Exploring the unknown: On the diversity of pachychilid freshwater gastropods in Vietnam (Caenogastropoda: Cerithioidea)

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Running head:

Vietnamese Pachychilidae

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Abstract

A revision of Vietnamese freshwater gastropods of the family Pachychilidae is presented based on the analysis of morphological characteristics and partial sequences of the mitochondrial genes of 16S rRNA (16S) and cytochrome c oxidase subunit I (COI). We found that in Vietnam two pachychilid genera occur, *Brotia* and *Sulcospira*. Of the eight to fifteen species reported by earlier authors, we can only confirm the presence of two species, *Sulcospira tonkiniana* and *S. tourannensis*. All further taxonomic names that were previously applied for Vietnamese pachychilids are considered either as junior synonyms of these two species or erroneous references to species from other regions of Southeast and South Asia. Additionally, we describe two new species of *Brotia* and four new species of *Sulcospira* and report another undescribed species with uncertain affinities. Patterns of morphological and genetic differentiation of the Vietnamese species and their affinities within the context of pachychilid phylogeny are briefly discussed.

Introduction

Vietnam boasts a rich biota with considerable levels of endemism. However, our knowledge of this diversity is still patchy and biased towards vertebrates whilst most invertebrate groups remain poorly documented (Sterlin et al. 2006). Pachychilid freshwater gastropods, which are the subject of this paper, serve as an example to illustrate how incomplete our current understanding of many invertebrate groups remains, regardless of their ecological importance and potential as ecological indicators. The basic knowledge of the freshwater gastropod fauna of Vietnam was established by French workers of the late 19th and early 20th century (e.g., Brot 1887; Fischer, 1891; Morlet 1893; Dautzenberg and Fischer 1906; 1908; Bavay and Dautzenberg 1910) and little work has been done since. However, workers at the time applied a highly polyphyletic concept and subsumed all cerithioidean freshwater gastropods under a single genus name, *Melania* Lamarck, 1799. This name is a junior synonym of *Thiara* Röding, 1798, though, and the majority of its former members has subsequently been dispersed among several families – not to mention many different genera (e.g., Glaubrecht, 1996). Of these 'melanid" families three are currently present in Vietnam: Thiaridae Troschel, 1857, Semisulcospiridae Morrison, 1952, and Pachychilidae Troschel, 1857. Due to the lack of modern systematic studies of Vietnamese freshwater gastropods, the familial affinities of the described '*Melania*' species remain unclear. Moreover, the French reports are biased

towards the region of northern Vietnam ("Tonkin" = Bac Bo in Vietnamese), whereas the faunas of both central Vietnam ("Annam" = Truong Bo) and southern Vietnam ("Cochinchina" = Nam Bo) have received only little attention. In the only recent work, Dang and Ho (2007) listed fifteen pachychilid species from Vietnam. This work, however, is based on the comparison of shells only and does not fully incorporate the knowledge of the SE Asian Pachychilidae, which has been accumulated in recent years. As a result some species were misidentified due to confusion among species with similar shells.

According to the latest systematic study (Köhler accepted), the Pachychilidae are represented by two genera in mainland Southeast Asia: *Brotia* H. Adams, 1866 and *Sulcospira* Troschel, 1858 (with the name *Adamietta* Brandt, 1974 considered a junior synonym of the latter). *Brotia* ranges from northeast India to Sumatra and Borneo (Köhler and Glaubrecht 2006). To the north its distribution is bounded by the Himalayas and their foothills. By contrast, *Sulcospira* as delimited by Köhler (accepted) occurs in the eastern part of Sundaland from Thailand in the west to Southern China in the east and Borneo and Java in the south. Currently it is unknown how far the genera extend into southern China and to what extent their distributions overlap in Laos, Vietnam, Cambodia, and eastern Thailand. Key diagnostic characters of these genera include certain features of the reproductive and embryonic shell morphology but not the teleoconch. Accordingly, *Brotia* is characterized by the presence of a wrinkled apical whorl of the embryonic shell while *Sulcospira* possesses a smooth and inflated apex. In addition, the pallial oviduct of *Brotia* lacks a seminal receptacle. This structure is present in *Sulcospira*, however. Previous accounts on Vietnamese freshwater snails are essentially based on shells and are therefore not helpful in deducing species affinities with respect to the above mentioned criteria. Studies of pachychilids from other regions of Southeast Asia that integrated more comprehensive morphological and molecular data showed that species are usually restricted to small areas, such as the drainages of single rivers (Glaubrecht and Köhler 2004; Köhler and Glaubrecht 2006). We anticipate that this observation will probably hold true also for other regions of Asia. This implies that a large number of undiscovered species may exist in areas that are not well covered by historical studies, such as the interior regions of central and southern Vietnam.

The goal of the current work is to place historical accounts into the context of a modern systematic framework in order to provide a basis for future efforts to document the pachychilid fauna of Vietnam completely and to assess its vulnerability to habitat losses and environmental changes that may occur due to current or future human activities.

Material and methods

This work is based on ethanol preserved material collected during two field trips undertaken by the authors in March and October 2006, respectively. Voucher specimens are deposited with the Museum für Naturkunde, Humboldt-Universität, Berlin (ZMB) and the Institute of Ecology and Bio-Resources, Vietnamese Academy of Science and Technology, Hanoi (IEBR). Historical and type material housed with the Muséum d'Histoire Naturelle, Paris (MNHN) and the Muséum d'Histoire Naturelle, Genève (MHNG) was also examined.

For statistical analyses, the shell parameters H (height of shell), B (breadth of shell), LA (length of aperture), WA (width of aperture) and BW (height of body whorl) were measured with a calliper. Anatomy was studied using a Leica MZ 8 stereo microscope with a camera lucida. Radular and embryonic shell morphology was studied using a scanning electron microscope; for a more detailed description of the applied methods see Köhler & Glaubrecht (2006).

Total DNA was extracted by application of a CTAB extraction protocol (Winnepenninckx et al. 1993). Foot muscle tissue was macerated at 60 °C in CTAB buffer containing Proteinase K. PCR amplifications were conducted in 25 µl volumes containing 1x PCR buffer, 200 µM each dNTP, 2.0 mM MgCl2, 0.5 µM each Primer, 1.25 units of Taq polymerase, and approximately 50 ng of DNA. After an initial denaturation step of 3 min at 96° C, 35 cycles each of 60 s at 94° C, 50-55° C, and 72° C were performed, followed by a final extension step of 5 min at 72° C. Partial sequences of the mitochondrial cytochrome oxidase subunit I gene (COI, 660 bp) and 16S ribosomal RNA gene (16S, ~826 bp) were amplified using the primers LCO 1490 (Folmer et al. 1994) and HCO2198 var. (Glaubrecht and Rintelen 2003) for COI and 16S-F (Wilson et al. 2004) and H3059 (Palumbi et al. 1991) for 16S, respectively. Both strands of the amplified gene fragments were directly cycle-sequenced on an ABI 377 automated sequencer in 10 µl volumes containing 2 µl of ABI Prism BigDye Terminator cycle sequencing reaction mix. Electropherograms were manually corrected for misreads and merged into one sequence file using BioEdit Version 5.0.1 (Hall 1999). Sequences have been deposited with GenBank (Tab. 1).

COI sequences were aligned by eye whilst 16S sequences were aligned with ClustalX version 2.0 (Larkin et al. 2007) in the multiple alignment routine using default settings and 'accurate search' followed by minor manual corrections. Phylogenetic analyses (Maximum Parsimony, MP; Bayesian Inference BI) were conducted for each gene fragment (COI, 16S) separately as

well as for a concatenated data set of 16S and COI. Parsimony analyses were performed using the Ratchet as implemented in WinClada ver. 1.00.08 (© Nixon, 1999-2002), with 1,000 iterations, 10 trees held at each iteration; bootstrap analyses under parsimony were also completed in WinClada, with 1,000 replicates. Bayesian analyses were conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). The generalized time reversible model of sequence evolution (GTR+I+Γ) was employed for a 5,000,000 generations Metropolis-coupled Markov chain Monte Carlo (4 chains, chain temperature = 0.2) for each gene. For the concatenated data set a partition was applied that allowed parameters to be estimated separately for 16S and each codon position of COI. Trees were sampled from the chain every 2000 generations. Trees from the first 2,000,000 generations were discarded as 'burn in'; posterior probabilities are indicated at the nodes of the Bayesian phylogram.

Systematics

Brotia H. Adams, 1866

Brotia H. Adams, (1866): 150. Type species (by monotypy): *Melania pagodula* Gould, 1847; Recent, Myanmar.

Antimelania Fischer & Crosse, (1892): 313. Type species (by subsequent designation of Pilsbry & Bequaert, 1927: 300): *Melania variabilis* Benson, 1836: Recent, India.

Taxonomic remarks and key characters: *Brotia* was originally established based on the round and multispiral operculum of the type species. A round and multispiral operculum, however, is only found in *B. pagodula* and a few other species – among them the two Vietnamese species described below. Species with round opercula of about eight whorls form two different clades within the genus, one of them being comprised by the Vietnamese and Laotian species (Köhler accepted). Most *Brotia* species have an oval operculum with about four whorls (Köhler and Glaubrecht 2001; 2006) similar to that found in other pachychilid genera, such as *Sulcospira*. Since the late 19th century, a vast number of species have been placed in *Brotia*, often without sufficient knowledge of their morphology (e.g., Brot 1874-79; Martens 1897; 1900; Martens and Thiele 1908; Abbott 1948; Brandt 1968; 1974; Davis 1971). In a recent revision Köhler and Glaubrecht (2006) restricted the concept of *Brotia* to pachychilid species with (1) a subhaemocoelic brood pouch, (2) an open pallial oviduct with a free medial lamina that comprises a deep, ciliated spermatophore bursa and lacks a seminal receptacle, and (3) an embryonic shell that exhibits a wrinkled apex, which is formed due to a shrinking yolk-sac and delayed calcification during embryonic development. This revised concept led to the exclusion of about 50% of all species previously subsumed under this generic name. *Brotia* as so delimited forms a monophyletic group in mitochondrial trees (Köhler et al. 2004; Köhler accepted). It ranges from northeast India in the west to Borneo in the southeast. However, its limits in the northeast remain unclear. Recently, evidence has been presented for its occurrence in Laos (Köhler 2008). The present paper presents the first confirmed reports of *Brotia* in Vietnam thereby extending the known range of the genus towards the east. Whether *Brotia* also occurs in southern China remains to be clarified

because the affiliation of species reported from this area awaits confirmation with respect to the key morphological features identified by Köhler & Glaubrecht (2006).

Brotia annamita n. sp.

Figs. 2A-C, 3A-B.

Material examined.

Holotype: Nghe An Prov., Thanh Chuong District, creek in Tam Quang commune, W of Con Cuong, foothills of Truong Son Mts., small mountain creek in forested area of the Truong Son Mountains, several km off road No. 7 from Do Luong to the Lao border, Ca River drainage, 18°48'N 105°20'E; coll. F. Köhler, Oct. 2006 (ZMB 114.376a, Fig. 2A).

Paratype: Same as holotype (ZMB 114.376b, Fig. 2B).

Description.

Shell (Fig. 2A-B): Medium sized (Tab. 2); broadly conical, very robust. Spire eroded with four remaining whorls. Whorls well and evenly rounded in diameter, separated by shallow suture. Sculpture consists of eight well developed spiral lirae at base of shell and two less developed spiral lirae at periphery of body whorl that become oblique on subsequent whorls; axial elements consist of regularly spaced ribs on second and third whorl, ribs become partly oblique on body whorl; small nodules form where spiral lirae and axial ribs meet. Aperture wide, narrow where outer lip meets parietal wall of shell. Outer lip well rounded, angularly produced below, sharp, slightly whitish inside. Inner lip slightly thickened, yellowish. Colour of shell chestnut brown, inside of aperture translucent with banding pattern formed by outer lirae.

Operculum (Fig. 2C): Round, about 7 mm in diameter with 4.5 regular whorls.

Anatomy: Living animal with brownish skin marbled by yellow patches, preserved animal greyish to black. Both specimens studied are females; brood pouches contain numerous (50+) egg capsules. Midgut unknown.

Radula (Fig. 3A-B): Ribbon 19.3 mm long with 131 rows of teeth (6.8 rows/mm) (n=1). Central tooth with rounded base, slightly broader than long, with concave upper rim and convex basal rim; cutting edge consisting of well developed central cusp and three accessory cusps on each side that taper in size; glabella with concave lateral margins, broad, not extending the lower margin of tooth. Lateral tooth with short lateral extension at outer side; cutting edge with large main cusp, one accessory cusp on inner and two to three ones on outer side. Inner and outer marginal teeth differ in shape. Inner one curved, slightly broader, with broadly pointed main cusp on outer side and smaller accessory cusp on inner side. Outer marginal teeth with external flange; broadly pointed main cusp on outer side, smaller inner accessory cusp.

Remarks.

Unlikely to be confounded with any other *Brotia* species or, indeed, any Vietnamese pachychilid owing to its marked sculpture, robust and shell, broadly conical shape, and angular aperture.

