA; G–K: Paralectotypes ZMB 200.103; L–O: Sumatra, Lake Singkarah (ZMB 200.118). Scale bar 10 mm.

Melania stricticosta Martens, 1897: 39, 40, pl. 2, figs. 22–26 ("See Singkarah, Sumatra" = Lake Singkarah, Sumatra), lectotype and two paralectotypes of *M. stricticosta* ZMB 200.102, 16 paralectotypes ZMB 200.103, two potential paralectotypes ZMB 200.151 (designated by Köhler & Glaubrecht, 2002a) (Fig. 72 E); types seen.

Taxonomy and Systematics

Brot described this species from Lake Singkarah, West-Sumatra (Indonesia) using a manuscript name of Boettger. From the same locality, Martens (1897) described not only a new var., *laevis*, but also two new species, *M. papillosa* and *M. stricticosta*, for subtle conchological differences. Examination of the type series shows that the named taxa only delineate conchological varieties of a single, albeit somewhat variable species. The different morphs occur frequently and syntopically in the lake with intermediate forms, and there is no evidence that they represent distinct species.

Material Examined

Indonesia, Sumatra: Lake Singkarah (ZMB 76.290-4, 200.118, 200.126, 200.155, 200.157-60).

Differential Diganosis

Shell small, thin but solid, conical to elongate turreted, pronounced sculpture of either strong spiral ridges, axial ribs, or combination of both; frequently with spiny nodules where spiral lines and axial ribs meet; operculum round; embryonic shells frequently with two spiral rows of nodules from second whorl on.

Description

Shell (Fig. 72): Small, relatively thin to delicate, broadly conic to elongate turreted, up to five flattened whorls; more or less prominent spiral lines or cords, especially at base, in some specimens dominating; mostly with strong axial ribs. In several specimens axial rows of three to four tubercles where spiral lines and axial ribs meet. Colour yellowish brown to olive brown. Aperture widely oval, well rounded to slightly produced below. Size: H = 12–36 mm, B = 6–16 mm.

Embryonic Shell (Fig. 73): Conical turreted, three to four whorls; fine vertical growth lines, in many specimens, double spiral row of distant, rounded nodules from second whorl on. Average proportions: H = 2.9 mm, B = 1.7 mm, HA = 0.17 mm, BA = 0.32 mm, DA = 0.66 mm (for n = 6).

Operculum: Round, up to eight whorls, central nucleus, smaller than aperture.

Radula (Fig. 71B): Ribbon with about 100 rows of teeth. Rachidian with slightly concave upper rim by only slightly inflated lateral corners. Cutting edge with main cusp flanked

FIG. 73. Embryonic shell morphology of *B. verbecki*. SEM image of shell removed from brood pouch (Sumatra, Lake Singkarah; ZMB 200.118). Scale bar = 1 mm.

by three smaller denticles on each side. Glabella rather straight at its basal end, with concave lateral margins. Lateral teeth with main cusp flanked by two to three smaller denticles. Inner and outer marginals with two pointed cusps, the outer one being broader.

Stomach: Typical, as in *B. citrina* (Fig. 4); except for both typhlosoles unfused at entire length of style sac.

Reproductive System

Females contained between 19 and 75 shelled juveniles (n = 4), forming cohorts with heights between 1.5 and 2.5 mm.

Distribution

Indonesia, West-Sumatra: Only known from Lake Singkarah.

Brotia wykoffi (Brandt, 1974)
(Figs. 74–76)

Brotia (*Senckenbergia*) *wykoffi* Brandt, 1974: 184, pl. 13, fig. 41 ("Creek at Sai Yok, Kanchanaburi Province"), holotype SMF 197268, four paratypes RMNH 55244/4; types seen.
Brotia wykoffi – Köhler & Glaubrecht, 2002a: 152.

FIG. 74. Shell morphology of *B. wykoffi* (Thailand, Nam Tok; ZMB 200.132).

Taxonomy and Systematics

Brandt (1974) affiliated this species to *Senckenbergia* Yen, 1939, and treated this taxon as a subgenus of *Brotia*. However, *Senckenbergia* is not considered a pachychilid (Köhler & Glaubrecht, 2002a).

Material Examined

Thailand: Prov. Kanchanaburi, Sai Yok Falls 2 (Sai Yok NP), Nam Tok, 14°26.3'N, 98°51.0'E (ZMB 200.131-2).

FIG. 75. Embryonic shell morphology of *B. wykoffi*. SEM images of embryonic shell removed from brood pouch (Thailand, Nam Tok; ZMB 200.132); apical and front view. Scale bar = 1 mm.

Differential Diganosis

Shell smooth, rather small, thin but solid, conical turreted; whorls flattened, only slightly convex; colour olive brown with lightly green spiral bands; aperture inside olive green with yellowish bands.

Description

Shell (Fig. 74): Small, thin but solid; spire conical turreted, up to eight flattened, only slightly convex whorls; smooth sculpture except for growth lines, weak spiral lirae at base. Colour olive brown with lightly green spiral bands. Aperture relatively narrow, angled below, pointed above, inside olive green with yellowish bands. H = 22–30 mm, B = 9–11 mm.

Embryonic Shell (Fig. 75): Broadly ovate, three whorls; smooth sculpture; aperture wide. Average proportions: H = 2.0 mm, B = 1.4 mm, HA = 0.15 mm, BA = 0.28 mm, DA = 0.62 mm (for n = 10).

Operculum: Round, up to eight regular whorls, central nucleus.

Radula (Fig. 76): Ribbon with about 120 rows of teeth. Rachidian with slightly concave upper rim by inflated lateral corners. Cutting edge with one main cusp flanked by two smaller denticles on each side. Glabella straight at its base, relatively long, with straight lateral margins. Laterals with main cusp flanked by two smaller denticles on each side, rather long lateral extensions. Inner and outer marginal teeth with two to three pointed cusps, the outer one being broader.

Stomach: Typical, as in *B. citrina* (Fig. 4); except for both typhlosoles unfused at entire length of style sac.

FIG. 76. Radular morphology of *B. wykoffi*. SEM image of segment viewed from above (Thailand, Nam Tok; ZMB 200.132). Scale bar = 100 µm.

Reproductive System

A female contained 21 juveniles of more or less of same size (ZMB 200.232).

Distribution

Thailand: Prov. Kanchanaburi, known only from type locality (Sai Yok Falls, Nam Tok).

Habitat

Small, tangled, swift stream discharging into Kwae Noi River.

Remarks

Somewhat similar to *B. dautzenbergiana*, which is much larger, juveniles more slender.

INCERTAE SEDIS

In the following, a number of taxa are listed that are members of the Pachychilidae, as can be judged from features of their shell, operculum and/or radula. However, an unequivocal affiliation with *Brotia* is not possible for lack of crucial information on diagnostic features, such as embryonic shell morphology or reproductive anatomy. Because the species originate from localities where other pachychilid genera may occur, such as, for example, *Adamietta*, we refrain from a formal treatment under *Brotia*, although it appears plausible for most of the following taxa that they are members of this genus.

Brotia (?) *angulifera* (Brot, 1872) (Figs. 77A, B)

Melania (*Pachychilus*) *angulifera* Brot, 1872: 32, pl. 2, fig. 9 ("Java"), lectotype and paralectotype MHNG, coll. Brot (designated by Köhler & Glaubrecht, 2002a) (Figs. 77A, B); types seen; Brot, 1875: 51, 52, pl. 6, fig. 5.

Brotia angulifera – Köhler & Glaubrecht, 2002a: 126, 127, fig. 1B.

Taxonomy and Systematics

Benthem Jutting (1956) considered this species a synonym of "*B. testudinaria*". However, the shells of both species are easy to distinguish. *B. angulifera* is considered here a distinct species. Details of soft body, radula, and embryonic shell remain unknown, which hin-

ders a systematic decision. Not identical with *Melania* (*Plotia*) *scabra* var. *angulifera* Martens, 1897.

Differential Diganosis

Shell conical turreted, one colour dark greenish to olive brown with convex, rounded to slightly shouldered whorls, sculptured by fine spiral lirae; spiral depression below suture.

Description

Shell (Figs. 77A, B): Medium sized, oval to conical turreted, solid, with six convex, well rounded to slightly shouldered whorls, narrow suture; with fine spiral lirae and faint vertical growth lines. Colour greenish to olive brown. Body whorl comparatively large. Aperture medium sized, oval, well rounded, slightly produced below. Columella thick. Size of lectotype: H = 33 mm, B = 14 mm.
Embryonic shell morphology, Operculum, Radula, Soft body anatomy: Unknown.

Distribution

Indonesia: Java, the type locality as only known record.

Brotia (?) *assamensis* (Nevill, 1885)
(Figs. 77C, D)

Melania (*Acrostoma*) *assamensis* Nevill, 1885: 271 ("Delaima River, North Cachar"), four syntypes IMC, according to Nevill (1885); types not seen.
Tiara (*Acrostoma*) *assamensis* – Preston, 1915: 31.
Paracrostoma assamensis – Köhler & Glaubrecht, 2002a: 128.

Taxonomy and Systematics

Specimens in the BMNH apparently were not available to Nevill (1885), who mentioned only four specimens in the IMC. Placed in *Paracrostoma* because of a close similarity to its type species, *P. huegeli* (Philippi, 1843), by Köhler & Glaubrecht (2002a) and because *Acrostoma* Brot, 1870, is a synonym of *Paracrostoma* Cossmann, 1900 (Köhler & Glaubrecht, 2002a). However, *Paracrostoma* is endemic to southern India (federal states of Karnataka, Kerala, and Tamil Nadu) and most likely does not occur in Assam, from where otherwise some *Brotia* species are known (unpubl. data). For this circumstantial evidence, we suggest to treat this species as a member of *Brotia*.

Material Examined

India: Assam, Delaima River, North Cachar (= Delaima River, N of Silchar; BMNH 19991534 (12 shells originating from the Godwin-Austen collection, same series as types; Figs. 77C, D).

Differential Diganosis

Shell elongate, conical with rounded whorls, surface smooth and glossy; one colour dark brown, sculptured by faint spiral lirae and growth lines only; aperture wide, angularly produced below; body whorl comparatively large compared to shell height.

Description

Shell (Figs. 77C, D): Medium sized, spire conically turreted, eroded, with three to five slightly convex to flattened whorls, sculpture smooth except for faint spiral lines and growth lines. All one colour, chestnut brown. Aperture elongate oval with produced to slightly angled lower margin, columellar margin inconspicuous, peristome sharp.
Embryonic Shell, Operculum, Radula, Soft Body: Anatomy unknown.

Distribution

India: Assam, Delaima River as the only known locality.

Remarks

Similar to *Paracrostoma huegeli*, but more slender in shape, coloration lacks spiral flames, body whorl not as inflated as in the former. *P. huegeli* lacks glossy surface.

Brotia (?) *beaumetzi* (Brot, 1887)
(Fig. 77H)

Melania beaumetzi Brot, 1887: 34, 35 ("Baie du Touranne", in error, replaced by "environs de Than Moi" by Dautzenberg & Hamonville, 1887 = Thanh Moi, about 200 km NE of Hanoi, Vietnam, 21°37'N, 106°32'E), holotype MNHN (Fig. 77H); type seen; Dautzenberg & Hamonville, 1887: 219; Fischer-Piette, 1950: 160, pl. 5, fig. 4.
Brotia beaumetzi – Köhler & Glaubrecht, 2002a: 129, fig. 1D.

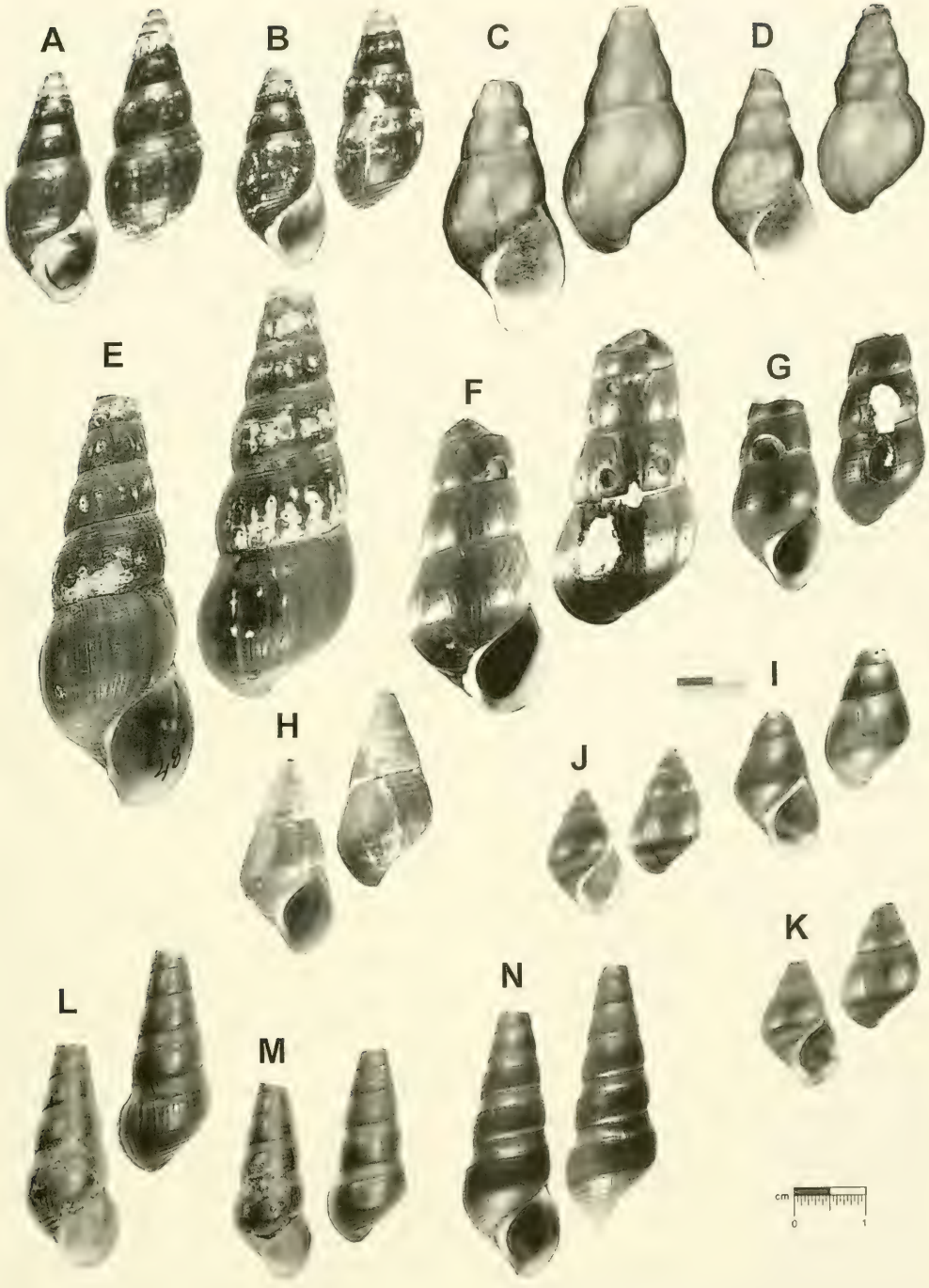

FIG. 77. Shell morphology of several species with uncertain classification. A: Lectotype of *M. angulifera* MHNG; B: Paralectotype MHNG; C–D: *Brotia* (?) *assamensis* (Assam, North Cachar, Delaima River; BMNH 19991534); E: Holotype of *M. borneensis* RMNH 71325; F: Lectotype of *M. cylindrus* MHNG; G: Lectotype of *M. subcylindrica* MHNG; H: Holotype of *M. beaumetzi* MNHN; I: Lectotype of *M. zonata* von dem Busch, 1842 ÜMB TK 271/1; J–K: Paralectotypes ÜMB TK 272/2; L–M: Syntypes of *Melania canaliculata* Reeve, 1869 (BMNH 20050105, ex coll. Cuming, = *Melania sooloensis* Reeve, 1860); N: *Brotia* (?) *solooensis* (Philippines, Sulu Islands; ZMB 59.161).

Taxonomy and Systematics

Brot (1887: 34) gave "Baie du Touranne" as the type locality. This was stated to be incorrect and replaced by "Environs de Than-Moi (leg. M. de Merlaincourt)" (Dautzenberg & Hamonville, 1887), which corresponds to the label of the type material. The species was transferred to *Brotia* by Köhler & Glaubrecht (2002a) because of its characteristic shell.

Differential Diganosis

Shell small, robust, broadly conical with flattened whorls; thin, regularly spaced spiral lirae; distinct by its conical shape, tiny size, keeled basal whorl, fine and regular spiral sculpture.

Description

Shell (Fig. 77H): Small, conical turreted with five flattened whorls, inconspicuous suture; regular spiral lirae. Colour light brownish olive. Aperture oval, angled, produced below, pointed above. Size of holotype: H = 20 mm, B = 10 mm.
Embryonic shell morphology, Operculum, Radula, Soft body anatomy: Unknown.

Distribution

Vietnam: Type locality only known record.

Brotia (?) *borneensis* (Schepman, 1896)
(Fig. 77E)

Melania borneensis Schepman, 1896: 137, 138, pl. 2, fig. 4 ("Borneo"), holotype RMNH 71325 (Fig. 77E); type seen.
Brotia borneensis – Köhler & Glaubrecht, 2002a: 130.

Taxonomy and Systematics

Transferred to *Brotia* by Köhler & Glaubrecht (2002a) because of its characteristic shell. Next to *B. praetermissa*, it would be the second *Brotia* species from Borneo.

Differential Diganosis

Shell relatively large, highly turreted with convex, well rounded whorls, sculptured by spiral lines most conspicuously below suture, and faint axial growth lines; aperture wide, well produced below.

Description

Shell (Fig. 77E): Large, spire elongate turreted, five remaining, regularly rounded, convex whorls; shell solid to thick; colour yellowish-olive; upper whorls sculptured by numerous spiral striae, more conspicuous on last whorl, growth lines inconspicuous. Aperture ovate, well rounded, produced below, pointed above; columellar margin thin, moderately curved; inferior of aperture bluish white. Size of holotype: H = 54.7 mm, B = 20.6 mm.
Embryonic shell morphology, Operculum, Radula, Soft body anatomy: Unknown.

Distribution

Borneo: Type locality only known record.

Brotia (?) *cylindrus* (Brot, 1886)
(Figs. 77F, G)

Melania cylindrus Brot, 1886: 92, 93, pl. 6, figs. 7, 7a ("Siam" = Thailand), lectotype and two paralectotypes MHNG, paralectotype MCZ 11268 (designated by Köhler & Glaubrecht, 2002a) (Fig. 77F); types seen.
Melania subcylindrica Brot, 1886: 102, 103, pl. 6, figs. 2, 2a ("Chine" = China), lectotype and two paralectotypes MHNG (designated by Köhler & Glaubrecht, 2002a) (Fig. 77G); types seen.

Taxonomy and Systematics

The two taxa described by Brot (1886) were considered identical for their similar shell and assigned to *Brotia* by Köhler & Glaubrecht (2002a). Because the types represent the only available material and most morphological properties are unknown, systematics is uncertain.

Differential Diganosis

Turreted shell, truncated spire, well-rounded whorls, sculptured by regularly spaced, fine spiral lines; aperture well rounded, relatively small; one colour dark brown to black.

Description

Shell (Figs. 77F, G): Highly turreted, frequently truncated after second or third whorl, whorls well rounded in diameter, sculptured by regularly spaced, fine spiral lines; aperture well

rounded below, relatively small compared to body whorl; one colour dark brown to black. Shell size: H = 27.5–42 mm, B = 13.5–19.7 mm.

Embryonic shell morphology, Operculum, Radula, Soft body anatomy: Unknown.

Distribution

Vague: "Siam" and "China" as only known records.

Brotia (?) *sooloensis* (Reeve, 1859)
(Figs. 77L, M)

Melania canaliculata Reeve, 1859: pl. 6, species 31 (*non M. canaliculata* Say, 1821) ("Sooloo Islands" = Sulu Islands, Philippines); two syntypes BMNH 20050105 (Sulu Islands, ex coll. Cuming) (Fig. 77L, M), types seen.

Melania sooloensis Reeve, 1860: errata; Brot, 1870: 281; Brot, 1875: 105, 106, pl. 14, fig.3.

Brotia sooloensis – Köhler & Glaubrecht, 2002a: 147, 148.

Taxonomy and Systematics

The name *M. sooloensis* was employed by Reeve (1860: errata) as replacement name for *M. canaliculata* Reeve, 1859, being preoccupied by *M. canaliculata* Say, 1821. The systematic affinity is suspicious due to unknown properties of soft body, embryonic shell, radula, and operculum. Herein preliminarily affiliated with *Brotia*, but this treatment requires critical revision as the Zulu Archipelago, Philippines, is not within the range of *Brotia* as defined here.

Material Examined

Philippines: Sulu Islands (MHNG, coll. Taylor; ZMB 59.161); Cagayan (MHNG; coll. Norris); Isabella (MHNG; leg. Semper), herein restricted to Isabela, Basilan (6°41'N, 118°58'E).

Differential Diganosis

Shape of shell unmistakable; in particular elongate spire, stepped whorls, subsutural depression or shoulder.

Description

Shell (Fig. 77L–N): Elongate turreted, solid but not thick, up to six whorls, deep suture, mostly truncated tip; whorls well rounded at base, upper whorls convex but more flattened than basal ones; subsutural depression, most prominent on last two or three whorls; smooth sculpture, basal spiral ridges, faint growth lines, faint spiral lines; surface glossy. Aperture oval, well rounded below. Shell size: H = 31–38 mm, B = 13–15 mm.

Embryonic shell morphology, Radula, Soft body anatomy: Unknown.

Distribution

Reports on this species refer to Sulu Islands, Philippines; neither known from Mindanao nor Borneo. Two islands in the Sulu Sea are named Cagayan. The island Cagayan-Sulu (material in MNHG) in N of Borneo (Sarawak), more than 300 km W of Sulu archipelago (6°59'N, 118°28'E); Cagayan Island in central Sulu Sea is even more remote, between Palawan and Negros (9°35'N, 121°28'E), about 600 km NW of the Sulu archipelago. Occurrence on both islands seems dubious and requires confirmation.

Remarks

Somewhat similar are species of *Pseudopotamis* (Glaubrecht & Rintelen, 2003). Well preserved material of *B. sooloensis* is needed to clarify its systematic position.

Brotia (?) *spinata* (Godwin-Austen, 1872)

Melanoides spinata Godwin-Austen, 1872: 514, pl. 30, figs. 1, 1a ("Kopili River, North Cachar hills, a tributary of the Brahmaputra" = Kopili River, Jaintia-Khâsi hills N of Silchar, federal state of Meghalaya, India); types not seen; Hanley & Theobald, 1874: pl. 109, fig. 1.

Melania spinata – Brot, 1875: 89, 90, pl. 10, figs. 2, 2a.

Melania (*Melanoides*) *spinata* – Nevill, 1885: 261.

Brotia spinata – Köhler & Glaubrecht, 2002a: 148 [partim].

Taxonomy and Systematics

Type material was not traced. Shell only known from original figure. Attributed to *Brotia* by Köhler & Glaubrecht (2002a) as being typical for *Brotia*. Geographical distribution well within range of the genus. Köhler & Glaubrecht (2002a) assumed that *B. binodosa* is conspecific for the similar shell. However, in their re-

vision of the Kaek River species flock, Glaubrecht & Köhler (2004) show that *B. binodosa* is endemic to central Thailand and, thus, not conspecific with *B. spinata*.

Differential Diganosis

Highly turreted shell with two spiral rows of spiny nodules supported by more or less prominent spiral cords; body whorl large compared to shell; aperture wide, produced below.

Distribution

India, Meghalaya: Known from type locality only.

Remarks

Similar to *B. binodosa*, which has a more slender shell.

Brotia (?) zonata (Benson, 1836)
(Figs. 77I–K)

Melania zonata Benson, 1836: 747 (no figure); types not seen.
Melania zonata Busch, 1842 – In: Philippi, 1842: 3, pl. 1, fig. 12 ("Bengalia"), lectotype ÜMB TK 271/1, two paralectotypes ÜMB TK 272/2 (designated by Knipper, 1958) (Figs. 77I–K); types seen.
Melanella zonata – H. Adams & A. Adams, 1854: 296.
Brotia zonata – Köhler & Glaubrecht, 2002a: 152.

Taxonomy and Systematics

Benson described this species from a collection of freshwater shells originating from Bengal and Sylhet, but did not explicitly mention a type locality. *Melania zonata* Busch (1842) was stated to be junior synonym by objective homonymy (Reeve, 1859; Brot, 1875; Knipper, 1958; Köhler & Glaubrecht, 2002a).

Differential Diganosis

Shell rather small, broadly conical, truncated after third whorl, strong, sculpture smooth except for growth lines, glossy surface, two chestnut brown spiral bands, aperture widely oval and well produced below.

Description

Shell (Figs. 77I–K): Relatively small, broadly conical with three whorls, shell robust; sculpture smooth except for faint growth lines, body whorl comparatively large; colour greenish brown with chestnut brown spiral bands; aperture oval, wide inside whitish with brown bands.
Embryonic shell morphology, Radula, Soft body anatomy: Unknown.

Distribution

India, Bangladesh: Bengal.

Remarks

Similar to *B. pseudosulcospira* and *B. microsculpta* in its smooth and conical shell; the spiral brown band being unique, though.

MOLECULAR GENETICS

Sequence Analysis

Separate sequence alignments comprise 646 bp (COI) and 826 bp (16S), respectively. Plotting rates of transitions (s) and transversions (v) against sequence divergence for both genes separately indicates that sequences are not saturated and, thus, accommodate phylogenetic analyses. A partition homogeneity test as implemented in PAUP* showed that the two data partitions (COI and 16S) are not significantly incongruent at the 99% level ($P < 0.01$). The analysis software MrModeltest (Nylander, 2002) revealed an invariant + gamma distributed model of sequence evolution (GTR+I+Γ; Gu et al., 1995) as the best fitting model for both sequence data sets. Accordingly, this model was chosen to calculate pair wise genetic distances shown in Table 5. The model was also implemented in distance based analyses (NJ and BI). Pair wise genetic distances were calculated separately for each of the partial genes. With one exception, in COI infraspecific distances usually do not exceed 16% (in *B. citrina*) and mostly range between 0 and 6%. The high sequence divergence in *B. sumatrensis* is very striking. Since a similar divergence is not observed in 16S, we assume that the one sequence of *B. sumatrensis* high-

TABLE 5. Genetic distances (GTR+I+Γ) within and between *Brotia* species for COI (upper rows) and 16S (lower rows) (N = number of sequences analysed per species; printed in bold = infraspecific distances; * dubious value, see discussion).

		N	*armata*	*binodosa*	*citrina*	*dautzen-bergiana*	*henriettae*	*herculea*	*micro-sculpta*	*pagodula*	*peninsularis*	*solemiana*	*suma-trensis*	*torquata*
armata	COI		**0–0.07**	0–0.06	0.17–0.26	0.19–0.22	0.18–0.22	0.19–0.24	0.00–0.06	0.24–0.29	0.16–0.20	0.06–0.08	0.17–1.38*	0.17–0.19
	16S	6	**0–0.03**	0.01–0.03	0.14–0.18	0.21–0.23	0.16–0.17	0.17–0.20	0.01–0.02	0.17–0.19	0.14–0.15	0.02–0.03	0.10–0.12	0.11–0.12
binodosa	COI			**0–0.15**	0.16–0.26	0.20–0.21	0.18–0.22	0.22–0.25	0–0.06	0.24–0.28	0.16–0.20	0.05–0.08	0.17–0.20	0.13–0.14
	16S	5		**0–0.02**	0.14–0.17	0.0–0.23	0.15–0.16	0.17–0.19	0–0.02	0.17–0.19	0.13–0.14	0.01–0.02	0.10–0.12	0.11–0.12
citrina	COI				**0–0.16**	0.17–0.20	0.19–0.25	0.18–0.25	0.17–0.26	0.14–0.18	0.14–0.18	0.18–0.27	0.17–2.41*	0.18–0.26
	16S	2			**0–0.10**	0.19–0.22	0.19–0.20	0.14–0.19	0.14–0.17	0.10–0.15	0.17–0.18	0.14–0.18	0.20–0.22	0.20–0.24
costula	COI		0.22–0.24	0.22–0.23	0.19–0.25	0.25	0.25–0.26	0.25–0.26	0.22–0.24	0.23	0.17–0.19	0.24	0.18–1.36*	0.16–0.18
	16S	1	0.10–0.11	0.10–0.11	0.16–0.17	0.25–0.26	0.16	0.19	0.10	0.20	0.16	0.10	0.14	0.14
dautzenbergiana	COI					**0–0.02**	0.22	0.16–0.17	0.20–0.22	0.21–0.24	0.15	0.18–0.20	0.19–1.68*	0.16–0.18
	16S	3				**0–0.02**	0.20–0.22	013–0.15	0.20–0.23	0.20–0.22	0.23–0.24	0.19–0.21	0.23–0.24	0.27–0.29
henriettae	COI						**0–0.05**	0.20–0.23	0.19–0.21	0.22–0.24	0.17–0.21	0.18–0.22	0.17–1.32*	0.17–0.20
	16S	2					**0–0.02**	0.17–0.19	0.15–0.16	0.21–0.22	0.17–0.19	0.15–0.16	0.18–0.19	0.21–0.22
herculea	COI							**0–0.09**	0.20–0.24	0.21–0.27	0.15–0.16	0.21–0.25	0.23–1.92*	0.21–0.25
	16S	5						**0–0.05**	0.16–0.18	0.18–0.20	0.17–0.20	0.15–0.16	0.20–0.21	0.22–0.24
microsculpta	COI								**0–0.05**	0.26–0.29	0.16–0.20	0–0.08	0.17–1.38*	0.17–0.20
	16S	3							**0–0.02**	0.16–0.19	0.13–0.15	0–0.02	0.10–0.11	0.10–0.12
pagodula	COI									**0–0.13**	0.17–0.18	0.24–0.28	0.19–1.58*	0.18–0.23
	16S	2								**0–0.10**	0.18–0.20	0.15–0.18	0.20–0.23	0.23–0.25
peninsularis	COI										**0–0.02**	0.16–0.20	0.17–1.55*	0.16–0.17
	16S	2									**0–0.005**	0.12–0.13	0.20	0.18–0.19
pseudosulcospira	COI		0.00–0.06	0.02	0.14–0.17	0.22	0.18–0.22	0.21–0.25	0.02 0.06	0.27 0.28	0.16 0.19	0.06 0.07	0.18–1.29*	0.17 0.19
	16S	1	0.00–0.04	0.01–0.03	0.15–0.17	0.22–0.23	0.16	0.19–0.20	0.01–0.02	0.18–0.20	0.15	0.01–0.02	0.11	0.12
solemiana	COI											**0–0.06**	0.17–1.31*	0.16–0.18
	16S	2										**0–0.01**	0.10	0.10
sumatrensis	COI												**0–0.49***	0.10–0.98
	16S	2											**0–0.004**	0.08–0.09
torquata	COI													**0–0.06**
	16S	2												**0–0.03**
verbecki	COI		0.17–0.21	0.17	0.20–0.29	0.20	0.19–0.20	0.23–0.24	0.17–0.20	0.23–0.25	0.21–0.22	0.18–0.19	0.10–0.93*	0.06
	16S	1	0.10–0.11	0.11	0.18–0.21	0.25–0.26	0.20–0.21	0.20–0.21	0.10–0.11	0.21–0.22	0.19	0.10	0.08	0.05–0.06
wykoffi	COI		0.21–0.22	0.21–0.22	0.19–0.25	0.20–0.22	0.19–0.21	0.21–0.23	0.21–0.22	0.24–0.27	0.17	0.18–0.21	0.19–1.57*	0.07
	16S	1	0.10–0.17	0.17	0.18–0.20	0.24	0.13	0.19	0.17	0.23–0.25	0.18	0.17	0.22	0.25–0.26

TABLE 6. Sequence data analysed in this study with GenBank accessions and inventory numbers.

Genus	Species	Inventory No.	Origin	COI	16S
Adamietta	*A. hainanensis*	ZMB 200.301	Hong Kong	AY 330827	AY 330778
	A. housei	ZMB 200.165	Thailand	AY 330823	AY 330774
	A. provisoria	ZMB 200.053	Borneo	AY 242951	AH 012869
	A. testudinaria	ZMB 190.415	Java	AY 330825	AY 330777
		ZMB 190.416	Java	AY 330826	AY 330776
		ZMB 200.099	Java	AY 330824	AY 330775
		ZMB 200.100	Java	AY 242950	AY 242949
Brotia	*B. armata*	ZMB 200.193	Thailand	AY 330853	AY 330810
		ZMB 200.252	Thailand	AY 330854	AY 330809
		ZMB 200.254	Thailand	AY 330834	AY 330808
		ZMB 200.265	Thailand	AY 330855	AY 330806
		ZMB 200.268	Thailand	AY 330837	AY 330807
		ZMB 200.268a	Thailand	AY 330856	AY 330811
	B. binodosa	ZMB 200.192	Thailand	AY 330857	AY 330815
		ZMB 200.202	Thailand	AY 330859	AY 330819
		ZMB 200.267	Thailand	AY 330860	AY 330818
		ZMB 200.269	Thailand	AY 330861	AY 330820
		ZMB 200.328	Thailand	AY 330858	AY 330816
	B. citrina	ZMB 200.207	Thailand	AY 330829	AY 330798
		ZMB 200.212	Thailand	AY 330830	AY 330799
	B. costula	ZMB 112.660	Nepal	DQ 284985	DQ 284986
	B. dautzenbergiana	ZMB 200.226	Thailand	AY 330831	AY 330802
		ZMB 200.229	Thailand	AY 330832	AY 330800
	B. henriettae	ZMB 200.210	Thailand	AY 330845	AY 330793
		ZMB 200.221	Thailand	AY 330846	AY 330794
	B. herculea	ZMB 200.206	Thailand	AY 330841	AY 330787
		ZMB 200.209	Thailand	AY 330842	AY 330789
		ZMB 200.219	Thailand	AY 330843	AY 330790
		ZMB 200.220	Thailand	AY 242972	AY 242971
		ZMB 200.253	Thailand	AY 330844	AY 330788
	B. microsculpta	ZMB 200.191	Thailand	AY 330836	AY 330805
		ZMB 200.200	Thailand	AY 330833	AY 330804
		ZMB 200.266	Thailand	AY 330835	AY 330803
	B. pagodula	ZMB 200.205	Thailand	AY 330847	AY 330795
		ZMB 200.208	Thailand	AY 172453	AY 172443
	B. peninsularis	ZMB 200.046	Thailand	AY 330850	AY 330792
		ZMB 200.242	Thailand	AY 330841	AY 330791
	B. pseudosulcospira	ZMB 200.196	Thailand	AY 330862	AY 330797
	B. solemiana	ZMB 200.174	Thailand	AY 330849	AY 330814
		ZMB 200.203	Thailand	AY 330848	AY 330812
	B. sumatrensis	ZMB 200.116	Sumatra	AY 330838	AY 330784
		ZMB 200.119	Sumatra	AY 330840	AY 330785
	B. torquata	ZMB 200.117	Sumatra	AY 330864	AY 330781
		ZMB 200.121	Sumatra	AY 330865	AY 330782
	B. verbecki	ZMB 200.118	Sumatra	AY 330863	AY 330779
	B. wykoffi	ZMB 200.232	Thailand	AY 330866	AY 330796
Paracrostoma	*P.* spec.	ZMB 200.318	South India	AY 330821	AY 330770
	P. spec.	ZMB 200.322	South India	AY 330822	AY 330773
Jagora	*J. asperata*	ZMB 200.311	Philippines	AY 172447	AY 172439
	J. dactylus	ZMB 200.109	Philippines	AY 172444	AY 172438

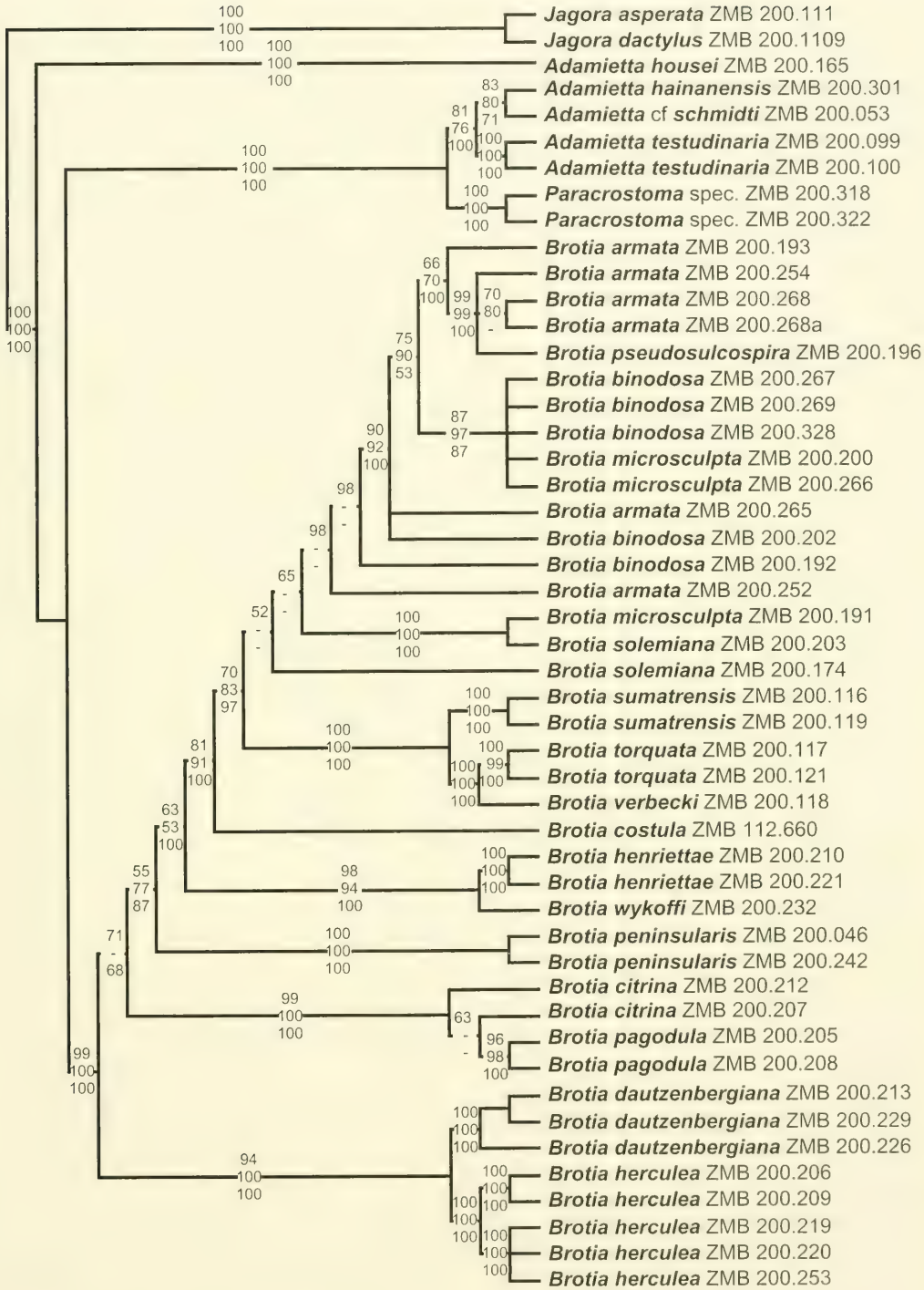

FIG. 78. Confidence limits on the topology of the MP strict consensus cladogram of concatenated data set of 16S and COI expressed by branch support values mapped on respective branches (above: MP bootstrap values, middle: NJ bootstrap values, below: BI posterior clade probabilities).