Etymology.

This species is named after the Annamite Mountains, or Truong Son Mountains in Vietnamese.

Distribution.

Known only from the type locality, Nghe An Province (Fig. 1).

Brotia hoabinhensis n. sp.

Figs. 2D-H, 3C-F, 4, 5.

Material examined.

Holotype: Hoa Binh Prov., Tan Lac District: Stream in Muong Khen, 20°37.088'N 105°16.21'E; coll. F. Köhler, Oct 2006 (ZMB 114.470a; Fig. 2D).

Paratypes: Same as holotype (ZMB 114.470b, 4 specimens, Fig. 2E; ZMB 114.153, 8 juvenile specimens, Figs. 2F-G; IEBR, 4 juvenile specimens).

Description.

Shell (Fig. 2D-G): Large (Tab. 2); Elongate conical, robust. Spire highly turreted, tip almost not eroded, seven to eight remaining whorls. Whorls widely rounded in diameter, slightly flattened above periphery; separated by shallow suture. Sculpture somewhat variable, predominantly smooth. Spiral lirae always present at base, mostly indistinct; some specimens exhibit more or less distinct spiral lirae on body whorl that become oblique on subsequent whorls. Axial ribs mostly absent, only exceptionally indistinct axial ribs or folds present at upper whorls; irregularly spaced. Colour uniformly light brown; broad, dark brown spiral band on upper third of whorls may be present, rather indistinct; especially visible in juveniles. Aperture moderately wide, slightly angularly produced below. Outer lip well rounded, sharp, translucent.

Operculum (Fig. 2H): Round, up to 8 mm in diameter with eight regular, narrow whorls.

Juvenile shell (Fig. 4): Brood pouch of one female contained 95 developed juveniles ranging from 2.2 mm (2 whorls) to 3 mm (3 whorls) in height as well as ~30 egg capsules. Embryonic shell sculpture smooth except for regular growth lines and wrinkled apical whorl.

Radula (Fig. 3C-F): Ribbon about 22.3 (± 1.9) mm long with 172.5 (±0.5) rows of teeth (7.8 ± 0.6 rows/mm) (n=2). Central teeth broader than long, with concave upper rim and convex basal rim; cutting edge consisting of well developed central cusp and three accessory cusps on each side that taper in size; glabella with concave lateral margins, not extending the basal rim of tooth. Outer side of lateral teeth with short lateral extension; cutting edge with large main cusp and two tapering accessory cusps on inner and three on outer side. Inner and outer marginal teeth differ in shape. Inner ones curved, slightly broader, with broadly pointed main

cusp on outer side and smaller accessory cusp on inner side. Outer marginal teeth with external flange; broadly pointed main cusp on outer side and smaller inner accessory cusp.

Anatomy: Living animal dark grey with some yellow pigment spots.

Midgut (Fig. 5): Openings to digestive gland (dgd) and oesophagus (oes) in close proximity, separated by inconspicuous fold; sorting area (sa) consists of regularly spaced, quite narrow folds and well developed and broad outer crescent septate pad (icp), inner one faintly developed (ocp); thin marginal fold (mf); thick lateral fold (lf).

Etymology.

This species is named after the Hoa Binh Province in which it was found.

Remarks.

Shell differs from *B. annamita* by having more whorls, an elongate shape and lack of axial sculpture. The sympatric *S. collyra* described below has a similarly elongate shell but differs by having a weaker spiral sculpture, presence of axial sculptural elements in some shells, presence of an oval operculum with about four whorls.

Distribution.

Known only from the type locality, Hoa Binh Province (Fig. 1).

Sulcospira Troschel, 1858

Sulcospira Troschel, 1858: 117-118. Type species (by monotypy): *Melania sulcospira* Mousson, 1849; Recent, Java.

Adamietta Brandt, 1974: 171-172. Type species (by monotypy): *Melania housei* I. Lea, 1856; Recent, Thailand.

Taxonomic remarks and key characters: Originally established for the Javan endemic *Melania sulcospira*, this taxon has been neglected by subsequent authors possibly due to the unavailability of material of the type species. However, *Sulcospira* was the first name introduced for a pachychilid genus-group taxon and it certainly has to be considered. Köhler and Glaubrecht (2005) compiled the available data on the type species and showed that *Sulcospira* differs markedly from *Brotia* by the presence of a smooth and conspicuously inflated apical whorl of the embryonic shell. Based on comparative anatomical and molecular analyses of the SE Asian Pachychilidae, Köhler (accepted) identified this smooth, inflated apical whorl of the embryonic shell and the presence of a seminal receptacle in the pallial oviduct as anatomical key characters of a clade of subhaemocoelic brooders among Asian pachychilids. Accordingly, we suggest applying the name *Sulcospira* to this group and that the name *Adamietta* be considered a junior synonym. The following Vietnamese pachychilid species that are evidently not members of *Brotia* exhibit anatomical features (smooth apical whorls of embryonic shells, presence of a seminal receptacle) that are consistent with placement within *Sulcospira*.

Sulcospira tonkiniana (Morlet, 1887)

Figs. 7-10.

Melania verbecki var. *tonkiniana* Morlet, 1887 [“1886”]: 264-265 (published in January) (“environs de Dong-song et Lang-son; leg. Jourdy”). Lectotype (present designation) and paralectotype (MHNG, coll. Brot).

Melania beaumetzi Brot, 1887: 34, 219 (published in March) (“mare environs Thanh Moi). Lectotype (by designation of Fischer-Piette, 1950: 156) MNHN.

Melania hamonvillei Brot, 1887: 32-34 (published in March) (“Tonkin”); Morlet, 1893: 154; Fischer & Dautzenberg, 1905: 141; Fischer & Dautzenberg, 1906: 164. Lectotype (by designation of Köhler and Glaubrecht, 2002: 137) and 14 paralectotypes MNHN, 9 paralectotypes Brot coll., MNHG.

Brotia hamonvillei – Köhler & Glaubrecht, 2002: 137, fig. 2C; Dang & Ho, 2007: 5, fig. 6.

Melania siamensis – Morlet, 1891: 234; Bavay & Dautzenberg, 1910: 7-10 (not *M. siamensis* Brot, 1886).

Melania aubryana – Dautzenberg & Fischer, 1908: 196; Bavay & Dautzenberg, 1910: 4-7, fig. 8 (not *Melania aubryana* Heude, 1889).

Melania aubryana var. *elongata* Bavay & Dautzenberg, 1910: 5, pl. 1b, fig. 9 ("Tonkin").

Melania aubryana var. *robusta* Bavay & Dautzenberg, 1910: 6, pl. 1b, fig. 10 ("Tonkin").

Melania aubryana var. *attenuata* Bavay & Dautzenberg, 1910: 6, pl. 1b, fig. 11 ("Tonkin").

Melania aubryana var. *paupera* Bavay & Dautzenberg, 1910: 6, pl. 1b, fig. 12 ("Tonkin").

Melania aubryana var. *polygonalis* Bavay & Dautzenberg, 1910: 6-7, pl. 1b, fig. 13 ("Tonkin").

Melania siamensis var. *nodosa* Bavay & Dautzenberg, 1910: 10, pl. 1b, fig. 14 ("Tonkin").

Melania siamensis var. *laevigata* Bavay & Dautzenberg, 1910: 10, pl. 1b, fig. 15 ("Tonkin").

Melania hugeli [sic !] – Dautzenberg & Fischer, 1906: 411 (not *M. huegelii* Philippi, 1843).

Semisulcospira aubryana – Dang & Ho, 2007: 6, fig. 11 (not *M. aubryana* Heude, 1889).

Brotia jullieni – Dang & Ho, 2007: 5, fig. 5 (not *M. jullieni* Deshayes, 1874).

Brotia siamensis – Dang & Ho, 2007:5, fig. 8 (not *M. siamensis* Brot, 1887).

Taxonomic remarks.

The species as delineated herein shows remarkable plasticity in the shell, which caused taxonomic difficulties. The first validly introduced name is the variety name '*tonkiniana* Morlet, 1887'. Although being widely ignored by subsequent authors, it is has priority over the younger names *M. hamonvillei* Brot, 1887 and *M. beaumetzi* Brot, 1887. Pages 257ff of volume 34(4) of the 'Journal de Conchyliologie', which contain the description of '*tonkiniana*', were published in January 1887 whereas pages 1-93 of volume 35(1), which contain the descriptions of *M. hamonvillei* and *M. beaumetzi*, were published in March of the same year (Winckworth 1936; Fischer-Piette 1937).

Morlet (1887) established the new name as variety of the Sumatran species *M. verbecki* Brot, 1886 (currently *Brotia verbecki*; see Köhler and Glaubrecht, 2006) in reference to the similar shell. However, the two taxa are not conspecific. Moreover, Morlet (1887) dealt with juveniles only and was apparently not aware of this. In the description Morlet (1887) states that he sent the shells to A. Brot, a keen specialist of the group, in order to request his opinion as to the identity of the shells. Accordingly, the only type specimens currently known to exist are the two shells housed with the Brot collection of the MNHG. No types were found in the type collection of the MNHN (Héros, pers. comm.). The larger specimen is herein designated as the lectotype of *Melania verbecki* var. *tonkiniana* in order to preserve stability of nomenclature according to the stipulations of § 74.7 of the Code of Zoological Nomenclature (ICZN 1999).

The description of *M. hamonvillei* is based on much larger, mature, and markedly turreted shells with a pronounced spiral sculpture (however some of the type specimens are more or less smooth). The type locality of *M. hamonvillei* is herein restricted to Thanh Moi (Lang Son Prov., 21°63'N 106°33'E) in reference to the type label.

As for '*tonkiniana*' the description of *M. beaumetzi* Brot, 1887 is based on a juvenile shell. The only known type specimen was considered as the holotype by Fischer-Piette (1950). Following Article 74.6 of the Code, this statement is considered as a valid lectotype designation. Brot stated "...I only have two specimens...", which indicates that another type specimen may exist. The type locality was first stated to be the 'Touranne River' but is in fact Thanh Moi (Lang Son Prov.) as subsequently corrected by Brot (1887: 219) and also given on the label. Consequently, the type localities of *M. hamonvillei* and *M. beaumetzi* are identical and *M. beaumetzi* is believed to represent a juvenile shell of the former species. Both taxa fall within the range of *S. tonkiniana* as delineated herein with respect to its distribution and shell morphology. If directly compared with each other, the types of the three nominal taxa *M. tonkiniana*, *M. hamonvillei*, and *M. beaumetzi* look so different that they would probably not be considered conspecific at first glance. However, the study of larger series of museum specimens from various localities in Northern Vietnam suggests that the shell morphology of this species varies greatly between and within populations. It is therefore assumed that the different taxa recognized by previous workers describe (although sometimes extreme) shell forms. The shell shape of *S. tonkiniana* is considered to vary from broadly conical to conically turreted, shell thickness from solid to robust, shell colour from brownish to almost black, and sculpture from being dominated by marked ribs (predominantly on upper whorls) to being

dominated by spiral lirae (mostly on lower whorls). The transition from spiral to axial sculpture is observed even within single specimens.

Irrespective of the marked differences in the shell, all examined samples from a wide range of northern Vietnamese habitats cluster closely together in a mitochondrial phylogeny (Fig. 22). This suggests that gene flow exists or recently existed between these populations. We suggest considering these populations as belonging to a single, morphologically variable and relatively wide spread species in absence of evidence that we are dealing with a species complex associated with phenomena, such as incomplete lineage sorting or introgressive hybridization.

This species has been confounded with other extralimital species that are currently considered as distinct, such as the Thai *M. siamensis* (Brot, 1886) (currently *Brotia siamensis*; see Köhler and Glaubrecht 2006), *M. aubryana* Heude, 1889 (current status unclear) from Guizhou Prov. (China), and *M. huegelii* Philippi, 1843 (currently *Paracrostoma huegelii*; see Köhler and Glaubrecht 2007) from Southern India. In addition, the Laotian species *Brotia mariae* Köhler, 2008 was not recognized by former authors as distinct because of its similar shell but affiliated with '*M. siamensis*'. A further taxon from Tonkin, *Melania krempfi* Dautzenberg & Fischer, 1907 (from Ban Hao; Fig. 7V) is found within the geographical range of *S. tonkiniana*. Ban Hao is a name applied for a number of settlements across northern Vietnam and therefore the type locality cannot be exactly specified. The lectotype designated by Fischer-Piette (1950: 173) (Fig. 7X; size 25.2 x 11.2 mm) differs from typical shells of *S. tonkiniana* by the absence of a marked sculpture and the presence of dark colour patches. However, similar specimens (ZMB 114.471) were found to be widely congruent with *S. tonkiniana* with respect to their radular and midgut anatomy. They also cluster within the *S. tonkiniana* clade in the mitochondrial trees (Fig. 22). These observations suggest that *M. krempfi* should also be considered conspecific. For the time being, however, we refrain from formally synonymising *M. krempfi* with *S. tonkiniana* and prefer to consider its status as unclear.

Material examined.

Lectotype (Fig. 7 A) and paralectotype of *Melania verbecki* var. *tonkiniana*: Lang Son (MHNG). Lectotype (Fig. 7B) and 14 paralectotypes of *Melania hamonvillei*: Tonkin (MNHN), nine paralectotypes of *Melania hamonvillei* (Tonkin; MHNG, Brot coll.). Lectotype

(Fig. 7W) of *Melania beaumetzi*: Thanh Moi (MNHN). Three syntypes of *Melania aubryana* var. *attenuata*: Tonkin (MNHN) (Fig. 7D). Syntype of *Melania aubryana* var. *elongata*: That Khe, Tonkin (MNHN) (Fig. 7C). Three syntypes of *Melania aubryana* var. *obliterata*: Song Bang Giang, Cao Bang (MNHN) (Fig. 7F). Two syntypes of *Melania aubryana* var. *robusta:* Tonkin (MNHN) (Fig. 7E).

Historical museum material: Northern Vietnam: Tonkin (ZMB 53.337-8, 61.467-8, 62.135, 200.296; MHNH): Lao Cai (MHNH, ZMB 200.295); Su Yut (ZMB 62.136, MNHN); Phu Lang Thuong nr Thanh Moi (ZMB 94.915; MNHN); Lang Son (BMNH 1908.12.21.15-19); That Khé (BMNH 1907.5.20.158; RMNH; MNHN); Song Bang Giang, Cao Bang (MNHN); Long Tchiou Sang (MNHN); Deo Cat (MNHN), Ba Bé Lakes (MNHN; SMF 291328); Bac Kan (SMF 291327; MNHN); Bac Kan, rivière de Song Cau (MNHN); Cap Baké, près de Cap St. Jaques (MNHN); Noi Tap, affluent of Red River (MNHN); Région du Song Luc Nam, Haut Song Bo (MNHN); Lai Chu, Rivière Noire (MNHN); Riviere Noire près de Phong Tho (MNHN); Yen Tinh (MNHN); Nai Son (MNHN); Trinh Thuong (MNHN); Hanoi (MNHN); Yen Bai (MNHN); Song Ma (MNHN); Bac Kan, affluent of the Nang Khung (MNHN).

Alcohol preserved material: Lao Cai Prov.: River 10km W of Lao Cai, 22°25.408’N 104°01.579’E (ZMB 114.169). Bac Kan Prov.: Bac Kan Prov.: Phu Luong Distr., Dong Dang in Yen Ninh, 21°50.86’N 105°43.89’E (ZMB 114.170), Cho Moi, 21°52.922’N 105°46.47’E (ZMB 114.174), River parallel to highway Thai Nguyen - Bac Kan, 13km S of Bac Kan, 22°02.099’N 105°51.053’E (ZMB 114.182), River W of Bac Kan, 22°08.062’N 105°47.341’E (ZMB 114.184), Cho Dou Distr., Phuong Vien, 22°09.578’N 105°36.548’E (ZMB 114.185), 20 km S Ba Be Lakes, 22°16.124’N 105°34.671’E (ZMB 114.186), Creek nr Ba Be Lakes (ZMB 114.187), Ba Be Lakes, ferry port, 22°24.099’N 105°37.45’E (ZMB 114.188), Outflow of Ba Be Lakes 22°26.99’N 105°33.858’E (ZMB 114.192), Lang Na To Distr., The Thai Na Pac, Thuyen Ngau Son 22°24.08’N 105°52.95’E (ZMB 114.196). Cao Bang Prov.: Cao Bang River in Cao Bang, 22°20.00’N 106°15.50’E (ZMB 114.201). Ha Giang Prov.: Ha Giang River nr Pac Bo, Chinese border, 22°20’N 106°30’E (ZMB 114.205). Lang Son Prov.: Cao Loc Distr., Highway 4B Lang Son - Tien Yen, 21°49.014’N 106°49.95’E (ZMB 114.209).

Description.

Shell (Fig. 7): Mature shells medium sized (Tab. 3); variable in shape and sculpture from broadly conical to conically turreted; with between three and seven remaining whorls and eroded tip, shell very thick to robust. Whorls flattened in diameter, separated by shallow suture. Sculpture mostly well pronounced but almost smooth individuals also occur. Basal whorl sculptured by regularly spaced, prominent spiral lirae, which can become quite strong but may be rather thin. Some specimens possess only faint lines. Axial sculpture is most pronounced on uppermost whorls and consists of indistinct, regularly spaced, sometimes very strong ribs. It gradually replaces spiral sculpture over the last two to three teleoconch whorls. Axial sculpture may also be entirely absent. Spiral sculpture consists of strong and regularly spaced basal lirae, it may be present only on base of shell or extend onto the subsequent whorls. Nodules may form where axial ribs and spiral sculpture meet. Aperture wide, ovate, well produced below, with thick to sharp margin; inner side white. Colour varies from almost black to light brown.

Embryonic shell (Fig. 8): Embryonic shells extracted from dry shells (MNHN, Lao Cai) are approximately 1 mm high with a diameter of 0.8 mm and comprise almost two whorls. Apical whorl is slightly inflated. Sculpture of entire shell is smooth except for growth lines.

Operculum (Fig. 7W): Slightly oval comprising up to four whorls and sub-central nucleus, substantially smaller than aperture.

Radula (Fig. 9): Ribbon on average 17.3 (± 3.5) mm long with 141 (±25) rows of teeth (8.7 ±1.2 rows/mm) (n = 37). Central teeth with straight upper rim and lower rim made convex by slightly extending glabella; cutting edge consists of large main denticle flanked by two much smaller accessory cusps; glabella with straight lateral margins. Lateral teeth with broad main denticle flanked by three inner and two outer accessory cusps. Inner and outer marginal teeth similar in shape and size, except that inner ones have a larger main denticle. Both possess one broadly rounded main denticle and tiny inner accessory cusp.

Midgut (Fig. 10): Wide opening of digestive gland duct (dgd) distant oesophagus (oes) and separated by two small ridges lying anteriorly; sorting area (sa) broad, consisting of regularly spaced, rather broad ribs, outer (ocp) and inner crescent (icp) septate pads well developed, rather short, anteriorly connected to each other; lateral fold (lf) rather thin; marginal fold (mf) inconspicuous.

Reproductive features: Sex ratio of dissected specimens 4 males / 6 females / 1 parasitized specimen of unknown sex; brood pouches of two females contained numerous (100+) egg capsules, diameter approximately 0.5 mm.

Remarks.

Shell similar to *Brotia siamensis* (Thailand) and *Brotia mariae* (Laos) with respect to conical shape and conspicuous spiral sculpture. However, *Brotia* species differ in embryonic shell morphology and details of reproductive tract. Also similar is '*Melania*' *aubryana* from Guizhou Prov., China (not studied). *M. aubryana* is assumed to be distinct because of the great geographical distance separating the two taxa. Currently, it remains unclear whether *M. aubryana* is a pachychilid or a semisulcospirid species.

Distribution.

Widely distributed in mountainous regions of northern Vietnam ('Tonkin') and probably also in the neighbouring regions of southern China (Yunnan, Guangxi); river system of the Red (or Hong) River and its tributaries (Fig. 6).

Sulcospira tourannensis (Souleyet, 1852)

Figs. 11-13.

Melania tourannensis Souleyet, 1852: 543-544, pl. 31, figs. 4-7 ("la rivière de Touranne, en Cochinchine = Han River nr Da Nang, Central Vietnam); Brot, 1870: 281; Brot, 1874: 105, pl. 14, fig. 2. Lectotype (by designation of Köhler and Glaubrecht, 2002: 150) and 5 paralectotypes MNHN.

Brotia tourannensis – Köhler & Glaubrecht, 2002: 150-151, fig. 3N.

Taxonomic remarks.

Based on superficial shell similarity, Nevill (1885: 250-251) considered *Melania gloriosa* Anthony, 1865 from Pegu (Myanmar) as a junior synonym of *M. tourannensis* and described two new varieties from Myanmar (var. *compacta* and var. *beddomeana*). He also referred to a further junior synonym "*Melania peguensis*", which is a *nomen nudum* introduced by mistake (for details see Köhler and Glaubrecht 2006). Nevill's affiliations are all incorrect because the Vietnamese *S. tourannensis* does not occur in Myanmar. The three names *M. gloriosa*, *M. compacta*, and *M. beddomeana* are currently considered as synonyms of *Brotia herculea* (Gould, 1846) (Köhler and Glaubrecht 2006). *S. tourannensis* did not attract further attention from subsequent authors who dealt mainly with species from northern Vietnam. Like most pachychilids, *S. tourannensis* inhabits well-oxygenated head waters. It probably does not occur in the lower course of the Han River (= "Rivière de Touranne") and is not found at the type locality in estuarine waters close to Da Nang. The species description was based on empty shells that were probably flushed downstream by running water or brought by locals to the town as a source of food. Based on the shell, Köhler & Glaubrecht (2002) erroneously assumed that this species is a member of *Brotia*.

Material examined.

Lectotype (Fig. 11A) and five paralectotypes: MNHN (la rivière de Touranne, en Cochinchine) (Fig. 11B-E).

Historical museum material: Quang Nam: Phuc Son (BMNH 1901.12.12.112-4; SMF 291994); Quang Nam Prov. (MNHN).

Alcohol preserved material: Quang Nam Prov.: Thanh My SW Da Nang, 15°45.32'N 107°50.21'E (ZMB 114.364), Phuc Son Distr., Cam Duc, Cai River drainage, 15°27.71'N 107°28.11'E (ZMB 114.366), Nam Giang Distr., Cai Dy between Thanh My and Kon Tum, 15°38.90'N 107°49.90'E (ZMB 114.369), Dong Giang Distr., Song Con between Prao and Da Nang, 15°57.74'N 107°45.74'E (ZMB 114.370), creek crossing Ho Chi Minh Highway, Cai River drainage, 15°18.47'N 107°43.83'E (ZMB 114.377); Gia Lai Prov.: creek crossing the Ho Chi Minh highway c. 100 km S Buon Ma Thuot, 13°24.54'N 108°05.49'E (ZMB 114.378), Hue Prov., Aluoi Distr., creek nr Aluoi, road to Prao, Rao Lac River drainage, 16°12.61'N 107°17.11'E (ZMB 114.380).

Description.

Shell (Fig. 11): Medium sized (Tab. 3); elongate, turreted with truncated eroded tip and usually three to four remaining whorls. Whorls broadly rounded in diameter with more or less conspicuous depression below upper suture being visible especially on body whorl of mature specimens. Whorls separated by shallow suture. Sculpture consists of spiral lirae at base of the shell, only; axial ribs lacking; shell surface smooth except for microradials and growth lines. Aperture pronouncedly produced below, oblong-ovate in shape. Outer lip sharp, inner lip inconspicuous; inside white. Shell colour chestnut brown.

Operculum (Fig. 11L): Markedly oval comprising up to four whorls and a sub-central nucleus.

Radula (Fig. 12): Ribbon on average 16.3 (±4) mm long, with 150 (±28) rows of teeth (9.83 ±1.8 rows/mm) (n = 5); rows sparsely arranged. Central teeth with straight to slightly concave upper rim and basal rim made convex by the slightly extending glabella; cutting edge consists of large, triangular main denticle flanked by two accessory cusps on each side, glabella with straight lateral margins and rounded base. Lateral teeth with broadly triangular main denticle flanked by two to three outer and one to two inner cusps. Inner marginal teeth slightly broader than outer ones; both supporting a broad, rounded main denticle with one or two accessory cusps on their inner side.

Anatomy: Skin dark grey, heavily blotched by yellowish pigment spots. Animal comprises up to three whorls; testis comprises the dorsal parts of the last two whorls. Columellar muscle broad and short.

Midgut(Fig. 13): Openings of digestive gland duct (dgd) and oesophagus (oes) rather distant, separated by a small ridge lying anterior to the opening of the digestive gland duct; sorting area (sa) very broad consisting of narrowly spaced ribs, outer (ocp) and inner (icp) crescent septate pads well developed, not connected to each other; marginal (mf) and lateral folds (lf) thin.

Reproductive features: Sex ratio of dissected specimens 4 males / 3 females; brood pouches of two females contained several hundred egg capsules with a diameter of about 0.5 mm.

Remarks.

Can be distinguished from all Vietnamese species treated above by its entirely smooth, large shell. For further comparisons, check the following species descriptions.

Distribution.

Central Vietnam, Quang Nam and Da Nang Provinces, Cai River drainage, southward possibly extending into the provinces of Gia Lai and Dak Lak (Fig. 1).

Sulcospira dakrongensis n. sp.

Figs. 14A-E, 15A-B, 16.

Material examined.

Holotype. Quang Tri Prov., Dakrong River near Dakrong, 20 km S Dakrong Bridge, 16°34.41'N 106°57.59'E; coll. F. Köhler, Oct 2006 (ZMB 114.367a, Fig. 14A).