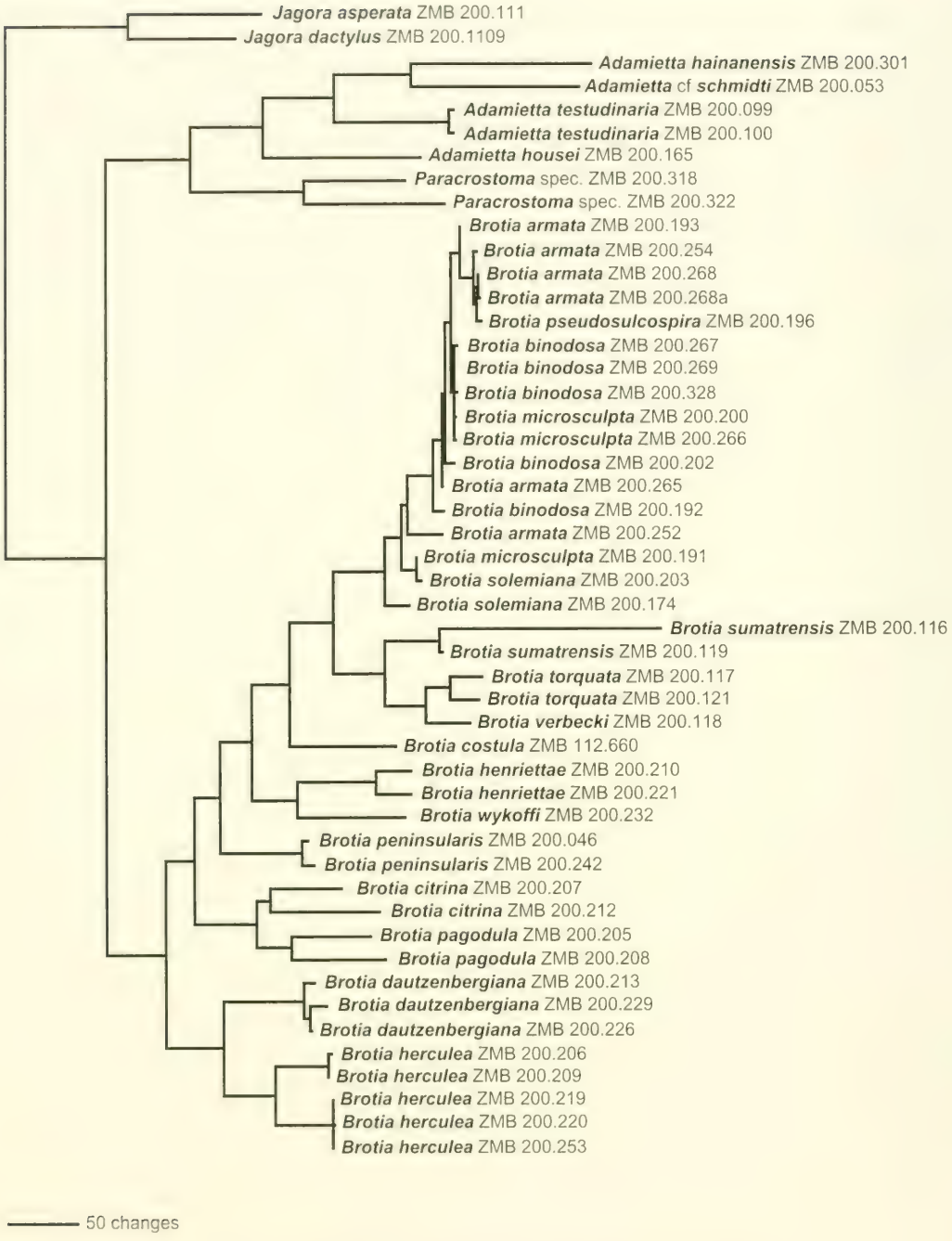

FIG. 79. Bayesian inference phylogram of concatenated data set of 16S and COI.

lighted as genetically very distinct is deficient. It will thus not be considered in further discussion.

Pair wise genetic distances between different *Brotia* species range between the maxima of 8 and 29% when considering species from outside the Kaek River, and between 0 and 8% when comparing endemic species in the Kaek River.

In COI, pair wise genetic distances between species from different genera are even higher, with 28–40%, when comparing *Jagora* and *Brotia*, 20–47% when comparing *Adamietta* and *Brotia*, and 24–42% when comparing *Paracrostoma* and *Brotia*.

Similar genetic distances are observed also in 16S with an infraspecific level of sequence divergence of up to 10%, interspecific distances of up to 8% among Kaek River species and up to 25% between other *Brotia* species as well as divergence rates between 30 and 56% when comparing species of different genera with each other.

Phylogenetic Analyses

The concatenated sequence data set was analysed using MP, NJ, and BI methodology.

603 positions of the concatenated data set with a total length of 1,472 bp are constant, 222 variable but parsimony uninformative, and 647 variable and parsimony informative. MP analysis delivers 6 most parsimonious trees; the strict consensus tree is shown in Fig. 78 (numbers mapped on the tree indicate branch support for the depicted topology by MP bootstrap values [above lines], NJ bootstrap values [on lines], and Bayesian posterior clade probabilities [below lines], respectively). All trees were rooted with species of *Jagora* as outgroup since this genus is among the most basal groups among the Pachychilidae (Köhler et al., 2004). The topologies of two distance based trees, the NJ phylogram (not shown) and the BI phylogram (Fig. 79), do widely correspond to the MP tree. However, in contrast to the MP tree, these reconstruction show both *Adamietta* and *B. citrina* as monophyletic groupings, the latter being sister to *B. pagodula*.

All trees corroborate the monophyly of *Brotia* as delineated according to morphological characteristics in respect to the other pachychilid genera included into the analysis, that is, *Jagora*, *Paracrostoma*, and *Adamietta*. The monophyly of *Adamietta* is not unambiguously corroborated since it is not shown as a monophylum in the MP tree. However, this is not relevant in regard to the monophyly of *Brotia*. Within *Brotia* some well supported subgroupings are shown, such as the Kaek River species flock, and a Sumatra clade. Only the species of the Kaek River species flock do not appear as monophyletic entities in either of the trees. These species also show low genetic distances, as has been mentioned above.

DISCUSSION

Evaluation of Morphological Characters

(1) Adult Shell

Traditionally, in classifications and taxon descriptions of gastropods the shell is emphasized. It bears many characters that are most convenient for taxonomic purposes and that are accessible even from dry material and fossils (for examples, Smith, 1981; Ridgeway et al., 1998). Also in pachychilids, shell features are essential to distinguish species, and morphometry is often useful for species discrimination. On the other hand, the shell may particularly be prone to environmental pressures such as wave action (Reid, 1986: 8 for *Littorina*) or predation (Vermeij & Covich, 1978) by birds (Reed & Janzen, 1999) or by crabs and crayfish (Reid, 1992; Warner, 1996). Therefore, divergent shells may represent just phenotypic variation. In addition to that, even relatively complex shell structures may have evolved in analogy as has been discussed for the clausilial apparatus in the Clausiliidae (Moorsel et al., 2000).

Shell features, such as shape, size, thickness, and sculpture, vary considerably among species of *Brotia*. This diversity provoked earlier authors to describe many new species based solely on the shell, and a few of them carried this to excess by introducing a vast number of taxonomic names for subtle conchological differences (e.g., Nevill, 1885). This procedure reflects the essentialist view of many systematists at this time (see Haffer, 1997 for examples from ornithology; Glaubrecht, 2004, for malacology).

Since the 1930's, when authors began to acknowledge the existence of intraspecific variation, it has frequently been assumed that

Brotia species are remarkably plastic not only in their phenotypic appearance. Similar taxa have in the following been considered conspecific, which has bloated the synonymies (e.g., Rensch, 1934; Benthem Jutting, 1956; Brandt, 1974). However, in many cases it remained unclear (and unattended) to which extent shell parameters really varied within single species. Most recent data suggests that intraspecific variability of morphological characters including the shell frequently was overemphasized, which has lead to erroneous taxonomic conclusions. This has been exemplified also for other pachychilids, such as *Jagora* by Köhler & Glaubrecht (2003). Consequently, one of the main results of the current study is the conclusion that in *Brotia*, 20th century authors have frequently gone too far in synonymizing taxa for exhibiting a similar shell. Instead, a quite contrasting picture is revealed herein showing that *Brotia* species in general are much more restricted be means of their morphological variability as well as their distributional range than assumed before.

Shell Shape: Most pachychilid species have highly turreted shells with about up to 12 whorls. This feature is found in all major clades as a predominant character. Few species have conical or even globular shells, such as *B. armata*, *B. paludiformis*, or *B. pagodula*. These species live attached to stones and boulders in swiftly flowing streams while other species are found buried in or crawling on substrata of all kinds.

It has been shown by Urabe (1998) for *Semisulcospira reiniana* that individuals inhabiting riverine habitats have a more conical shell than specimens from stagnant waters as a phenotypic response to environmental pressures. Although this observation refers to phenotypic responses only, a conical shell can be considered as adaptation to strong water currents repeatedly obtained by Asian pachychilids.

Size and Thickness: In general, shell size and thickness may be controlled by the availability of nutrients (Frömming, 1956), but also by the harshness of physical environmental factors (Vermeij, 1972), parasitism (Wright, 1966), or predation (Zipser & Vermeij, 1978; Reimchen, 1982; Reid, 1986). Nevertheless, there is substantial evidence that shell growth rate and adult size are also under genetic control (Vermeij, 1980). In *Brotia*, variability in shell size among conspecific specimens of same age is considered lower than formerly supposed. Only in few cases, shells may vary for about the twofold between populations from different environments: Specimens of *B. torquata* from Lake Manindjau are considerably smaller than those from adjacent rivers. In other cases, however, inhabitants of lakes are larger than riverine forms (e.g., *B. sumatrensis* from Lake Toba). A possible explanation could include the limitation of certain nutrients due to interspecific competition in one case and the presence of predators, such as shell crushing crabs as discussed for *Tylomelania* in Sulawesi (Rintelen et al., 2004) or simply the fact that large shells are prone to dislodgement in rivers but not in lakes in the other case.

Sculpture: Freshwater gastropods in general are notorious for their plasticity in form and sculpture (e.g., Davis, 1971; Fretter & Graham, 1984; Urabe, 2000). Similarly, among *Brotia* shell sculptures vary considerably and are used as a conspicuous feature to distinguish among species. Shells may be completely smooth or sculptured by strong axial ribs, spiral cords, spiny nodules, and/or spines. The degree of intraspecific variability, however, seems to differ greatly. In general, variation of the shell morphology, and thus also sculpture, has been considered to have a genetic basis and a strong sculpture shall be adaptive against predators or physical environmental factors (e.g., West & Cohen, 1996). It has been shown that sculptured shells are more tolerant of a crushing load than are smooth shells with the same shell mass (Urabe, 2000). Some studies have further demonstrated that shell morphology shows a great deal of phenotypic plasticity controlled by physical or biological factors (e.g., De Wolf et al., 1997), such as the substratum (Urabe, 2000). While phenotypic plasticity within single species has not been addressed in this study, it can be confirmed that shell form and sculpture are correlated to the substratum: species with smooth shells were always found on sandy or pebble substrata, whereas species with armed shells live on gravel, stony bottoms or sit on boulders (Glaubrecht & Köhler, 2004, for *Brotia* species of the Kaek River). It is assumed that a sculpture not only prevents the animals from being preyed upon, which seems to be a rather imaginary threat when sitting directly in the water current, but

from the influence of physical forces. A well-developed sculpture, however, is unfavourable when crawling in the sand as it would increase the friction with the substratum. Accordingly, different shell sculptures may have evolved as result of ecological and morphological diversification, in some cases induced by competitive interaction between the different species.

Colour: In *Brotia* shell colour is uniform, from yellowish brown to olive brown, dark brown or almost black and overall not very helpful for species recognition. In some species, dark spiral bands may be present; axial flames that can be observed in other pachychilids, such as *Pachychilus*, *Adamietta,* and *Paracrostoma*, are generally lacking.

(2) Embryonic Shell

Brotia shows a remarkable modification of the ontogeny that is also imprinted in the embryonic shell structure (Köhler & Glaubrecht, 2001): In early ontogenetic stages, soft tissue protrudes from the apical whorl of the forming shell. This tissue is believed to have nutritive function for the encapsulated embryo. A secondary shell layer closes at the apex not before this tissue is entirely consumed. Protruding tissue and uncalcified apex was first noted by Morrison (1954) in embryonic shells of "*Brotia baccata*" (= *B. henriettae*). The uncalcified apex was called by him an "open" or "soft apex" and stated to be a characteristic feature of *Brotia*. Subsequently, Solem (1966: 16, fig. 1) depicted several embryonic stages of *B. binodosa* with protruding soft tissue and open apex; this was followed by a report an "asymmetric" apical portion of the juvenile shell of *B. episcopalis* (Davis, 1971: fig. 11).

A storage structure similar to the tissue observed in *Brotia* was described for numerous other "prosobranchs", functioning as a substructure for the formation of the digestive gland (Fioroni & Schmekel, 1976: 129 ff.). The "yolk sac" of *Brotia*, which originates from the yolk supply of the egg capsule, is believed to be of same morphological and functional origin.

Riedel (1993) has hypothesized that delayed calcification of the apical whorl and a shrinking visceral mass in *Melanoides tuberculata* result in a wrinkled shell structure. This pattern is also observed in *Brotia*, in which the process of shell calcification is retarded and overlaps with the shrinking of the yolk sac by consumption of nutritive material. However, while delayed shell calcification is known from a number of gastropods (Eyster, 1986: 224–226), among them also some Thiaridae (Riedel, 1993; Glaubrecht, 1996), nutrition via a large, protruding yolk sac is unique among freshwater gastropods in the pachychilid genera *Brotia* and *Jagora* (description of the latter: Köhler & Glaubrecht, 2003). However, the phylogenetic relationships between these two genera indicate that open apex and protruding yolk sac have evolved independently (Köhler et al., 2004). This is suggested also by a different appearance of the apical portion of the embryonic shell in the two taxa. While in *Brotia* the apical whorl is wrinkled and appears irregular when viewed from above, in *Jagora* it is comprised by a lid-like structure that does not resemble a whorl at all (figured in Köhler & Glaubrecht, 2003).

Embryonic shells of all other Asian Pachychilidae can easily be distinguished from *Brotia* by the lack of wrinkles. A comparative overview of different embryonic shell morphologies in the Pachychilidae is provided by Köhler & Glaubrecht (2005). Consequently, in all other pachychilid taxa shell calcification is not retarded, but complete and continuous, a protruding yolk sac is not present.

Operculum

Next to the shell, the operculum is a feature that has long been used as diagnostic character for the classification of "melaniid" gastropods. For example, Troschel (1857–58) based his classification of the "Melaniidae" in part on opercular features. P. Sarasin & F. Sarasin (1898) distinguished between "Neomelanien" and "Palaeomelanien" on basis of a different operculum. Later, all palaeomelanian species were transferred to *Brotia* by Thiele (1928, 1929). While this decision has proven erroneous, the two species groups delineated by P. Sarasin & F. Sarasin (1898) still are considered to largely represent groups recognised by modern systematics: Pachychilidae and Thiaridae, respectively (Glaubrecht, 1999). Even taxa more closely related to the Pachychilidae, such as *Faunus ater* and the Melanopsidae, possess a paucispiral operculum (Houbrick, 1991; Glaubrecht, 1996). Consequently, a multispiral operculum with a central or subcentral nucleus is considered as autapomorphy of the Pachychilidae. Within this family, however, operculum morphology is a conservative character, and only in some species it may be used for species determination.

Radula

In general, the molluscan radula is considered a conservative character with little variation on the species level (Fretter & Graham, 1994). Nevertheless, the importance of radular characteristics, at least in higher level classifications, has been acknowledged early on (Troschel, 1856–1863; Thiele, 1928, 1929–1935). At high levels of taxonomic hierarchy, several of the radular patterns first described by 19th century morphologists still correspond largely or entirely with monophyletic clades recognised by modern cladistic analyses. Also at lower levels, recent cladistic analyses of morphology have frequently included radular characters (Glaubrecht, 1996; Reid, 1996; Ponder & Lindberg, 1997; Simone, 2001; Strong, 2003). Though, it became evident that radular characters, as any other morphological feature, may be prone to adaptation, parallelism and convergence and that intraspecific variability and plasticity may be considerable (Padilla, 1998; Reid & Mak, 1999; Reid, 2000). Therefore, before radular features can be used in phylogenetic studies, the extent of intraspecific variation must be carefully assessed, as is standard practise for shell characters.

The pachychilid radula is of the generalised taenioglossate type. Each row consists of a central rachidian, flanked on each side by a lateral and an inner and outer marginal tooth. All these teeth bear a number of cusps. Comparison of radulae of different pachychilid genera, such as *Pachychilus* (Troschel, 1858: pl. 9; Fischer & Crosse, 1892: pl. 49, fig. 14; Simone, 2001: figs. 95, 96), *Doryssa* (Simone, 2001: figs. 89–92), *Potadoma* (Glaubrecht, 1996: pl. 5, figs. 7, 8), *Jagora* (Köhler & Glaubrecht, 2003), *Sulcospira* (Troschel, 1858: pl. 9, fig. 6; Köhler & Glaubrecht, 2005), *Pseudopotamis* (Glaubrecht & Rintelen, 2003), and *Tylomelania* (Rintelen & Glaubrecht, 2005), shows little variation of radula patterns within the family. Nonetheless, it has also been shown that denticle shape and size as well as radular length may vary considerable even between closely related species if they occur in sympatry but feed on different substrata (Glaubrecht & Köhler, 2004; Rintelen et al., 2004; Rintelen & Glaubrecht, 2005).

The generalized radular pattern observed in most *Brotia* species comprises a central tooth with a well-developed glabella and a cutting edge comprising one main denticle flanked by up to three accessory cusps that taper in size, a lateral tooth exhibiting a glabella and a main denticle flanked frequently by two inner and two to three outer accessory cusps, as well as the inner and outer marginals, each with two cusps. These cusps may be rather of the same size or the outer cusp is enlarged. There are several other structures, for example, lateral extensions of the central and lateral tooth or a lateral flange of the marginal teeth that show a certain degree of variability among different species. Furthermore, the shape of the glabella of the main denticle varies among species. In general, the range of variation within *Brotia* is rather small, though, and only rarely some radular features are species specific. Most conspicuous modifications of the radula are connected to the substratum (Glaubrecht & Köhler, 2004; Rintelen et al., 2004). In rock-dwelling species, cusps may be enlarged, blunt or broadly round (e.g., in *B. pagodula*), whereas species living on soft substrata may possess much smaller denticles as well as radular teeth (e.g., *B. microsculpta*).

Gross Anatomy

The general appearance of the soft body and general organisation of the mantle cavity is rather constant among southeast Asian pachychilids and corresponds largely to the description given for *Brotia.* A feature typical for the Pachychilidae is the smooth mantle edge, which clearly differs from the papillated mantle edge found in Thiaridae. Among Pachychilidae, *Jagora, Tylomelania, Melanatria*, and *Pachychilus* differ from *Brotia, Adamietta,* and *Paracrostoma* in possessing a fleshy flap at the inner surface of the mantle roof. It has been suggested that this flap has a function for the formation of clutch masses during egg laying; therefore, it would have no function in viviparous species (Houbrick, 1991). In *Jagora,* it still might be functional, perhaps to prevent egg capsules and juveniles from becoming dislocated from the mantle cavity in which they are retained. Another structure connected to reproduction is the genital groove at the right side of the head, which is found not only in pachychilids, but also Melanopsidae and Potamididae. While in egg laying species, this groove is involved in egg deposition, in *Brotia* it is needed to transfer eggs from the pallial oviduct to the brood pouch (Fig. 5F).

Reproductive Organs: These are the most informative for pachychilid systematics (Köhler et al., 2004). Although all Asian Pachy-

chilidae are viviparous, brooding structures are not homologous among several groups. The subhaemocoelic brood pouch found in *Brotia* was first mentioned by Martens (1897: 29). Later, Moore (1899: 161, 162; pl. 14, fig. 13; pl. 16, fig. 2), Morrison (1954: 383), Davis (1971: 69), and Köhler & Glaubrecht (2001) described this pouch in more detail. A homologous brood pouch is found in *Adamietta* (Brandt, 1974) and *Paracrostoma* (unpubl. data) and is considered as a synapomorphy of the Asia mainland clade among the Pachychilidae (Köhler et al., 2004).

No homologous incubatory structures are possessed by other Asian Pachychilidae or other freshwater cerithioideans. The Philippine pachychilid *Jagora* broods in the mantle cavity (Köhler & Glaubrecht, 2003), while pachychilid *Pseudopotamis* and *Tylomelania* possess a uterine brood pouch (Glaubrecht & Rintelen, 2003; Rintelen & Glaubrecht, 2005). Since oviparity is suggested to represent a plesiomorphic character state in the Pachychilidae, brooding in turn must have evolved three times independently in this family (Köhler et al., 2004). In the Thiaridae and viviparous Planaxidae, a subhaemocoelic brood pouch very similar to that of *Brotia* is found. While this brood pouch was discussed as representing a possible synapomorphy of a clade comprising Planaxidae and Thiaridae (e.g., Houbrick, 1988; Glaubrecht, 1996; Simone, 2001), recent phylogenetic studies suggest that these are convergent (Lydeard et al., 2002; Köhler et al., 2004), The presence of a subhaemocoelic brood pouch in *Brotia* was furthermore a reason for erroneously placing *Brotia* within the Thiaridae (e.g., Morrison, 1954; Benthem Jutting, 1956; Brandt, 1968, 1974).

Other informative structures of the reproductive morphology include the pallial oviduct and the arrangement of the gonads. Among Asian Pachychilidae, *Brotia* possesses the simplest pallial oviduct. *Paracrostoma* differs by a distinct organisation of the sperm gutter (unpubl. data), which is located more posteriorly. *Adamietta* possesses a seminal receptacle in addition to a spermatophore bursa, which is present also in *Brotia* (Köhler & Glaubrecht, 2001, for the *Brotia testudinaria* group). Again, *Jagora*, *Pseudopotamis*, and *Tylomelania* possess oviduct morphologies that significantly deviate from *Brotia* (Glaubrecht & Rintelen, 2003; Köhler & Glaubrecht, 2003; Rintelen & Glaubrecht, 2005).

Stomach: Midgut morphology recently emerged as an yet untapped source of phylogenetic information, at least when groups of higher taxonomic ranks are compared (e.g., Simone, 2001; Strong, 2003). Various features of the stomach, such as a laminated crescent sorting area with two adjacent crescent and septate thickenings, a lateral and marginal fold, a single digestive gland duct, and two crescent ridges posterior to the opening of the digestive gland duct are considered synapomorphic among Pachychilidae (Strong & Glaubrecht, 1999). However, these features show little variation among the different genera as can be judged from the figures and descriptions for *Potadoma* (Binder, 1959), *Pachychilus* (Simone, 2001), *Jagora* (Köhler & Glaubrecht, 2003), and *Tylomelania* (Rintelen & Glaubrecht, 2005), and we were not able to identify characters that can be considered as diagnostic for species of *Brotia*.

Molecular Phylogeny of *Brotia*

The number of species included into phylogenetic analyses of molecular data is limited because of the restricted availability of material suitable for sequencing. For instance, it was not possible to extract high molecular DNA from preserved museum material. Nonetheless, mitochondrial DNA from a total of 48 samples of 16 Thai and Sumatran *Brotia* taxa, as well as 6 further pachychilid taxa from Asia mainland, were sequenced and analysed. Sequences of two *Jagora* species were included as outgroup representatives.

The mitochondrial trees unambiguously corroborate the monophyly of *Brotia* as restricted herein by morphology with regard to other pachychilid genera included in the analyses (i.e., *Jagora*, *Paracrostoma*, *Adamietta*). In this respect, it is important to bear in mind that also the concepts of the latter two genera – *Paracrostoma* and *Adamietta* – are subject to changes in regard to previous treatments, for example, by Solem (1966) and Brandt (1968, 1974). For instance, some species that were affiliated with *Paracrostoma* because of their concial shell were transferred to *Brotia* by Glaubrecht & Köhler (2004). *Paracrostoma* is now restricted to its type species, *P. huegelii*, and some yet undescribed species (Köhler, unpubl. data) endemic to southern India.

While on generic level the phylogeny strongly supports the classification based on the morphology, problems mainly occur as to the identification of some species-level taxa, in particular among the Kaek River radiation (Figs. 78, 79). This radiation comprises at least seven species recognized by a divergent shell and radular morphology, such as *B. armata*, *B. binodosa*, and *B. microsculpta*. However, sequence divergence among these taxa is very low, which is considered the main reason for the observed mismatch between the topology of the mitochondrial gene tree and the presumed species identity of these taxa as based on their morphology. Low genetic divergence indicates a relatively recent origin of the Kaek River radiation, and incomplete lineage sorting is the most likely explanation for the unresolved mitochondrial gene tree (Glaubrecht & Köhler, 2004). In order to get better resolved molecular reconstructions, it has been suggested to analyse different genetic markers and to use a different methodology, that is, AFLP genotyping.

Looking beyond the Kaek River species flock, all other *Brotia* species recognized by their morphology are also resolved as monophyletic entities in the mitochondrial gene trees. There is only one exception, *B. citrina*, the two sequences of which are shown as a paraphylum in the MP tree. In the distance-based trees, however, these sequences cluster together as a sister pair, which supports our treatment of the two populations as being conspecific. The mismatch in the MP tree therefore is no reason to doubt in the correct determination of *B. citrina*.

Infraspecific sequence divergence among *Brotia* species calculated unter the GTR+I+Γ model of sequence evolution does not exceed a maximum of 16% in COI and 29% in 16S, but mostly values are clearly smaller. Not considered is the unusual high sequence divergence of one of the two sequences of *B. sumatrensis*, which is caused by numerous peculiar substitutions in this sequence. Since a similar divergence is not observed in 16S, technical failure in sequencing cannot be ruled out.

Rates of sequence divergence reported here for *Brotia*, although difficult to compare since different models of gene evolution were applied by different studies, does exceed the limits observed in other freshwater cerithioideans (e.g., Pleuroceridae; Lydeard et al. 1997; Holznagel & Lydeard, 2000), but is similar to infaspecific sequence divergences observed in other Pachychilidae (e.g., Köhler & Glaubrecht, 2003; Glaubrecht & Rintelen, 2003).

Interestingly, in *Brotia* morphological disparity and genetic differentiation obviously are not linked to each other. Instead, two extremes are observed with the morphologically diverse but genetically rather undifferentiated Kaek River species flock on one hand and with species such as *B. citrina* and *B. pagodula* on the other, which show a low degree of morphological plasticity but a high degree of genetic differentiation. This phenomenon can probably be explained by strong competition and low prezygotic isolation (by means of geographical separation) between different sympatric taxa in the first case and absence of competition and relatively strong geographical separation between different conspecific populations in the latter case. This significant variation of infraspecific sequence divergences among *Brotia* shows with which problems approaches are fraught that aim at delimiting species only by the use of genetic distances (for further discussion of the merits and limits of DNA taxonomy the reader is refered to the contributions of, e.g., Lipscomb et al., 2003; Seberg et al., 2003; Tautz et al., 2003; Blaxter, 2004).

Systematic Implications

(1) Family Placement

The familiar placement of *Brotia* was subject to controversy caused by a mélange of rival systematic opinions as well as taxonomic difficulties. In an attempt to clarify the confusion, we shortly revise phylogenetic and systematic aspects on one hand and taxonomic issues on the other.

In the first attempts to classify what we call today cerithioidean freshwater gastropods all species were placed in a single group called Melanien or melanians, later also Melaniidae (e.g., Lamarck, 1822; Brot, 1874). This huge assemblage was subsequently subdivided into different groupings according to diagnostic features of their shell, operculum, and radula (e.g., Troschel, 1856–1863; Fischer & Crosse, 1891–1892; Thiele, 1928, 1929–1935); but Melaniidae were still considered a large monophylum. Fischer & Crosse (1891–1892) as well as Thiele (1928, 1929–1935) recognized six different lineages within the Melaniidae, among them a group already characterized by Troschel (1857) as "Pachychili" that comprises, for example, *Pachychilus*, *Potadoma*, *Melanatria*, and *Sulcospira*. This

group was ranked as a subfamily Pachychilinae of the Melaniidae according to the name introduced by Troschel. Morrison (1954), however, who strongly influenced most 20th century authors, recognized only three lineages and placed representatives of the "Pachychili" within two different clades, that is, the Pleuroceridae (*Pachychilus* and *Potadoma*) and the Thiaridae (*Sulcospira*, *Antimelania*, and *Brotia*). Later authors followed Morrison and treated Neotropical taxa as member of the Pleuroceridae (e.g., Vaught, 1989; Simone, 2001), but Asian taxa as Thiaridae (e.g., Solem, 1966; Davis, 1971; Brandt, 1968, 1974; Burch, 1980). This concept initially seemed to gain support even from a first cladistic analysis of morphological data presented by Houbrick (1988). In this analysis, which was to a large part based on morphological data presented by Morrison (1954), two major and independent freshwater lineages within the Cerithioidea were recognized, that is (1) Pleuroceridae + Melanopsidae and (2) Thiaridae. *Brotia* was affiliated with the latter for possessing a subhaemocoelic brood pouch. First doubts in this view have been raised by another cladistic analysis of morphological data (Glaubrecht, 1996), which revealed a new group besides Thiaridae and Melanopsidae (while Pleuroceridae were not included): the Pachychilidae. However, in this study only the oviparous taxa *Pachychilus*, *Doryssa*, *Melanatria*, and *Potadoma* were subsumed under the Pachychilidae, whereas the viviparous *Brotia* still was considered a thiarid. A third cladistic analysis of morphological data (Simone, 2001) supports the existence of exactly this monophyletic freshwater group comprising *Pachychilus* and *Doryssa* as being clearly distinct from the Thiaridae (with *Melanoides* and *Aylacostoma*). However, in this study the (wrong) name "Pleuroceridae" was employed for this lineage.

Molecular genetic studies helped much to clarify aspects of cerithioidean phylogeny. The most comprehensive phylogeny based on mitochondrial sequence data was so far presented by Lydeard et al. (2002). This study provided further evidence for the existence of at least three distinct freshwater lineages, (1) the Thiaridae, (2) the Melanopsidae + Pleuroceridae, and (3) an unnamed group comprising *Pachychilus* and *Paracrostoma*. This clear evidence unfortunately was obscured by application of a misleading taxonomy: Although forming a distinct lineage, *Pachychilus* and *Paracrostoma* were uncritically treated as members of Pleuroceridae and Thiaridae, respectively. As a consequence, all freshwater cerithioidean lineages were seemingly rendered polyphyletic, while a more restricted application of names would have unmistakably shown that they are in fact all monophyletic.

Direct comparison of the different phylogenetic studies is complicated by their deviant taxon composition. However, a closer look reveals that there is strong evidence for the existence of a monophyletic freshwater lineage beside the (1) Thiaridae and (2) Pleuroceridae + Melanopsidae, constituted by taxa such as *Pachychilus*, *Doryssa*, or *Paracrostoma* (Glaubrecht, 1996; Simone, 2001; Lydeard et al., 2002). All three studies failed to name this lineage properly, though. The names Thiaridae and Pleuroceridae although formerly used certainly are not available for this group, since they refer to the other two freshwater clades.

As the oldest name for this "new" lineage the name "Pachychili" was introduced by Troschel (1857) and later used as Pachychilinae by Fischer & Crosse (1891). Thiele (1921), who believed the name Pachychilinae to be invalid since he erroneously considered the generic name *Pachychilus* Lea, 1850, for neotropical "melaniids" as being preoccupied by *Pachychila* Eschscholtz, 1831, also recognized this taxon but suggested "Melanatriinae" as a replacement name. For a different reasoning against the validity of the name "Pachychilidae" with Troschel (1857) as author, see Bouchet & Rocroi (2005) as well as the introductory remarks in this article.

In contrast to Thiele (1921, 1925, 1928) we consider the name Pachychilidae as available and valid. Consequently, Melanatriinae is a synonym of Pachychilidae (Köhler & Glaubrecht, 2002, 2002a).

Eventually, Thiele (1925: 83) noticed that *Brotia* is member of this group besides, for example, *Pachychilus* and *Melanatria*, based on radular and opercular features. This is supported by a molecular phylogeny showing the close affinity of the Asian taxa, such as *Brotia*, with the Neotropical taxa, such as *Pachychilus*. This provided strong evidence for the existence of the clade named Pachychilidae (Köhler et al., 2004). As a consequence, the view of Morrison (1954) and Houbrick (1988), who strongly emphasized features of the soft body, in particular of the reproductive tract, on the systematic position of *Brotia* is refuted.

Morphological comparison of *Brotia* with other freshwater cerithioideans reveals that it shares as a synapomorphic character a widely corresponding operculum and radular morphology with oviparous pachychilids, such as *Pachychilus*. This also means that a subhaemocoelic brood pouch in *Brotia* has evolved in convergence to a similar structure found in the Thiaridae (Köhler & Glaubrecht, 2001; Köhler et al., 2004).

(2) Phylogenetic Relationships among Asian Pachychilidae

Traditionally almost all Asian pachychilid species sooner or later were attributed to *Brotia* by one or the other author. This was done in absence of a phylogenetic reconstruction, which would allow to identify autapomorphic features and lead to an inflated concept of *Brotia*, which in its conventional understanding by Rensch (1934), Abbott (1948), Benthem Jutting (1956), Brandt (1968, 1974), and Davis (1971) is rendered a polyphyletic grouping.

In a preliminary study, Köhler & Glaubrecht (2001) identified four different species groups among what was previously considered as constituting *Brotia*, which most conspicuously are characterized by peculiarities of their reproductive tract, their incubatory anatomy, and their embryonic shell. In concert with molecular genetic analyses it has been shown that these groups represent independent and monophyletic evolutionary lineages. The conspicuous morphological differences between and different evolutionary histories of these lineages justify the treatment as independent genera (Köhler & Glaubrecht, 2003; Glaubrecht & Rintelen, 2003; Köhler et al., 2004; Rintelen & Glaubrecht, 2005). According to this revised and more specific concept, *Brotia* is here restricted to pachychilid species possessing diagnostic characteristics, such as a wrinkled apical whorl of the embryonic shell and a simple pallial oviduct with a deep, ciliated spermatophore bursa but without a seminal receptacle. Besides *Brotia* there are six further pachychilid genera mainly recognized on basis of a divergent reproductive and embryonic shell morphology. Some of them have already been systematically revised, such as (1) *Jagora* endemic to the Philippines (Köhler & Glaubrecht, 2003), (2) *Tylomelania* endemic to Sulawesi (Rintelen & Glaubrecht, 2005), (3) *Pseudopotamis* endemic to the Torres Strait Islands (Glaubrecht & Rintelen, 2003), and (4) *Sulcospira* endemic to Java (Köhler & Glaubrecht, 2005). Irrespective of the fact that a formal revision of the two remaining genera, (5) *Adamietta* and (6) *Paracrostoma*, still is pending, it is suggested on basis of a molecular phylogeny of the Pachychilidae that they are also distinct (Köhler et al., 2004). This suggestion is corroborated by published and also unpublished morphological data (Köhler & Glaubrecht, 2001; Köhler, unpubl. data).

Together with these latter two genera *Brotia* forms a monophyletic lineage, the Southeast Asia mainland clade, which is characterized by possession of a subhaemocoelic brood pouch as synapomorphic feature (see Köhler et al., 2004).

Revised Concept of *Brotia*

What remains of *Brotia* under the restricted concept, still is a diverse group comprising at least 27 species that ranges from northeast India through Bangladesh, Myanmar, Thailand, and the Malaysian Peninsula to Sumatra, Borneo, and perhaps even Java. Systematic affinities of eight additional species remain to be clarified.

A subdivision into three subgenera as suggested by Brandt (1974) is refuted by the current study. Brandt suggested ranking two taxa, *Paracrostoma* and *Senckenbergia*, as subgenera of *Brotia*. This treatment is supported neither by morphological nor by molecular genetic data. In fact, *Paracrostoma* represents a monophyletic group closely related to *Brotia* but definitely distinct, as is revealed by the mitochondrial phylogeny (Figs. 78, 79; Köhler et al., 2004). All Thai species affiliated with *Paracrostoma* by Solem (1966) and Brandt (1968, 1974) are members of *Brotia* since they are not closely related to *Paracrostoma* from southern India but cluster together within *Brotia* (Glaubrecht & Köhler, 2004).

Type species of *Senckenbergia* is *Melania pleuroceroides* Bavay & Dautzenberg, 1910, a species from the Yangtze-Kiang. This species was stated to possess an operculum similar to *Semisulcospira*, which is a pleurocerid (Yen, 1939: 55). Since the Yangtze-Kiang is far out of the range of *Brotia*, and since also the operculum of its type species is of a pleurocerid type, *Senckenbergia* cannot be considered as a member of *Brotia*. A species originally assigned to *Senckenbergia* by Brandt (1974) is herein treated as *Brotia wykoffi* in regard to its morphology and position in the molecular trees.

In comparison to concepts used by former revising authors, that is, mainly Brandt (1968, 1974), the current study shows that assumptions on the morphological variability and geographical range of single species were exaggerated. For instance, Rensch (1934), Benthem Jutting (1956), and Brandt (1974) believed *B. costula* to be a highly variable species that occurs across entire Southeast Asia from India to the Philippines and even on some oceanic islands. It has been shown, however, that this species is much more restricted in its occurrence and also in respect to its morphological properties. Still, there are a number of named forms that preliminary remain as synonyms of this as well as of other species, although their distinct shells might indicate that they in fact represent independent species. This holds true, for example, for *B. reevei* (treated as synonym of *B. herculea*) and *B. elongata* (treated as synonym of *B. henriettae*). However, any decision on the status of these and other named forms in absence of properly preserved material would be rendered rather a matter of opinion. For this reason and in order to not further complicate the taxonomy of this group, we here follow the usual treatment of those taxa by former authors. In this respect, we are convinced that future studies will be able to recognize further, yet vaguely defined or unknown species within *Brotia*.

Conclusions

In summary, 27 species of *Brotia* are recognized in this work and eight additional species are presented with uncertain affinities. Using morphological and molecular data, the characteristics of *Brotia* are specified, and many species are newly delimited. Former systematic concepts are discussed and corrected accordingly. The current study results in an altered and more restricted concept of *Brotia* in comparison to former suggestions. It further shows that the subdivision into several subgenera as suggested by Brandt (1974) is erroneous. The new systematic concept is relevant also from a biogeographical perspective. While it has been assumed before that the range of *Brotia* covers almost entire South and Southeast Asia, it now becomes clear that its distribution is actually much more restricted. Thus, *Brotia* appears to be distributed mainly to the west of continental Southeast Asia ranging from northeast India (Assam, Sikkim, Meghalaya) and Bangladesh to central Thailand and the Malaysian Peninsula in the east. It is in the latter area where *Brotia* reaches its highest diversity. In the south, Sumatra, Java, and Borneo, comprising parts of former Sundaland, are within its distributional area. Among these three areas, Sumatra supports the highest diversity of species, forming a monophyletic subgroup, while from Java and Borneo only few species are known. As a rule, reports from Java and Borneo are not confirmed by collections after about 1920. If and how far the distribution of *Brotia* ranges towards the east of continental Asia (to Laos, Cambodia, southern China, and Vietnam) remains to be studied.

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ECOGENETICS OF SHELL SCULPTURE IN *ONCOMELANIA* (GASTROPODA) IN CANALS OF HUBEI, CHINA, AND RELEVANCE FOR SCHISTOSOME TRANSMISSION

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ABSTRACT

Oncomelania hupensis in China is well known as the intermediate host of the human blood parasite *Schistosoma japonicum*. There are three subspecies on the mainland of China with discrete patterns of distribution: above the Three Gorges of the Yangtze River (*O. h. robertsoni*) in Yunnan and Sichuan provinces; below the Three Gorges along the Yangtze River drainage (*O. h. hupensis*) with an incursion into Guangxi Province; and Fujian Province along the coast (*O. h. tangi*). Of these taxa, only *O. h. hupensis* has ribbed shells. Until now, *O. h. hupensis* has been shown to be dimorphic, with ribbed-shelled aggregates of individuals on flood plains and smooth-shelled populations in habitats elevated above the effects of floods or removed from the effects of severe annual floods by barriers. Molecular population genetics and anatomical studies have shown that there are no significant genetic differences between the two *O. h. hupensis* morphs; they belong to the same species (Davis et al., 1999b; Shi et al., 2002). Evidence to date has also shown that the ribbed-shelled aggregates of individuals are not true populations and are highly susceptible to infection with the parasite, whereas smooth-shelled populations have lesser potential to be infected, grading to total resistance.

We recently found in two canals of Hubei, well buffered from the annual Yangtze River floods, isolated populations that are truly polymorphic, with three to five classes of shell sculpture. The two canals were significantly different in their polymorphisms in 2001 (single sample per canal) and in 2004 (multiple samples within canals). We know the history of the construction of these canals (14 and 21 years ago, respectively), and the only available pathway of colonization of these canals (from the Yangtze River through the Guan Yin flood gate into the primary Hong Chou Canal). The colonizing snails were most probably derived from strongly ribbed snails of the adjacent flood plains. The changes from heavily ribbed to nearly smooth had to occur within the short span of 14 to 21 years. There were significant differences within canals in 2004 when multiple samples were taken.

The purpose of this paper is to present base-line data on this first reported case of shell sculptural polymorphism within *O. h. hupensis*, with the hypothesis that in the absence of sever flooding selection, this taxon will rapidly change from heavily-ribbed shells to slightly-ribbed to the smooth-shelled condition. Further, these changes give insight into questions of population evolution and coevolution with *Schistosoma japonicum*, in which smoothness is associated with genetic stability (defined in Davis, 1999a) that leads to the reduced potential to transmit the parasite (under coevolutionary pressure) and, in some instances, the evolved refractiveness to transmission.

Key words: schistosomiasis, *Schistosoma japonicum*, China, polymorphism, *Oncomelania*, evolution, population structure, coevolution, Red Queen.

INTRODUCTION

Oncomelania hupensis in China is well known as the intermediate host for the blood fluke *Schistosoma japonicum* afflicting man and other mammals. There are three subspecies with discrete patterns of distribution on the mainland of China (reviewed in Davis,

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1992, 1994; Davis et al., 1995, 1999a). All three transmit *Schistosoma japonicum* strains. *Oncomelania hupensis hupensis* is found throughout the Yangtze River drainage system below the Three Gorges of the river. *Oncomelania h. robertsoni* is located in the highlands and mountains of Yunnan and Sichuan provinces above the Three Gorges. *Oncomelania h. tangi* lives in coastal areas of Fujian Province, isolated from the Yangtze River by a mountain range.

Oncomelania with its two species, and *O. hupensis* with several subspecies distributed in Japan, Philippines, Celebes, Taiwan, are taxa that, with one exception, have smooth shells, as seen in the other genera of the family Pomatiopsidae (Davis, 1979, 1980, 1992; Davis et al., 1999a). The exception is found in populations of *O. hupensis hupensis* of the flood plains of the Yangtze River and its tributaries. Such flood-challenged populations have prominent ribs. Additionally, flood-plain *O. h. hupensis* has longer, heavier shells than the other subspecies, especially *O. h. robertsoni*, with very small shells and no varix (special thickening of the outer shell lip).

Until now, *O. h. hupensis* has been shown to be dimorphic, with ribbed-shelled aggregates of individuals on flood plains and smooth-shelled populations in habitats elevated above the effects of flooding. Molecular population genetics and anatomical studies show that there are no significant genetic differences between the smooth-shelled and ribbed-shelled populations; they belong to the same subspecies (Davis et al., 1995, 1999b, using allozymes; Shi et al., 2002, using mitochondrial CO1 gene sequencing). Based on breeding genetics, ribbing in *Oncomelania hupensis* is controlled by a single locus (Mendelian inheritance of a single gene) where ribs are dominant, smooth recessive, and with multiple alleles of that gene (Davis & Ruff, 1973). Likewise, size is controlled by alleles at a single locus.

All evidence indicates that ribbing is an evolved response to heavy annual flooding, that is, ribbing is maintained by natural selection. The hypothesis is that increased size and ribbing of flood plain individuals confer a selective advantage by way of strengthening the shells and enabling flotation to survive flooding (reviewed in Davis et al., 1999a, b).

We are currently studying the ecogenetics of *Schistosoma* transmission in two selected inner "tertiary" canals of Hubei, because they have an environment that is the most buffered from the ravages of the annual floods of the Yangtze River. Additionally, numbers of these canals, at the same or lower elevation as the Yangtze River, are relatively recently constructed and thus provide an opportunity to study a number of unique factors impacting disease transmission.

In October 2001, while selecting study sites, we found populations of *Oncomelania hupensis hupensis* in two unconnected canals, 2.7 km apart, that had shells that were polymorphic for ribbing. These canals were chosen because they are part of a *Schistosoma japonicum* endemic area, with infected snails in these canals, and the canals are far removed from the influence of the Yangtze River. We scored shells from a single population from each canal for strength of ribbing and found that three to four classes of ribbing could be identified. Further, the populations had significantly different frequencies of the morphs (Table 1, $P > 0.0001$).

The purpose of this paper is to present initial base-line data derived from analysis of shell ribbing in populations along these two Hubei canals (both the 2001 and 2004 data), and to demonstrate that within populations there are polymorphisms that we hypothesize to be stages of loss of ribbing in the absence of flooding selection. Further, the polymorphisms found give insight into questions of population evolution and coevolution involving (1) the timing of reversion from ribbing to smoothness; (2) the coevolution of *Oncomelania hupensis hupensis* with *Schistosoma japonicum*, in which smoothness is associated with genetic stability (defined in Davis et al., 1999a) leading to the reduced potential to transmit the parasite, and in some situations, the evolved refractiveness to transmission.

TABLE 1. X^2 comparison of shell polymorphisms of shell ribbing on *Oncomelania hupensis hupensis* snails from two Hubei Canals in 2001. N = number of shells from living snails. Data given as % of N. $P > 0.0001$. See text for details.

	Ma Ling (N = 101)	Gu Hu (N = 105)
M	13.9	43.8
SL	61.3	40.0
S-/S	24.8	16.2

METHODS

Canal Locations and Descriptions

The canals are located in the administrative villages of Gu Hu and Ma Ling of Sha Shi District, Jingzhou City, Hubei. The Gu Hu Canal (30°19.129'N, 112°23.386'E at mid-canal) is 1.6 km long. It is oriented E–W (95°–275° true). The Ma Ling Canal (30°19.578'N; 112°21.270'E at mid-canal) is 1.48 km long, with a N–S orientation (187°–8° true). Both canals are absolutely straight. We divide the Ma Ling Canal into two zones, the dividing point being where the canal is interrupted by a wider canal, the Wu Yi Canal. The right angle intersection is open in all four directions. Water from the Ma Ling-Wu Yi juncture flows through a pipe under the road, which parallels the Wu Yi Canal, to flow N 1 km to dead-end at the end of zone 1 of the Ma Ling Canal. At high water, this northern zone acts as a drainage canal. If water levels get too high, water is pumped from the northern end to the immense Si Hu Canal on the other side of a high dyke. The Si Hu, built pre 1960, is a major drainage canal flowing east. Zone 2 runs south of the Wu Yi Canal and at low water it is separated from the Wu Yi Canal by a very low, man-made earthen dam that holds back water of zone 2 to form a duck pond. Further south, the standing water meanders between low banks with thick marsh grass providing an ideal marshy environment for snails. Zone 2 is about 280 m long.

Both canals are about 4 to 5 m wide. The canals are separated by 2.7 km, with the Gu Hu Canal due E from the Ma Ling Canal. The unconnected canals are 2.4 km S of the vast Chang Lake and 12 km E of the Yangtze River. The canals in question are less than 22 years old. The Ma Ling Canal was built in 1984 and Gu Hu Canal in 1992.

Sampling and Scoring

Polymorphism data were an unintended and surprising byproduct of the primary purpose of our research on the long-term consequences of environmental change on the genetics and infectivity patterns of snails in recently constructed and highly protected canal systems. Data were taken from the 2001 mass collec-

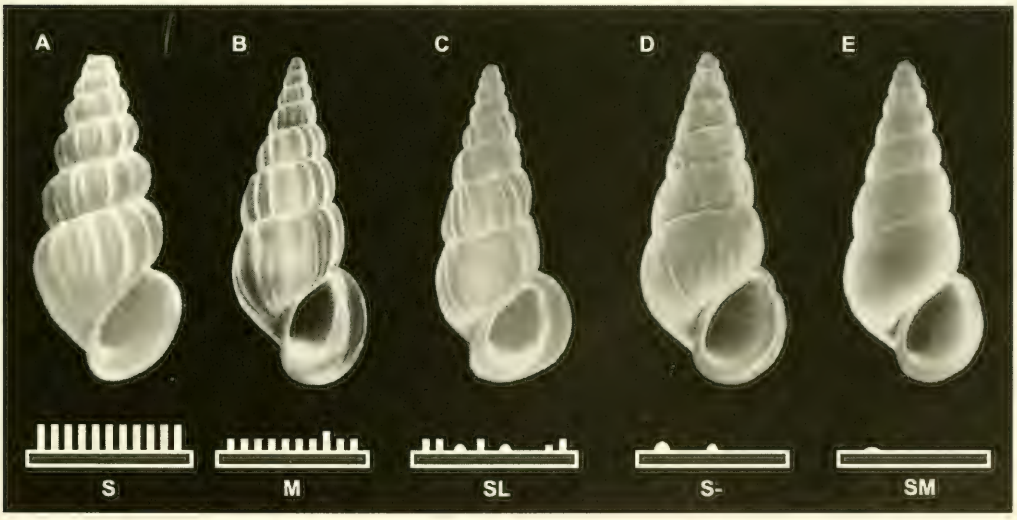

FIG. 1. Degrees of shell ribbing of *Oncomelania hupensis hupensis*. The schematic drawings (below) make clear the degree of ribbing strength and height that are very difficult to adequately portray in photographs or SEM pictures of shells (above). A: Strong ribs (S); B: Medium ribs (M); C: Slightly ribbed (SL); D: Trace of ribbing (S-); E: Smooth (SM). In D and E the "bumps" on the shell may be low swellings of a growth line or slightly elongated very low nodes. In smooth shells, the shell may be entirely smooth or, in few individuals, there may be one or two very low nodes or swellings indicating a highly degraded rib.

tion of snails taken to see if the sites were fitting for future study and from the experimental study initiated in 2004.

For our primary purpose, snails were collected each collection period (twice a year) from a 2 m^2 (a half frame, see Davis et al., 2002) positioned at 20 randomly selected sites on either side of the canal, enabling estimates of snail density per m^2 for each canal. Where there were ten or more snails per 4 m^2 (a frame) or $\geq$ 2.5 snails per m^2, (snails pooled from both sides of the canal) there were sufficient snails to score shells for one of five possible shell sculpture conditions (Figs. 1A–E): smooth (SM), smooth with negligible trace of ribs (S-), slightly ribbed (SL), medium ribbed (M), and strongly ribbed (S). Strong ribs are the type of ribbing found on the Yangtze River flood plains (heavy shells, tall and thick ribs regularly positioned on each whorl).

Of the 40 sites sampled, only five had ten or more snails per frame. Smooth shells vary from having a completely smooth shell surface to one which may have slight irregularities as a low swelling (bump) or an irregular growth line Figure 1E. Between these extremes (smooth or heavily ribbed) are three intermediate conditions (1) Negligible ribbing (Fig. 1D): the shell surface varies from completely smooth to having one or two scattered irregular nodes, or low thin rib lines on the body and penultimate whorl indicating the position where a rib might develop. (2) Slightly ribbed (Fig. 1C): The shell surface has some irregularly placed low, thin ribs with some rib-nodes (undeveloped ribs). (3) Medium ribbed (Fig. 1B): The penultimate and body whorls have regularly positioned fully developed low ribs on the entire whorls. These ribs are considerably lower than those found on heavily ribbed shells.

Statistical Analysis

Microsoft Excel was used for X^2 analyses of morph frequencies. Given the small numbers in some cells, the Fisher Exact Test was used.

RESULTS

Results of Initial 2001 Exploratory Canal Examinations

On 25 October 2001, we collected snails from the first $^1/_3$ of each canal closest to the main road. The snails were collected from a small area, about 90 m^2 along one side of each canal for the purpose of seeing what they looked like and if they were infected. Examination with a dissecting microscope at relatively low power showed the shells to have different patterns of ribbing. We could easily discern four types that we classified at that time as (1) smooth to trace of ribs (= types D and E, Fig. 1), (2) slightly ribbed, (3) medium ribbing. There were no strongly ribbed shells. The results of the scoring and the X^2 analysis given in Table 1 show a highly significant difference between the canals ($P > 0.0001$). The Fisher Exact Tests did not change the results. Ma Ling had significantly more slightly ribbed shells and significantly fewer medium ribbed shells than Gu Hu. This was the first time, to our knowledge, that such polymorphisms within populations of *Oncomelania* were found to exist.

Results of the September 2004 Collection

Scores for the four Ma Ling and one Gu Hu sites are given in Table 2 both for actual numbers of snails scored and % of snails in each

TABLE 2. Scoring snails for all sites for number and % snails with each morph category. S = strong ribs; M = medium ribbing; SL = slightly ribbed; S- = trace of ribbing; SM = smooth.

	Ma Ling				Gu Hu
	Site 1 (N = 15)	Site 17 (N = 12)	Site 19 (N = 122)	Site 20 (N = 96)	Site 16 (N = 77)
S	0	0	2 (1.6%)	0	0
M	15 (100%)	1 (8.3%)	31 (25.4%)	39 (40.6%)	38 (49.4%)
SL	0	10 (83.3%)	74 (60.7%)	52 (54.2%)	37 (48.1%)
S-	0	1 (8.3%)	14 (11.5%)	5 (5.2%)	2 (2.6%)
SM	0	0	1 (0.8%)	0	0

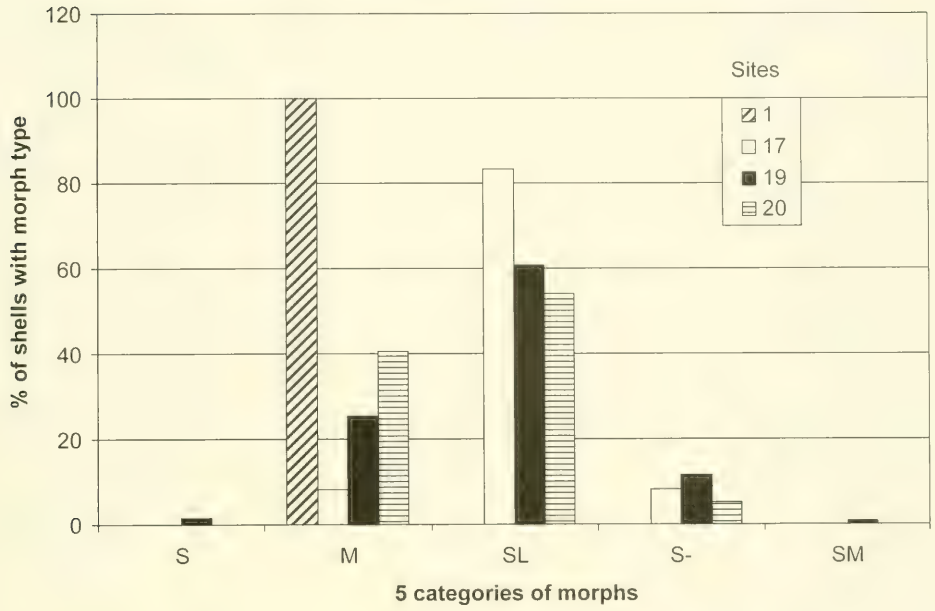

FIG. 2. The percent of morph types in the Ma Ling sites. S = strongly ribbed; M = medium ribbing; SL = slightly ribbed; S- = trace of ribbing; SM = smooth.

of the five morph classes. The percentage of each morph type in the four Ma Ling sites is graphed (Fig. 2). Ma Ling site one, at the northern end of the canal (zone 1, 1.0 km from site 17 close to the Wu Yi intersection), was unique in having only medium-ribbed shells, but the number of snails collected (15) was low. The remaining sites were separated by distances ranging from 25 m to 50 m between them. Snails from site 17 (zone 1 close to the Wu Yi

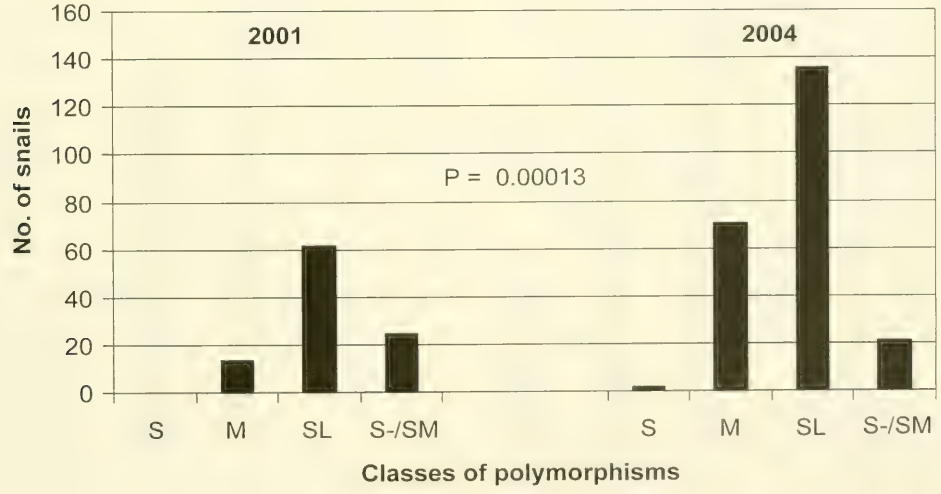

FIG. 3. Comparing Ma Ling snails from 2001 and 2004 (sites 17, 19, 20 combined data) for numbers of snails in each of four shell ribbing classes. See Fig. 2 for abbreviations. X^2 = highly significant difference.

TABLE 3. Cross comparison of canals and localities for years 2001 and 2004 to determine level of significant differences among sites for polymorphic classes of shell sculpture. **NS** = not significant; **BNS** = barely not significant; HSD = highly significant difference; SD = significant difference; VSD = very significant difference.

	2001 Ma Ling	2001 Gu Hu	2004 Gu H	2004 Ma Ling 1	2004 Ma Ling 17	2004 Ma Ling 19	2004 Ma Ling 20
2001 Ma Ling	-	HSD	HSD	HSD	**NS**	SD	SD
2001 Gu Hu		-	SD	HSD	SD	VSD	SD
2004 Gu Hu			-	HSD	SD	VSD	**NS**
2004 Ma Ling 1				-	HS	HSD	HSD
2004 Ma Ling 17					-	**NS**	**NS**
2004 Ma Ling 19						-	**BNS (0.060)**
2004 Ma Ling 20							-

canal intersection) were unique in having 83% slightly ribbed shells (but again low numbers, i.e., 12). Sites 19 and 20 were in zone 2. A cross comparison of sites for significant differences (Table 3) yielded only five comparisons that were not significantly different (or barely not significantly different. Sites at the southern end of the Ma Ling Canal in 2004 (sites 17, 19, 20)

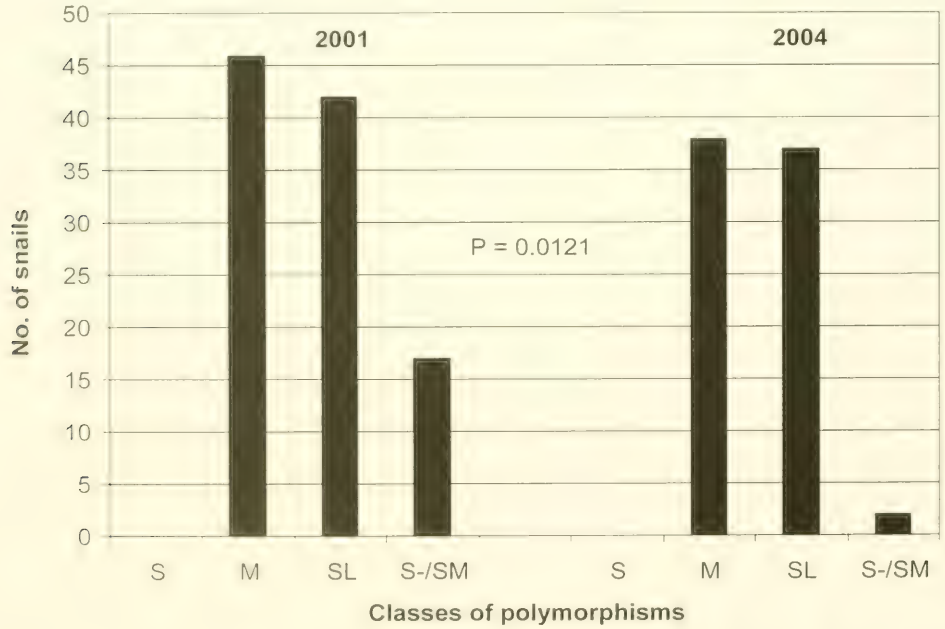

FIG. 4. Comparing Gu Hu snails from 2001 and 2004 for shell polymorphisms. See Fig. 2 for abbreviations. X^2 = significant difference but barely so.

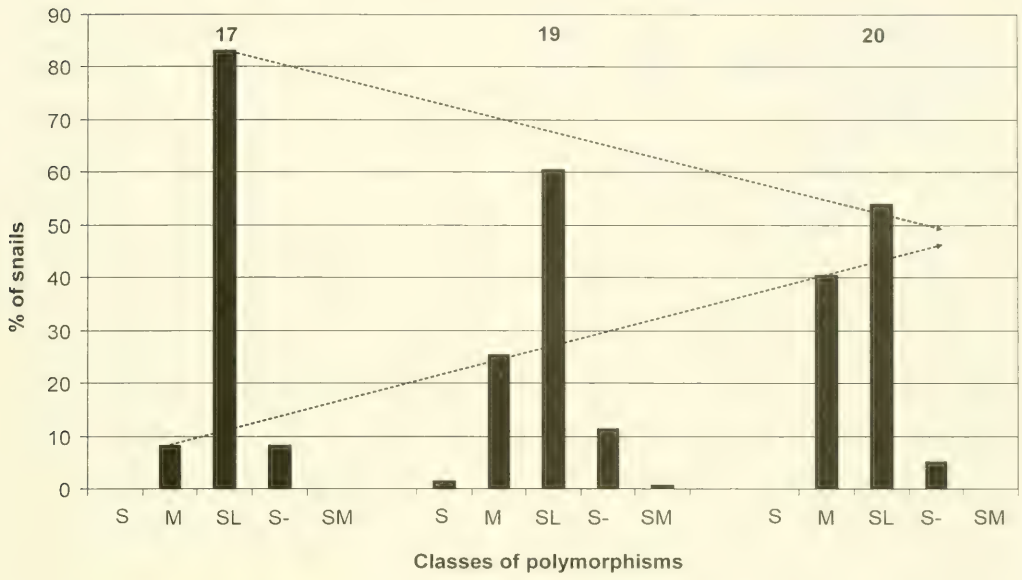

FIG. 5. Comparing Ma Ling 2004 sites 17, 19, 20 for five classes of shell ribbing polymorphisms. The % of each class is shown. See Fig. 2 for abbreviations. Dashed arrows indicate trend for decreasing slightly ribbed moving up the canal and increasing medium ribbed snails.

were not significantly different or bordering not significantly different.

To enable cross comparisons of 2001 and 2004 data, we combined data S- and SM from 2004. We also combined data from Ma Ling 17, 19, and 20, as 2004 populations were either not significantly different or barely significantly different. The Ma Ling snails from 2001 were highly significantly different from those of 2004 (Fig. 3). The 2004 population had fewer S- class and more M class snails. The Gu Hu snails from 2001 and 2004 were significantly different but barely so (Fig. 4). Most noticeable was the decrease in 2004 of S- class snails.

In comparing Ma Ling southern canal populations (17, 19, 20), one notices two distinct trends. Moving from north to south and across the larger perpendicular canal, there is distinct decrease in percentage of snails of the slightly ribbed class (Fig. 5). There is a distinct increase in snails of the medium-ribbed class.

Entirely smooth snails are thus far rare in these canals.

DISCUSSION

The major findings of this study are as follows. (1) For the first time shell sculptural polymorphism at a single site is reported in *Oncomelania.* (2) The polymorphism in degree of ribbing from strongly ribbed to smooth-shelled individuals in a relatively new canal environment is not static. Changes occur over a short period of time, that is less than 22 years, from strong ribbing to reduced strength of ribbing, including loss of ribbing. Further, morph frequency changes have occurred over the short span of 2–3 years. (3) The occurrence of shell sculpture polymorphism in isolated populations provides the first demonstrable linkage between ribbed-shelled and smooth-shelled *O. hupensis hupensis* populations, the rate with which the transition from the ribbed to smooth states can occur, and enables a clearer understanding of population genetic structure and the potential for populations to transmit *Schistosoma japonicum.*

Shell Sculptural Polymorphism and Environmental Selection

Polymorphism here is apparently dependent on a relatively new man-made environment that is affecting the genetic structure of these populations. Stability and removal from the annual floods of the Yangtze are the keys to change. Individuals in these relatively isolated canal habitats are changing from the heavily ribbed morphotype seen along the banks of

the Yangtze River, where their recent ancestors originated. Ribbing, as a derived flood-induced character, must be an energetically expensive character to maintain, as it is lost so quickly once the environmental enforcement is removed.

These canals are low land environments. Flow of water into the canals from the Yangtze is controlled by flood gates. The origin of the canal snails in this large region must be from the Yangtze River flood plains, dispersing into canals as the canals were built. The flotation of living snails from the Yangtze through the river embankment portals has been well documented (Xu & Fang, 1988; Xu et al., 1989, 1993; Yang et al., 1992). The only plausible origin for these snails is from the Yangtze River through the Guan Yin flood Gate into the primary Hong Chou Canal, hence to the north trending secondary Nan Bei Canal. The Nan Bei Canal is piped under (reverse siphoning) the Huge Shi Gong (that takes waste water away from Sha Shi City) to flow north to Malin Village. The Wu Yi Canal turns west off of the Nan Bei Canal to transect the Ma Ling Canal of our study about a km away.

Now we see deep in the tertiary interior canals that there has been a considerable change over the past 20 or so years since these particular canals were made. In 2004, only 0.47% of the snails had strong ribs, while 41% had slightly irregularly ribbed shells, and 5% were close to smooth. The significant differences between canals and between years within a given canal demonstrate a dynamic process, such as seen in Figure 5 showing discrete trends of increasing and decreasing morph frequencies between relatively closely positioned sites. We do not know the reason(s) for these short-term differences (or trends). In these canals, snails have low vagility and are subject to regular perturbation by man and animals. Differences could be due to founder effects or local selective pressures of micro-area effects.

The compelling argument is that flooding selection drives the selection for alleles favoring development of stronger shells, larger size, and ribs. Predation does not appear to drive these genotypes. The only known predators of *Oncomelania hupensis* snails are ducks and perhaps some predatory fish. But in the presence of ducks, *Oncomelania* in the southern zone of the Ma Ling Canal do not have strong ribs; they mostly have slightly ribbed shells (> 50%) and there are many (9%) nearly smooth (S-) shells. It is unlikely that fish are a factor as adult *Oncomelania* is amphibious, living in the ecotone between water and dry land, a habitat not accessible to fish.

Ecology, Population Genetics of *Oncomelania hupensis hupensis* and the Transmission of *S. japonicum*

While there is no direct genetic linkage between shell sculpture and the potential to transmit *Schistosoma japonicum*, there are significant differences between strongly ribbed-shelled aggregates of *O. hupensis hupensis* and smooth-shelled populations with regard to both population genetic structure and the potential to transmit *Schistosoma japonicum*. Empirical data have shown ribbed-shelled aggregates of snails to be both genetically unstable (thus not true populations) and highly susceptible to infection with *Schistosoma japonicum*. Smooth-shelled populations have this far been shown to be genetically stable and have low to no capacity to transmit *S. japonicum* (Davis et al., 1999a; Wilke et al., 2000; Shi et al., 2002). The infectivity capacity has been hypothesized to be driven through coevolution with *S. japonicum*, with infectivity differences the basis for invoking the Red Queen hypothesis of coevolution of Van Valen (1973) (Davis, 1980, 1992: 193).