Paratypes. Same as holotype (ZMB 114.367b, 35 specimens, Fig. 14B). Dakrong River near Dakrong; coll. F. Köhler, Oct 2006 (ZMB 114.146, 4 specimens, Fig. 14C). Quang Tri Prov., Aluoi Distr., upper course of Dakrong River nr Hong Thuy, 16°34.41'N 107°04.11'E; coll. F. Köhler, Oct 2006 (ZMB 114.371, 56 specimens, Fig. 14D; IEBR, 10 specimens).

Other ethanol preserved material: Quang Nam Prov., creek crossing the Ho Chi Minh Highway between Aluoi and Thanh My, 16°03.38'N 107°28.88'E; coll. F. Köhler, Oct 2006 (ZMB 114.379), Quang Nam Prov., near Aluoi; coll. F. Köhler, Oct 2006 (ZMB 114.143). Dakrong River (IEBR).

Description.

Shell (Fig. 14A-D): Comparatively small, broadly conical in shape; spire comprising two to four whorls (Tab. 3). Body whorl large compared to upper whorls; whorls well rounded in diameter only very slightly depressed below the upper suture. Sculpture generally smooth except for fine and regular axial growth lines and rather inconspicuous spiral lirae at the base.

Aperture wide, oval, angularly produced below, inside white; peristome sharp, parietal callus inconspicuous. Colour chestnut brown.

Operculum (Fig. 14E): Oval comprising up to four whorls and a sub-central nucleus.

Radula (Fig. 15A-B): Ribbon on average 15.2 (±2.1) mm long with 169.5 (±11.4) rows of teeth (11.2 ±1.3 rows/mm) (n = 5); rows densely arranged. Central teeth with squarish base, upper rim straight, basal rim made concave by slightly extending glabella; glabella broad with straight lateral margins; central cusp of cutting edge flanked by two accessory cusps on each side. Lateral teeth with one broadly triangular main cusp and two accessory cusps on each side, short lateral extensions. Inner and outer marginals of similar shape and size, hooked, with one broadly oval main denticle and an accessory cusp on the inner side.

Anatomy: Skin dark grey, no yellow pigment spots observed. Animal comprises up to three whorls; testis occupies the dorsal parts of the last two whorls. Columellar muscle comparatively long.

Midgut (Fig. 16): Openings of digestive gland duct (dgd) and oesophagus (oes) rather distant, separated by a small and short ridge lying anterior to the wide opening of the digestive gland duct; sorting area (sa) narrow; outer (ocp) and inner (icp) crescent septate pads well developed, rather thin, not connected with each other; marginal fold (mf) thin, lateral fold (lf) inconspicuous; crescent fold (cf) below digestive gland opening elongate.

Reproductive features: Sex ratio of dissected specimens 2 males / 4 females; brood pouches of two females contained numerous (100+) egg capsules with a diameter of about 0.5 mm.

Remarks.

Differs from *S. tourannensis* by much smaller shell, broadly conical shape, inflated body whorl, and lack of subsutural depression.

Etymology.

This species is named after the Dakrong River, in which it is found.

Distribution.

Headwaters and mid-streams of rivers in the river systems of the Dakrong - Quang Tri and Bo River, Provinces of Quang Tri, Than Thien-Hue, and Quang Nam (Fig. 1).

Sulcospira quangtriensis n. sp.

Figs. 14F-J, 15C-D, 17.

Material examined.

Holotype. Quang Tri Prov., Cam Lô District, Krong Klang, 16°42.02'N 106°52.56'E; coll. F. Köhler, Oct 2006 (ZMB 114.372a, Fig. 14F).

Paratypes. Same as holotype (ZMB 114.372b, 5 specimens, Fig. 14G-H; IEBR, 2 specimens). Quang Tri Prov., Cam Lô District, creek near Cam Lô, 16°45.34'N 106°51.27'E; coll. F. Köhler, Oct 2006 (ZMB 114.373, 2 specimens). Quang Tri Prov., 35 km N Khe Sanh, 16°45.45'N 106°34.33'E; coll. F. Köhler, Oct 2006 (ZMB 114.368, 10 specimens, Fig. 14I).

Description.

Shell (Fig. 14F-I): Medium sized; conically turreted, with usually five to six remaining whorls and an eroded tip (Tab. 3). Whorls well rounded in diameter, not or very slightly flattened below suture, body whorl comparatively large and slightly inflated. Sculpture smooth except for axial growth lines and inconspicuous spiral lines. Aperture wide, slightly flaring, basally produced, outer lip well rounded and sharp, inner lip inconspicuous, inside white. Periostracum smooth, glossy, uniformly chestnut brown.

Operculum (Fig. 14J): Oval comprising up to four whorls and a sub-central nucleus.

Radula (Fig. 15C-D): Ribbon on average 14.6 (±2.7) mm long with 161 (±27) rows of teeth (12.5 ± 2 rows/mm) (n = 3); rows sparsely arranged. Central teeth with squarish base, upper rim straight to slightly concave, basal rim concave by pronouncedly extended glabella, glabella broad with straight lateral margins, central cusp of cutting edge flanked by two accessory cusps on each side. Lateral teeth with one broadly triangular main cusp and two

accessory cusps on each side, short lateral extensions. Marginal teeth hooked, with one broadly oval main denticle and one or two accessory cusps on the inner side. Inner and outer marginals of very similar shape and size.

Midgut (Fig. 17): Openings of digestive gland duct (dgd) and oesophagus (oes) both wide, distant from each other, separated by a long ridge lying anterior to the wide opening of the digestive gland duct; sorting area (sa) very narrow consisting of regularly spaced ribs, outer (ocp) and inner (icp) crescent septate pads well developed, anteriorly connected to each other; marginal (mf) and lateral fold (lf) rather thick; crescent fold (cf) below digestive gland opening elongate.

Remarks.

Differs from *S. dakrongensis* by its taller and more slender shell; its body whorl being not as inflated as in the former. *S. vietnamensis* has a thicker shell, and a different dentition pattern of the radula. *S. tourannensis* differs by the presence of a subsutural depression. Dang & Ho (2007: Fig. 1) figured shells from central Vietnam under the name "*Paracrostoma solemiana* Brandt, 1968" that may be conspecific.

Etymology.

This species is named after Quang Tri Province in which it is found.

Distribution.

Quang Tri River system (Fig. 1).

Sulcospira vietnamensis n. sp.

Figs. 14K-N, 15E-F, 18.

Material examined.

Holotype. Quang Binh Prov., Minh Hoa Distr., river in Hoa Hop between Yen Thanh and Tan Binh, 17°52.49'N 105°52.02'E; coll. Köhler, Oct 2006 (ZMB 114.365a, Fig. 14K).

Paratypes. Quang Binh Prov., Minh Hoa Distr., river in Hoa Hop between Yen Thanh and Tan Binh, 17°52.49'N 105°52.02'E; coll. Köhler, Oct 2006 (ZMB 114.365b, 10 specimens; IEBR, 3 specimens). Quang Binh Prov., Tuyen Hoa District, river in Thanh Hua at bridge of Ho Chi Minh Highway between Huong Khe and Hung Nguyen, 17°59.13'N 105°49.88'E; coll. Köhler, Oct 2006 (ZMB 114.375, 7 specimens).

Other ethanol preserved material: Nghe An Prov., Thanh Cuong District, creek in Tam Quang village, foothills of Truong Son Mts.; 18°48'N 105°20'E; coll. Köhler, Oct 2006 (ZMB 114.374).

Description.

Shell (Fig. 14K-M): Medium sized, conical; spire with usually four or five (up to eight) remaining whorls and an eroded spire (Tab. 3). Whorls well rounded in diameter; separated by a relatively deep suture; body whorl slightly inflated. Sculpture smooth except for inconspicuous axial growth lines. Aperture oval, wide to slightly flaring with well rounded periphery, rounded below; peristome strong, parietal callus thin. Colour light brown to dark brown; when background colour is light a dark spiral band may be visible on upper part of the whorl.

Operculum (Fig. 14N): Oval comprising up to four whorls and a sub-central nucleus.

Radula (Fig. 15E-F): Ribbon on average 28 (±4) mm long with 288 (±16) rows of teeth (10.3 ±0.9 rows/mm) (n=2); densely arranged. Central teeth with squarish base, upper rim straight, basal rim concave by slightly extending glabella, glabella broad with straight lateral margins, central cusp of cutting edge flanked by two accessory cusps on each side; cutting edge with one very broad and triangular main denticle and one accessory cusp. Lateral and marginal teeth with completely reduced accessory cusps, the remaining main cusp being broadly spatulate. Inner and outer marginals similar in shape and size.

Midgut (Fig. 18): Openings to digestive gland duct (dgd) and oesophagus (oes) distant from each other; lateral fold (lf) very thick with thickened anterior end next to the outer crescent

septate pad (ocp), which is well developed but comparatively small; inner crescent septate pad (icp) well developed, not connected with outer septate padl; marginal fold (mf) thin.

Remarks.

Most similar to *S. dakrongensis* but shell more elongate and thicker, suture deeper, aperture not as angular below. Radula markedly different from all other known Vietnamese species by absence or large reduction of accessory teeth and broadly spatulate shape of main denticles.

Etymology.

This species is named after Vietnam.

Distribution.

Gianh River system, Quang Binh Prov., and Ca River system, Nghe an and Ha Tinh Provinces (Fig. 1).

Sulcospira collyra n. sp.

Figs. 19-21.

Material examined.

Holotype. Hoa Binh Prov., Cao Phung District, Binh Tanh community, creek in Giang village, 19°55'N 105°30'E; coll. F. Köhler, Oct 2006 (ZMB 114.135a, Fig. 19A).

Paratypes. Hoa Binh Prov., Cao Phung District, Binh Tanh community, creek in Giang village, 19°55'N 105°30'E; coll. F. Köhler, Oct 2006 (ZMB 114.135b, 265 specimens, Fig. 19B-C; IEBR, 5 specimens).

Other ethanol preserved material: Hoa Binh Prov., Tan Lac Distr., Stream in Muong Khen; 20°37.088'N 105°16.21'E; coll. F. Köhler, Oct 2006 (ZMB 114.472; ribbed form).

Description.

Shell (Fig. 19A-E): Medium sized, elongately turreted, slender shape, tip only slightly eroded with usually seven remaining whorls (Tab. 3). Whorls flattened in diameter, with inconspicuous sub-sutural depression; suture shallow. Shell may be entirely smooth except for regularly spaced axial growth lines or may have a sculpture consisting of well developed axial ribs that are prominent particularly in upper whorls; additionally, faint spiral lirae may be visible particularly on last whorl. Aperture comparatively small, ovate, slightly angularly produced below; outer lip well rounded, sharp; inner lip inconspicuous; inside white. Colour light brown, sometimes marbled.

Operculum (Fig. 19F): Oval, with up to 4 whorls and sub-central nucleus.

Radula (Fig. 20): Ribbon on average 19.2 (± 2.4) mm long with 153 (±7) rows of teeth (8.1 ±0.8 rows/mm (n=6). Central teeth with squarish to rounded base, straight upper rim, and convex lower rim by slightly extending glabella; cutting edge with main denticle and two accessory cusps on each side; glabella with straight lateral margins. Lateral teeth with one accessory cusp on each side of the pointed main denticle. Inner marginal teeth slightly broader than outer ones; marginal teeth possess one broad main denticle and a smaller inner accessory cusp.

Midgut (Fig. 21): Openings of digestive gland duct (dgd) and oesophagus (oes) distant; conspicuous ridge anterior to opening of digestive gland duct; sorting area (sa) broad consisting of regularly spaced ribs, outer (ocp) and inner (icp) crescent septate pads well developed, inner one narrow, both anteriorly connected to each other; marginal (mf) and lateral fold (lf) rather thin.

Remarks.

Two populations of this species were examined. Shells of the type population are generally smooth whereas specimens of a second population, which co-occurs with *B. hoabinhensis*, are markedly ribbed (compare Figs. 19 A-C with D-E). Except for the markedly distinct shell sculpture, both populations show broadly corresponding morphology with respect to radula and midgut. In addition, both populations yielded identical mitochondrial 16S and COI

sequences. The lack of morphological and mitochondrial differentiation suggests that the two shell morphs are conspecific. Juveniles and sub-adults of the co-occurring *B. hoabinhensis* differ by a more conical shape and the presence of a dark spiral colour band. Adults of *B. hoabinhensis* are much larger and lack the conspicuous axial sculpture present in co-occurring specimens of *S. collyra* (ZMB 114.472). Dang & Ho (2007: Fig. 1) figured shells under the name "*Adamietta delavayana* (Heude, 1889)" without locality data that may well be conspecific.

Etymology.

The name refers to the elongate shell of this species (collyra [Latin] = elongated).

Distribution.

Hoa Bing and likely Ninh Binh Province (Fig. 1).

Sulcospira (?) n.sp.

Fig. 22.

Shells from Lao Cai, in the far northwest of Vietnam, that are kept in the MNHN under the names "*Melania huegelii*" and "*Melania delavayana*" are considered to belong to an as yet undescribed pachychilid species. Neither the name *M. huegelii* (from southern India) nor the name *M. delavayana* (from Guangdong Prov., China) is believed to be correctly applied because distributional areas of pachychilid species are usually small. Even the closest area of Guangdong is about 1,000 km from Lao Cai. This species resembles the Chinese *M. delavayana* (of which only the original figure is known) in having a large, highly turreted shell. As anatomy of this species is unknown due to the unavailability of preserved material, its affinities remain uncertain and we refrain from naming it.