Origin of Ribbing, Genetic Instability and Infectivity

Historically, the evolutionary developments have been: (1) The plesiomorphic state (primitive or basic state) is being small and with smooth shell (Davis, 1979). (2) With evolving river systems and dispersal down the Yangtze River of the smooth-shelled morph into the new environment of the evolving Yangtze River and onset of annual monsoon-floods, there evolved the presence of ribs, a thicker shell, and increased size to cope with environmental challenge. Of all *Oncomelania* taxa, only the Yangtze River drainage (and derived drainages) developed ribbing on the shells. (3) Below the Three Gorges of the Yangtze River and dispersing up into habitats not affected by flooding, as well as dispersing to Taiwan and Japan, the snails reverted to (or maintained) a smooth shell. (4) Becoming smooth and living in isolation from immigration enables genetic stability, a requirement for the Red Queen to operate. In isolation, and normal inbreeding, smooth-shelled individuals evolve under selection pressure of the parasite from

being highly susceptible to the parasite to lowered susceptibility at the population level to totally resistant to infection.

Genetic Structure and the Transmission of *S. japonicum*

Population genetic stability vs. instability was defined (Davis et al., 1999a) using MtDNA sequence data. Low haplotype diversity (1 or 2 haplotypes per ≥ 10 individuals collected from a small area (e.g., 100 m^2) is a surrogate for Hardy-Weinberg equilibrium or normal panmixis within a population over a period of years, that is, stability. Instability is indicated by high haplotype diversity for the same conditions above, where 6–10 haplotypes are found in ≤ 10 individuals. Instability is indicative of aggregates of individuals ("populations") of recent immigration; that is, these are not part of a normal interbreeding population. Snails are swept together from different locations, carried by flood waters. Hardy Weinberg is not attained. The example was given (Davis et al., 1999a) where five "populations" around the shores of Dong Ting Lake, all subjected to severe annual flooding, had heavy ribbing. These had 6–10 haplotypes for ten individuals thus all were unstable. The sixth population came from an elevation between 100 and 500 m. The shells were smooth and had two haplotypes per ten individuals, that is, genetic stability. All the ribbed snail populations were highly susceptible to schistosome infection. The smooth-shelled population was not and could not be infected (Li, Hunan Institute of Parasitic Diseases, personal communication).

A study of *O. hupensis hupensis* populations along the Yangtze River from Hunan and Hubei provinces through to Zhejiang and Jiangsu provinces involved questions of population evolution, haplotype diversity and ecology (Wilke et al., 2000). The data indicated that ribbing is associated with annual floods along the flood plains of the Yangtze River, where snails are swept into aggregates of snails with high haplotype diversity. In areas not affected by flooding, the snails were generally smooth and genetic diversity decreased significantly. The one Jiangsu population was smooth and living essentially at sea level in a water network (one haplotype in six individuals). One Zhejiang population (elevation of 100 m) had a trace of ribbing and low haplotype diversity. One Anhui population living in the lowlands but potentially removed from flooding had slightly ribbed shells and three haplotypes per ten individuals.

A study re-visiting the Miao River (Shi et al., 2002) involved haplotype analysis and ecological setting to examine both the question of smooth-shelled vs. ribbed shelled and the relationship between these morphs and infectivity. There was a clear trend for decreasing haplotype diversity upstream from the mouth of the river. Nucleotide-sequence diversity was > 0.015 at sites A and B close to the mouth of the river and where the snails had heavily ribbed shells; it was < 0.0085 at site G at the top of the river, where the snails were smooth (sites above the flood level with smooth-shelled populations were D–G). With regard to infectivity, the down-stream ribbed-shelled "populations" had higher infection rates and higher susceptibility to infection with *S. japonicum* than did upstream smooth-shelled populations. The higher infectivity of down-stream "populations" was attributed to the importation and mixture of snails (i.e., aggregates) of different genotypes of snails and schistosomes in flooded areas increasing the possibility of multiple infections by schistosomes of different genotypes. In upstream populations, low infectivity is probably due to isolation and attaining equilibrium, with the parasite at low frequencies of infection.

The Red Queen and Decreasing Infectivity

The Red Queen pertains to co-evolution in which the impact of a parasite on the host (in this case the intermediate snail host) elicits a genetic response of the host to repel the parasite. This in turn generates a genetic response in the parasite to overcome the defense of the host. Through time, the interaction becomes highly specific and convoluted. For this to happen, the snail population must be, in fact, a true population with little or no immigration, that is, in Hardy-Weinberg equilibrium (genetically stable). There are three possible end results of this "genetic war". (1) The parasite wins, and the snail population goes extinct (witnessed in the decline and local extinction of *Hydrobia truncata* in New England, USA (Davis et al., 1988). (2) The snail wins, and the parasite becomes extinct in the snail population. (3) The snail infectivity rate decreases until some equilibrium is reached at a low level of infectivity.

We have uncovered a number of situations in which the smooth-shelled snail population has gone to fixation for completely warding off the parasite. Davis & Ruff (1973) hybridized smooth totally refractive *Oncomelania hupen-*

sis from Taiwan with highly susceptible ribbed-shelled *O. hupensis* from the mainland of China. The hybrids could be infected; there was indeed a genetic component to transmission. On Taiwan, there are *Oncomelania hupensis* populations that are totally refractive, others are susceptible to non-human *Schistosoma japonicum*, and one population is not naturally infected with any schistosome but can be infected with all allopatric strains of *S. japonicum*.

We have found one Anhui smooth-shelled population that is totally refractive to infection. The refractive population above Dong Ting Lake was mentioned. The Miao River study demonstrated the highly infectious nature of the genetically unstable downstream ribbed-shelled snails in contrast to the much less susceptible upstream smooth-shelled populations.

There is a large literature on cross infectivity studies, based on the pioneering work of DeWitt (1954) involving permutations and combinations of *S. japonicum* from different localities and countries and *Oncomelania hupensis* from the corresponding localities. A sampling of a few papers on cross susceptibility studies are Moose & Williams (1963), Chi et al. (1971), He et al. (1991), Lin et al. (1994), Hong et al. (1995), and Sheng et al. (1995). These studies show that there is considerable evidence for evolutionary divergence among allopatric populations with regard to the genetic potential to transmit an allopatric *S. japonicum*. The results range from complete incompatibility to partial compatibility involving allopatric pairs.

We continue to maintain the hypothesis that genetically unstable aggregates of ribbed-shelled snails are more highly susceptible than isolated populations of smooth-shelled snails because the mixing of snails, due to importation by flooding (with an array of genotypes relative to schistosome success or failure at infecting these snails), facilitates high success in infecting snails. In such an environment, the schistosomes have a "menu" of genotypes to choose from with regard to their success in infecting the snail. Given the unstable nature of the mixture of the moment, one sees little opportunity for selective pressures to act on these genotypes relative to the process of speciation or emerging new disease. However, the mixture is a potent cocktail of genotypes that present a dangerous situation relative to importing the mixture, with all its genetic diversity, to a new environment. In genetically stable populations that are isolated and where the Red Queen is in action, it is possible that selective pressures on allopatric stable populations could drive speciation and emerging disease.

Oncomelania hupensis robertsoni – A Different Evolutionary Trajectory

Oncomelania hupensis robertsoni has a different history and involvement with *Schistosoma japonicum* than *O. h. hupensis*. *Oncomelania hupensis robertsoni* is highly divergent genetically from *O. h. hupensis* (Davis et al., 1998; Wilke et al., 2000, 2006). As described above, this taxon, living in the mountains of Sichuan and Yunnan provinces, closer to the area of origin of the genus than *O. hupensis hupensis*, is not affected by the great annual floods of the Yangtze River, and has small, smooth shells and no varix. The varix, the thickening of the outer lip seen in *O. h. hupensis*, is equal to the terminal rib seen in all *O. hupensis hupensis* populations, smooth or ribbed. Of note is that thus far no population of *O. h. robertsoni* has been found to be refractive to infection with the Yunnan-Sichuan strain of S. *japonicum*. A different dynamic seems to be at work here. It is also noted that *robertsoni* lives on the banks of small streams, irrigation ditches, and the base of retaining walls of agricultural terraces. These are generally in a high gradient environment, where heavy rain wash snails downward. Following such rains, the snails, which are negative geotropic and negatively rheotropic, move relentlessly upward. The net result is hypothesized to be considerable genetic mixing within a drainage system, that is, resulting in genetically unstable aggregates of snails with high susceptibility to *S. japonicum*. Preliminary data support the instability argument as of 13 populations studied in Wilke et al. (2006), 40 of 66 specimens had different haplotypes, and of 24 specimens studied, each had a unique AFLP fingerprint. These data indicate a lack of population structure due to great heterogeneity, not due to uniform panmixia.

A great deal of work must be done with *O. h. robertsoni* on population genetics, population structure, natural patterns of infection, and laboratory infectivity studies before one can say much more. A tentative hypothesis is that *robertsoni* maintains the plesiomorphic receptiveness to infection and those environmental-population factors do not promote the Red Queen.

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TAXONOMIC DISTRIBUTION AND PHYLOGENETIC UTILITY OF GENDER-ASSOCIATED MITOCHONDRIAL GENOMES IN THE UNIONOIDA (BIVALVIA)

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ABSTRACT

Unionoid bivalves exhibit a great diversity in reproductive characteristics. However, the lack of a robust phylogeny severely restricts evolutionary interpretations regarding the genesis and consequences of reproductive character state diversity within the order. The apparent high fidelity of unionoidean doubly uniparental inheritance of mtDNA (DUI), where distinct female- (F) and male-(M) transmitted mtDNA genomes are present, may allow for multiple, independent mtDNA-based estimates of phylogeny and thus contribute to the generation of more robust estimates of unionoid evolutionary history. However, the current lack of knowledge regarding mtDNA transmission patterns in the Etherioidea severely hampers our ability to evaluate the potential of DUI for explicating unionoid phylogeny. This situation prompted us to address the following questions in this study: (1) Is DUI found in the Etherioidea? (2) What is the relative phylogenetic utility of F, M, and concatenated F + M cytochrome *c* oxidase subunit I (*cox1*) sequences for elucidating higher level unionoid evolutionary relationships? (3) What can trees derived from F and M sequence analyses tell us about the evolution of unionoid DUI and other reproductive characters?

Forty-seven species representing all six families within the Unionoida were evaluated, using PCR-based methods, for the presence of DUI. Phylogenetic analyses were carried out on unionoid species for which complementary F and M *cox1* DNA sequences were available as well as on a much more taxonomically inclusive F *cox1* data set. We determined that (1) the Etherioidea likely lacks DUI; (2) M and F + M *cox1*-based analyses provide better resolved estimates of unionoidean relationships than do F *cox1*-based analyses; and (3) the F and M non-concatenated *cox1* inclusive phylogenetic analyses suggest the inference that (a) the presence of DUI, glochidial larvae, and endobranchous brooding are the ancestral unionoid character states, (b) both DUI and glochidial larvae were lost in the ancestral etherioidean lineage, (c) margaritiferids are closely related to unionids and exhibit a derived suite of morphological characteristics, and (d) a clarification of the evolutionary dynamics of unionoid DUI and other reproductive characteristics will require a robust phylogeny for the order that is based on multiple data sets.

Key words: DUI, *cox1*, mtDNA, Hyriidae, Margaritiferidae, Unionidae, Iridinidae, Mycetopodidae.

INTRODUCTION

Freshwater unionoid bivalves exhibit significant taxonomic diversity (~175 genera) and a broad geographic distribution that includes all continents, with the exception of Antarctica (Simpson, 1896, 1900, 1914; Haas, 1969; Starobogatov, 1970). Following Parodiz & Bonetto (1963), the bivalve order Unionoida is comprised of six families contained within two superfamilies of freshwater mussels (Superfamily Etherioidea: Etheriidae, Iridinidae and Mycetopodidae; Superfamily Unionoidea: Hyriidae, Margaritiferidae, and Unionidae). However, the concept of the Etheriidae as a monophyletic group containing all cemented unionoid bivalves has been rejected (Bogan & Hoeh, 2000), thus its usage herein is applied only to the genus *Etheria*.

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Unionoid bivalves also exhibit great diversity, among higher taxa, in their reproductive characteristics, such as the morphology of their parasitic larvae as well as the larval brooding location. There are two types of parasitic unionoid larvae: the bivalved glochidium (Hyriidae, Margeritiferidae, and Unionidae) and the univalved lasidium (Iridinidae and Mycetopodidae) (Wächtler et al., 2001). It has been suggested that the extreme morphological divergence between these two types of larvae indicates that the unionoidean and etherioidean bivalves represent independently derived freshwater lineages and thus the Unionoida is a polyphyletic assemblage (Parodiz & Bonetto, 1963). However, the results of recent unionoid phylogenetic analyses reject the latter hypothesis (e.g., Hoeh et al., 2001; Roe & Hoeh, 2003). In addition to the extreme distinctions in larval morphology, unionoid higher taxa also exhibit differences in larval brooding location. Unionoids use three general brooding locations. Tetragenous brooders utilize all four ctenidia as marsupia (Margaritiferidae and some Unionidae), endobranchous brooders utilize only the inner two ctenidia

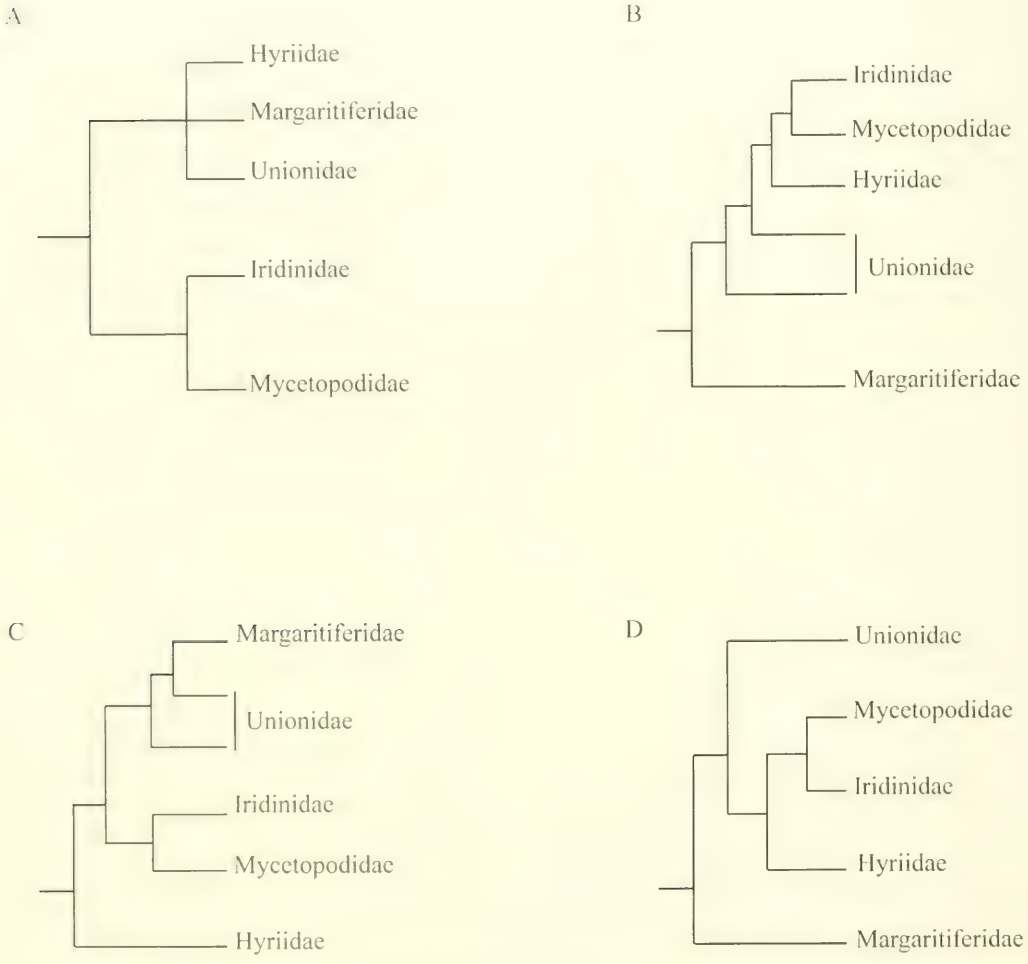

FIG. 1. Simplified representations of unionoid familial relationships based on the A: Classifications of Simpson (1900) and Parodiz & Bonetto (1963); B: Phylogenetic analyses of morphological characters after Graf (2000) and Hoeh et al. (2001); C: Phylogenetic analysis of combined morphological and molecular characters after Hoeh et al. (2001); D: Phylogenetic analysis of morphological and molecular characters after Roe & Hoeh (2003).

(Hyriidae, Iridinidae, and Mycetopodidae), and ectobranchous brooders utilize only the outer two ctenidia (some Unionidae). Increasing our understanding regarding the evolution of this diversity in reproductive structures has been a major, if largely unrealized, goal of recent unionoid phylogenetic studies (e.g., Graf & Ó Foighil, 2000; Hoeh et al., 1998a, 2001).

Numerous hypotheses of unionoid evolutionary relationships, based on analyses of both morphological and DNA characters, have been published recently (Bogan & Hoeh, 2000; Graf & Ó Foighil, 2000; Hoeh et al., 1998a, 2001; Graf, 2000; Roe & Hoeh, 2003). Despite this flurry of recent studies, when comparing hypotheses of unionoid familial relationships, it becomes apparent that there is little agreement (Fig. 1). Parodiz & Bonetto's (1963) classification mirrors that of Simpson (1896, 1900), in describing Mycetopodidae + Iridinidae (Simpson's Mutelidae) as fundamentally distinct from the Unionidae + Margaritiferidae + Hyriidae (Simpson's Unionidae) (Fig. 1A). Recent phylogenetic analyses offer neither corroboration of this view nor significant among-analysis congruence. Analyses of morphological characters presented by Graf (2000) and Hoeh et al. (2001) both return the Margaritiferidae as the basal unionoid lineage, a paraphyletic Unionidae, and the Hyriidae + Mycetopodidae + Iridinidae as a derived lineage (Fig. 1B). In contrast, both the molecular (i.e., *cox1* DNA sequences) and the combined analysis of morphological and molecular data presented by Hoeh et al. (1998a, 2001) (Fig. 1C) place the Hyriidae as the basal unionoid lineage, with the Unionidae returned as paraphyletic. Yet another combined analysis of morphological and molecular (i.e., *cox1* DNA sequences) characters (Roe & Hoeh, 2003; Fig. 1D), this time using binary coding of the morphological data and a posteriori character weighting, returns the Margaritiferidae as basal and a monophyletic Unionidae as sister to a Hyriidae + Iridinidae + Mycetopodidae clade. As is readily apparent from the above comparisons, ambiguity still remains when attempting to explain the evolution of unionoid diversity. This difficulty results from the fact that the only degree of stability exhibited across all of the relationship hypotheses above is that the Mycetopodidae and Iridinidae are always returned as closely related. Importantly, the topological positions of the Hyriidae and Margaritiferidae do not remain stable across analyses and thus, we are left with the lack of a well-resolved phylogeny for the Unionoida. This situation severely restricts evolutionary interpretations regarding the genesis and consequences of reproductive character state diversity within the order.

A largely underutilized set of phylogenetically informative characters exists in the male-transmitted mtDNA genomes within the Unionoidea, as a consequence of the presence of doubly uniparental inheritance of mtDNA (DUI) in that taxon. DUI has been observed in two orders of marine bivalves (Mytiloida: Skibinski et al., 1994; Zouros et al., 1994; Hoeh et al., 1996; and Veneroida: Passamonti & Scali, 2001) and the freshwater bivalve superfamily Unionoidea (Hoeh et al., 1996; Liu et al., 1996). In species with this type of mtDNA inheritance, there are distinct female-(F) and male-(M) transmitted genomes. Typically, females are homoplasmic for the F genome, whereas males are heteroplasmic, that is, they contain both the F and M genomes (Skibinski et al., 1994; Zouros et al., 1994). In males, these two distinct mtDNA genomes segregate by tissue type. The F genome predominates in somatic tissues while the M genome is concentrated in spermatogenic tissues (Stewart et al., 1995; Garrido-Ramos et al., 1998). Therefore, taxa possessing DUI transmit two distinct mtDNA genomes. Females pass on their F genome to both male and female progeny while males transmit their M genome only to male progeny. While earlier mtDNA-based analyses of unionoid phylogeny largely made use of F genome sequences (e.g., Graf & O' Foighil, 2000; Hoeh et al., 1998a, 2001), more recent phylogenetic analyses of both F and M genome DNA sequences, from exemplar species representing the Hyriidae, Margaritiferidae, and Unionidae, have produced evolutionary trees with distinct F and M clades exhibiting very similar topologies (Curole & Kocher, 2002, 2005; Hoeh et al., 2002). The observed reciprocal monophyly of these topologically similar F and M clades, in conjunction with fossil evidence, suggests that DUI has been operating at a high level of fidelity in the Unionoidea for more than 100 my (Curole & Kocher, 2002, 2005; Hoeh et al., 1996, 2002).

The fidelity of the DUI system is sometimes compromised in mytiloids. Evidence supporting this view comes from phylogenetic analyses (e.g., Hoeh et al., 1997) and breeding studies (Fisher & Skibinski 1990; Zouros et al., 1994; Rawson et al., 1996; Saavedra et

al., 1997; Quesada et al., 1999; Ladoukakis et al., 2002). For example, taxonomically more inclusive phylogenetic analyses of F and M genomes in mytiloids have failed to recover distinct F and M clades (e.g., Hoeh et al., 1996, 1997). As one example, some *Mytilus* M genomes appear more closely related to F genomes than to other similarly transmitted genomes (Hoeh et al. 1997). Failure to inherit the M genome may result in recruitment and masculinization of the F genome to function as a newly derived "M" genome (Hoeh et al., 1996, 1997). Initially after masculinization, the F and the newly derived "M" genome have identical DNA sequences. Subsequently, divergence between the F and "M" genome begins *de novo* (Hoeh et al., 1996, 1997). Additionally, mytiloid masculinization events have been observed in laboratory crosses (Zouros et al., 1994; Saavedra et al., 1997) as well as in natural populations (Fisher & Skibinski, 1990; Rawson et al., 1996; Quesada et al., 1999; Ladoukakis et al., 2002). This presents a problem when using F and M genomes in a complementary manner for mytiloid phylogenetic analyses as non-orthologous comparisons could result. To date, feminization, or recruitment of an M genome to function as the F, has not been observed or inferred for any taxa with DUI. Unlike the situation in mytiloids, masculinization events have not been documented for the Unionoidea (Hoeh et al., 1996, 2002; Curole & Kocher, 2002, 2005). This apparent high fidelity of unionoidean DUI may allow for multiple, independent mtDNA-based estimates of phylogeny and genetic variation within the order (Hoeh et al., 2002; Krebs, 2004).

The presence of DUI in the hyriid, margaritiferid, and unionid specimens sampled to date prompted us to address the following questions in this study: (1) Is DUI found in the Etheriidae, Iridinidae and Mycetopodidae? If so, DUI likely represents the ancestral mtDNA transmission pattern for unionoid bivalves. If not, DUI presence/absence data may be informative regarding unionoid familial relationships. (2) What is the relative phylogenetic utility of F, M, and concatenated F + M cytochrome *c* oxidase subunit I (*cox1*) sequences for elucidating higher level unionoid evolutionary relationships? (3) What can trees derived from F and M sequence analyses tell us about the evolution of unionoid DUI and reproductive characters?

MATERIALS AND METHODS

Taxa sampled in this study for the presence of a male genome included 47 species representing all six families within the Unionoida (Table 1). Gender was determined by microscopical examination of gonadal tissues. Total genomic DNA was isolated from either somatic (mantle or foot) or testis tissue using the Qiagen DNeasy animal kit. An approximately 710 bp fragment of *cox1* was amplified from both the F and M mtDNA genomes using modified versions of the universal *cox1* primers (Folmer et al., 1994): LCO22me2 5'-GGTCAACAAAYCATAARGATATTGG-3'; HCO700dy2, 5'-TCAGGGTGACCAAAAAAYCA-3'. To efficiently screen for the presence of the M genome, largely gender-specific *cox2* primers were used to amplify the *cox2-cox1* fragment used by Curole & Kocher (2002). These primers were chosen due to the size difference exhibited between the F and M *cox2-cox1* fragments as described by Curole & Kocher (2002). The "male-specific" *cox2* primer was UNIOCOII.2 (Curole, 2004) and a "female-specific" primer (UNIOCOII.2b, 5'-CAGTGRTATTGRRVDTAYGA-3') was derived from the UNIOCOII.2 primer and other unionid F sequences available from GenBank. Both "gender-specific" primers were paired with HCO700dy2 to amplify the *cox2-cox1* fragment. These primers typically amplified approximately 1.1 Kbp of *cox2-cox1* from F genomes and approximately 1.7 Kbp from M genomes. PCR reactions consisted of 1X Qiagen PCR buffer, 0.2 mM each dNTP, 0.5μM each primer, and Qiagen *Taq*. Reactions using the *cox1* primer pair were cycled at 94°C for 60 s, 40°C for 60 s, and 72°C for 60 s for a total of 40 cycles and reactions using the male-specific *cox2* primer were cycled at 94°C for 60 s, 50°C for 60 s, and 72°C for 120 s for a total of 40 cycles. Reactions involving the female-specific *cox2* primer followed the same profile given above for the male specific primer but were annealed at 46°C. Sequencing template purification was carried out following Folmer et al. (1994). The *cox1* fragment yielded 619 bp of sequence via cycle sequencing with Perkin Elmer AmpliCycle Sequencing Kits using ddNTP-dNTP ratios optimized for automated sequencing. Sequences were obtained from both strands of the *cox1* fragment and the dye-labeled *cox1* sequencing primers were of the same sequence as the PCR prim-

TABLE 1. Taxa evaluated for the presence/absence of F and M *cox2-cox1* amplicons; + = amplification successful, - = amplification failed, NA = amplification not attempted.

Family	Species	*cox2-cox1* amplicon F	*cox2-cox1* amplicon M
Unionidae	*Actinonaias ligamentina*	+	+
	Amblema plicata	+	+
	Anodonta californiensis	+	+
	Cyprogenia aberti	+	+
	Cyrtonaias tampicoensis	-	+
	Dromus dromas	+	+
	Ellipsaria lineolata	+	+
	Elliptio dilitata	+	+
	Epioblasma brevidens	+	+
	Fusconaia flava	+	+
	Glebula rotundata	+	+
	Hamiota subrotundata	+	+
	Lampsilis cardium	+	+
	Lampsilis hydiana	+	+
	Lampsilis powellii	+	+
	Lampsilis reeveiana	+	+
	Lampsilis siliquoidea	+	+
	Lampsilis straminea	+	+
	Lampsilis streckeri	+	+
	Lampsilis teres	+	+
	Leptodea fragilis	+	+
	Leptodea leptodon	+	+
	Ligumia recta	+	+
	Medionidus conradicus	+	+
	Obovaria olivaria	+	+
	Popenaias popeii	+	+
	Potamilus alatus	+	+
	Potamilus capax	+	+
	Potamilus ohiensis	+	+
	Potamilus purpuratus	+	+
	Ptychobranchus fasciolare	+	+
	Toxolasma glans	+	+
	Truncilla truncata	+	+
	Venustaconcha ellipsiformis	+	+
	Villosa iris	+	-
	Villosa lienosa	+	+
	Villosa villosa	+	+
Margaritiferidae	*Cumberlandia monodonta*	+	+
	Dahurinaia sp.	-	+
	Margaritifera margaritifera	-	+
Hyriidae	*Hyridella menziesi*	+	+
Iridinidae	*Chambardia rubens*	+	-
	Mutela dubia	+	-
Etheriidae	*Etheria elliptica*	+	-
Mycetopodidae	*Anodontites guanarensis*	+	-
	Tamsiella tamsiana	+	-
Neotrigoniidae	*Neotrigonia margaritacea*	-	NA

ers. The 3' portion of the M *cox2-cox1* fragment was sometimes sequenced, using the HCO700dy2 sequencing primer, to confirm M *cox1* sequences generated by the *cox1* primer pair. Sequences were visualized using Li-Cor 4200L-2 and 4200S-2 DNA sequencers and initial base calls were made by e-Seq v 2.0. Contiguous sequences were assembled and verified using AlignIR v2.0 and final sequence alignments were completed manually with MacClade v4.0. GenBank accession numbers for the *cox1* sequences generated and/or analyzed herein are given in Table 2. All testis extraction-derived *cox1* sequences were added to a matrix containing confirmed F and M *cox1* sequences and phylogenetic analyses were used to test the putative M status of the newly generated sequences. Subsequent to the initial attempts to amplify the M *cox2-cox1* fragment from their testis-derived total DNAs, multiple attempts were made to amplify an M mitochondrial fragment from members of the Iridinidae and Mycetopodidae using two, intragenic universal primer pairs (i.e., *cox1* and *16S* [LR-J-12887, LR-N-13398; Simon et al., 1994]).

Three complementary F and M sequence data sets, populated by the unionoid species from which both F and M *cox1* sequences were obtained, were analyzed using the maximum likelihood (ML) and maximum parsimony (MP) algorithms contained in PAUP* (v.4.0b10; Swofford, 2001). Bayesian inference (BI) analyses were carried out with MrBayes v3.0b4 (Huelsenbeck & Ronquist, 2003). The complementary F and M genome *cox1* sequences were analyzed both individually and in an F + M concatenated manner. Recent literature indicates that a total evidence approach can produce the best tree topologies (e.g., Collin, 2003; Creer et al., 2003; Hassanin & Douzery, 2003; Schwarz et al., 2003). Thus, the F + M *cox1* concatenated trees were used as the best estimates of the phylogenetic relationships among the unionoid sequences examined. Additional phylogenetic analyses were carried out, using the BI and MP algorithms, on an inclusive non-concatenated *cox1* DNA sequence data set that contained a much broader taxonomic sampling of the available F *cox1* sequences as well as all available M *cox1* sequences. Modeltest (v. 3.6: Posada & Crandall, 1998) was used to determine which model best fit the F, M, and F + M sequence data. The GTR + G + I model was used in all BI and ML analyses. *Neotrigonia margaritacea* (Trigonioida) *cox1* sequences were used to root the trees derived from the complementary data set analyses (e.g., Hoeh et al., 1998a), while a much broader sampling of taxa was used to root the trees derived from analyses of the inclusive data set (e.g., Hoeh et al., 2002).

A total of 29 *cox1* sequences were included in the complementary data set phylogenetic analyses while 105 sequences were present in the inclusive non-concatenated M and F data set. Each of the four BI analyses consisted of 10 chains, 5 million generations, and a 2 million generation burn-in. PAUP* was used to select, from among all of the 1,000 saved BI trees from each of the complementary data set analyses, the topologies with the highest log likelihood scores. Due to the saturation of third position transitions (e.g., Hoeh et al., 1998a), all MP analyses were conducted on transformed *cox1* sequences such that third position transitions were excluded from analyses. Multiple random terminal taxa addition sequence runs, combined with global branch rearrangement options, were employed when generating topologies, from the complementary data sets, via the ML and MP algorithms. These options increased the probability of finding the actual best topology under each of these two optimality criteria (e.g., Hendy et al., 1988; Maddison, 1991). Standard non-parametric bootstrap (Felsenstein, 1985) analyses were carried out to evaluate the level of support for particular nodes obtained from the ML (1,000 bootstrap replicates) and MP (10,000 bootstrap replicates; 100,000 fast-heuristic replicates for the inclusive data set) analyses. A parsimony-based ILD test (Farris, 1994), as implemented in PAUP*, was used to test for incongruence between the F and M *cox1* sequences.

RESULTS AND DISCUSSION

What is the Taxonomic Distribution of DUI within the Unionoida?

Definitively M genome *cox2-cox1* fragments were amplified from 40 species representing three (Hyriidae, Margaritiferidae, and Unionidae) of the six unionoid families (Table 1). Sequences from *cox1* confirmed that the long *cox2-cox1* PCR fragments obtained from testis-based DNA extractions were from M genomes. However, testis-based DNA extrac-

TABLE 2. Source taxa and GenBank accession numbers for the *cox1* DNA sequences used in phylogenetic analyses.

Family	Species	GenBank Accession No. F	M
Unionidae	*Actinonias ligamentina*	AF231730	AF406796
	Cyrtonaias tampicoensis	AF231749	AF406798
	Fusconaia flava	AF231733	AF406799
	Gonidea angulata	DQ206792	DQ206794
	Lampsilis teres	AF406803	AF406794
	Ligumia recta	AF231748	AF406795
	Potamilus purpuratus	AF406804	AF406797
	Pseudodon vondembuschianus	DQ206793	DQ206795
	Pyganodon fragilis	AF406805	AF406800
	Pyganodon grandis	AF231734	AF406801
Margaritiferidae	*Cumberlandia monodonta* 1	AY785393	AY785397
	Cumberlandia monodonta 2	AF156498	
	Cumberlandia monodonta 3	AF156497	
	Cumberlandia monodonta 4	AY579131	
	Dahurinaia dahurica 1	AY579123	
	Dahurinaia dahurica 2		AY785400
	Dahurinaia dahurica 3		DQ241802
	Margaritifera auricularia 1	AY579125	
	Margaritifera auricularia 2	AF303312	
	Margaritifera auricularia 3	AF303313	
	Margaritifera auricularia 4	AF303315	
	Margaritifera falcata 1	AY579126	
	Margaritifera falcata 2	AY579128	
	Margaritifera falcata 3	AY579127	
	Margaritifera laevis	AY579124	
	Margaritifera margaritifera 1	AF303319	
	Margaritifera margaritifera 2	AF303320	
	Margaritifera margaritifera 3	AF303336	
	Margaritifera margaritifera 4	AF303341	
	Margaritifera margaritifera 5	AY579129	
	Margaritifera margaritifera 6	AY579130	
	Margaritifera margaritifera 7	AF303331	
	Margaritifera margaritifera 8	AF303332	
	Margaritifera margaritifera 9	AF303338	
	Margaritifera margaritifera 10	AF303340	
	Margaritifera margaritifera 11	AF303335	
	Margaritifera margaritifera 12	AF303337	
	Margaritifera margaritifera 13	U56847	
	Margaritifera margaritifera 14	DQ060171	
	Margaritifera margaritifera 15	AF303339	
	Margaritifera margaritifera 16		AY785399
	Margaritifera margaritifera durrovensis 1	AF303344	
	Margaritifera margaritifera durrovensis 2	AF303345	
	Margaritifera margaritifera durrovensis 3	AF303346	
	Margaritifera margaritifera durrovensis 4	AF303347	
	Margaritifera margaritifera durrovensis 5	AF303342	
	Margaritifera margaritifera durrovensis 6	AF303343	

(*continues*)

(continued)

Family	Species	GenBank Accession No. F	M
Hyriidae	*Alathyria jacksoni* 1	AY386977	
	Alathyria jacksoni 2	AY386981	
	Alathyria jacksoni 3	AY386970	
	Alathyria jacksoni 4	AY386974	
	Castalia stevensi	AF231736	
	Diplodon deceptus	AF231736	
	Hyridella australis	AF305367	
	Hyridella depressa 1	AF156496	
	Hyridella depressa 2	AF305368	
	Hyridella menziesi 1	AF231747	
	Hyridella menziesi 2		AF406802
	Lortiella rugata	AF231746	
	Velesunio ambiguus 1	AF305371	
	Velesunio ambiguus 2	AF305372	
	Velesunio ambiguus 3	AY211582	
	Velesunio ambiguus 4	AY211586	
	Velesunio angasi	AF231743	
	Velesunio sp. 1	AY387018	
	Velesunio sp. 2	AY386999	
	Velesunio sp. A 1	AY211550	
	Velesunio sp. A 2	AY211554	
	Velesunio sp. B 1	AY211558	
	Velesunio sp. B 2	AY211566	
	Velesunio sp. D 1	AY211587	
	Velesunio sp. D 2	AY211598	
Iridinidae	*Chambardia rubens* 1	DQ241807	
	Chambardia rubens 2	DQ241808	
	Chambardia rubens 3	AY785389	
	Mutela dubia 1	DQ241805	
	Mutela dubia 2	AY785388	
	Mutela dubia 3	DQ241806	
	Mutela rostrata 1	AY785387	
	Mutela rostrata 2	DQ241804	
Mycetopodidae	*Acostaea rivolii*	AF231739	
	Anodontites guaranensis	AY785383	
	Anodontites trigonus	AF231738	
	Monocondylaea minuana	AF231745	
	Tamsiella tamsiana	AY785384	
Etheriidae	*Etheria elliptica* 1	DQ241803	
	Etheria elliptica 2	AF231739	
Outgroup taxa	*Albinaria turrita*	X71393	
	Dentalium sp.	U56843	
	Drosophila yakuba	X03240	
	Katharina sp.	U56845	
	Lepetodrilus elevatus	U56846	
	Neotrigonia margaritacea	U56850	
	Solemya velum	U56852	

tions from all five species representing the Etheriidae, Iridinidae, and Mycetopodidae failed to yield the expected long M *cox2-cox1* fragment. Regarding the total DNAs extracted from representatives of these three families, all PCR attempts resulted in amplification of an F genome fragment from both mantle and testis DNA extractions. Subsequent sequencing and phylogenetic analyses of these fragments confirmed that identical sequences (all from F genomes) had been amplified from both mantle and testis extractions from the same individuals. Furthermore, the *cox1* and *16S* intragenic primer pairs failed to produce an M genome fragment. This corroborates the failure of the intergenic *cox2-cox1* primers to amplify an M fragment from the sampled etherioidean individuals.

The Etheriidae, Iridinidae, and Mycetopodidae represent closely related taxa, and have typically been given distinct superfamilial status, Etherioidea (Parodiz & Bonetto, 1963; Hoeh et al., 1998b, 2001; Bogan & Hoeh, 2000; Roe & Hoeh, 2003). Given their phylogenetic propinquity, it is not surprising that representatives of these three families would produce similar, yet unexpected, results: failure to yield M genome amplicons. There appear to be three possible explanations for the failure of representatives of the Etheriidae, Iridinidae and Mycetopodidae to yield M fragments. (1) Recent masculinization events have occurred such that the newly recruited "M genomes" (originally F genomes) are amplified. (2) The primers failed to anneal to the M sequence due to the rapidly evolving nature of the M genomes (Rawson & Hilbish, 1995; Stewart et al., 1995; Curole & Kocher, 2002; Hoeh et al., 2002; Krebs, 2004). (3) These taxa do not possess DUI.

Mitochondrial DNA masculinization events in DUI-containing taxa were first postulated for, and later supported with data from, *Mytilus* by Hoeh et al. (1996, 1997). Subsequently, other investigators have corroborated the existence of the mtDNA masculinization process in mytiloid but not in unionoid bivalves (e.g., Zouros et al., 1994; Hoeh et al., 1996, 2002; Saavedra et al., 1997). However, if the masculinization hypothesis is to be invoked as the explanation for our observations, we would expect to observe distinct etherioidean M mtDNA sequences that are more closely related to F sequences than to other M sequences. Our repeated observations of identical mtDNA sequences from testis- and mantle-derived DNA extractions from each etherioidean individual examined do not meet these expectations. Immediately after a masculinization event, it is predicted that the F and new "M" (i.e., recently converted from the female- to the male-transmission route) genomes will be identical. However, under the masculinization hypothesis, it is extremely unlikely that we would have observed identical *cox1* sequences from separate mantle and testis DNA extractions from individuals representing five species as this would require multiple independent, approximately simultaneous, and relatively recent masculinization events.

The M genomes in both unionoid and mytiloid bivalves have a significantly greater rate of substitution relative to that estimated for the corresponding F genomes (Skibinski et al., 1994; Hoeh et al., 1996, 2002; Liu et al., 1996; Stewart et al., 1996; Quesada et al., 1998; Krebs, 2004). This may be due to a higher mutation rate for the M genomes, smaller effective population size for the M genomes, positive selection for the M genomes, relaxed selection for the M genomes, or a combination of these processes (Stewart et al., 1996; Passamonti et al., 2003). Nevertheless, an elevated rate of substitution has been suggested as the explanation for the occasional failure of universal mtDNA primer pairs to amplify M genomes (Rawson & Hilbish, 1995; Stewart et al., 1995; Curole & Kocher, 2002; Hoeh et al., 2002; Krebs, 2004). Our use of three distinct, conserved primer pairs to attempt amplification of etherioidean M genomes, with failure to do so in each instance, suggests that either (1) all of the sampled etherioid specimens have very divergent M mtDNA sequences for *16S*, *cox1*, and *cox2* or (2) these specimens lack DUI. We believe that multiple failed M-fragment amplification attempts, using both intra- and inter-genic conserved primer pairs, render the former hypothesis unlikely.

We thus believe that it is likely that DUI is absent from the Etherioidea. This leads to the question of whether the absence of DUI in this superfamily indicates a loss in the ancestral etherioidean lineage or a gain of DUI in the ancestral unionoidean lineage (after Parodiz & Bonetto, 1963). Unfortunately, due to the lack of a robust unionoid phylogeny and information regarding the presence/absence of DUI in *Neotrigonia*, there remains no *a priori* way to rigorously evaluate which condition is apo-

morphic and thus, the phylogenetically informative character state. If DUI was derived within the Unionoida, then it may represent an apomorphy for the Unionoidea, whereas a loss of DUI in the common etherioidean ancestor would represent an apomorphy for the etherioids and the presence of DUI would represent a plesiomorphy for the Unionoida. Robust inferences regarding the evolutionary dynamics of unionoid DUI depend upon the existence of a robust phylogeny for the Unionoida. As mentioned previously, phylogenetic analyses to date, utilizing partial F *cox1* sequences (Folmer fragment), have been unable to robustly resolve unionoid familial relationships. However, M *cox1* sequences have demonstrated the ability to increase topological resolution when analyzed alone or in conjunction with F *cox1* sequences (Hoeh et al., 2002). Utilization of the relatively new model-based Bayesian phylogenetic methods appears to reveal additional phylogenetic signal contained within existing F *cox1* sequences.

What Can Analyses of Complementary F and M *cox1* Sequences Tell us About Higher Level Unionoidean Relationships?

Failure to amplify M genome fragments from any etherioid taxa obviously prevents us from conducting any taxonomically inclusive M genome-based higher level phylogenetic analysis of unionoid relationships. Given this serious limitation, what can analyses of complementary F and M *cox1* sequence data sets tell us about higher level unionoidean (*sensu* Parodiz & Bonetto, 1963) relationships? Phylogenetic analyses of F *cox1* sequences recover *Hyridella* as the basal unionoidean lineage; however, the Unionidae are not recovered as monophyletic (Fig. 2). Specifically, *Cumberlandia*, a margaritiferid, is depicted as the sister taxon to *Fusconaia* and this placement renders the Unionidae paraphyletic. Nodal support levels for the interfamilial relationships are relatively low as seen in previously published F *cox1* analyses (e.g., Hoeh et al., 2001). Robust intergeneric nodal support values are only observed for the clade containing the following four lampsiline taxa: *Actinonaias*, *Lampsilis*, *Ligumia*, and *Potamilus*.

Analyses of M *cox1* sequences recovered a robustly supported, monophyletic Unionidae but the relationships among the hyriid, margaritiferid, and unionid taxa were not well resolved (Fig. 3). As in the topology obtained from the F *cox1* analysis, *Hyridella* is represented as a descendent of the primary unionoidean cladogenic event. In general, the nodal support values derived from analyses of M *cox1* sequences are significantly improved over those of the F *cox1* analysis presented herein (Fig. 2). These results were foreshadowed by previous comparative analyses of F and M sequences (e.g., Hoeh et al., 2002; Krebs, 2004).

Concatenating F and M *cox1* sequences was legitimized by the lack of significant incongruence between the F and M sequences (as indicated by the ILD test, $p = 0.271$). Phylogenetic analyses of the concatenated F and M *cox1* sequences (Fig. 4) produced a topology very similar to that produced by the M *cox1* analyses (Fig. 3). This result was anticipated due to the greater number of parsimony-informative sites in the M *cox1* sequences (Hoeh et al., 2002) and the lack of significant incongruence between the F and M sequences. However, a fundamental difference between the results of the M and F + M analyses is the latter's increased nodal support for margaritiferids (represented herein by *Cumberlandia*) as the sister taxon to the Unionidae. This result strongly supports the basal position of hyriids (represented by *Hyridella*) within the Unionoidea. This basal placement of hyriids is independently supported by Graf's (2002) analyses of *28S* sequences, which also lacked representatives of the Etherioidea.

The basal position of *Hyridella* in all of the analyses presented herein is in conflict with the placement of the Margaritiferidae as the basal unionoid lineage as depicted in the morphology-based trees of Graf (2000) and Hoeh et al. (2001), as well as in the total evidence-based tree presented in Roe & Hoeh (2003). However, our topology is consistent with the molecular and total evidence-based topologies of Hoeh et al. (1998b, 2001) as well as with the hypothesis that the relatively "simple" anatomy of margaritiferids is a derived rather than an ancestral condition as often postulated. For example, the loss of ctenidial water tubes in margaritiferids may be the end result of selection for the release of a significantly larger conglutinate mass than that of its ancestor.

If the relative evolutionary relationships postulated from the complementary F and M concatenated *cox1* data set analyses are maintained in subsequent more taxonomically inclusive phylogenetic analyses of the Unionoida, where might a monophyletic Etherioidea attach to our unionoidean tree? Two previously presented hypotheses of unionoid

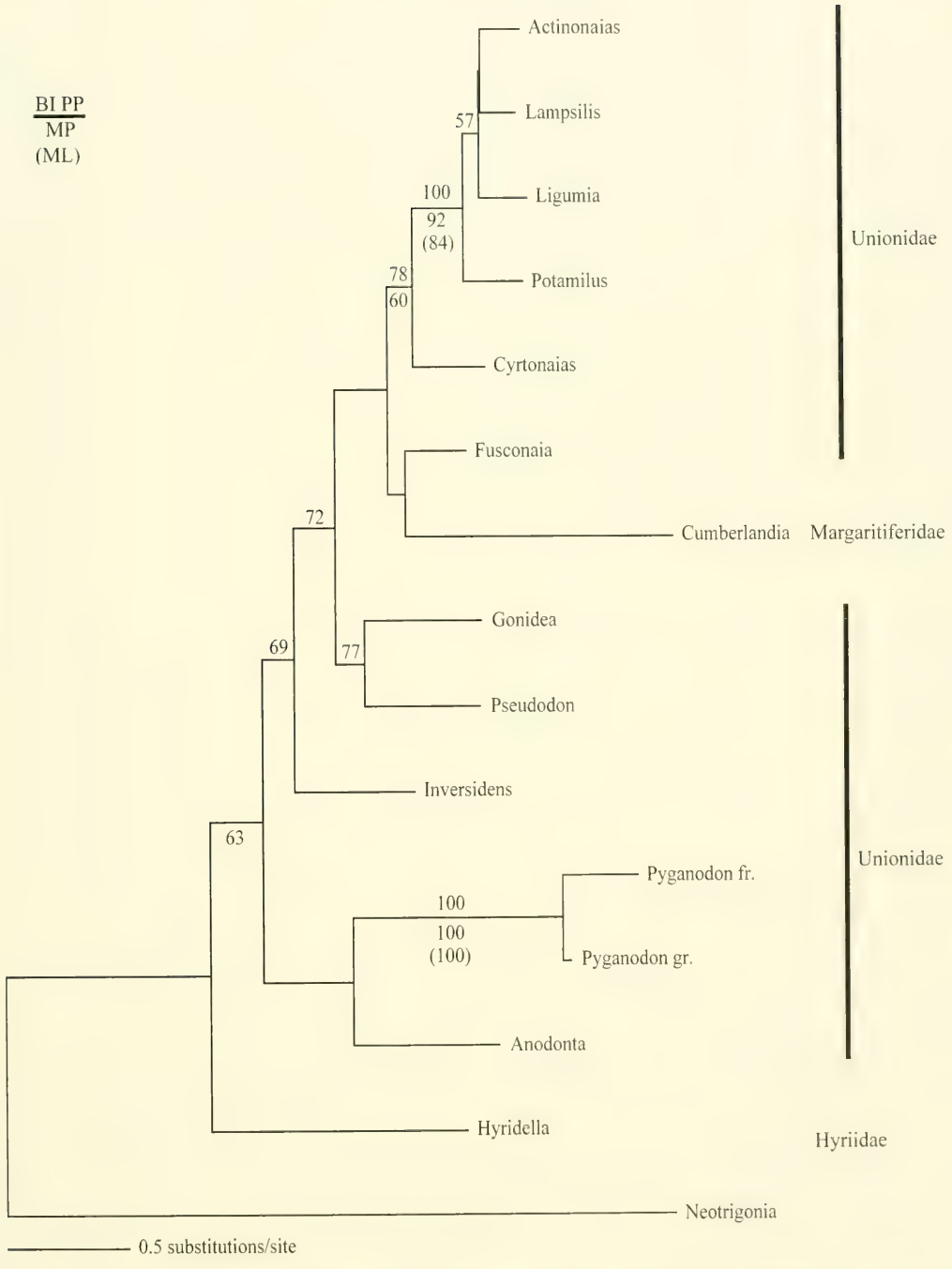

FIG. 2. Best tree found by Bayesian analyses of F *cox1* sequences (619 bp). When > 50, Bayesian posterior probabilities (x100) presented above internodes, MP bootstrap values and ML bootstrap values (in parentheses) are presented below internodes.

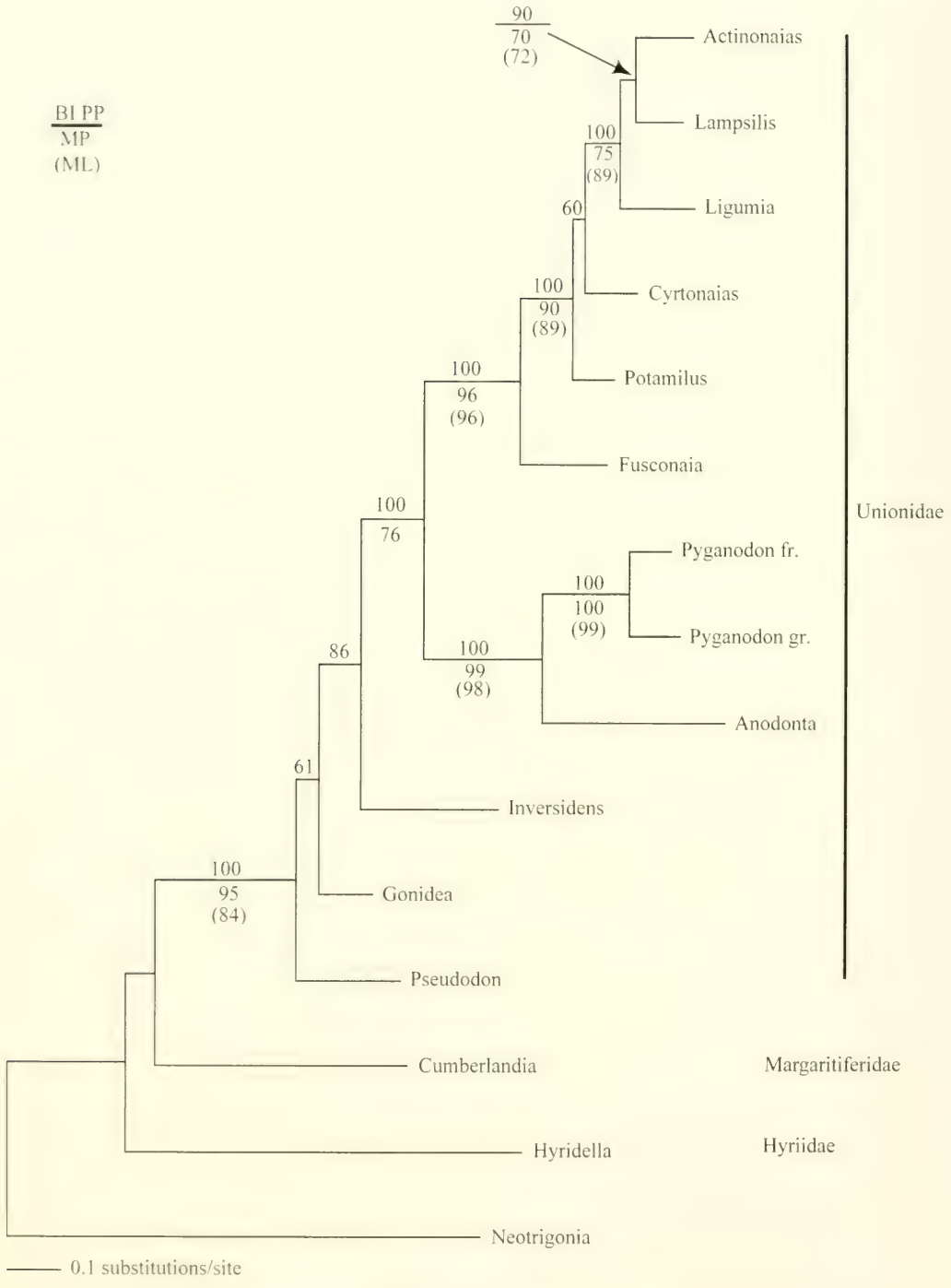

FIG. 3. Best tree found by Bayesian analyses of M *cox1* sequences (619 bp). When > 50, Bayesian posterior probabilities (x100) presented above internodes, MP bootstrap values and ML bootstrap values (in parentheses) are presented below internodes.

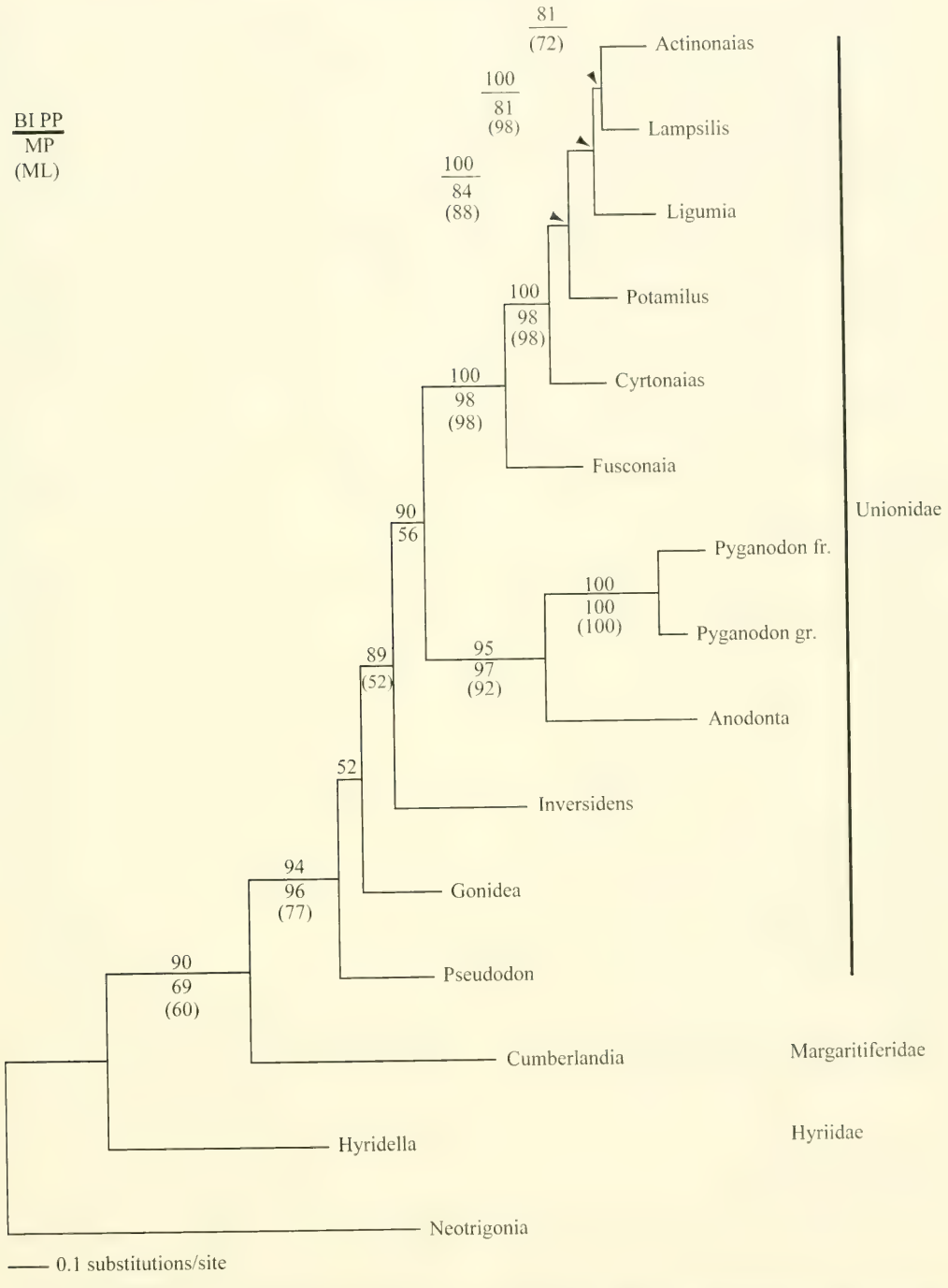

FIG. 4. Best tree found by Bayesian analyses of concatenated F + M *cox1* sequences (1238 bp). When > 50, Bayesian posterior probabilities (x100) presented above internodes, MP bootstrap values and ML bootstrap values (in parentheses) are presented below internodes.

higher level relationships are consistent with the relative relationships presented herein. One possibility is that the etherioidean lineage is the sister taxon to a monophyletic Unionoidea (Fig. 5A). This topology is consistent with the Parodiz & Bonetto (1963) unionoid classification. Deductions from this topology regarding DUI evolutionary dynamics are dependent on whether or not the outgroup, *Neotrigonia*, possesses DUI. If *Neotrigonia* lacks DUI, this topology would be consistent with the hypothesis of an ancestral unionoidean gain of DUI. Alternatively, if *Neotrigonia* possesses DUI, this topology would be consistent with a loss of DUI in the ancestral etherioidean lineage. Under this topology, endobranchy is hypothesized as the ancestral unionoid brooding strategy but the two principal larval character states cannot be polarized. In addition, under this topology, the Parodiz & Bonetto (1963) "independent invasions of freshwater" hypothesis for unionoidean and etherioidean bivalves is not robustly rejected.

An alternative unionoid topology, that would maintain the relative evolutionary relationships represented in the trees from the complemen-

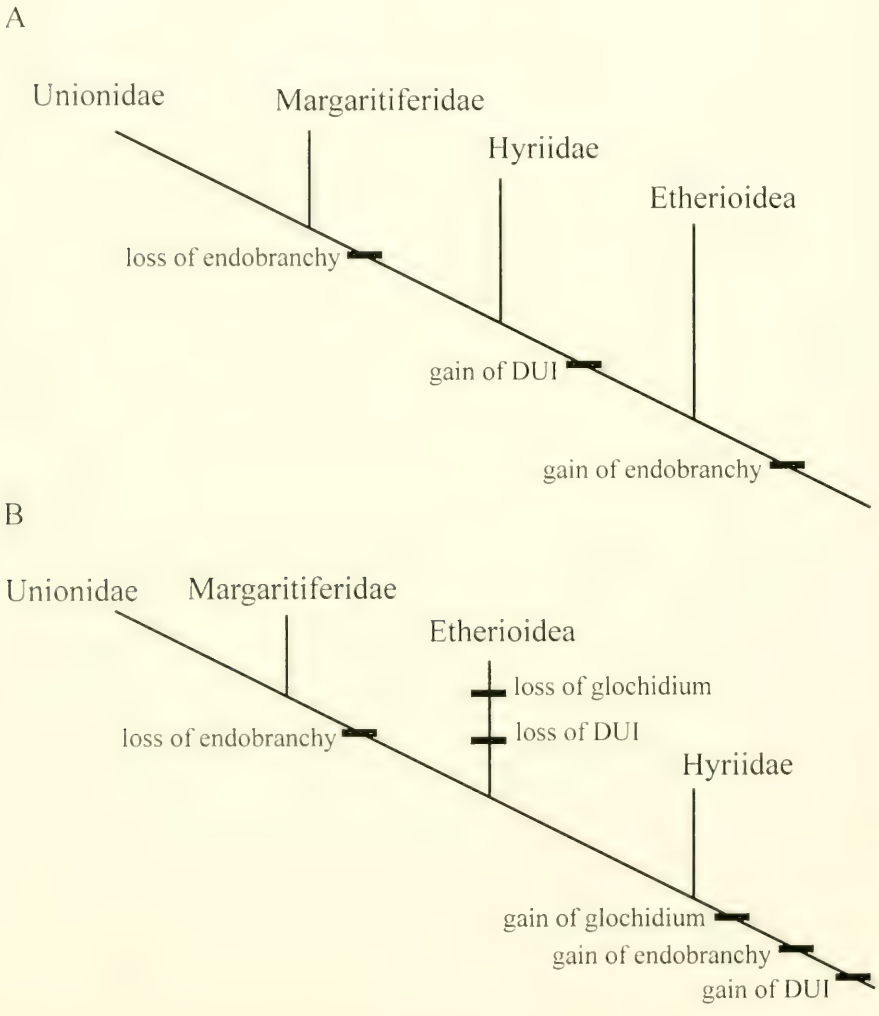

FIG. 5. Hypothesized character state transitions for reproductive characters exhibited by unionoid bivalves. A: Hypothesized familial relationships after Parodiz & Bonetto (1963); B: Hypothesized familial relationships after Hoeh et al. (2001). The two DUI character state optimizations displayed herein are based on the assumption that *Neotrigonia* lacks DUI.

tary analyses, is that the Etherioidea is sister taxon to the margaritiferid + unionid clade (Fig. 5B). This particular unionoid topology was supported by previous analyses using F *cox1* sequences (Hoeh et al., 1998b, 2001). This topological placement would support the following hypotheses: (1) the ancestral etherioidean lineage lost DUI, (2) the glochidium is the ancestral larval type for the Unionoida, and (3) endobranchy is the ancestral brooding condition for the Unionoida. Additionally, this particular unionoid topology would reject the monophyly of the Unionoidea (*sensu* Parodiz & Bonetto, 1963) as well as Parodiz & Bonetto's "independent invasions of freshwater" hypothesis for unionoidean and etherioidean bivalves.

What Can Taxonomically Inclusive Analyses of Non-concatenated F and M *cox1* Sequences Tell us About Higher Level Unionoid Relationships?

The topology of the F genome portion of the inclusive BI analysis (Fig. 6) strongly supports the hypothesis that etherioids are the sister taxon to a margaritiferid + unionid clade (PP = 93), thus rendering the Unionoidea paraphyletic. This topology is congruent with the hypothesis of unionoid relationships presented in Figure 1C (after Hoeh et al., 1998b, 2001). It also strongly supports the monophyly of margaritiferid, mycetopodid, and etherioid bivalves (PP = 100 for each clade) and the paraphyly of the Unionidae (PP = 90). In contrast, monophyly of the Hyriidae is weakly supported (PP = 63) and iridinid monophyly was not supported. The results from the M-genome portion of the inclusive BI-based phylogenetic analysis of the *cox1* DNA sequences (Fig. 6) strongly support the sister taxa status (PP = 96) for the margaritiferid and unionid clades (PP = 100 for each family). Furthermore, both the F and M genome portions of this BI analysis strongly support the evolutionary propinquity of *Gonidea* and *Pseudodon* (PPs = 98 and 100, respectively). The topology obtained from the concatenated F and M *cox1* sequence analysis (Fig. 4) is in agreement with the former but not the latter hypothesis. In general, the MP bootstrap analysis of the inclusive non-concatenated *cox1* data set produced much lower nodal support values than did the BI analysis (Fig. 6).

The BI analysis presented in Figure 6 strongly supports the character state dynamics hypothesized in Figure 5B: (1) The presence of DUI, glochidial larvae, and endobranchous brooding characterized the ancestral unionoid lineage, (2) the loss of DUI and glochidial larvae (i.e., the gain of standard maternal inheritance and lasidial larvae) occurred in the ancestral etherioidean lineage, and (3) the loss of endobranchy occurred in the ancestor of the margaritiferid + unionid clade. The hypothesis that the relatively "simple" anatomy of margaritiferids is a derived rather than an ancestral condition is also supported. Furthermore, the topology presented in Figure 6 strongly rejects the hypothesis that unionoidean and etherioidean bivalves represent independent invasions of freshwater habitat (i.e., unionoid bivalve polyphyly, as suggested by Parodiz & Bonetto, 1963). Reciprocal monophyly for the Etherioidea and Unionoidea, which would be consistent with the independent invasion hypothesis, is rejected by the topology presented in Figure 6.

At least two aspects of the phylogenetic results presented in Figure 6 should serve as a caution to any attempt to canonize these results: (1) only the BI analysis provided strong support for many of the higher level unionoid bivalve relationships discussed above and (2) the phylogeny for the Unionoida indicated in the F clade of Figure 6 is based on a relatively small number of nucleotides (a maximum of 619) from a single genetic locus (F *cox1*). In order to more rigorously evaluate these central yet, in our opinion, currently open questions regarding unionoid higher level relationships and character state evolutionary dynamics, we are currently investigating the efficacy of incorporating DNA sequences from additional F mitochondrial genes (e.g., F *cox2*). Including information from multiple female–transmitted unionoid mtDNA genes has the potential to increase the topological resolution of taxonomically inclusive analyses. In addition, nuclear genes (e.g., *28S*, *EF1-alpha*, *act42A*) as well as morphological characters are being investigated to assess their potential to facilitate the construction of a robust phylogeny for the Unionoida.

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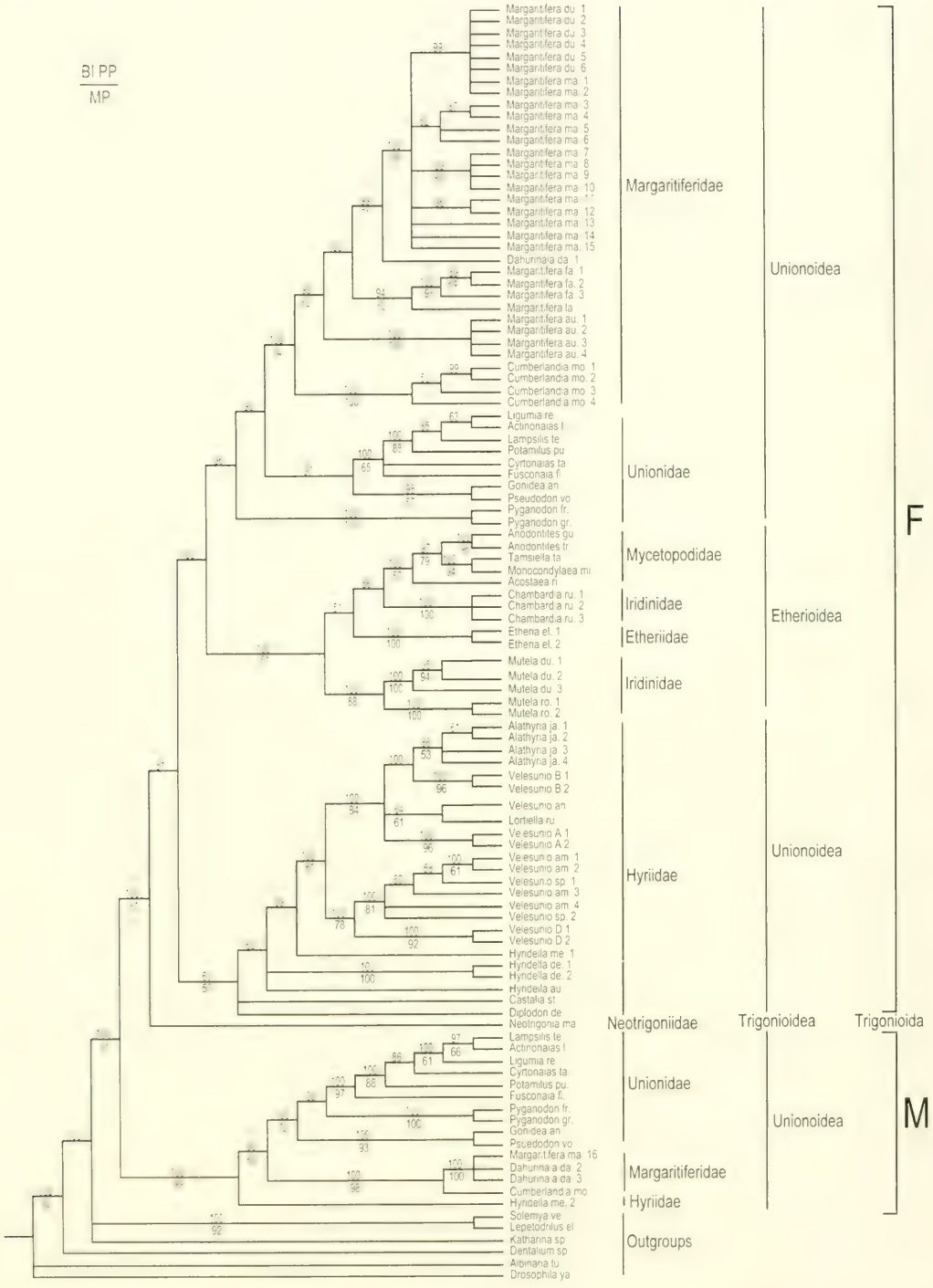

FIG. 6. Fifty percent majority rule consensus tree, with posterior probabilities (x100, above internodes), obtained from the inclusive Bayesian analysis of non-concatenated *cox1* F and M DNA sequences. When > 50, MP bootstrap values (100,000x, fast-heuristic search) are presented under the internodes.

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RESEARCH NOTES

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THE HISTORICAL MISIDENTIFICATION OF *MARGARITIFERA AURICULARIA* FOR *M. MARGARITIFERA* (BIVALVIA, UNIONOIDEA) EXPLAINED BY THEIR ICONOGRAPHY

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ABSTRACT

Throughout its history, *Margaritifera auricularia* has been confused with its relative *M. margaritifera*. This paper compiles the early iconography of *M. auricularia* and reproduces the illustrations of this species. Our objective is to not only recapture the many interesting images of *M. auricularia*, but also to examine the historical errors that led to the confusion between the two species. After selecting valid representations of *M. auricularia* and its true synonyms, we see that this confusion has existed since Spengler (1793) first described the species. Indeed, we show that the first published image of *M. auricularia*, by Draparnaud (1805), was erroneously labeled as an image of *M. margaritifera*. We also reproduce several previously undiscovered illustrations of juvenile specimens of *M. auricularia*, as well as some interesting figures of *M. margaritifera* that were published before its description by Linnaeus (1758). One of these illustrations, Magnus (1555), is probably the first known image of a freshwater mussel.

FIRST DESCRIPTION OF *M. AURICULARIA* AND ITS EARLY MISIDENTIFICATION WITH *M. MARGARITIFERA*

The giant freshwater mussel, *Margaritifera auricularia*, is one of two European species of *Margaritifera*. Before its present rarity, it lived in the large, muddy rivers of western Europe and North Africa (Araujo & Ramos, 2000), whereas its relative *M. margaritifera* inhabited the smaller, colder rivers of northern Europe and North America. The characteristics of the fluvial habitat of *M. auricularia* have made it difficult to gather specimens. Thus, not only was this species discovered later, but it is less well known than *M. margaritifera*, which has been exploited since Roman times for its capacity to produce small pearls (Bonnemère, 1901).