Molecular genetics

Three different data sets of mitochondrial sequences were examined: The partial 16S sequence data set consisted of 36 sequences with total a length of 821 aligned nucleotide positions. The COI data set contained 35 sequences with a total length of 660 bp. These data sets differed slightly in taxon composition because in a few cases we were unable to amplify both gene fragments. The concatenated data set of 16S and COI consisted of 33 sequences. *S. collyra* is represented only by 16S sequences and is therefore absent from the COI and concatenated sequence data sets. In addition to the Vietnamese species, each data set contained sequences of the two Philippine pachychilids *Jagora asperata* and *J. dactylus*. that were used as outgroup to root the tree. *Jagora* was chosen as outgroup because it occupies a basal position within the Pachychilidae (Köhler et al. 2004).

In order to infer the phylogenetic relationships of the Vietnamese Pachychilidae, we employed maximum parsimony as implemented in the MP Ratchet, and Bayesian Inference. MP Ratchet analyses revealed 107 equally parsimonious trees with a length of 627 steps for 16S; in the strict consensus tree 12 nodes collapsed. For COI 44 equally parsimonious trees with a length of 54 steps were found; in the strict consensus also 12 nodes collapsed. The analysis of the concatenated data set revealed 6 equally parsimonious trees with a length of 1,071 steps; 5 nodes collapse in the strict consensus tree. All three MP trees revealed broadly identical topologies that differed only with respect to the branching pattern within the clade of *S. tonkiniana* – all tips of this branch being very close to each other. The same holds true for the BI trees. Consequently, only two trees are shown here, the BI phylogram and MP strict consensus tree obtained for 16S, which are most complete with respect to taxon sampling (Fig. 22). The trees produced by applying the two different methods correspond widely with respect to the overall branching pattern but differ in some details. Trees show a consistent bifurcation between the *Brotia* and *Sulcospira* clades. All species appear as monophyletic clades with considerable branch support. Within *Sulcospira* three main clades are recognized: *S. tonkiniana* from northern Vietnam, *S. collyra* from Hoa Binh (northern central Vietnam), and species from central to southern Vietnam (*S. vietnamensis*, *S. quangtriensis*, *S. dakrongensis*, *S. tourannensis*). However, while the relationships between these three clades are unresolved in the BI tree, the MP suggests a sister group relationship between *S. collyra* and *S. tonkiniana*, both together forming the sister group of the central to southern Vietnam clade. Additionally, the MP tree suggests the following relationships within the southern clade: *S. tourannensis* (*S. vietnamensis* (*S. dakrongensis* and *S. quangtriensis*)) whereas in the

BI phylogram the relationships between *S. tourannensis*, *S. vietnamensis*, and the sister pair of *S. dakrongensis* and *S. quangtriensis* are not resolved.

Discussion

Phylogeny and systematics.

The taxonomy and systematics of cerithioidean (= 'melaniid') freshwater gastropods have been subject to substantial changes in recent decades (Glaubrecht 1996; 1999; Lydeard et al. 2002). Modern works recognize several families now included in the predominantly marine Cerithioidea that have colonized freshwater biotopes independently (Glaubrecht 1999; Lydeard et al. 2002; Köhler et al. 2004). Yet, the implications of the modern systematic concept for the Vietnamese '*Melania*' species described in the late 19th and early 20th century have not been addressed. Only recently has it been demonstrated that one of the neglected Vietnamese freshwater gastropods, the semisulcospirid '*Melania*' *jacqueti* Dautzenberg & Fischer 1906, occupies a basal position within the Semisulcospiridae and represents a 'missing link' between the Asian and western North American members of this family and the eastern North American Pleuroceridae (Strong and Köhler accepted).

Within the Pachychilidae, which form another part of the former melaniid assemblage, currently two genera are recognised in mainland SE Asia, *Brotia* and *Sulcospira*, both of which occur in Vietnam (Köhler accepted). *Brotia* is well defined by means of key morphological characters (reproductive and embryonic shell features) and its monophyly is robustly corroborated by analyses of mitochondrial sequences (Köhler et al. 2004; Köhler submitted). Both Vietnamese *Brotia* species cluster well within the genus clade and form a sub-clade with species from Laos (Köhler submitted). In contrast, the monophyly of *Sulcospira* as a whole is not as well supported. In mtDNA phylogenies it is shown as the sister group of *Brotia* and is deeply divided into three main clades. The monophyly of *Sulcospira* is supported by BI and ML but rejected by MP analyses of partial 16S sequences. However, all members of *Sulcospira* show a widely congruent anatomy (except for the shell) and none of these principal clades is characterized by obvious key anatomical features. The widely conserved anatomy was the reason for not formally naming the three clades of *Sulcospira* and for suggesting synonymy of the name *Adamietta* Brandt, 1974, which was

established as a monotypic genus for *Melania housei* I. Lea, 1856. This species has a basal position within *Sulcospira* (Köhler accepted). The species of *Sulcospira* from Vietnam and southern China form a separate clade that occupies a more derived position and is itself bifurcated into two sub-clades – one containing the species from northern Vietnam/southern China and one those from central to southern Vietnam. The bifurcation into a northern and a central to southern Vietnamese clade is also found in the present study.

The deep phylogenetic splits between the main *Sulcospira* clades (one of which represents the Vietnamese and southern Chinese species) suggest that they have remained separated for a considerable period of time. The sister group of the Vietnam/southern China clade contains endemic species from Java and northeastern Borneo. The divergence of this group may date back to the mid Tertiary, when for an extended period lowered sea levels and humid climate may have enabled less vagile freshwater invertebrates, such as pachychilids to disperse across Sundaland (e.g., Tjia 1980; Whitmore 1987; Heaney 1991; 1999; Renner et al. 2001; Köhler and Glaubrecht 2007).

Morphological and genetic differentiation of Vietnamese pachychilids.

There does not appear to be a consistent pattern in the morphological and genetic differentiation of Vietnamese pachychilids. The new *Brotia* species are each represented by only a single population; therefore little can be inferred for these two species. They are however easily recognisable by their shell and also genetically well differentiated. This is evident from observed genetic p-distances in 16S of 2.1 to 3.4% between each other and with respect to their most closely related congener *Brotia mariae* from Laos. Rates of intra- and interspecific differentiation are variable. There is a marked discrepancy between the species from northern Vietnam (i.e. *S. tonkiniana*) and those from central to southern Vietnam (*S. collyra*, *S. tourannensis*, *S. dakrongensis*, *S. quangtriensis*) with the former showing a remarkable range of shell variation that is accompanied by an almost complete lack of genetic differentiation in the examined mitochondrial markers. There are several possible explanations for this observation. First, morphologically distinct forms could represent different species, perhaps within a species complex. Existence of several species or forms was assumed by most previous authors, who applied various names to more or less well defined morphs. However, this procedure was highly arbitrary and each author delineated taxa differently. This becomes especially obvious when historical material kept in museum

collections (in particular MHNH) is studied. A variety of taxon names, such as *M. siamensis*, *M. hamonvillei*, *M. aubryana* or *M. huegelii*, have been erratically applied for museum lots without clear boundaries between these so delimited taxa becoming evident. The significant problems experienced by former authors in consistently delineating different forms are also indicated by the introduction of a large number of variety names by Bavay and Dautzenberg (1910). These only added to the nomenclatorial confusion.

On the other hand, the absence of genetic differentiation between populations (16S: p-distances of 0 to 0.03%) does not provide incontrovertible evidence in favour of the hypothesis that all populations are truly conspecific. Other studies of freshwater gastropods have revealed that mtDNA markers may have limited utility in assessing status at the species level. For example, in Korean *Semisulcospira* populations mt haplotypes were found to be more geographically than taxonomically structured (Lee et al. 2007). Faced with similar phenomena in the pachychilid taxa *Brotia* and *Tylomelania*, Glaubrecht and Köhler (2004) and Rintelen et al. (2004) respectively, suggested that mismatches between gene trees and species trees may be caused by incomplete lineage sorting or introgressive hybridization. Consequently, in case of *S. tonkiniana*, we cannot rule out that we are dealing with a complex of (cryptic) species. In summary, because the lack of genetic differentiation provides evidence for recent and extensive gene flow between populations and since morphological characters except of the shell show low variability, we assume that the different shell forms are ecophenotypic morphs.

A similarly plastic shell has been found in the northern Vietnamese semisulcospirid gastropod *Hua jacqueti* (see Strong and Köhler accepted). *H. jacqueti* and *S. tonkiniana* not only show similar patterns with respect to a variable shell but also co-occur in the large drainage system of the Red River where they inhabit a wide range of habitats that differ with respect to water depth, current, pollution, and substrate. We believe that locally differentiated selection may help to maintain the variety of shell morphs across a large distributional range despite the occurrence of gene flow. That in principal shell shape and sculpture in freshwater gastropods may change in response to varying substrates, for instance, has been demonstrated for *Semisulcospira reiniana* by Urabe (1998, 2000).

In species from central and southern Vietnam we observed a contrasting situation: Genetically relatively well differentiated species (p-distances in 16S of 3-7% between and <3% within species) exhibit quite similar shells. Other than in northern Vietnam, the species of the *S. tourannensis* clade in central Vietnam occur under allopatric conditions in isolated river systems. We therefore postulate that geographical isolation is the main factor of speciation

operating in this region. Since the species are closely related and still inhabit very similar environments, shell morphology apparently remained close to the plesiomorphic condition.

Taxonomy and Diversity.

This paper is based on the examination of eight pachychilid species from two genera and one species with uncertain affinities. Six of these species are described as new. Compared with previous accounts of freshwater gastropods this number may seem small. French authors of the late 19th to early 20th century recognized at least eight species in Vietnam that are considered as pachychilids by their shells (*M. aubryana*, *M. beaumetzi* , *M. delavayana*, *M. hamonvillei*, *M. huegelii*, *M. krempfi*, *M. siamensis*, *M. tourannensis*). Recently, Dang & Ho (2007) even reported on 15 pachychilid species from Vietnam. However, many previous references are considered as incorrect or dubious for various reasons. In a number of cases Vietnamese pachychilids were confounded with similar species from other regions of South and Southeast Asia. Accordingly, references to e.g. '*M. huegelii*' (from India), '*M. gloriosa*' (from Myanmar), '*M. siamensis*' (from Thailand), and '*M. aubryana*' and '*M. delavayana*' (from China) are herein not accepted as correct names for Vietnamese pachychilids. Recent revisions of pachychilids in other regions of South and Southeast Asia showed that species usually have restricted distributions, implying that the above mentioned taxa do not occur in Vietnam (Köhler and Glaubrecht 2001; 2002; 2006; 2007; Glaubrecht and Köhler 2004). This fact alone reduces the number of accepted names for Vietnamese pachychilids considerably. Secondly, two validly described taxa (*M. hamonvillei, M. beaumetzi*) are herein synonymised with *S. tonkiniana* and the status of a third taxon (*M. krempfi*) is considered dubious. The fact that after a careful revision only two introduced names are accepted as being available for valid Vietnamese pachychilid species (*S. tonkiniana*, *S. tourannensis*) demonstrates the paucity of our current knowledge of this group. We are aware of the fact that the current report being largely based on material collected during two field trips is far from being complete. Further species reported from Vietnam by Dang and Ho (2007), such as *Sulcospira housei* (I. Lea, 1856) and *S. dautzenbergiana* (Morlet, 1884), are not included here because preserved material suitable for genetic and morphological studies was unavailable. Given the facts that at most half of the country's area was covered by the field trips and that most species occupy relatively small ranges, we estimate that this effort has documented no more than about half of the true diversity of Vietnamese pachychilids. There is no reason to expect that pachychilids should be exceptional with respect to the state of our knowledge, and

promise that similar levels of undiscovered diversity are to be found in other macro-invertebrates.

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Table captions

Table 1. Samples studied with details on distributions, voucher numbers of sequenced specimens and Genbank accession numbers.

Table 2. Shell sizes of Vietnamese *Brotia* species[mm].

Table 3. Shell sizes of Vietnamese *Sulcospira* species [mm].

Figure captions

Figure 1. Distribution of pachychilid species in central and southern Vietnam. □ = *B. hoabinhensis*; ■ = *B. annamita*; ♦ = *S. collyra*; ● = *S. vietnamensis*; ○ = *S. quangtriensis*; ▲ = *S. dakrongensis*; ▼= *S. tourannensis*.

Figure 2. Shells and opercula of new *Brotia* species. **A**. *B. annamita*, holotype, ZMB 114.376a. **B**. *B. annamita*, paratype, ZMB 114.376b. **C**. Operculum, *B. annamita*, paratype, ZMB 114.376b. **D**. *B. hoabinhensis*, holotype, ZMB 114.470a. **E**. *B. hoabinhensis*, paratype ZMB 114.470b. **F-G**. *B. hoabinhensis*, paratypes, ZMB 114.153 (subadult). **H**. Operculum, *B. hoabinhensis*, paratype, ZMB 114.470b. Scale bars = 10 mm.