Margaritifera margaritifera was first described by Linnaeus (1758) as *Mya margaritifera*. *Margaritifera auricularia* was originally named as *Unio auricularius* by the Danish malacologist Lorentz Spengler (1793: 54–55), who erroneously cited the East Indies as its type locality. Although Spengler did not illustrate *U. auricularius*, his description of its large dorsal teeth and the hinge clearly differentiate it from *M. margaritifera*. Lamarck (1819) described *Unio sinuata* (Fig. 1), which today is considered to be a synonym of *M. auricularia*.

Despite Spengler's description, both European species of the genus *Margaritifera* have been misidentified many times, and the first author to do so was, curiously enough, Spengler himself. In his original description, he cited a figure in by Martin Lister's *Historiae conchyliorum* (1686: fig. 149) as an illustration of *Unio auricularius*. However, Lister's figure shows the inside of a large, very sinuate *M. margaritifera* valve with pronounced cardinal teeth, and which at first glance resembles a valve of *M. auricularia* (Fig. 2). To confirm this, we tried unsuccessfully to find this specimen. Lister used shells from several collections to illustrate his book, mainly from his collection and that of William Courten. According to Wilkins (1953), the Courten collection was acquired by Hans Sloane, and the Sloane collection later became the nucleus of the British Museum collection, now in The Natural History Museum. Nevertheless, this *M. margaritifera* valve is not among the shells in the Sloane collection that were illustrated by Lister (Wilkins, 1953). It is possible that this valve was part of the Lister collection that was first owned by the Ashmolean Museum, and which

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FIGS. 1–5. FIG. 1: One of the syntypes of *Unio sinuata* Lamarck (MHNG 1086/75). Inscriptions by Lamarck are found in the interior of the valves; FIG. 2: Lister (1686: sheet of "plates", each a separate woodcut) with several freshwater bivalves and one right valve of *M. margaritifera* in pl. 149 (bottom). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 3: A fishery of *M. margaritifera* by Magnus (1555). By permission of the Biblioteca Nacional, Madrid, Spain; FIG. 4: The illustration of *M. margaritifera* (upper left corner) by Pontoppidan (1755). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 5: Type specimen and original label of *M. auricularia* from the Spengler collection.

was later moved to the Oxford University Museum of Natural History. However, Dance (1986) reported that none of the shells attributed to the Lister collection were there.

Simpson (1900) attributed Lister's figure to *M. margaritifera*, and Haas (1909), one of the most important researchers on freshwater mussels, also discovered Spengler's error, realizing that the lateral teeth were absent. This also meant that *M. margaritifera* had been illustrated by Lister nearly a century prior to its description by Linnaeus. There were at least two other authors who illustrated *M. margaritifera* before Lister. The first of these was probably Olaus Magnus (1555), a Swedish geographer, archbishop of Upsala and author of *Historiae de gentibus septentrionalibus*. His illustration of a catch of *M. margaritifera* (Fig. 3) was the first rough image of this species and perhaps the first ever of a freshwater mussel. Pontoppidan (1755), a bishop of Bergen, also illustrated *M. margaritifera* in his *The natural history of Norway* (Fig. 4). (This same figure was probably in the original 1753 edition, but we have not had an opportunity to examine it.) Other pre-Linnean authors, including Rondelet (1555) and Boussuet (1558), illustrated specimens of such other freshwater mussels as *Anodonta*.

Haas (1913) confirmed true identity of *Unio auricularius* in his paper on the *Unio* species described by Spengler. In an attempt to prevent future misidentification, he illustrated Spengler's polished specimen in the Natural History Museum of Copenhagen (Fig. 5).

Several years prior to this, two European authors contributed to the confusion with their interpretation of freshwater mussel fossils discovered in Britain. Jackson & Kennard (1909) mistakenly attributed *M. auricularia* shells from Pleistocene sediments of the Thames River to *Unio* (*Margaritana*) *margaritifer* (Linnaeus) (= *M. margaritifera*). (*Margaritana* is an objective synonym of *Margaritifera*.) These authors noted the extraordinary size of the shells and concluded that "*Unio margaritifer* was living abundantly in the Thames". Haas (1910) and Jackson (1911) soon rectified this error when they confirmed that the fossils were actually *Unio sinuatus* (Lamarck) (= *M. auricularia*).

Just like their European counterparts, North American malacologists have also been confused by these *Margaritifera* species. For instance, Simpson (1900) used the names *Margaritana margaritifera* (Linnaeus) and *Margaritana crassa* (Retzius, 1788) to refer to *M. auricularia*. Several years later, Kennard et al. (1925) suggested that this confusion was caused "partly through misidentification and partly because the later observers relied on the figures of their predecessors more than on their texts but chiefly because successive writers borrowed the synonymy of their forerunners without checking it". Despite this observation, however, they also continued to make the same errors themselves. According to these authors, the *Mya margaritifera* from Schröter's *Die Geschichte der Flüssconchylien* (1779: pl. 4, fig. 1) represents *M. auricularia* when, in fact, it is *M. margaritifera*. It is likely that they did not examine this figure, given that they considered their identification "unmistakable because of the strong lateral teeth and the peculiarities of the anterior muscular scars". These characters are absent in the above mentioned engraving, which clearly illustrates a specimen of *M. margaritifera*. After reading the authors' commentaries on another figure, we are certain that either they did not carefully study or did not understand Schröter's book. Schroter's specimen of *Mya testa crassa* is not, as they claim, a medium-sized specimen of *M. margaritifera*, but rather a normal specimen of *Unio crassus* (Fig. 6).

We see then that the confusion began with Spengler's erroneous interpretation of Lister's figure and was later complicated by the equally incorrect interpretation of *Mya testa crassa* (Schröter) by Kennard et al. (1925). Simpson (1900: 677, note 4) makes the same error by including *Mya testa crassa* (Schröter) as a synonym for the species *Margaritana crassa* (Retzius) in his records of *M. auricularia*. The confusion was perhaps caused by usage of the Latin *crassus* (meaning "very thick"), by both Lister, in his caption below the figure of *M. margaritifera* (*Musculus niger, omnium longe crassisimus, conchae longae species Gesn. Aldrov.*), and by Spengler in his description of *Unio auricularius* (*Testa crassa, oblonga, etc.*).

More interesting information is revealed about Lister's figure in his *Historiae animalium Angliae* (1681), some years prior to *Historiae conchyliorum* (1686). Here, Lister illustrates the same *M. margaritifera* valve that appears in the later work, along with valves from two other molluscs – *Unio pictorum* and *Anodonta* sp. The description of the *M. margaritifera* valve is only slightly different from that which appeared in *Historiae conchyliorum*: "Black

FIGS. 6–9. FIG. 6: Plate 2 of Schröter (1779). *Mya testa crassa* in fig. 2 (upper left corner) is actually *Unio crassus*. By permission of the British Library; FIG. 7: Blainville's (1827: pl. 67, fig. 3) figure of *M. auricularia* (middle). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 8: Plate 10 of Draparnaud (1805). This is the first known illustration of *M. auricularia* (middle and bottom left). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 9: Plate 23 by Dupuy (1851) representing one adult specimen (top) and the first known figure of a *M. auricularia* juvenile (middle) in figs. 7a and 7c, respectively. Fig. 7b (left) depicts the hinge of the adult. By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain.

mussel, entire shell very thick and very strong, from long shelled species after Gesner and Aldrovandi" [*Musculus niger, omnium crassissima et ponderosissima testa, conchae longae species Gesn. Aldrov.*]. However, further information written below the figure plainly pertains to *M. margaritifera*. For instance, Lister says: "It is sometimes fished with net in the deep whirpools of the Tees River in Yorkshire, not so far from Dinsdale" [*In profundis voraginibus Fluvii Tees agri Eboracensis, non longe a Dinsdale, rete aliquando expiscatur*]. We know today that only *M. margaritifera* lives in Yorkshire Rivers.

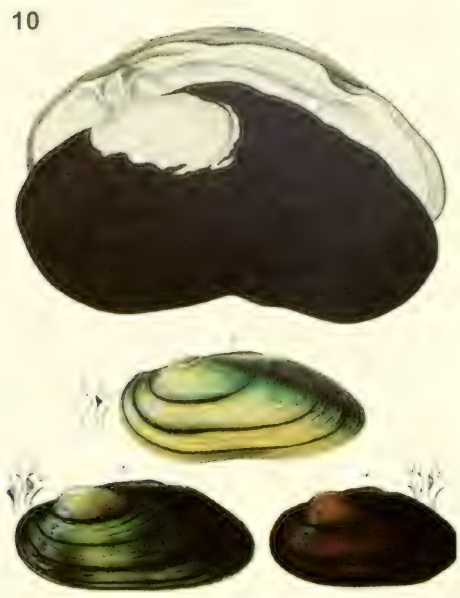
ICONOGRAPHY OF *MARGARITIFERA AURICULARIA*

We have reviewed all the early books on shells and malacology listed by Caprotti (1994) and Barbero (1999) (Table 1), as well as Simpson's (1900) list of synonyms for *Margaritana margaritifera* and *M. crassa*. Having confirmed that *Mya testa crassa* (Schröter) did not correspond to *M. auricularia,* the next author on Simpson's list to illustrate the species was Blainville (1827: pl. 67, fig. 3). In a lithography showing naiads (Fig. 7), Blainville identified the giant freshwater pearl mussel as *Unio sinuata* (or moulette sinuée). Nevertheless, Azpeitia (1933) discovered that another author, Draparnaud (1805), illustrated *M. auricularia* several years prior in his *Histoire naturelle des mollusques terrestres et fluviatiles de la France* (Fig. 8). This image went unnoticed because Draparnaud misidentified both species of *Margaritifera* and labeled his image *Unio margaritifer*, Moulette margaritifera, or Moule du Rhin, although its real identity can be proven by the hinge teeth. Locard (1895) also reported this mistake in his *Étude sur la collection conchyliologique de Draparnaud*: "Draparnaud has made an error in respect of this species. His *Unio margaritifer*, cited by him as *Mya margaritifera* after Linné and Müller, really is the *Unio sinuatus* of Lamarck. We have specimens proceeding from the Loire River which are exactly similar to the one figured by him."

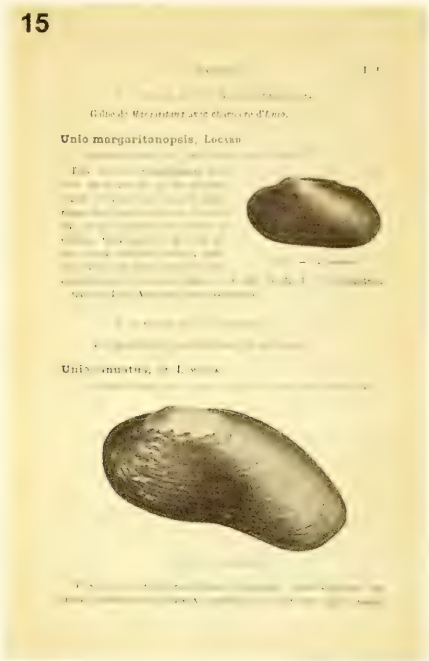
The next authors on Simpson's list to illustrate *M. auricularia* were Dupuy (1851) (Fig. 9), who drew the first known figure of a juvenile *M. auricularia*, Küster (1855) (Fig. 10), Rossmässler (1855) (Fig. 11), Moquin-Tandon (1855) (Fig. 12), Drouet (1857) (Fig. 13), G. B. Sowerby II (1868) (Fig. 14), and Locard (1893) (Fig. 15). Simpson also makes reference to: Bruguière (1797: pl. 248) [as "Deshayes, 1827"], Pfeiffer (1821), Rossmässler (1836, 1838, 1856), and Hanley (1856), but with the exception of Rossmässler (1856), the figures of these authors do no depict *M. auricularia*. Simpson (1900) wrote that the alleged *M. auricularia* specimens illustrated by Bruguière (1797) "look something like a heavy inflated *Lampsilis alatus* Say" [now *Potamilus alatus* (Say, 1817)]. In any event, the figured outline

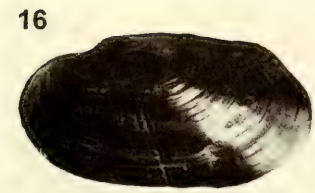
TABLE 1. Historical illustrations of *M. auricularia*.

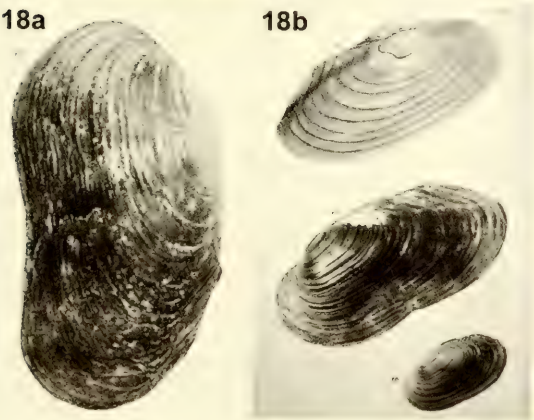
Author	Date	Figure(s)	Cited as
Draparnaud	1805	pl. 10, fig. 19	*Unio margaritifera*
Blainville	1827	pl. 67, fig. 3	*Unio sinuata*
Dupuy	1851	pl. 23, fig. 7a–c	*Unio sinuatus*
Küster	1855	pl. 37, fig. 1	*Unio sinuatus*
Rossmässler	1855	pl. 70, fig. 853	*Unio sinuatus*
Moquin-Tandon	1855	pl. 48, fig. 1	*Unio sinuatus*
Drouet	1857	pl. 2	*Unio sinuatus*
Sowerby	1868	pl. 62, fig. 311	*Unio sinuatus*
Locard	1893	figs. 163, 164	*Unio margaritanopsis* & *U. sinuatus*
Haas	1913	fig. 1	*Unio auricularius*
Haas	1916	fig. 1	*Margaritana auricularia*
Kennard et al.	1925	pl. 21, figs. 1–3	*Margaritana auricularia*
Haas	1929	figs. 181, 182	*Margaritifera auricularia*
Germain	1930	pl. 26, fig. 609, 615	*Margaritana auricularia* & *M.? margaritanopsis*
Azpeitia	1933	pl. 12, figs. 65, 66; pl. 13, fig. 67	*Margaritana auricularia*
Huckriede & Berdau	1970	pl. 1	*Margaritifera auricularia*
Fechter & Falkner	1990	color photo, p. 255	*Pseudunio auricularius*
Falkner	1994	photo, fig. 1	*Pseudunio auricularius*



FIGS. 10–13. FIG. 10: Plate 37 of Küster (1848). Top, *M. auricularia*; FIG. 11: Plate 70 by Rossmässler (1835) showing the hinge and a left valve of *M. auricularia*. By permission of the Österreichische Nationalbibliothek; FIG. 12: Plate 48 of Moquin-Tandon (1855). Fig. 1 (top) is *M. auricularia*. By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 13: Figure of *M. auricularia* in plate 2 by Drouet (1857). By permission of the Natural History Museum Picture Library.







FIGS. 14–18. FIG. 14: Plate 62 by G. B. Sowerby II (1868). Fig. 311 (middle) is *M. auricularia*. By permission of the British Library; FIG. 15: Page 151 of Locard (1893) showing a juvenile (top) and an adult specimen of *M. auricularia* (figs. 163 and 164, respectively). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 16: The juvenile specimen of *M. auricularia* figured by Haas (1916). By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 17: Plate 26 of Germain (1930). Figs. 609 (top) and 615 (bottom right corner) depict an adult and a juvenile specimen of *M. auricularia*. By permission of the Museo Nacional de Ciencias Naturales, Madrid, Spain; FIG. 18a: *M. auricularia* in Azpeitia (1933: pl. 12); FIG. 18b: Adult (middle) and juvenile (bottom) specimens of *M. auricularia* in Azpeitia (1933: pl. 13).

of the shell and the presence of two siphons are characters that are completely absent in margaritiferids. The image by Pfeiffer (1821) is, in fact, *Potomida littoralis* (Lamarck, 1801), and it is the same species that Rossmässler (1836: pl. 13, fig. 195) drew and labeled *Unio sinuatus*. Rossmässler's (1838: pl. 35, fig. 493) figure of *Unio gargottae* Philippi, 1836, actually depicts *M. margaritifera*, and Rossmässler's (1856: pl. 80, fig. 853) is the same *M. auricularia* he illustrated in 1855. Lastly, the shell illustrated by Hanley (1856) identified as *Unio crassissimus* Hanley, 1843, another synonym of *M. auricularia*, may or may not be *M. auricularia*, as it is one of 60 very small illustrations of freshwater mussels on the same plate. It is interesting to note that *Unio margaritanopsis* Locard, 1893 (Fig. 15), is really a juvenile *M. auricularia*. Haas (1913, 1916, 1929) (Fig. 16), Kennard et al. (1925), Germain (1930) (Fig. 17), and Azpeitia (1933) (Fig. 18a, b) are the last historical authors to figure the species. Curiously, three of these four authors illustrated juvenile specimens. Haas (1916) and Azpeitia (1933) did so intentionally, but Germain assigns this juvenile as the type for a different species – *Margaritana margaritanopsis* (Locard), from the locality of Aiguillon, Lot et Garonne, the same locality of Locard's synonymous *Unio margaritanopsis*. The first of the figures by Haas (1913) depicts the polished type specimen from the Spengler collection, whereas the second (Haas, 1929) was reproduced from the figure by Dupuy (1851).

Some fossil valves were figured by Huckriede & Berdau (1970), but a new illustration of Recent *M. auricularia* did not appear until almost 60 years after the image by Azpeitia (1933), a color photo in Fechter & Falkner's (1990) guide to European land and freshwater molluscs. Several years later, Falkner (1994) photographed Spengler's type specimen of *M. auricularia* and designated it as the lectotype of the species *Pseudunio auricularius*. (*Margaritifera auricularia* is the type species of *Pseudunio* Haas, 1910, a subgenus sometimes used for it.) Since the rediscovery of *M. auricularia* in Spain, and after almost 60 years without records, many new illustrations have depicted this endangered species in all stages of its development (Araujo et al., 2002), illustrations that are very different from the earlier, yet charming lithographies and hand-colored engravings.

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A FIELD STUDY OF THE LIFE HISTORY OF THE ENDEMIC HAWAIIAN SNAIL *SUCCINEA NEWCOMBIANA*

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Although the Hawaiian land snail fauna is noted worldwide for its diversity and endemism, little is known about the life histories and ecology of most of the endemic Hawaiian species. Hadfield et al. (1993) described the life histories of a few achatinelline tree snail species and, more recently, the life history of a succineid, *Succinea thaanumi*, has been described in laboratory (Rundell & Cowie, 2003) and field studies (Brown et al., 2003a, b). More information on the ecology and life histories of land snail radiations would allow comparisons with other molluscs, such as the freshwater molluscs described by Dillon (2000), and would increase our understanding of allopatric and sympatric speciation (Coyne & Orr, 2004).

In this paper, we report on the life history of *Succinea newcombiana*. In contrast to *S. thaanumi*, which is found on the eastern side of the island of Hawaii from Volcano Village to the Hilo Forest Reserve, *S. newcombiana* is found on the northern side of the island in the Kohala Forest Reserve. The two habitats differ in that *S. thaanumi* is found in rainforests including Puu Makaala, where we previously studied *S. thaanumi*, and which receives a median annual rainfall of 4,000 mm (Giambelluca & Sanderson, 1993), whereas *S. newcombiana* is found in a cloud mist environment where the median annual rainfall is only 2,000 mm (Giambelluca & Sanderson, 1993). Although not as abundant or as widespread as *S. thaanumi*, *S. newcombiana* is relatively common compared to other Hawaiian land snails. The current study was conducted in the Kohala Forest Reserve in a 10 m^2 plot at an elevation of 907 m (20°3.339'N, 155°37.515'W). The understory consisted primarily of an alien torch ginger of the family Zingiberaceae; hapuu, a Hawaiian tree fern (*Cibotium* sp.); and ieie (*Freycinetia* sp.). The overstory consisted primarily of ohia lehua, *Metrosideros polymorpha*, and was less dense than the overstory of Puu Makaala.

Data were collected from 25 January 2003 to 18 January 2004 for a total of 42 observations (1/03 = 1; 2/03 = 2; 3/03 = 2; 4/03 = 2; 5/03 = 3; 6/03 = 6; 7/03 = 4; 8/03 = 5; 9/03 = 4; 10/03 = 4; 11/03 = 5; 12/03 = 3; 1/04 = 1). More observations were made during the summer months because we were following egg masses. All plants, up to 1.8 m high, in the study area were examined for the presence of snails and egg masses by at least two observers during each observation. Maximum shell length of all snails was measured with a ruler *in situ* with a minimum amount of contact. Additionally, we recorded snail activity. In the past, we recorded activity based on whether a snail was extended out of its shell (Brown et al., 2003a). However, because *S. newcombiana* could not completely retract their bodies into their shells, we based activity on whether or not the snail's eye stalks were retracted. We recorded the snail's placement on a plant: top or bottom of a leaf, petiole, flower or stem. Only a few snails were observed on the petioles, flowers or stems of the plants, so we did not include these data in the behavioral analyses. Number of egg masses found and the number of embryos in each mass were recorded. Temperature and humidity data were gathered during each observation period with a RadioShack temperature/humidity gauge.

To examine the relationship between behavior and the microclimate variables across the 42 observations, we computed simple correlations between the total number of observed snails, the number of snails found on the top of a leaf with their eye stalks in, the number of snails found on the top of a leaf with their eye stalks out, the number of snails found on the bottom of a leaf with their eye stalks in, the number of snails on the bottom of a leaf with their eye stalks out, the total number of snails with their eye stalks out regardless of their location on a plant, and the microclimate variables of temperature and humidity.

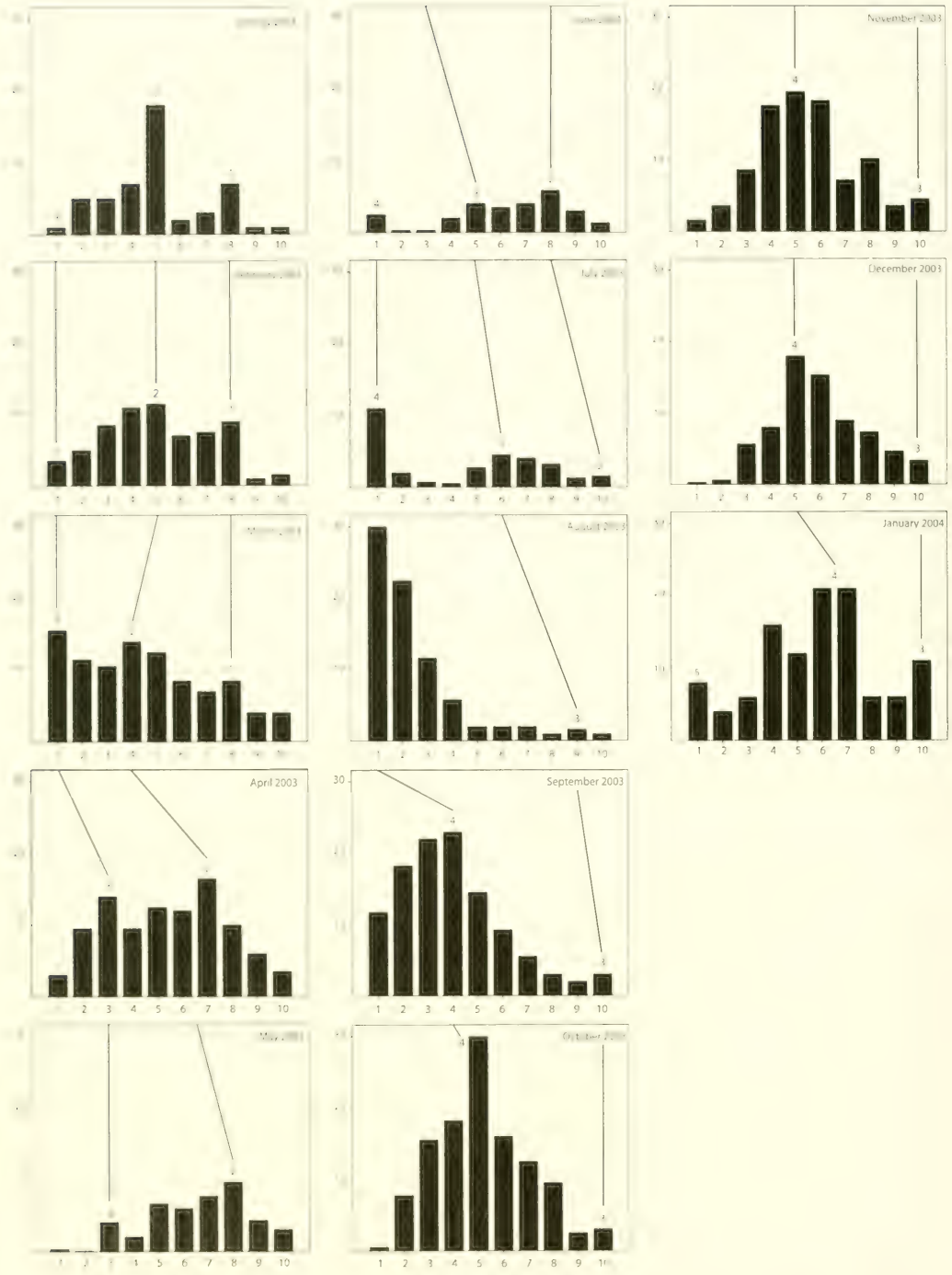

FIG. 1. Monthly frequency distributions of snail size. The Y-axis is the average number of snails of a particular size observed across a month (Number of observations per month: 1/03 = 1; 2/03 = 2; 3/03 = 2; 4/03 = 2; 5/03 = 3; 6/03 = 6; 7/03 = 4; 8/03 = 5; 9/03 = 4; 10/03 = 4; 11/03 = 5; 12/03 = 3; 1/04 = 1). The X-axis is snail shell size in mm. In August 2003, there were 52 snails 1 mm in size rather than 30.

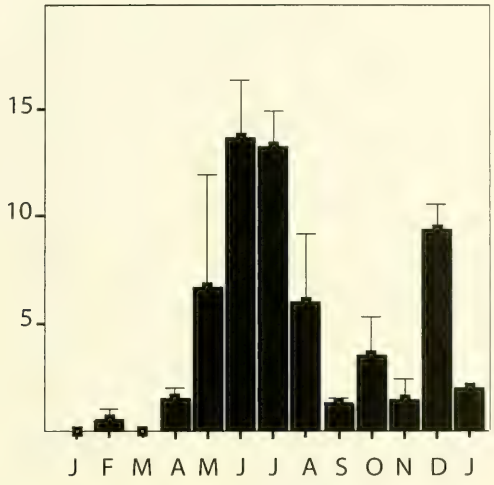

FIG. 2. Frequency distribution of the mean number of egg masses observed across a month. The letters on the X-axis are the first letters of a month beginning with January. Bars represent the standard errors of the mean.

The snails and their egg masses were found primarily on the alien ginger plants. Snail sizes varied across the year and showed two distinct lineages (Fig. 1). In January 2003, there were three cohorts of snails: cohort 1 contained adult snails, cohort 2 contained half-grown snails, and cohort 3 contained newly emerged snails. As the months proceeded, the adult snails in cohort 1 disappeared. Snails in cohort 2 grew from January through July, laid eggs in June and July (Fig. 2) and disappeared in August. Snails in cohort 3 emerged from their eggs masses from January through March, grew from March through August, laid eggs in December (Fig. 2), and formed a cohort of adult snails in January 2004 similar to cohort 1 observed in January 2003. From July through September, snails emerged from egg masses laid by cohort 2 and became cohort 4. Cohort 4 snails grew from August to January 2004 and formed a cohort of half-grown snails similar to cohort 2 observed in January 2003. Finally, cohort 5 consisted of snails emerging from egg masses laid by cohort 3 in December and was similar to cohort 3 observed in January 2003 (Fig. 1). Therefore, we observed two lineages: lineage 1 was formed from cohorts 1, 3 and 5; lineage 2 from cohorts 2 and 4.

Egg masses were translucent like those of *S. thaanumi* (Brown et al., 2003b). The number of new egg masses declined from August through November but increased dramatically in December (Fig. 2), followed by a second decrease in January 2004. Although many fewer egg masses were laid in December (n = 28) than from May to August (n = 185), the December egg masses contained significantly more embryos (Fig. 3) than the May to August masses ($X^2_{(3)} = 22.16$; $p < 0.0001$).

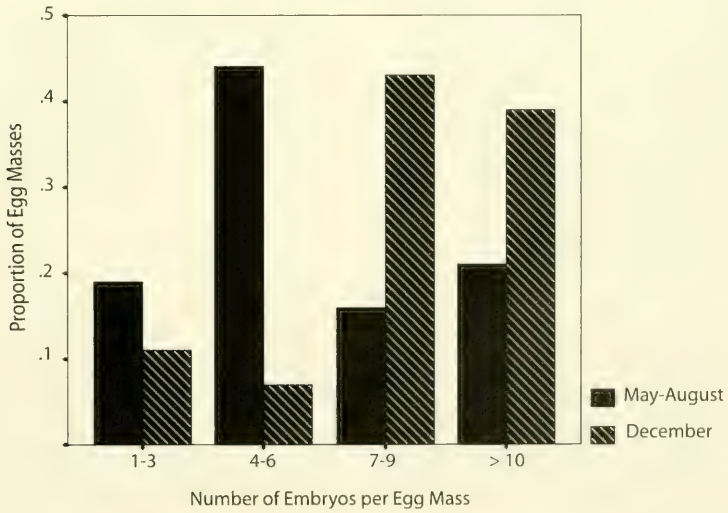

FIG. 3. Bar graph of the number of embryos in a clutch of eggs during the two major laying seasons.

We interpreted the above growth patterns for *S. newcombiana* as indicating an annual, semelparous life cycle for two snail lineages. Most snails probably lived about 12 months. These life cycles were similar to the life cycle of *S. thaanumi* (Brown et al., 2003a), but the data on *S. thaanumi* reflected a single snail lineage. The egg masses of the two lineages of snails also differed. Snails in cohort 2 laid more egg masses with fewer embryos, whereas snails in cohort 3 laid fewer eggs masses with more embryos per mass. At present, we do not know if the two lineages have different haplotypes.

Mating was observed five times during the study: 2/1/03, 5/22/03, 6/16/03, 6/30/03, and 7/7/03. As we found with *S. thaanumi* (Brown et al., 2003a), the smaller snail acted as the male (succineids are hermaphrodites), but, unlike *S. thaanumi*, we observed mating only between dyads (no triads as we previously observed with *S. thaanumi*).

Snail behavior was related to the microclimates of the study area. Snails were more likely to be found on the bottom of a leaf with their eyes stalks in when temperature was higher ($r = 0.66$; $p < 0.01$; $N = 42$ for all correlations) and humidity was lower ($r = -0.72$; $p < 0.01$). Snails found on the tops of leaves were also more likely to have their eye stalks in when temperature was higher ($r = 0.49$; $p < 0.01$) and humidity was lower ($r = -0.50$; $p < 0.01$). Snails with their eye stalks out that were active on the plants, however, were found at all temperatures ($r = -0.03$) and humidities ($r = 0.07$). This differed noticeably from our observations of *S. thaanumi*. In high temperature and low humidity conditions, we seldom observed active *S. thaanumi*, but we often observed active *S. newcombiana* in direct sunlight and low humidity conditions. The total number of snails observed was also not related to temperature ($r = 0.18$) or humidity ($r = -0.16$). Again, this differed from our previous observations of *S. thaanumi*, for which we observed fewer snails as the temperature increased, suggesting that the snails moved to a different part of their habitat. *Succinea newcombiana* cannot retract into its shell, whereas *S. thaanumi* can do so. Because of its inability to retract into its shell, one might conclude that *S. newcombiana* is more susceptible to high temperature and low humidity than *S. thaanumi*, but this was not the case. These behavioral differences to high temperature and low humidity might be related to the different ecotypes the two species occupy. The population *S. newcombiana* is found in a cloud mist forest with relatively less rainfall but more mist, whereas populations of *S. thaanumi* are found in rainforest habitat with relatively more rainfall and less mist. *Succinea newcombiana* might have lost the ability to retract into its shell because of the presence of abundant moisture in the air.

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We thank Naio Vivas and Kensley Raigeluw for help in initially finding the snails and B. Kalani Spain for help in data collection. Additional thanks go to Robert Cowie for identifying *S. newcombiana* for us. This research was funded, in part, by NSF grant 0223040.

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NATICID BOREHOLES ON A TERTIARY CYLICHNID GASTROPOD FROM SOUTHERN PATAGONIA

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INTRODUCTION

The fossil communities are in clear disadvantage when it comes to study the interactions among the species included in them. However, predation on shells gives us the possibility to examine at least some of these interactions. The diameter of the borehole, its placement, and the volume of the prey are parameters that can be easily recorded. Diverse combinations of these may allow inference about the size of the predator and the time spent on the perforation (Kitchell et al., 1981).

The morphology of two types of boreholes was described by Carriker & Yochelson (1968) that later Bromley (1981) described as ichnofossils. Taylor (1980) and later Bromley (1981) pointed out that externally wide and internally narrow predation marks with paraboloid walls are produced by naticid gastropods (*Oichnus paraboloides*), whereas those cylindrical with non-beveled edges are assigned to predators belonging to the Muricidae (Carriker, 1981).

Kelley (1988) developed a model in which predation of the Miocene naticids is stereotyped and predictable. The observed pattern of predation was revealed when analyzing the selection of perforation location and the size of the predators by means of the perforation diameter. Kitchell et al. (1981) showed that the main purpose of such patterns is an adaptive behavior to maximize energy efficiency and to select by prey size.

GEOLOGICAL SETTING

The material studied was collected in Neogene rocks exposed along the Atlantic coast of southern Patagonia. This is the first record of drilled gastropod shells from the Monte León Formation. Along the coast there are spectacular almost continuous outcrops of Tertiary rocks from the mouth of the Río Negro in northern Patagonia to the Straits of Magellan, and many authors have visited this area as they contain a very rich fauna of continental mammals and marine invertebrates. These beds have been subdivided based on their fossil content. These subdivisions – as well as the ages proposed for these rocks – have been a matter of great controversy, which has not yet been completely resolved.

The samples containing the studied material come from a locality along the southern margin of the Santa Cruz River first visited by Charles Darwin and by him called Mount Entrance (Fig. 1). They were collected in a very thin shelly bed of loose sediment lying within the Monte Entrada Member of the Monte León Formation (Fig. 2). The unit is richly fossiliferous, but the smaller specimens have generally escaped attention. The extraordinary abundance of *Kaitoa* in this particular bed has been overlooked, as most existing collections have only a few specimens. The Monte León Formation was formally introduced by Bertels (1970, 1978), and she subdivided it into a lower member (Monte Entrada) and an upper one (Monte Observación). The age of these rocks has been amply discussed and is presently believed to be late Oligocene to more probably earliest Miocene (Barreda & Palamarczuk, 2000).

Kaitoa patagonica (Ihering, 1897) (Fig. 3) is a small cylichnid first described from the Superpatagonian beds of Yegua Quemada, in the province of Santa Cruz and included in *Bulla*. However, the shell shape, ornamentation and columellar features suggest its affinities lie with *Kaitoa* Marwick, 1931 (type species *Kaitoa haroldi* Marwick, 1931), a genus described originally from Altonian (late early Miocene) rocks in New Zealand and which according to Beu & Maxwell (1990) occurs there from the Otaian (mid-early Miocene) to the Waipipian (early-late Pliocene). The occurrence of taxa peculiar to Australasia or Antarctica in South America has been variously recorded and this is just another example of such a connection (Beu et al., 1997).

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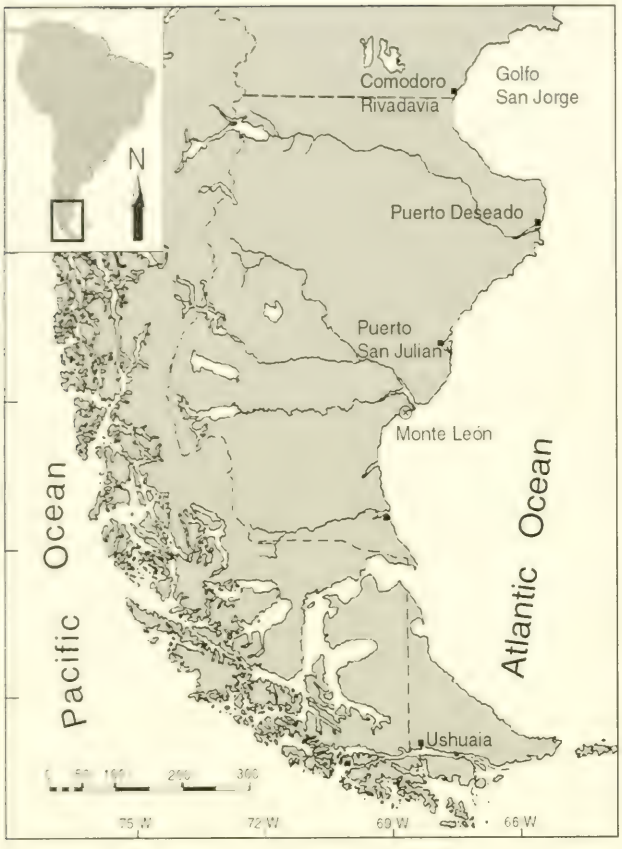


FIG. 1. Location map of the sampling area.

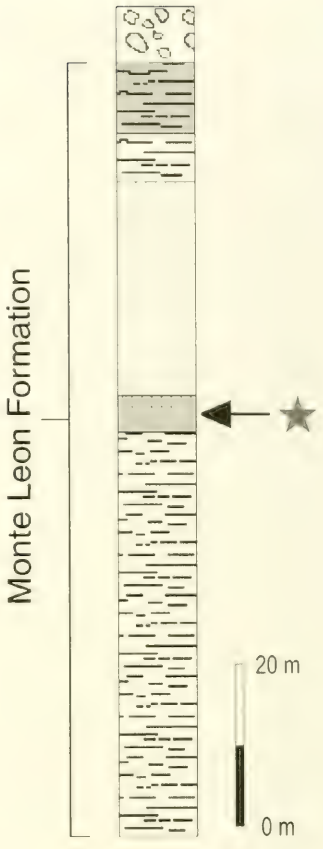


FIG. 2. Schematic Section of Monte León Formation.

MATERIALS AND METHODS

A total of 873 specimens of *Kaitoa patagonica* were considered in this study (Figs. 3–9). Internal and external diameters of 242 boreholes were measured using a stereoscopic microscope. The total length was measured in all 873 specimens, including those that were perforated. These three parameters were used to build a frequency table. SEMs pictures were done at MACN with a Philips XL30. All pictures were digitally processed.

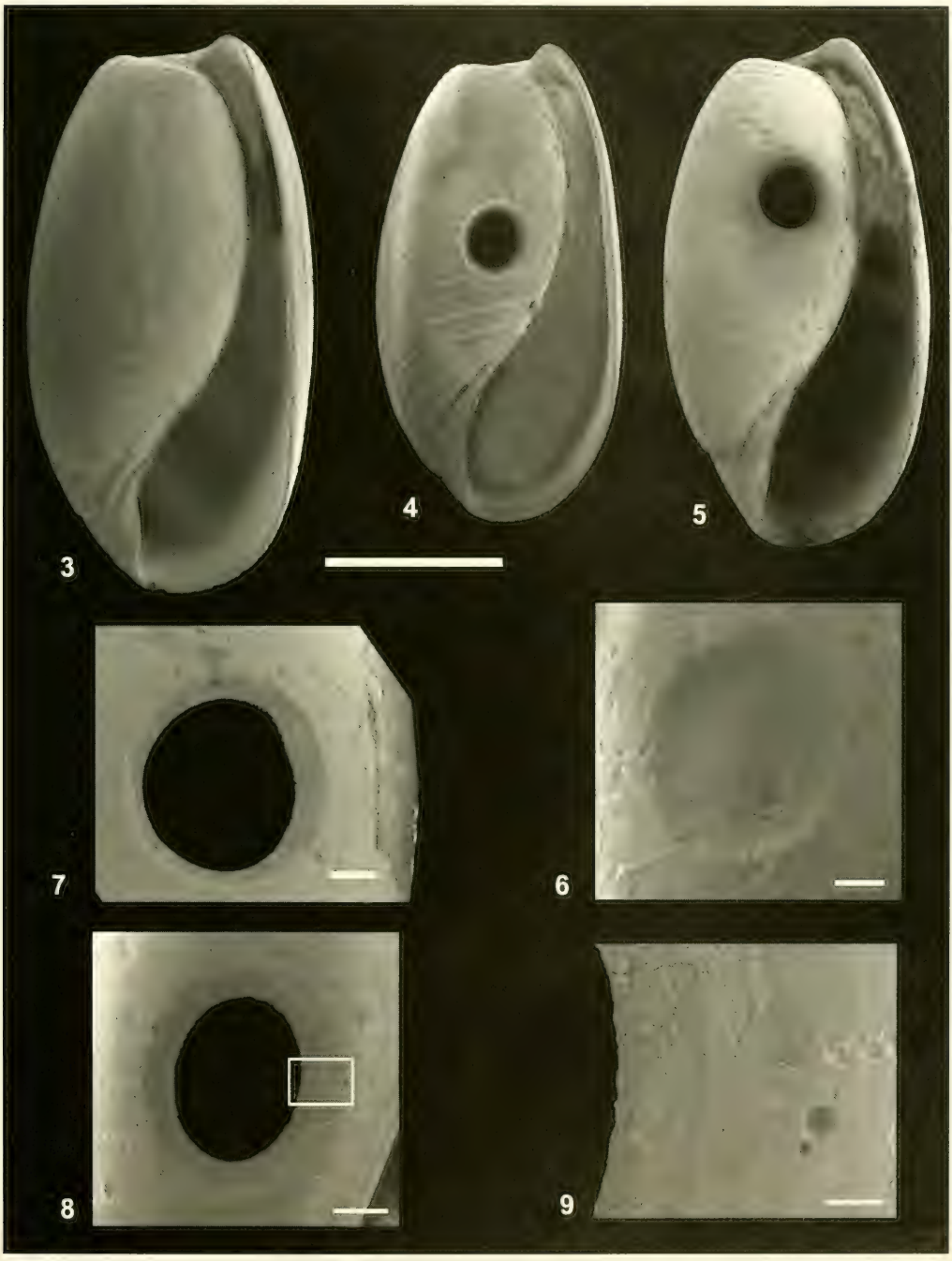
The studied material is housed in the Departamento de Ciencas Naturales, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, under the numbers GHUNLPam26500, 26501, 26502 and 26503, for the illustrated specimens and GHUNL Pam26504 for the others.

RESULTS

All boreholes showed the same morphology. The perforations are conical, with the larger diameter on the external surface of the shell and the smaller diameter on the internal surface (Fig. 4).

Of all the boreholes measured in *Kaitoa patagonica*, 90% are placed on an area of the last whorl near the inner lip, that is, on the central part of the apertural side of the shell. The rest were found on the dorsal side of the shell (Fig. 5).

A very low percentage of incomplete boreholes were observed in the population. This does not allow us to draw any conclusion about predator behavior. However, the drilling mechanism was recognized due to the presence of a slightly prominent central boss (Fig. 6).



FIGS. 3–9. *Kaitoa patagonica* (Ihering, 1897). FIG. 3: UNLPam 26500; FIGS. 4–5: Two drilled specimens, UNLPam 26501, 26502. Scale bar = 2 mm (FIGS. 3–5); FIG. 6: Detail of an incomplete borehole, UNLPam 26503. Scale bar = 200 µm; FIGS. 7–8: Two complete boreholes. Scale bar = 200 µm; FIG. 9: Detail of the square from Fig. 8. Scale bar = 50 µm.

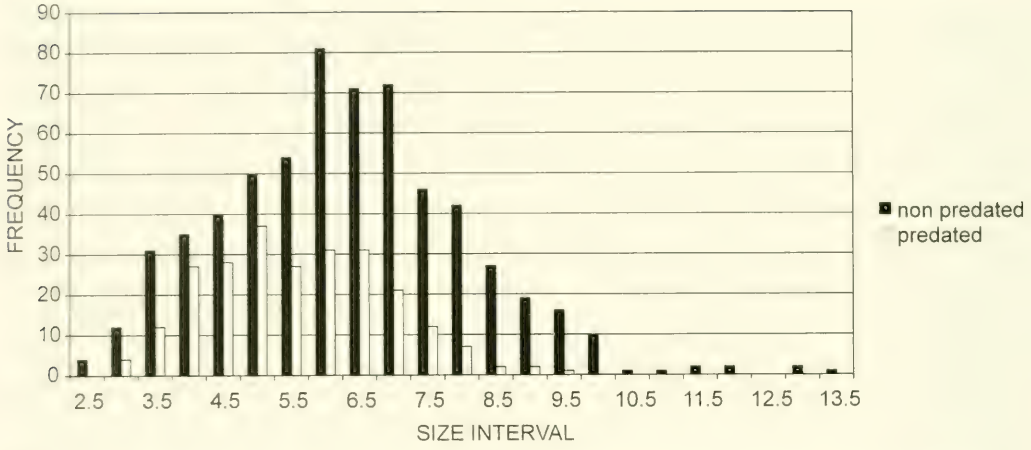


FIG. 10. Size intervals vs. number of specimens (predated and non-predated) of *Kaitoa patagonica*.

The size distribution curve of the population is normal in both predated and non-predated individuals. The most frequent size in the population is 6–6.5 mm of total length, whereas the most predated size is 5–5.5 mm of total length (Fig. 10). The distribution of borehole sizes is normal. The most frequent borehole size is 0.6–0.8 mm considering its internal diameter (Fig. 11). This borehole size curve is displaced to the left.

Correlation between internal diameter and the size-range of the population was analyzed with the software Statistica v. 4.0. The result was not significant ($R^2 = 0.1257$; $p < 0.000$), but there is a trend suggesting that the larger predators produced holes with a larger diameter (Fig. 12).

DISCUSSION

The conical shape of the perforations agrees with the morphology of boreholes referred to gastropods belonging to the Naticidae

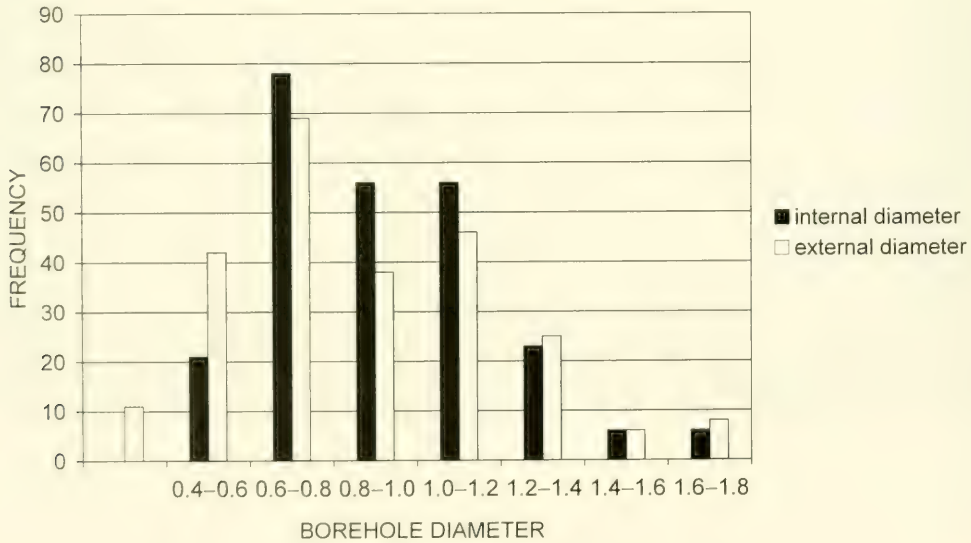


FIG. 11. Distribution of diameters of boreholes present in *Kaitoa patagonica*.

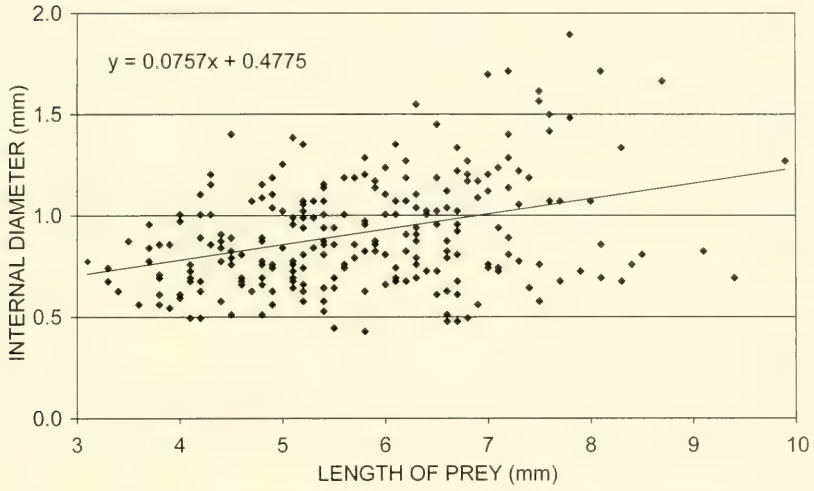


FIG. 12. Total shell length of prey vs. internal diameter of the borehole.

(Carriker & Yochelson, 1968; Taylor et al., 1980, among others). Among the species of this family described from coeval rocks in the same area are *Polinices santacruzensis* Ihering, 1897, and *Natica subtenuis* Ihering, 1897. These species were based on generally poorly preserved large adult shells, which seem unlikely to have been responsible for the borings on *Kaitoa*. There are numerous small juvenile naticid shells in this unit, but until further data become available on the different stages of the species described on the basis of large specimens, we cannot ascertain to which of them they may belong. Therefore, the identity of the *Kaitoa*-borer must remain as yet uncertain.

Borehole diameter provided an excellent tool to estimate the size of the predator. Such a size selection is a common behavior in naticids (Calvet i Catà, 1989). As reported here, the most abundant size in the population is not the most intensely attacked by the predator (Fig. 10). The reason for this discrepancy may lie in the fact that the predator could have been the very small, equally abundant naticid juvenile that appears in the same beds as *Kaitoa patagonica*. These presumably could prey on *Kaitoa patagonica* up to a certain size, but were somehow prevented of attacking the larger specimens, whether because of morphological constraints or because of a faster growth of the opisthobranch compared to naticids.

The non-predated population may constitute a size-refuge, such as those described for other groups of mollusks commonly attacked by naticids (Kabat, 1990; Pastorino & Ivanov, 1996).

A location selection for perforations (Hofmann & Martinell, 1986) is very well defined in most drilled shells of the Patagonian species. The area adjacent to the parietal callus carries the largest percentage of the perforations. This ellicits the question as to why it is so if the normal way of living is with the apertural side down. The possible answer to this may rest in the way in which the predator manipulated the prey. Additionally, this place is the easiest way to kill the prey because beneath the adapertural part of the shell, the most exposed, is where the foot is retracted, whereas a perforation on the ventral side assures the predator better chances of reaching vital organs and therefore enhancing its possibilities of killing the prey with minimum effort.

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GEOTACTIC BEHAVIOUR OF *DREISSENA POLYMORPHA* (BIVALVIA)

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ABSTRACT

Zebra mussel movement was studied in the laboratory, on a glass slope inclined at 2, 3, 4 or 8° to the bottom, in darkness and in the light (the latter on the steepest slope only). Small mussels (< 10 mm) climbed upward on the 4 or 8° slopes in darkness (negative geotaxis) but showed no preferences on the other slopes and in the light. Large mussels (> 12 mm) moved downwards on the 4 and 8° slopes (also in the light) and showed no preferences in the other treatments.

INTRODUCTION

Dreissena polymorpha (Pallas, 1771), the zebra mussel, is a gregarious bivalve that strongly influences freshwater ecosystems and hydrotechnical devices (Lewandowski, 2001; O'Neill, 1997). Its distribution is mainly determined by dispersal and settlement of planktonic larvae (Lewandowski, 2001; Kobak, 2004), but may also be affected by post-settlement movement of mussels: they move upwards to avoid poor chemical conditions at the base of a colony (Burks et al., 2002) and prefer shaded or dark substrata (Kobak, 2001; Toomey et al., 2002).

A cue that could be useful for a moving mussel is gravity. It provides information to an animal about its orientation in space, independent of photoperiod and geographic location. In the field, zebra mussels may prefer either the lower (Walz, 1973; Lewandowski, 2001) or upper (Marsden & Lansky, 2000) substrate side. Such distribution could result from both geo- and phototaxis, as well as the effects of water flow or predation. To test the influence of gravity upon mussels, I studied their movement on a series of slopes in the laboratory. In the light of my field research (Kobak, 2004), I hypothesized that small mussels would move upwards and illumination would reverse this behaviour because of negative phototaxis. I also expected that large mussels, less mobile than small ones, would prefer the easier, downward direction.

MATERIALS AND METHODS

Mussels were collected by a diver from a dam wall of the Włocławek Dam Reservoir (the Vistula River, central Poland) and kept in a 500 l aquarium filled with aerated, settled tap water, at ca. 20°C. Only individuals that reattached themselves in this aquarium were tested. They were used only once, not sooner than two weeks and not later than three months after collecting. The tested individuals were divided into small mussels (mean shell length ± SD: 7.3 ± 1.29 mm, range: 3.3–9.9 mm) and large ones (15.3 ± 1.55 mm, range: 12.2–22.6 mm).

The experiment was run in a glass tank (480 x 230 mm, water level: 240 mm) with settled (24 h) tap water (19–22.5°C). A 400 x 230 mm glass plate was put into the tank, with one of its longer edges resting on the bottom and the other leaning against the wall (Fig. 1). The aerator was placed below the plate level to avoid mussel disturbance by air bubbles. The mussels (13 individuals per tank in a single trial) were put onto the central long axis of the plate, with their long axes parallel to the tank's longer edge. Each mussel was covered with a glass tunnel (width and height: 25 mm, length: 220 mm, outlets closed with 1 mm nylon mesh) to avoid the impact of conspecifics on their behaviour (Mörtl & Rothhaupt, 2003).

To study geotaxis in the dark, I tested mussels on slopes inclined at 2, 3, 4 or 8° to the bottom, in a tank covered with a cardboard

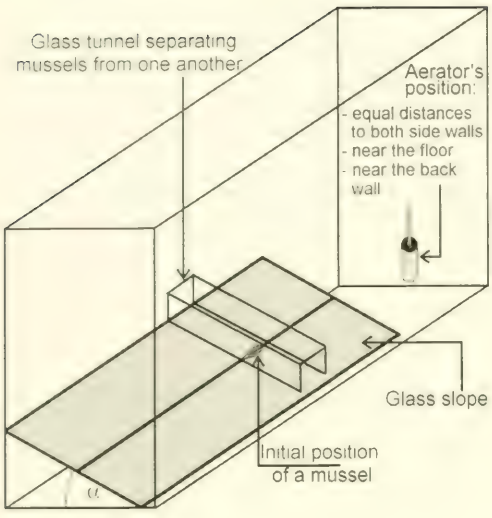

FIG. 1. Experimental tank. α in various treatments is 2, 3, 4 or 8°.

box. Illumination under this box was below the detection limits of the luxometer (Sonopan L-20A) (i.e., < 0.1 lx). To examine the impact of light on geotaxis, I tested mussels on a constantly illuminated slope (16 W bulb 0.5 m above the surface, incident illuminance at the surface ca. 700 lx). Light was used only in experiments involving the steepest slope (8°), the most likely to evoke geotaxis.

To check whether mussels could passively slide down the slope, 20 empty shells of each size group, filled with aquarium silicon glue to imitate a live mussel's shape and weight, were put on the steepest slope (8°) in various positions (lying on the ventral or side shell surface, with the front, back, or side pointing down). I observed no passive relocations of these shells.

I carried out ten 48-hour trials (13 mussels in each) for each treatment and size group. They were run consecutively, in a random sequence. The slope direction relative to the laboratory room was changed in the successive trials. At the end of each trial, distances moved by the mussels (measured to their anterior ends) were determined to the nearest 1 cm (the scores were from -11 at the bottom to +11 at the top).

Numbers of mussels moving in opposite directions were compared using t-tests for paired data with the sequential Bonferroni correction.

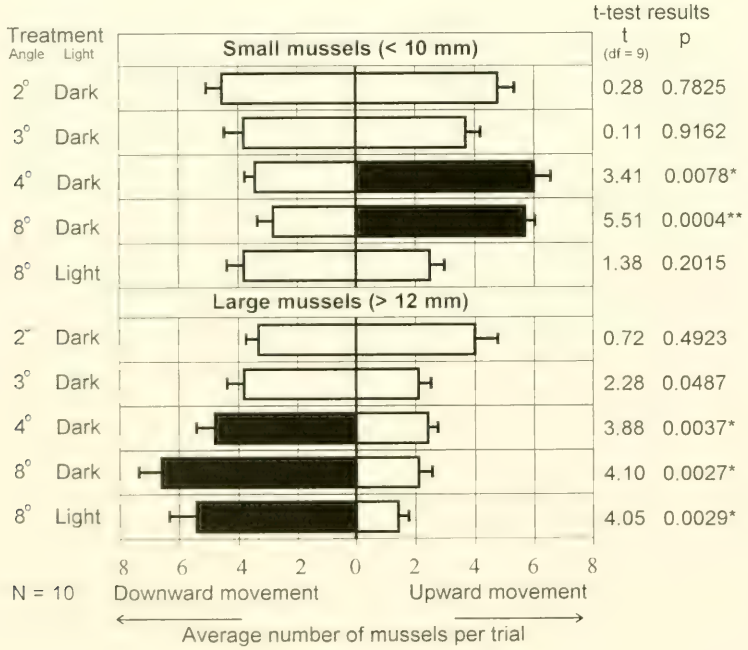

FIG. 2. Direction of zebra mussel movement on different slopes. Error bars are standard errors of mean. Black bars indicate a significant preference for one direction in a given treatment. The asterisks show statistical significance of the t-tests for paired data after applying the sequential Bonferroni correction: *p < 0.05, **p < 0.01.

Differences among the treatments were tested using the two-way ANOVA (factors: mussel size and slope type) of individual distances moved by the mussels (with downward distances coded as negative values). The Bonferroni-adjusted pairwise t-tests were used as post-hoc comparisons. Mussels that neither moved nor attached themselves to the plate were regarded as being in a poor physical condition and not analysed.

RESULTS

On the darkened 4 and 8° slopes, the small mussels tended to move upwards, while the large individuals preferred the downward direction. In the light preferences of the small mussels disappeared, while the large ones retained their downward preference. I observed no directional reactions on the other slopes (Fig. 2).

The interaction between mussel size and slope was significant in the ANOVA of the distances ($F_{4, 1014} = 7.97$, $p < 0.001$). The distances moved by the small mussels on the darkened 4 and 8° slopes differed significantly from those measured in the other treatments, as well as from the distances moved by the large individuals in the same conditions. In the case of the large mussels, only the distances moved on the 2 and 8° slopes differed significantly from each other (Fig. 3).

DISCUSSION

To my knowledge, negative geotaxis of metamorphosed bivalves has not been reported so far. Uryu et al. (1996) observed positive geotaxis of a mussel *Limnoperna fortunei*. Negative geotaxis of small zebra mussels, found here, could account for the aggregations of recruits along the upper edge of vertical settlement plates deployed in the field (Kobak, 2004).

Negative geotaxis could be beneficial in a dense colony, where water quality is poor. Burks et al. (2002) found an upward movement of mussels apparently stimulated by chemical gradients within a colony (e.g., oxygen and nitrate). In the present study, mussels moved upwards, although they were kept at low density and separated from one another, so such a gradient did not appear. Thus, in certain conditions, upward movement may occur without any chemical stimuli. In the field, negative geotaxis could help mussels to find a suitable site at the top of a colony. However, zebra mussels are photophobic (Kobak, 2001; Toomey et al., 2002), which is contradictory to negative geotaxis: climbing up means approaching the light source. Photophobic behaviour may explain why the upward movement of small mussels disappeared under illumination in the present study. Uryu et al. (1996) observed a light-induced change in

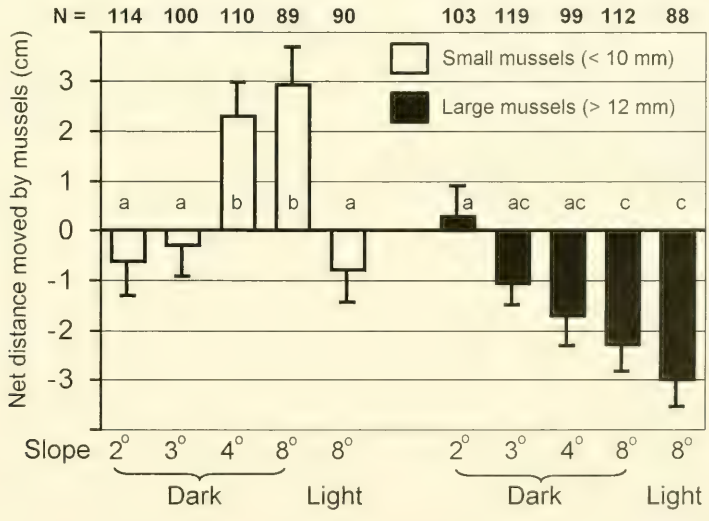

FIG. 3. Average net distances moved by zebra mussels on different slopes. Downward distances were counted as negative values. Treatments labelled with the same letter did not differ significantly from one another (Bonferroni-adjusted t-tests). The values above the chart are the numbers of mussels analysed in each treatment.

behaviour of *L. fortunei*, which chose no direction in darkness and moved downwards in light. For both species, illumination reduced upward movement. Previously, I have shown that light (ca. 700 lx) was a stronger cue than gravity: small mussels avoided the upper, illuminated part of the slope when only its lower half was darkened (Kobak, 2002).

Large mussels move less frequently and over shorter distances than smaller mussels, probably due to their heavier bodies (Toomey et al., 2002). Thus, larger individuals may prefer the downward direction because it demands less effort. Older mussels often bear other individuals attached to their shells, which further limits their locomotion and makes it less likely to be crucial to their survival.

A number of studies (e.g., Kobak, 2001, 2002; Burks et al., 2002; Toomey et al., 2002), including the present one, show that small zebra mussels can use multiple environmental cues to select an attachment site by crawling over substratum. Thus, active movement of settled individuals may be an important factor affecting mussel distribution in the field.

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LETTERS TO THE EDITOR

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VALID UNTIL SYNONYMIZED, OR INVALID UNTIL PROVEN VALID? A RESPONSE TO DAVIS (2004) ON SPECIES CHECK-LISTS

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The recent publication of taxonomic authority lists for the non-marine mollusks of France (Falkner et al., 2002) and of north and northwest Europe (Falkner et al., 2001) has elicited critical comments from Davis (2004). I have been involved in both works, for the French list as instigator and editor, for the European list as a provider of supraspecific nomenclature (Bank et al. 2001). The purpose of the present response is to place both lists in perspective, to justify the editorial decisions taken, and to defend the rationale behind the scientific decisions made.

On Checklists and Taxonomic Authority Lists

Taxonomic authority lists or checklists are as old as the science of systematics. However, unlike many other groups of animals, large or small, mollusks do not have a comprehensive catalogue of species, or even of names. The last academic attempt to list the Recent mollusks of the world was Tryon and Pilsbry's *Manual of Conchology*, now on average 100 years old. With an accumulated load of perhaps 500,000 names and a synonymy ratio that is matched probably only in butterflies, the compilation of a global mollusk checklist is not a small task. The result is that we do not even know whether the number of valid named Recent species of mollusks is on the order of 50,000 or 100,000, an uncertainty that is persistent throughout Recent and fossil biota but is seen as "particularly problematic" for mollusks (Hammond, 1995).

Information technology has suddenly made it much easier to compile and update species catalogues that reflect changes in knowledge and thus taxonomic instability, whereas, simultaneously, a growing corpus of legal texts and other documents, such as Red Lists, demand authoritative lists of names that will change little over time. Recently published regional checklists emphasize one or the other of these two approaches. For instance, Turgeon et al.'s (1998) list was compiled to provide an authoritative reference for U.S. federal and state conservation texts, and it emphasizes stability and established knowledge over scientific inquisitiveness and controversial opinions.

In Europe, there has been a long tradition of national checklists of non-marine mollusks (see, e.g., Bruyne et al., 1994; Manganelli et al., 1995; Kerney, 1999), but no continent-wide list has been published since Westerlund's catalogues of the 1870–1880s. In 1998, proposals were made to issue a taxonomic authority list of the land and freshwater metazoans of geographical Europe. With funding from the European Commission, Fauna Europaea was formally initiated in 2000 for a period of four years, and Ruud Bank was chosen to be the "Group Coordinator" (in Fauna Europaea parlance) for the gastropods. At the onset, it was estimated that there would be on the order of 3,000 valid molluscan terminal taxa (species and subspecies; see below), and it was also recognized that, because of the chaos caused by the Nouvelle Ecole, the French fauna was the major stumbling block in compiling a list of valid taxa. I then decided to contract Gerhard Falkner and Theo Ripken, both with an extensive knowledge of the French fauna, to produce a taxonomic authority list for France. The result is the Falkner et al. publication appeared in March 2002, but the species list had already been made available for the CLECOM [Check List of the European COntinental Molluscs] catalogue (covering the countries of northern and northwestern Europe), involving Falkner and Ripken as co-authors and published the year before on the occasion of the World Congress of Malacology in Vienna in August 2001. In turn, the CLECOM catalogue became the core of the Fauna Europaea checklist, released electronically in October 2004 (Bank, 2004). Although the three products are embedded within each other and have complementary scientific contents, they differ in format in addition to geographical scope. The French checklist comes

with 110 pages of endnotes that justify taxonomic or nomenclatural decisions, or report new faunistic records. The CLECOM and Fauna Europaea lists do not have associated notes, the former is available on paper and electronically, and the latter only in electronic form.

I should like to emphasize that taxonomic checklists are only as good as the quality of the science that is behind them. Davis' (2004) criticisms focus in particular on the poor taxonomy of hydrobioids in the French and CLECOM checklists. I do not disagree. But the culprits are the systematists themselves, past and present, who have been and are establishing new taxa without proper qualifications and comparisons. The French checklist was about to go to press when Bernasconi (2000) published a work on the *Bythinella* of southwestern France in a non-peer reviewed publication, with the description of seven new species. As editor of the list, I agreed with Falkner that the new species should be given the benefit of the doubt and be listed as valid until synonymized. Falkner et al. (2001, 2002), CLECOM and Fauna Europaea thus list 37 valid species of *Bythinella* from France, but the French checklist emphasizes (endnote 78, page 86): "Bernasconi's results and our own will have to be tested by using molecular characters. In the meantime, segregating morphotypes at the rank of species will better meet the needs of mapping and conservation programs that motivated the compiling of the present list." I believe that highlighting the problems does a better service to science than suppressing them. I agree with Davis that one may, or even should, view the listing of 36 species and subspecies of *Bythiospeum* in Germany with disbelief. Davis' comment "I do not know of any molecular or detailed anatomical study that has looked at variability within and between populations to infer possible genetic breaks in taxa of *Bythiospeum*" is, in my view, not a criticism of list compilers but a criticism of those who add to the confusion by establishing still more new species based on inadequate character analysis and comparisons. To make a comparison with North America, I would suggest that a future edition of Turgeon et al.'s *Common and scientific names of aquatic invertebrates from the United States and Canada* should list *Physella hemphilli* D. W. Taylor, 2003, and *Physella winnipegensis* Pip, 2004, as valid, unless these nominal species have been synonymized; by doing so, Turgeon et al. would not be making a judgement on Taylor's (2003) or Pip's (2004) work. Checklists are simply reflections of the state of the art, and one should not "shoot the messenger" if the news is not good.

On Bourguignat and Revalidation of the Nouvelle Ecole's Nominal Taxa

Few personalities in the world of malacology have elicited so much criticism, and even hatred, as Jules-René Bourguignat. It is fair to recognize that Bourguignat had a sharp discriminating eye for characters, that his knowledge of the literature was immense, and that he had a network of correspondants that channelled large amounts of valuable material to him from all over the western Palaearctic (Kuiper, 1969). However, Bourguignat also had very personal views on what deserved to be ranked as a species, and he developed an undefensible system whereby he would rank as "species" specimens that would be diagnosable by three characters. This, in combination with a self-infatuated personality and personal attacks on his competitors, invited the wrath of established and influential European malacologists. Both Crosse and Kobelt used the journals they edited, *Journal de Conchyliologie* in Paris and *Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft* in Frankfurt respectively, to build a sanitary cordon around Bourguignat and his followers, the self declared "Nouvelle Ecole". Bourguignat retaliated with a gifted pen and ridiculed his enemies in "*Lettres Malacologiques*" and other polemic writings (Bourguignat, 1882). The two camps being at war with each other, Bourguignat's school would not listen to any, justified or unjustified, criticism and went on unchecked to establish thousands of new nominal species.

By the end of the 19th century, Locard recognized no less than 1850 valid non-marine mollusk species in the French fauna (Locard, 1893), among them no less than 506 species of unionids (versus eight now regarded as valid at the species level). The uncompromising attitude of the "Nouvelle Ecole" was mirrored in the other camp by the rejection *en masse* of Bourguignat's works and species. In the decades that followed, any species named by Bourguignat, Locard, Mabille, or Caziot, to name just a few, was *a priori* suspected to be synonymous of an earlier "classical" species, that is, a species recognized by British or Ger-

man authors. The question then asked was not "Is this a valid species or a synonym?", but "Which species is this a synonym of?" The first decades of the 20th century were thus a period of massive synonymization; to ridicule the insignificance of the species established by Locard, Coutagne (1929: 16) even created the word "locardies", a parallel to the "jordanons" of botanical literature. Germain started his career by co-authoring two papers with Locard, but later became the principal instrument of the synonymization of the Nouvelle Ecole's nominal species. Germain's two volumes of the *Faune de France* (Germain, 1931) represent the culmination of bringing the French non-marine fauna into harmony with its time. Because of the chaos caused by the Nouvelle Ecole's oversplitting, Germain's "normalization" was received with much relief and his new synonymizing was gladly and uncritically accepted by his contemporaries and followers. It was not until the 1970s that the French fauna received new, critical attention from malacologists from the Netherlands, Germany, Italy, and Spain. In particular, the Rijksmuseum in Leiden (today Naturalis) made southwestern Europe its area of excellence, resumed comprehensive field work, and started to critically re-examine the systematics of the land snails from France, Spain and Portugal based on solid population-based species concepts and using anatomical, and, later, molecular data. This led to the resurrection of several nominal species from the graveyard of synonymy, e.g., *Abida occidentalis* (Fagot, 1888), a local endemic from the central Pyrenees, resurrected from the synonymy of *A. pyrenaearia* (Michaud, 1831) (Gittenberger, 1973), and *Cernuella aginnica* (Locard, 1894), broadly distributed in southern France, resurrected from the synonymy of *C. virgata* (da Costa, 1778) (Clerx & Gittenberger, 1977). When revalidating *Trichia phorochaetia* (Bourguignat, 1864), endemic to the Grande Chartreuse and Vercors regions of the French Alps, Winter (1990) commented: "Notwithstanding the good description and figures provided by Bourguignat (1864), the species was placed by both Hesse (1921) and Germain (1930) in the synonymy of *Trichia villosa*, no doubt because of Bourguignat's reputation."