Figure 3. Radulae of new *Brotia* species. **A-B**. *B. annamita*, paratype, ZMB 114.376b. **D-E**. *B. hoabinhensis*, paratype, ZMB 114.470b. **F-G**. *B. hoabinhensis*, paratype, ZMB 114.153. Scale bars = 100 μm.

Figure 4. Embryonic shell of *B. hoabinhensis*, paratype, ZMB 114.470b. SEM photograph showing apical and front view. Scale bar = 1 mm.

Figure 5. Midgut of *B. hoabinhensis*, paratype, ZMB 114.470b. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 6. Distribution of pachychilids in northern Vietnam. ○ = *S. tonkiniana* (historical museum material and newly collected samples). Numbers indicate type localities of 1 = Lang Son, type locality of *M. verbecki* var. *tonkiniana*. 2 = Thanh Moi, type locality of *M. hamonvillei* and *M. beaumetzi*.

Figure 7. Shells and operculum of *S. tonkiniana*. **A**. Lectotype and paralectotypes, *Melania verbecki* var. *tonkiniana,* MNHG. **B**. Lectotype, *Melania hamonvillei*, MNHN. **C**. Syntype, *Melania aubryana* var. *elongata,* That Khe, MNHN. **D**. Two syntypes, *Melania aubryana* var. *attenuata*, Tonkin, MNHN. **E**. Two syntypes, *Melania aubryana* var. *robusta*, Tonkin, MNHN. **F**. Two syntypes, *Melania aubryana* var. *obliterata*, Cao Bang, Song Bang Giang, MNHN. **G**. Thanh Moi, MNHN. **H**. Nam Luat, MNHN. **I**. Haut Tonkin, MNHN. **J**. Bac Kan, ZMB 114.184. **K**. 20 km S Ba Be Lakes, ZMB 114.186. **L**. Phuong Vien, ZMB 114.185. **M**. Thuyen Ngau Son, ZMB 114.196. **N**. Cho Moi, ZMB 114.174. **O**. Creek nr Ba Be Lakes, ZMB 114.187. **P**. Bac Kan – Thai Nguyen, ZMB 114.182. **Q**. Lao Cai, ZMB 114.169. **R**. Ha Giang, ZMB 114.205. **S**. Tien Yen, ZMB 114.209. **T**. Ba Be Lakes outflow, ZMB 114.192. **U**. Ba Be Lakes, ZMB 114.188. **V**. Cao Bang, ZMB 114.201. **W**. Lectotype, *Melania beaumetzi*, Thanh Moi, MNHN. **X**. Lectotype, *Melania krempfi*, Tonkin, MNHN. **Y**. Operculum of *S. tonkiniana*, Ba Be Lakes outflow, ZMB 114.192. Scale bars = 10 mm.

Figure 8. Embryonic shell of *S. tonkiniana*, Lao Cai, MNHN. SEM photograph showing apical and front view. Scale bar = 200 μm.

Figure 9. Radula of *S. tonkiniana*. **A-B**. Thuyen Ngau Son, ZMB 114.196. **C-D**. Ba Be Lakes, ZMB 114.188. Scale bars = 100 μm.

Figure 10. Midgut of *S. tonkiniana*, Thuyen Ngau Son, ZMB 114.196. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 11. Shells and operculum of *S. tourannensis*. **A**. *Melania tourannensis*, lectotype, MNHN with label. **B-E**. Paralectotypes, MNHN. **F-G**. Quang Nam Prov., MNHN. **H-I**. Cam Duc, ZMB 114.366. **J**. Thanh My, ZMB 114.364. **K**. Aluoi, ZMB 114.380. **L**. Operculum, Ho-Chi-Ming Highway, ZMB 114.377. Scale bars = 10 mm.

Figure 12. Radula of *S. tourannensis*. **A-B**. Song Con, ZMB 114.370. **C-D**. Gia Lai Prov., ZMB 114.378. Scale bars = 100 μm.

Figure 13. Midgut of *S. tourannensis*, Cam Duc, ZMB 114.366. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 14. Shells and opercula of new *Sulcospira* species. **A**. *S. dakrongensis*, holotype, ZMB 114.367a. **B**. *S. dakrongensis*, paratype, ZMB 114.367b. **C**. *S. dakrongensis*, paratype, ZMB 114.146. **D**. *S. dakrongensis*, paratype, ZMB 114.371. **E**. Operculum, *S. dakrongensis*, paratype, ZMB 114.367b. **F**. *S. quangtriensis*, holotype, ZMB 114.372a. **G-H**. *S. quangtriensis*, paratypes, ZMB 114.372b. **I**. *S. quangtriensis*, paratype, ZMB 114.368. **J**. Operculum, *S. quangtriensis*, paratype, ZMB 114.372b. **K**. *S. vietnamensis*, holotype, ZMB 114.365a. **L-M**. *S. vietnamensis*, paratypes, ZMB 114.365b. **N**. Operculum, *S. vietnamensis*, paratype ZMB 114.365b. Scale bars = 10 mm.

Figure 15. Radulae of new *Sulcospira* species. **A-B**. *S. dakrongensis*, paratype, ZMB 114.367b. **C-D**. *S. quangtriensis*, paratype, ZMB 114.373. **E-F**. *S. vietnamensis*, paratype, ZMB 114.375. Scale bars = 100 μm.

Figure 16. Midgut of *S. dakrongensis*, paratype, ZMB 114.367b. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int,

opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 17. Midgut of *S. quangtriensis*, paratype, ZMB 114.368. See Fig. 5 for labelling of structures. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 18. Midgut of *S. vietnamensis*, paratype, ZMB 114.365b. See Fig. 5 for labelling of structures. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 19. Shells and operculum of *Sulcospira collyra*. **A**. Holotype, ZMB 114.135a. **B-C**. Paratypes, ZMB 114.135b. **D-E**. Ribbed form, Muong Khen, ZMB 114.472. **F**. Operculum, paratype, ZMB 114.135b. Scale bars = 10 mm.

Figure 20. Radulae of *Sulcospira collyra*. **A-B**. Paratype, ZMB 114.135b. **C-D**. Ribbed form, Muong Khen, ZMB 114.472.

Figure 21. Midgut of *Sulcospira collyra*, paratype, ZMB 114.135b. See Fig. 5 for labelling of structures. Abbreviations: cf, paired crescent folds; dgd, opening to digestive gland duct; gp, gastric pad; gs, gastric shield; int, opening to intestine; icp, inner crescent septate pad; lf, lateral fold; mf, marginal fold; ocp, outer crescent septate pad; oes, opening to oesophagus; sa, sorting area; ss, opening to style sac. Scale bar = 5 mm.

Figure 22. Shells of an undescribed species tentatively placed within *Sulcospira*, Lao Cai, MNHN. Scale bar = 10 mm.

Figure 23. Phylogenetic trees based on 16S sequences. A. Bayesian phylogram (numbers above branches indicate nodal support by Bayesian posterior clade probabilities). B. Strict consensus cladogram of 107 equally parsimonious MP trees (numbers above branches indicate nodal support by MP bootstrapping). □ = *B. hoabinhensis*; ■ = *B. annamita*; ☼ = *S. tonkiniana*; ♦ = *S. collyra*; ● = *S. vietnamensis*; ○ = *S. quangtriensis*; ▲ = *S. dakrongensis*; ▼= *S. tourannensis*.

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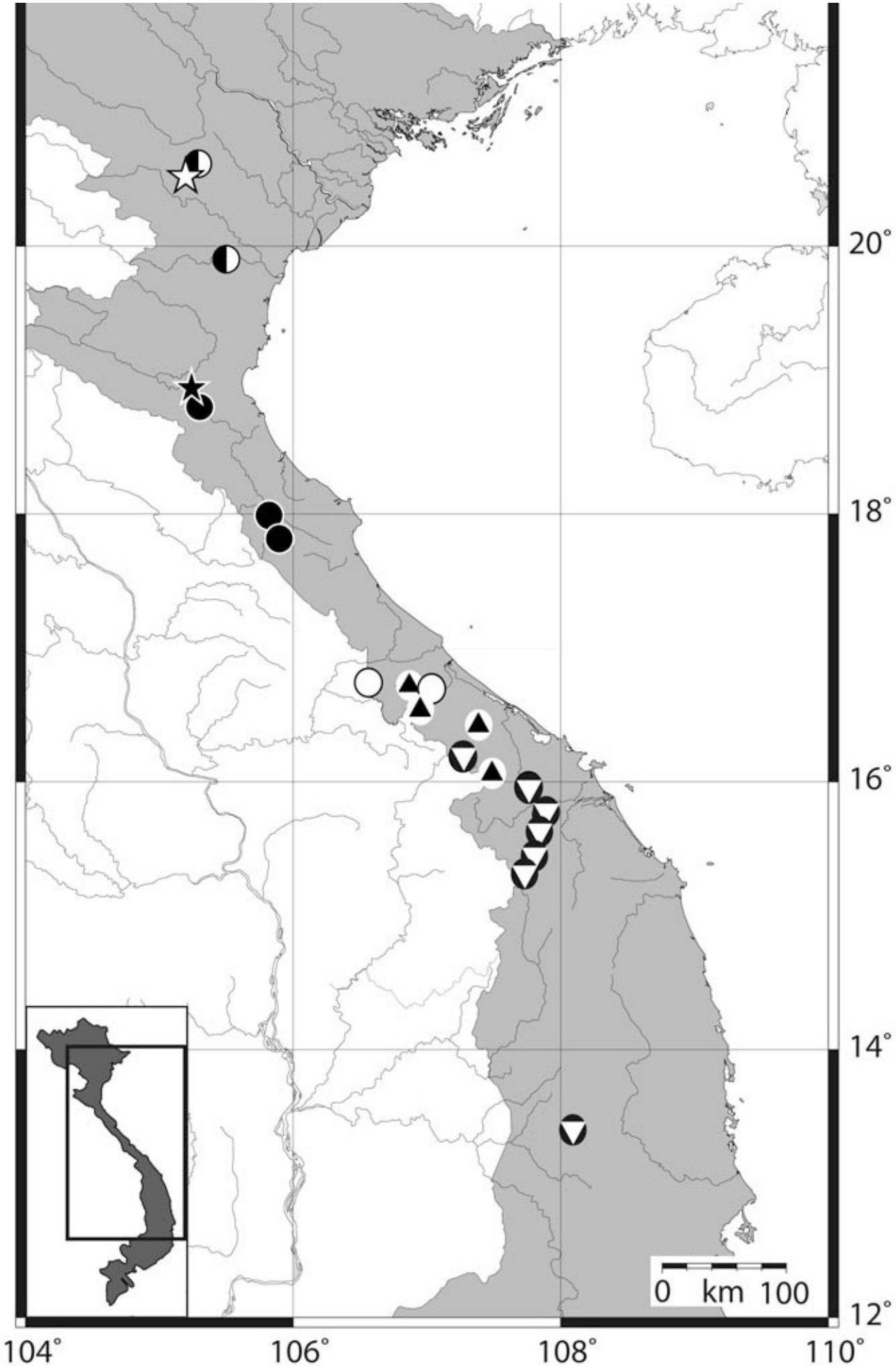
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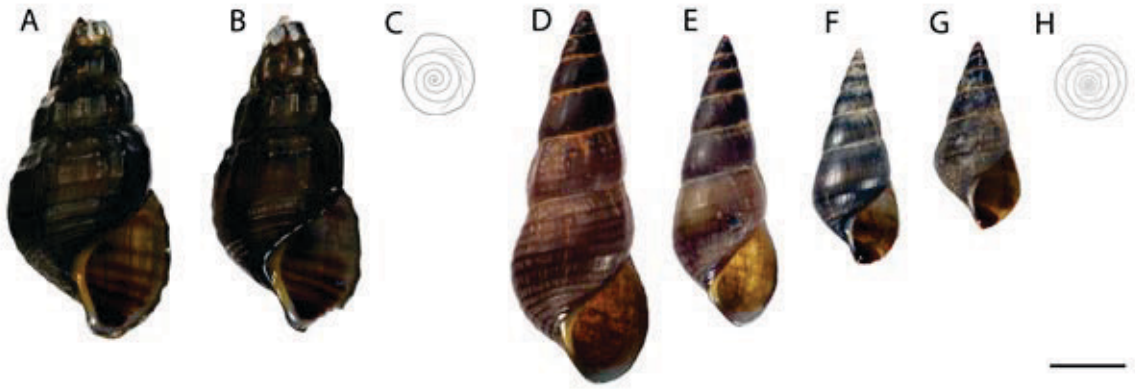

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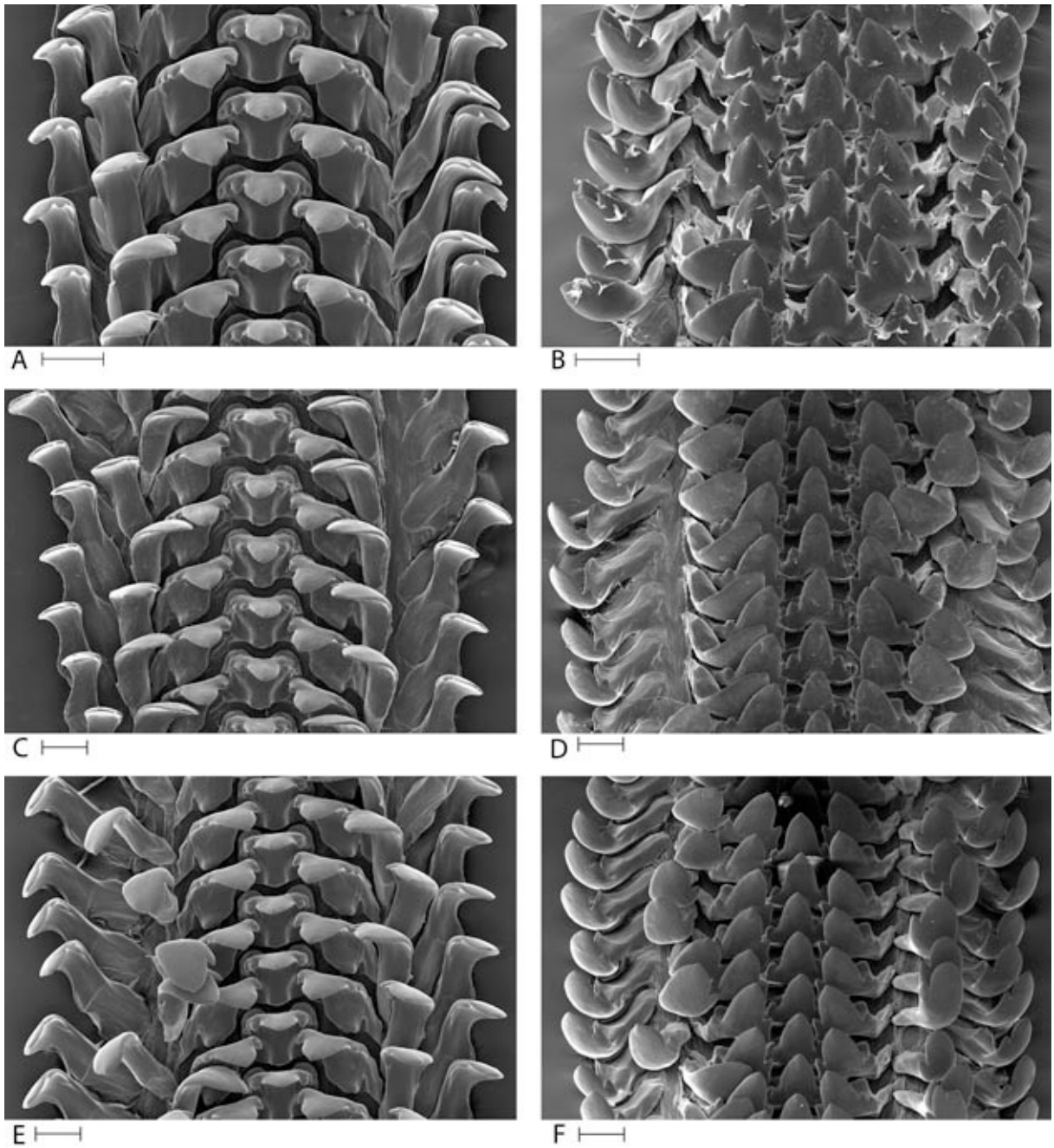

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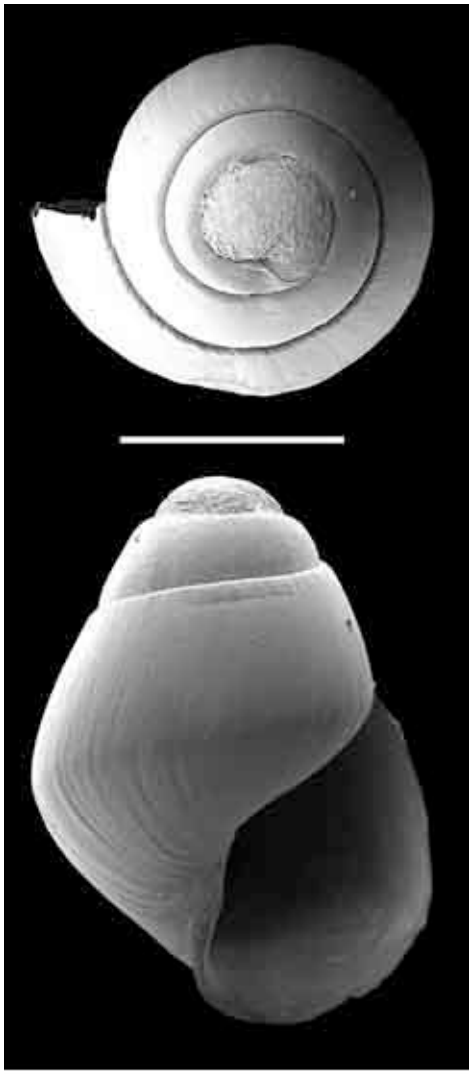

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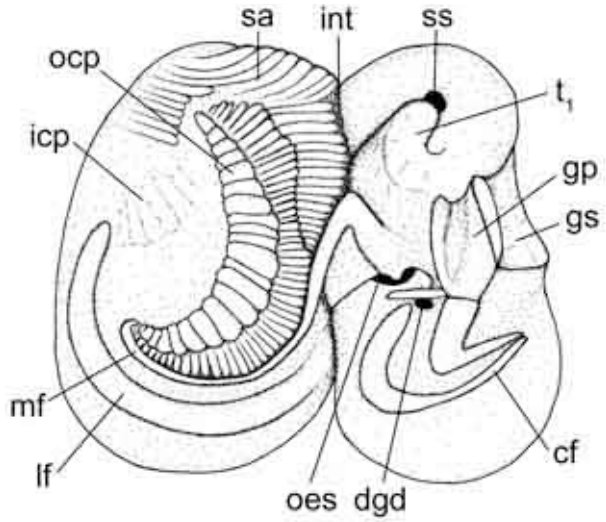

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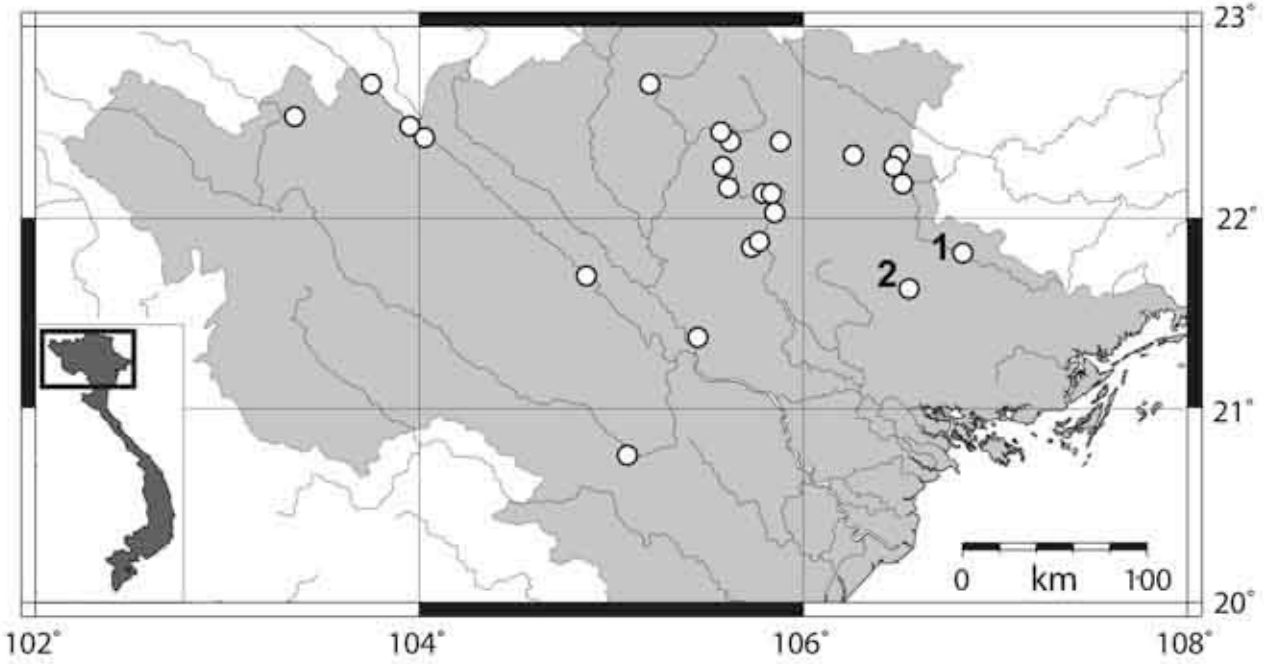

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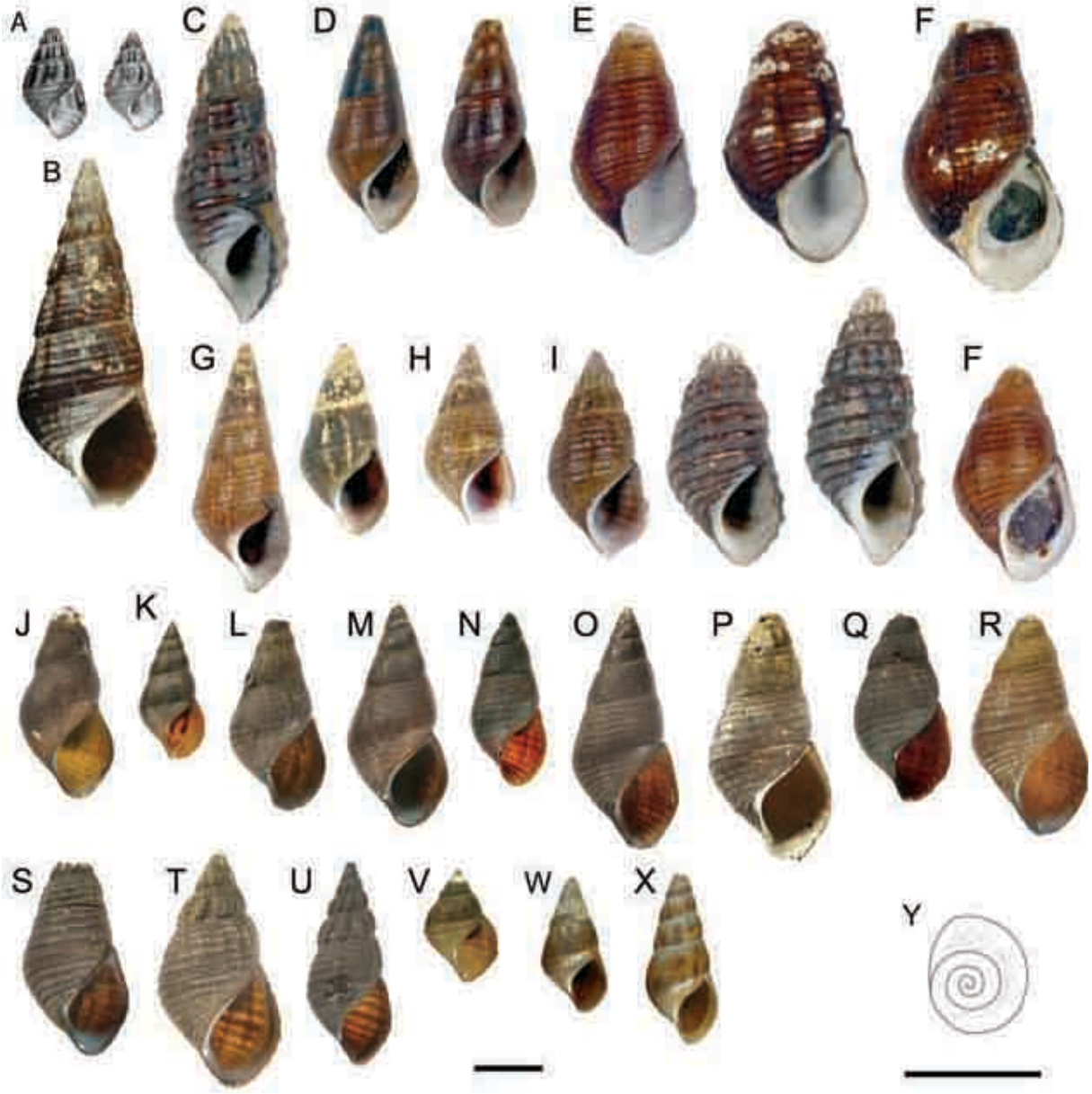