It became clear in the 1980s and 1990s that, among the many superfluous names produced by the Nouvelle Ecole, not everything was a synonym, and in fact Bourguignat and his followers had named some perfectly valid species, often local endemics from the Alps, the Pyrenees or the Mediterranean region. What Falkner and Ripken did when working up the French checklist was to critically re-examine as many nominal species as possible, based on the original collections, including types, of Bourguignat (in Muséum d'Histoire Naturelle de la Ville de Genève), of Locard (in Muséum National d'Histoire Naturelle, Paris), and of Caziot (in Muséum d'Histoire Naturelle de Nice), an approach that, surprisingly, no one had done systematically before. In the overwhelming majority of the nominal species they re-examined, they confirmed earlier accepted synonymies. However, this work also revealed a number of taxa that they suspected represent valid species: rather than pushing these into synonymy against the available evidence, they decided to give the benefit of the doubt to these taxa. For instance, the French checklist thus revalidated *Oxychilus colliourensis* (Locard, 1894) and *O. adjaciensis* (Caziot, 1904), based on historical as well as newly collected material. It also tentatively listed as valid, for example, *Limax granosus* (Bérenguier, 1900) and *Milax ochraceus* (Bérenguier, 1900) because of their distinctive anatomy, despite their not having been found in the last 100 years (but also, admittedly, they have not been searched for at the type localities). As the editor of the French checklist, I agreed that "giving their chance" to *Limax granosus* and *Milax ochraceus* as potentially valid species was more likely to lead to hypothesis testing and falsification, than continuing to obliterate them as doubtful synonyms (and then, as synonyms of what?).

To conclude on Bourguignat and the Nouvelle Ecole chapter, I would like to make a comparison with another (I am afraid, also French!) malacologist of the 19th century who has been the subject of much controversy on the other side of the Atlantic, I mean Constantin Schmaltz Rafinesque of course. It has been said that Rafinesque was his own worst enemy, and the same could be said of Bourguignat. Their published works were so controversial, their personalities were so unconventional, that they became ostracized to the point of suppression. For a long time, malacologists in the United States ignored Rafinesque's names, while European malacologists who dared declare any of Bourguignat's species as valid were stigmatized. Admittedly, Rafinesque and Bourguignat wreaked havoc on the systematics and nomenclature of North American freshwater

mollusks, and western Palearctic land snails, respectively. But the rules of nomenclature demand that names and works are evaluated on a case-by-case basis. Rehabilitating *Trichia phorochaetia* as a valid species is not a rehabilitation of its author, personality or method. After 130 years of blanket ostracism of Bourguignat, let's acknowledge that some valid species were indeed named by Bourguignat, even if such cases fuel incendiary judgements on zoological nomenclature: "In other sciences the work of incompetents is merely ignored; in taxonomy, because of priority, it is preserved" (Michener, cited by Gould, 1992).

On Subspecies

Continental European, especially Germanic authors, have a long tradition of systematics formally recognizing subspecies for discrete geographical variations within species, and the CLECOM and French checklists, as well as Fauna Europaea, clearly belong to this school. As regional checklists recently published in Europe vary in how they treat geographical variation, some background information is useful to place the Germanic tradition in perspective.

(1) For decades, many authors have used indiscriminately the concepts of "variety" and "subspecies", which is reflected to this day in the *International Code of Zoological Nomenclature* that regulates how varietal names can be rescued when applied to subspecies. In the 19th century and well into the 20th century, the concept of "variety" was used to designate any kind of variation (size, colour, sculpture), with or without a geographical component, and at any scale (local, regional or global). Taxonomic and nomenclatural practice shifted when evolutionary thought changed our understanding of variation. Taxonomists started to treat species as groups of populations, rather than collections of individuals, and they observed that morphological gaps could occasionally be overlaid with geographical gaps. This approach was first conceptualized in the 1930s by Bernhard Rensch and Ernst Mayr, at the time both working in the Berlin Museum. Mayr's well-known influence on bird systematics was a reflection of the immense impact of his teaching and writing on geographical variation. Less well known abroad is the role of Rensch, who had taken over in Berlin after Thiele, before becoming a professor at the University of Münster. Rensch had a considerable influence on German evolutionary systematics after WWII and, because he was also a malacologist (see, among others, Rensch, 1937), his impact on German malacology cannot be overestimated.

(2) It certainly is no accident that the study of geographical variation had a much higher resonance in the heart of alpine Europe than elsewhere in Europe. Most of northern Europe was covered by ice during the glacial periods of the Quaternary, and its current fauna and flora could become established there only as the land became free of ice: for example, in the British Isles, nearly all the species that live today arrived there less than 10,000 years ago. This recent colonization has two consequences: first, there are no endemic species in the British fauna and flora; and, second, there is no discernible geographical variation among the British populations of even broadly distributed species. If British (or Scandinavian) authors do not recognize subspecies, this is not because Rensch was wrong, but rather because there are no subspecies *within* the British (or Scandinavian) fauna.

By contrast, glaciations have created in alpine Europe a mosaic of refuges. Whereas the major valleys were occupied by glaciers, there emerged archipelagoes of unglaciated territories, for example, slopes facing south, nunataks, thermal areas, and populations isolated in these refuges had time to genetically diverge between two interglacial cycles. Superimposed on the complex topography and climatology of the mountain areas of southern Europe, the glacial cycles had the effect of breaking down territorial and genetic continuities, generating the many highly localized species and subspecies that today characterize alpine and Mediterranean Europe. The Alps thus naturally became the playground of malacologists applying the concepts of Mayr and Rensch's evolutionary systematics.

How we translate geographical variation into classification is not just an academic exercise in nomenclature, but is embedded in evolutionary biology and has consequences for management and conservation. Subspecies based on morphology (just as well as species) are hypotheses of genetic relationships between specimens considered representative of natural populations. In this respect, the allopatric subspecies of evolutionary systematists are the "Least-Inclusive Taxonomic Units" (LITU) of phylogenetic systematists (Pleijel & Rouse 2000). It does not really mat-

ter, and it is in fact a matter of personal choice, whether one wants to call these terminal taxa species or subspecies, and whether one bases such decisions in a Phylogenetic Species Concept or a Biological Species Concept. For that matter, Kottelat's (1995) "Pragmatic Species Concept" is not lurking far behind. I believe that George Davis and I do not stand far apart on this issue, and I agree that taxonomic principles inspiring the checklist compilers could have been made more explicit. In fact, the whole issue broadly falls within the hotly debated subject of taxonomic ranks. Isaac et al. (2004) have recently discussed how changes in species concept, rather than new discoveries, are leading to raising known subspecies to species level, with consequences on macroecology and conservation biology. What really matters is that these terminal taxa should be seen as biological/evolutionary/management units, rather than the esoteric fancy of a taxonomic splitter.

Davis (2004) defends the view that "it is inappropriate to name subspecies as a convenience and in the absence of well-founded data". None can disagree with him on this point, although we may disagree on what constitutes "well-founded data". However, I believe that Davis' criticisms are not aimed at the right target and do not do justice to the state-of-the-art of European non-marine molluscan taxonomy. The checklists compilers are not working in a nomenclatural *terra nullius*, and the names are in fact already out there in the 250 years of accumulated literature on European non-marine mollusks, however brilliant or pathetic, modern or outdated. In the checklists being discussed, the list compilers did *not* name any new subspecies, but they *recorded the use* of subspecies names in the latest authoritative publications on the subject. There is already a considerable body of literature on the geographical variation and the distribution of subspecies of continental European land snails. In this respect, the French checklist only reflects the state-of-the art of that existing literature. For instance, the subspecific taxonomy of *Chilostoma zonatum* (Studer, 1820) is based on Forcart (1933), that of *Abida secale* (Draparnaud, 1801) is based on Gittenberger (1973), and that of *Clausilia rugosa* (Draparnaud, 1801) is based on Nordsieck (1990). One of several taxonomic areas where the French checklist innovates is unionid systematics below the species level. In that family, taxonomic stability had been reached several

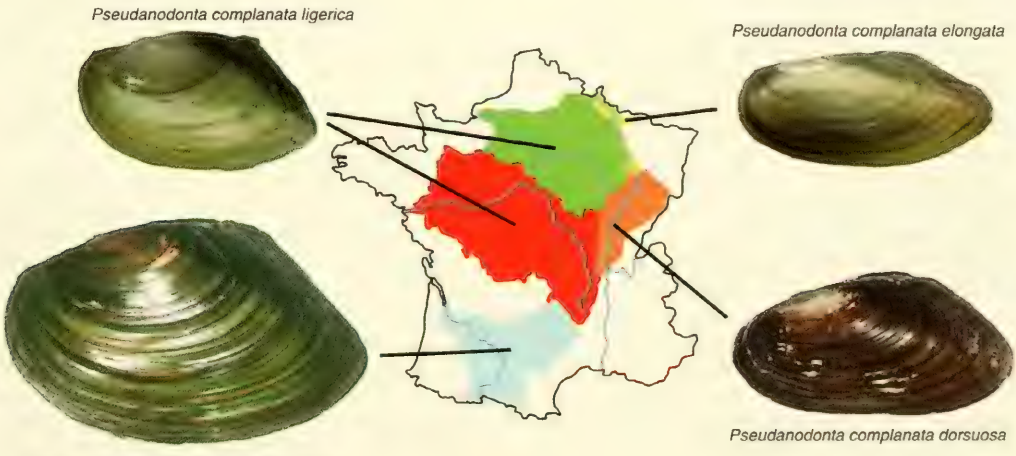

FIG. 1. Variation in European unionids has been historically disconcerting and difficult to analyze, because it includes a high within-population component and a more discrete geographical, between-population, component. By segregating discrete subspecies, the recently published French checklist hypothesizes that the morphologically recognizable forms from major drainages do have biological significance that must be taken into account into biodiversity inventories and management schemes. For instance, hypothetical populations reinforcements should avoid translocations of individuals between populations of *Pseudanodonta complanata* from the Moselle (a tributary of the Rhine), Seine-Loire, Garonne, and Saône (a tributary of the Rhône) drainages. [Copied from Bouchet, in Falkner et al., 2002: 12].

decades ago at the species level, with *all* of the hundreds of Nouvelle Ecole names ending up in synonymy. However, recent genetic work provides grounds for recognizing "subspecies" within these morphologically defined taxa. For instance, Badino et al. (1991) commented that a phenogram of genetic distances between populations of *Unio elongatulus* C. Pfeiffer, 1825 [now *U. mancus* Lamarck, 1819], and *Unio pictorum* (Linnaeus, 1758) showed an "almost perfect arrangement of populations according to the hydrographic basins". Recognition of "subspecies" within the unionids of France reflects the hypothesis that different hydrographic basins are inhabited by distinct genetic stocks, as evidenced by discrete morphological differences between basins (Fig. 1). As editor of the French checklist, I agreed with Falkner's decision to highlight such discrete differences by using subspecific names, so that ecologists and geneticists could test and challenge them, rather than merging all regional variants into a broad, apparently uniform pool. I had earlier (Bouchet et al., 1999) advocated subspecies to be an appropriate level for establishing lists of protected taxa under the European legal instruments.

Regional Species Checklists: What They Are Not

There are also points where I do agree with Davis, in particular classification, when he writes "Check lists should not be a vehicle for promoting the reconstruction of phylogenetic history or promoting a particular phylogenetic hypothesis" (Davis, 2004: 230). I agree that regional checklists are not the place to produce elaborate, finely dissected classifications. A classification using subfamilies, tribes and subgenera may be necessary when the purpose is to catalogue hundreds of species of Enidae from the Middle East and central Asia, but it certainly is not necessary when there are only six species of Enidae in the French fauna. This criticism of the inappropriateness of overly elaborate classifications in country or regional checklists probably is most founded in the case of hydrobioids which, in addition to the necessity of addressing their classification in a global context, are also undergoing a phase of profound re-evaluation (e.g., Wilke et al., 2001). The usefulness of national and regional checklists is because their compilers have an intimate knowledge of a usually highly fragmented local literature, both in space and time, dealing with taxonomic status and distribution of the terminal taxa. (Almost 64% of the 3,000 references in the French check-list are papers, pamphlets and books published in France.) However, the body of literature dealing with higher classification is of an entirely different nature, has no geographical borders, and is fast changing. In the currently very active phase of reevaluation of the phylogeny of the mollusks, any classification is certain to become rapidly outdated (Bouchet & Rocroi, 2005).

It should also be recognized that, in the case of the European fauna, regional species checklists are faced with special difficulties, because of the huge synonymy load, conflicting or parallel taxonomic schools, and centuries of accumulated literature, opinions, and mistakes. For fewer than 5,000 terminal taxa, there may be somewhere around 50,000 nomenclaturally available names. Under these circumstances, these European regional species checklists cannot be and were not intended to be taxonomic *revisions* nor comprehensive nomenclatural compilations, in which every name is listed and/or every taxonomic opinion is supported by facts and references. As such, the checklists are not themselves standard, falsifiable research products, although they do synthesize such research results.

Taxonomic "Authority" Lists: Authoritative to Whom? The Tyranny of Users

The discussion that is now taking place in *Malacologia* about the French and the CLECOM checklists raises the issue of the acceptance of such checklists by the rest of the malacological community *and* users in general. These two categories of users may in fact at times have diverging interests, and this is where problems of acceptance may arise. Just like the *International Code of Zoological Nomenclature*, taxonomic authority lists only work as long as there is a majority consensus to accept them. The International Commission on Zoological Nomenclature does not have a police to enforce violations of the Code, and the general adherence to the *Code* marginalizes those zoologists who might reject the *Code*. In other words, the community of systematic zoologists will only accept those rules that it is prepared to follow. In turn, the International Commission on Zoological No-

menclature is led to propose compromises between consistency, for example, the "priority rule", and established usage, that may inconsistently apply the rules.

Taxonomic "authority" lists face the same kind of dilemma. If they list only those taxa that are accepted by an overwhelming majority (95%?) of systematists, they will be seen as perpetuating the worst conservatism and will give the impression that everything is known and no further research in taxonomy is necessary. If they list nominal taxa that have not yet been properly scrutinized by peers, they run the risk of disseminating instability. However, the role of checklists is not primarily to give a false impression of stability where there is not. When passing judgement on the authoritativeness of a checklist, one should not underestimate the tyranny of non-specialist users whose demand for "stability" is legitimate when it concerns the nomenclature of reasonably understood biological entities, but is not legitimate when it closes the door to progress in knowledge, or even to ambiguity (see, e.g., Dubois, 1998). Likewise, compilers of checklists must sometimes find compromises between scientific rectitude and the expectations of users. I want to illustrate this point by an example involving George Davis' own research.

For nearly 100 years, British and Irish malacologists have argued over the taxonomic status of populations of *Margaritifera* living in the river Nore in Ireland. Whereas *Margaritifera margaritifera* is a soft-water species everywhere it lives in Europe, there are Irish populations in the Nore basin that live in hard water, that have subtle shell differences that led to their segregation as a different species, *Margaritifera durrovensis*. Until the advent of molecular techniques, it remained disputed and unresolved whether *Margaritifera durrovensis* was just a hardwater variant of *M. margaritifera*, or a distinct species. Subsequently, an allozyme study (Chesney et al., 1993) concluded that *M. durrovensis* was just an ecophenotypic variant. As a scientist, I of course accept the results of the molecular study, but as a conservationist I may understand the desire by Irish naturalists to treat the Nore river *Margaritifera* as a "conservation unit". In fact, Chesney et al. themselves noted that "the classification of *M. durrovensis* as an ecophenotype of *M. margaritifera* does not detract from its need to be conserved". However, how does one give special conservation consideration to a "*Margaritifera margaritifera* Nore basin conservation unit"? Legislators and regulators have answered that question by placing "*Margaritifera durrovensis*" on Annex 2 to the European Habitats Directive (the EU equivalent to the US Endangered Species Act). And they have done so in 1995, that is, *after* the results of the Chesney et al. (1993) study, which were known to the proponent of the listing. I am not, through this example, advocating that it was justified to place the "species" *Margaritifera durrovensis* or even a "subspecies" *Margaritifera margaritifera durrovensis* on a list of protected species. But the fact is that it was listed, despite the advice of scientists (including myself) consulted by their national regulatory authorities. How was this to be reflected in CLECOM? One course of action was to promote scientific rectitude, ignore the name *durrovensis* altogether, and run the risk of being viewed as "irrelevant" or "useless" by the agencies using taxonomic authority lists for management. Another course of action was to promote consistency between regulatory texts and taxonomic authority lists, list *Margaritifera durrovensis* as valid, and run the risk of being viewed as "incompetent" by professional systematists. After much debate among its authors, CLECOM chose a middle course, and listed *Margaritifera margaritifera durrovensis* as a valid subspecies. It would not take much for me to accept that this compromise is eminently disputable. Just as a classification does *not* reflect *all* the relationships between taxa (the tree does), names are, after all, no more than a tag that people – scientists or non-scientists – find convenient to use to designate a biological "entity" or "unit", and communicate about its attributes and properties. Listing *Margaritifera margaritifera durrovensis* in taxonomic authority lists is a convenient way to access information on its conservation status as well as the associated literature, including Chesney et al. (1993).

This example reminds us that taxonomists and compilers of taxonomic authority lists are not working in a sociological vacuum. There is pressure from non-scientists to have *names* to designate management entities, even when these do not correspond to sound biological units. Davis (2004) defends the idea of using "conservation units instead of dubious subspecies". I believe scientists may argue *ad nauseam* in academic journals on whether "conservation units" are concepts that should be preferred over "dubious subspecies". I am

afraid this is an esoteric debate that has lost sight of the sociology of users who prefer to use named entities over "conservation units". As the Irish *Margaritifera* demonstrates, one powerful reason is that only named entities can have a legal status (that may actually promote research on the status of the taxon in question). I believe that when there is a testable hypothesis that such an entity may be treated as a biologically significant unit *and* there already exists a name for it, then we should definitely use that name. When a name does not exist already, I agree that it may be disputable whether it is justified to establish one, and I would defend the view that it depends on the scientific as well socio-historical context. For three generations, malacologists working on the systematics of European land snails have extensively and consistently used trinominal nomenclature, whereas by contrast, the analysis of geographical variation of North American land snails, and/or the way this variation is traditionally expressed through names, has *not* led to trinominal nomenclature.

To conclude, I would like to proselitize on the recently produced checklists of French and European non-marine mollusks. They represent a huge effort of data collation and knowledge consolidation, and they represent the state-of-the art of species-level systematics by systematists who have a personal opinion on the validity of nominal taxa, rather than perpetuate the state-of-the-art of 30 or 70 years ago. That the current state-of-the-art is challenging so many entrenched usage traditions is a reflection of the health of non-marine molluscan systematics in Europe. That the current state-of-the-art is highlighting so many unresolved problems also reflects the need for more research. This is why I entitled my introductory chapter to the French checklist: Land and freshwater mollusks of France: a new taxonomic authority list, a new start, new perspectives [«*Mollusques terrestres et aquatiques de France: un nouveau référentiel taxonomique, un nouveau départ, de nouvelles perspectives*»]. To close with a dose of humility, I will quote Isaac et al. (2004) in their recent essay on "taxonomic inflation": "Taxonomic uncertainty is ultimately due to the evolutionary nature of species, and is unlikely to be solved completely by standardization. For the moment, at least, users must acknowledge the limitations of taxonomic lists and avoid unrealistic expectations of species lists."

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CHECK-LISTS AND CLECOM: A RESPONSE TO DAVIS (2004)

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Recently, Davis (2004) published a paper with the title "Species check-lists: death or revival of the Nouvelle École?". From his title it is clear that he considers the work of CLECOM as the revival of the Nouvelle École (since a "death" of that school – whatever it means – would not be worth mentioning). This is a serious indictment, and therefore we have taken the liberty to explain some of our work in a more detailed manner.

CLECOM

The acronym CLECOM stands for "Check-List of the European Continental Mollusca". The initiative dates back to June 1986, and in August 1995 the CLECOM Working Group was officially endorsed by the General Assembly of Unitas Malacologica (UM) at its 12th International Malacologial Congress in Vigo, Spain. The CLECOM Working Group acts under the umbrella and on behalf of UM; the Friedrich-Held-Gesellschaft (München) is the organisation responsible for the CLECOM Working Group (Falkner et al., 2001).

One of the primary aims of the CLECOM initiative is to produce a distributional check-list of accepted scientific names for all the non-marine molluscan (sub)species recognised in Europe. The first CLECOM list covers northern, western and central Europe (Falkner et al., 2001). The second CLECOM list gives a supraspecific classification of the European non-marine molluscs (Bank et al., 2001a). The third CLECOM list covers Macaronesia (Bank et al., 2002). The lists have been welcomed by many malacologists. In fact, recent works have adapted their nomenclature/classification to a large extent to the CLECOM lists (e.g., Alba et al., 2004; Glöer, 2002; Martínez-Ortí & Robles, 2003; Moorkens & Speight, 2001; Olsen, 2002; Šteffek & Grego, 2002; Vilella et al., 2003). By doing so, a much more uniform nomenclature becomes available within the malacological community: regional scientific names, that are still hampering communication approximately 250 years after Linnaeus, are more difficult to defend now. This will favor integration, for example, among databases. One such database is a product of the Fauna Europaea project (www.faunaeur.org), and uses the CLECOM lists as its basis for the Gastropoda.

Although there have been some cautionary comments regarding use of the CLECOM lists (e.g., Cameron, 2003), we have the impression that a certain level of traditionalism is involved. For example, Cameron (2003) still uses the name *Oxyloma pfeifferi* despite the fact that the epithet *pfeifferi* Rossmässler, 1834, has been replaced for taxonomic reasons by the older name *elegans* Risso, 1826, for nearly half a century (Forcart, 1956; see also ICZN Opinion 336, 1955). It is difficult to find a paper published by a non-British or non-Scandinavian malacologist after 1970 that uses the name *pfeifferi*. Davis (2004), however, questioned the scientific basis of the CLECOM lists as a whole. Although his criticism is only very general, we would like to respond to his comments.

Organismal and Molecular Taxonomists Should Combine Their Forces in Biodiversity Research

People have marvelled and puzzled over morphological diversity for thousands of years. According to Wheeler (2004) we now have the opportunity to put centuries of scholarship on morphology into perspective and share it with the world. One tool for achieving this is the preparation of species check-lists, which should be considered snapshots of our knowledge of the world's biodiversity at a given moment. Such check-lists are often needed

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to attain an overview of the data available in the ever increasing taxonomic and faunistic literature. In fact, check-lists and bibliographies often boost research, as they provide insight on the status of current knowledge.

Most of the taxonomic and faunistic literature is based on morphological studies. Professionals and amateurs make lists of species from particular locations by identifying species mostly with a field guide based on observable morphology. According to Davis (2004: 228), "creating taxa simply on the basis of shell characters (qualitative and quantitative) should always be done with extreme caution". He even states that "the situation may not substantially change even when other characters, anatomical, eco-ethological are added". Davis is advocating molecular research as the Holy Grail. This approach to biodiversity/systematics is a subject of serious concern (Lee, 2004; Wheeler, 2004; Will & Rubinoff, 2004; Wiens, 2004). We advocate that all disciplines of taxonomy should combine their forces to decipher evolutionary processes rather than each stressing its own superiority. What we do not advocate is the training of biologists who can only identify organisms after grinding them up and feeding them into a sequencing machine.

Sequences, although highly informative, are not *a priori* better than morphological, anatomical or eco-ethological characters. An example is the recently published molecular phylogeny of the western palaearctic Helicidae *sensu lato* (Steinke et al., 2004). The maximum likelihood phylogenetic tree (based on a combined dataset of COI, 16S, 18S and ITS-1 sequences) fits almost perfectly with the family/subfamily classification provided by CLECOM, a classification based on anatomical data. Where more than one species per genus was sampled, these genera were monophyletic. An exception was the nominal genus *Cernuella*, as the three species that were sequenced – *virgata*, *neglecta* and *cespitum* – are scattered within the phylogenetic tree of the Hygromiidae. Had the authors consulted the CLECOM check-list, they would have learned that *cespitum* is not a *Cernuella*, but belongs to the genus *Xerosecta*, subgenus *Xeromagna*, and that *neglecta* and *virgata* belong to different subgenera (*Xerocincta* and *Cernuella*, respectively) of the genus *Cernuella*. Unfortunately, scientists working with molecules often use old taxonomic frameworks in their publications.

Naming Species and the Biological Species Concept

Species concepts have been the subject of voluminous debate. We adhere to the biological species concept (BSC), although we admit that most of the entities that are currently recognized in malacology as "good species" are in fact morphospecies. It is widely accepted, however, that the vast majority of the currently accepted morphospecies correspond to biological species. In fact, we are not aware of any example in malacology of widely accepted morphospecies recognized half a century ago as living in Europe now being lumped together as a result of more "sophisticated" studies involving, for example, population genetics or molecular data. In fact, such studies often revealed higher diversity than was expected on morphological criteria, for example, because of the presence of cryptic species. The number of taxa in check-lists presented as "good species" should therefore be considered conservative rather than exaggerated.

Care should be taken with the doctrines known as the "Nouvelle École" of Bourguignat or "Starobogatovismus". Practitioners of both doctrines should realize that they do not contribute to a better understanding of biodiversity (Dance, 1970; Reischütz, 1994). However, as one can see from our CLECOM list, we have evaluated the numerous taxa recognized by the "Nouvelle École" of Bourguignat or the Starobogatov doctrine and found most taxa not to be taxonomically sound. Thus, only a few names have entered our lists. We would like to stress once more, that we have critically investigated, case by case, the evidence on which a morphospecies has been based before we decided to accept it in our check-list. So we did exactly what Davis describes as the necessary methodological procedure: sort through publications, look at voucher specimens and field records (in order to assess the biological validity of formally described taxa), consider contradictory information, make decisions, and ensure compliance with the International Code of Zoological Nomenclature. We agree with Davis that there is some subjectivity in this procedure. This subjectivity is likely to be minimized through the collaboration of independent specialists: as clearly stated in the introduction, the CLECOM check-list is not the product of a single person. We hope that Davis does not expect us to be in-

fallible. Of course, errors, wrong decisions, and omissions happened; and sometimes – to spare time – we were not critical enough and relied on secondary literature of which we judged the authors to be thorough and reliable. To correct such errors and to include new findings we formally institutionalised the instrument of Updates for the CLECOM-lists, of which the first appeared in 2001 (Bank et al., 2001b) and the second is now ready for printing.

The Use of Subspecies

It is well-known that many species vary in space. They may consist of (groups of) recognizable populations inhabiting different geographical areas. Such populations may have been initially described as separate species. They are combined as polytypic species, objectively whenever hybrid zones connect the diagnosable population groups, or subjectively on the – weak indeed – basis of similarity and analogy to comparable cases. In land snails the subspecies concept has resulted in a decrease in the number of so-called species. Given the importance of the polytypic species concept, it is remarkable that it is widely ignored in faunistic studies (but often not in taxonomical work!). Davis (2004: 229) argues that a check-list that is based on faunistic literature should ignore subspecies, as in faunistic literature the subspecies is widely neglected. However, our check-list is based on taxonomic literature. We see no good reason to ignore the subspecies level of biodiversity in faunistic studies. Subspecies in the BSC are terminal taxa in the phylogenetic species concept (see the response of Bouchet, 2006).

Too Many (Sub)Species?

Some authors feel uncomfortable about the large number of species and subspecies mentioned in the CLECOM list or in the Fauna Europaea database. An example is the large list of Clausiliidae from Greece. However, molecular data on some of the most speciose genera are in agreement with the large radiations that were unraveled by morphological studies (Schilthuizen, 1994; Moorsel, 2001; Uit de Weerd, 2004). The diversification of the helicid genus *Arianta*, based on morphological features and postulated over a decade ago (Gittenberger, 1991), has now been confirmed by molecular data (Gittenberger et al., 2004). Species complexes within the Arionidae, recognized on the basis of morphological and anatomical characters, are becoming confirmed by molecular data as well (Pinceel et al., 2004). We thus feel that in the vast majority of cases the CLECOM list reflects the biodiversity of continental molluscs as present in Europe. Of course, gaps in our knowledge will certainly result in incomplete or wrong data in our list. The list can only be as good as the science on which it is based. We still have a long way to go to achieve a complete assessment of the actual snail diversity of Europe. Currently, some 25 new (sub)species are described on a yearly basis from Europe: on average one new taxon every two weeks.

The Case of *Bythiospeum*

Bythiospeum is admittedly a major problem in the faunal lists (and Red Lists) of Germany in general and the federal states of Baden-Württemberg and Bayern in particular, which hampers the evaluation of the fauna with respect to its degree of endemism and the fixation of conservation priorities. So far, 58 names have been introduced to designate the different forms of *Bythiospeum* in Germany. The validity of these nominal taxa has been a matter of intensive discussion, but so far no consensus has been reached (also not among the CLECOM authors!). Obviously however, the three polytypic species complexes of Bolling (1966) are not a reflection of the true amount of differentiation in this genus in Germany. Nevertheless, Bolling was followed in the successive editions of the freshwater mollusc guide of Glöer et al. (1978–1992) and Glöer & Meier-Brook (1994–2003). Perpetuation of that practice was considered by us as unacceptable. We also did not follow the example of Boeters (1998), who simply provided a list of all available names. Although this exhaustive listing has the advantage of being objective, it is purely technical and by definition excludes any modification or incorporation of new biological results with the exception of the addition of new names. Such a list is surely useful but is not compatible with our aim of depicting "real world" biological diversity.

We will explain how we established the list of *Bythiospeum* taxa as presented in our CLECOM list. First, we accepted the hypothesis of Geyer (1908) that in several species parallel converging dwarf forms occur, which may be so different from their stem form that they give the impression of being distinct spe-

cies (in some cases syntopic occurrences of distinct species may really be hidden under this phenomenon). Second, we took into account the fact that several species can be clearly distinguished by their anatomy. In this context, it is of interest to note that the three species that are differentiated by Boeters (1984) were regarded by Bolling (1966) as belonging to one "species" (*acicula* sensu Bolling) and that two of them (*suevicum* and *exiguum*) represented his subspecies *clessini*. This shows that even a provisional maintainance of Bolling's system was impossible. In the Bavarian alpine foreland, *B. acicula* and *B. heldii* (occurring in the same hydrological system at Obernach on the Walchensee) are anatomically separable (Boeters, 2002). The other nominal species occurring in the Bavarian alpine foreland, namely *rougemonti*, *carychiodes*, *aciculoides* and *algoviensis*, were considered provisionally by us as synonyms of *acicula* or *heldii*; *B. alzense* was considered a separate species. For Baden-Württemberg, we accepted in general the classification of Geyer (1908), being the result of extensive and carefully planned fieldwork by Geyer, producing a hitherto unsurpassed wealth of material. The classification system was elaborated by Geyer in a self-critical way following the modern species concept (i.e., anticipating the biological species concept). He based his revision on thorough conchological examination but also stressed the need for anatomical studies. From his papers, it is clear that Geyer recognised variability and ecophenotypical variation, and that he had well-founded ideas on geographical barriers and the genetical coherence of populations. Geyer was also aware of the pitfalls of Bourguignatism, which he rejected: "Ich ... hütete mich aber vor der Art der neufranzösischen Schule, welche den Lebenszusammenhang mißachtet" (1908: 595). In our opinion, the work of Geyer is the best that has ever been done on *Bythiospeum* and we see no reasons to abandon his system, which is scientific because it allows falsification and amendment. We have revalidated *Bythiospeum pellucidum*, the type-species of the genus, which was shown by Boeters (1984) to be a good species, different from *B. quenstedti* to which it had been attributed by Geyer as a mere ecophenotype (Geyer, who named the species with inclusion of its alleged derivate *quenstedti*, was apparently unaware of the nomenclatural consequences, as were all his followers). Other deviations from Geyer are: (1) we neglected all subspecies that could not be attributed to geographical regions or were said to occur side by side in the same cave or spring; (2) we treated *lamperti*, *taxisi*, and *senefelderi* as full species, following Ehrmann (1933); (3) for Mainfranken, we followed the concept of two species (*clessini* and *puerkhaueri*) with several subspecies and provisionally treated *moenanum*, *elongatum* and *nolli* as synonyms, based on Noll & Hässlein (1952).

In summary, we rejected the *Bythiospeum* species constructs of Bolling and adopted the more traditional (non-Bourguignatian) classification of Geyer (1904–1908) with modifications based on arguments of later authors. Taking into account the relevant literature on this topic, we listed for Germany 26 species, 6 of them polytypic (a total of 36 names). We have thus "neglected" (synonymised) 22 names. This will certainly be the subject of further changes after additional research. But it remains to be demonstrated – by DNA sequencing for example – whether this is indeed a case of "splitting". In this context, it is of interest to note that Haase (1995), after a detailed morphometric study of the then eight recognized *Bythiospeum* taxa from Austria, accepted them all (albeit provisionally) as separate species.

Value of Molecular Data: Systematics of Hydrobiidae as an Example

We acknowledge the great value of molecular data for systematic work and, as a consequence, also for check-lists, and have used these data whenever possible. We are surprised to learn from Davis that we have ignored the molecular work from his group. He writes (2004: 230) "... the molecular work my group has done in the past ten years on several European taxa in the superfamily Rissooidea largely has been ignored by CLECOM. Instead, the rissooidean systematics of that list is still mainly based on traditional (mostly shell-based) data. It is not that the molecular data have been ignored, because they often contradict the findings of members of the CLECOM team. Rather, it is important that CLECOM incorporates new findings based on genetic data much more quickly, even if these findings are inconvenient". First, we would like to stress that our classification is not mostly shell-based but mainly based on

anatomical characters, namely on the genital organs from a large number of genera (as provided by Radoman, 1983). Second, we are well aware of the excellent work of Davis and co-workers on Rissooidea, and have integrated their results in our check-list. So we are eager to learn, where our list deviates from his work published *before* the beginning of 2001, the year that our list was published. Unfortunately, no example(s) is(are) given. An interesting paper by Wilke et al. (December 2001), with Davis as one of the authors, described the molecular systematics of the Hydrobiidae. This paper is the most recent work of Davis dealing with the classification of a part of the European taxa generally assigned to the Hydrobiidae. Interestingly, the presented classification essentially reflects the classification used by CLECOM. We noted the following differences: the Lithoglyphidae, Cochliopidae and Amnicolidae are dealt with by CLECOM as subfamilies within the Hydrobiidae, rather than families. As already stated by Wilke et al. (2001: 11), "There is no universal definition for "family"." In this context, it is of interest to note, that Davis et al. (1982, 1985) ranked these families, as CLECOM did, before 2001 as subfamilies. Thus, we "lumped" rather than "split" the suprageneric hierarchical units: certainly no revival of the "Nouvelle École"!

The subdivision of Hydrobiidae into several clades by Wilke et al. (2001) (that is, the maximum likelihood tree based on combined COI and 18S sequences – their fig. 3), also essentially reflects our classification. *Adrioinsulana*, *Pseudamnicola*, *Adriohydrobia*, *Hydrobia*, *Peringia* and *Ventrosia* [correct name: *Ecrobia*] cluster together, separate from *Graziana*, *Belgrandia*, *Horatia*, *Sadleriana*, *Orientalina*, *Hauffenia*, *Fissuria*, *Alzoniella*, *Avenionia* and *Islamia*. These two clades are designated Hydrobiinae and Belgrandiinae, respectively, in our classification. The only two surprises in their figure 3 are (1) *Mercuria*, which we classified within the Hydrobiinae, not Belgrandiinae, and (2) the classification of *Bythiospeum* within the Moitessieriidae. With respect to *Bythiospeum*, there are massive anatomical data suggesting that *Bythiospeum* and *Moitessieria* are not closely related (Boeters, 1972; Boeters & Gittenberger, 1990; Boeters, 2003). Unfortunately, the sequences are based on an unidentified "*Bythiospeum* spec." collected in southern France, which does not allow verification of the diagnostic characters given by Boeters (2003). The family status of the Moitessieriidae itself has been defended by some authors for many years and denied by others: here DNA demonstrates its value. All this shows the importance of a good (morphological) taxonomic framework to start with. Incorrect identifications result in wrong conclusions. With respect to *Mercuria*, we noticed that in the maximum likelihood tree based on the mitochondrial COI only (their fig. 2B), *Mercuria* seems to be more closely related to the Hydrobiinae than to the Belgrandiinae, whereas in the maximum likelihood tree based on the nuclear 18S (their fig. 2A) the reverse is seen. This is a good example of the well-know fact that gene trees and phylogenetic trees may differ significantly from one another. What is needed is an integrated approach. It is this approach that was used while preparing the CLECOM check-list.

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Taxa in **bold** are new; pages in *italic* indicate figures of taxa.

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