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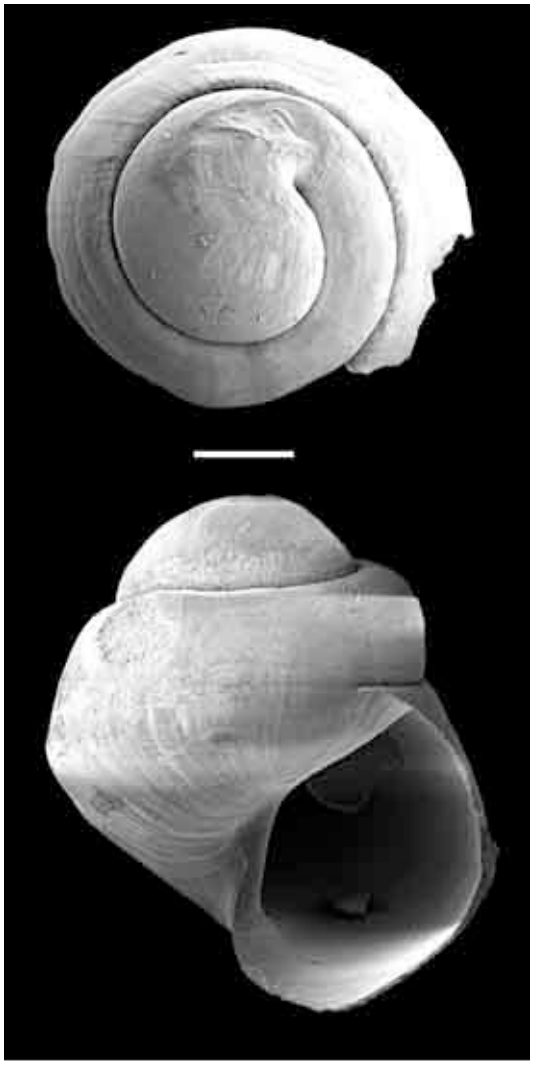

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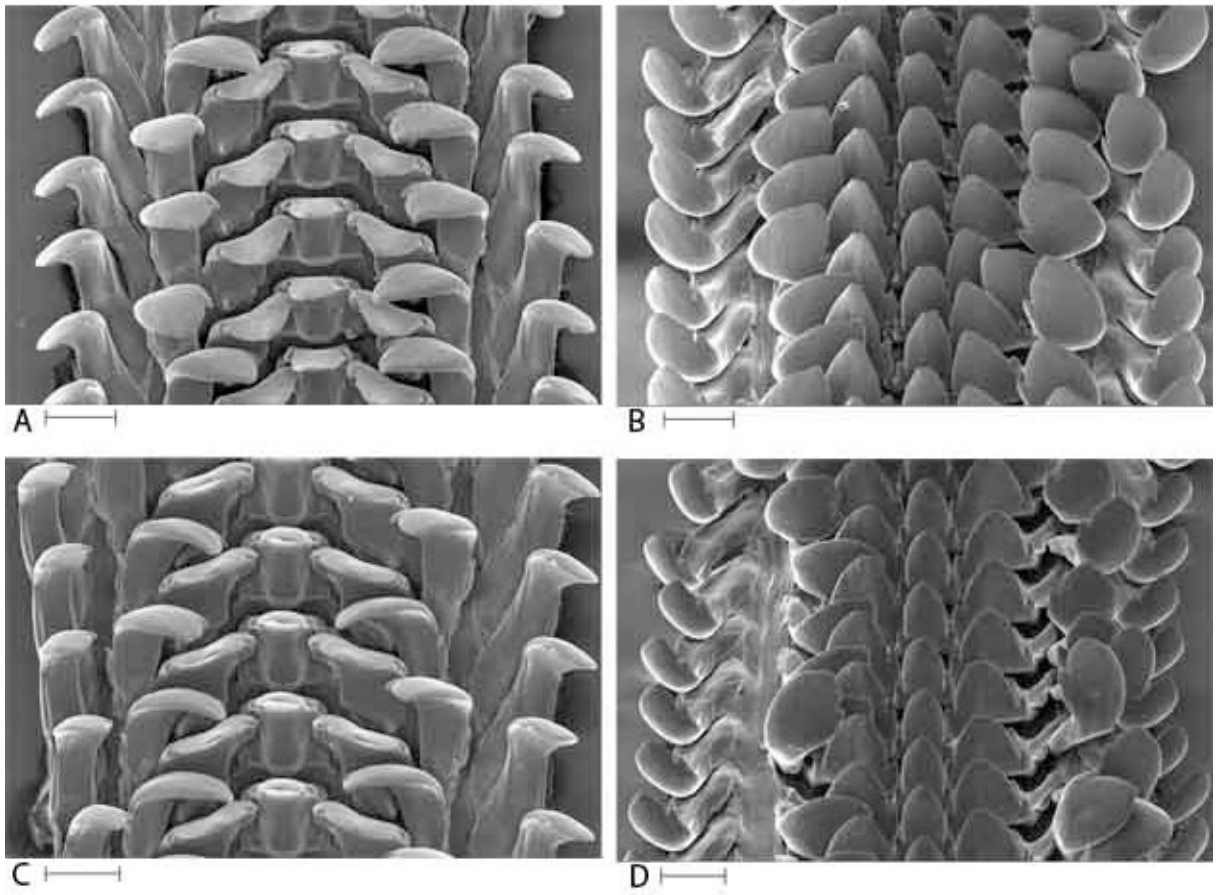

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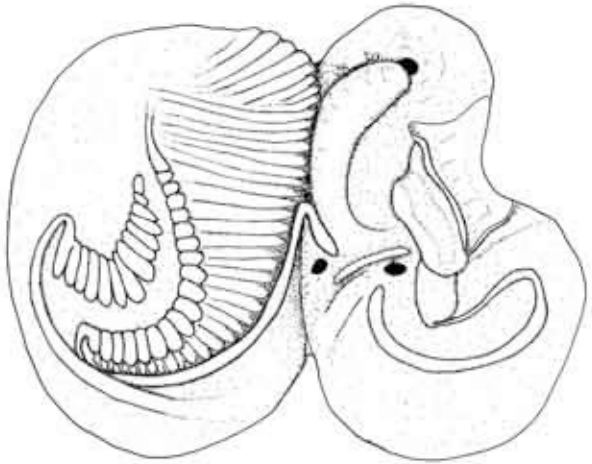

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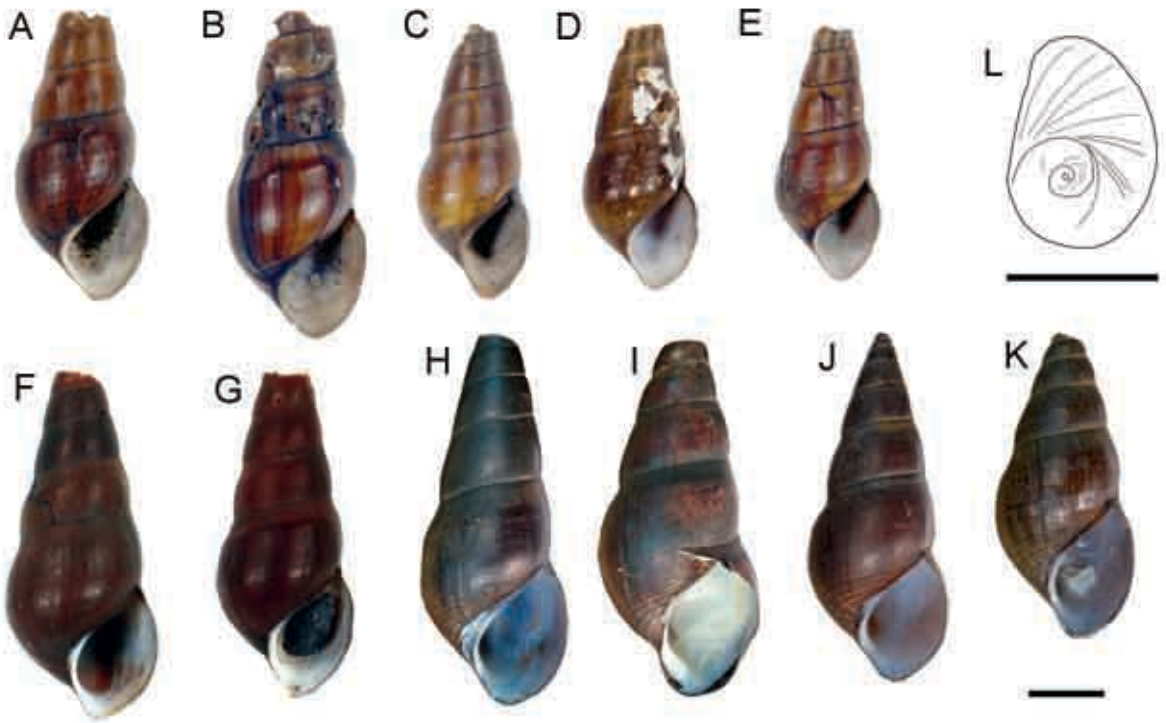

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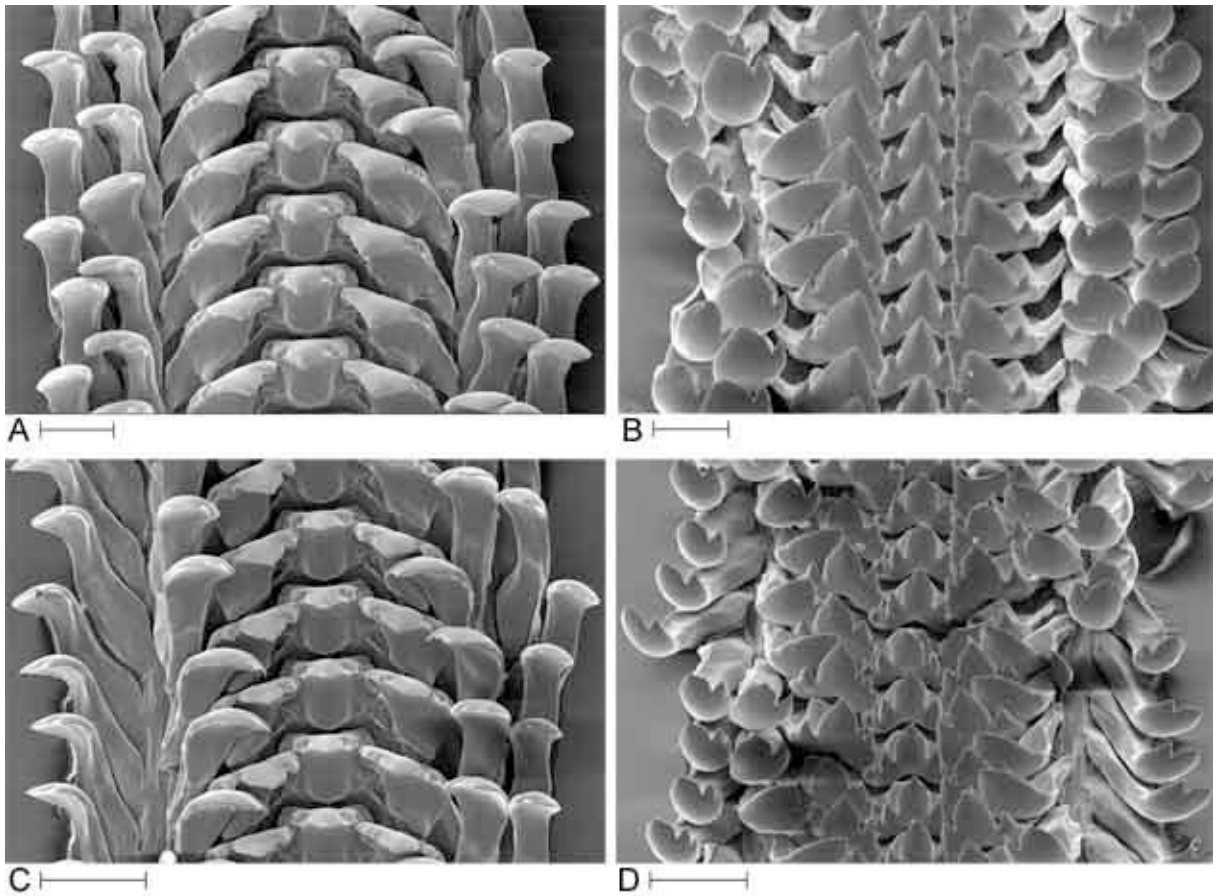

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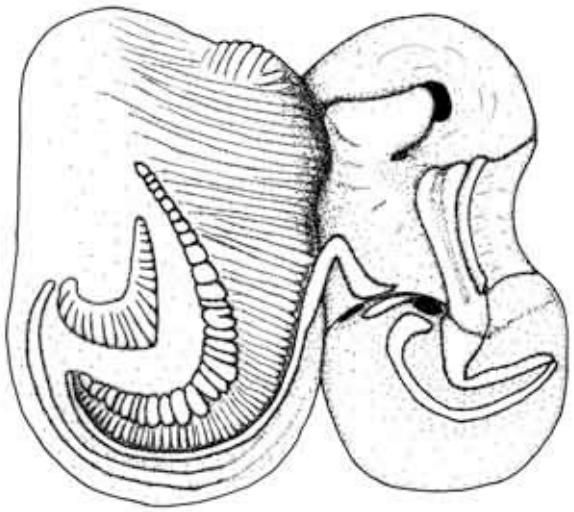

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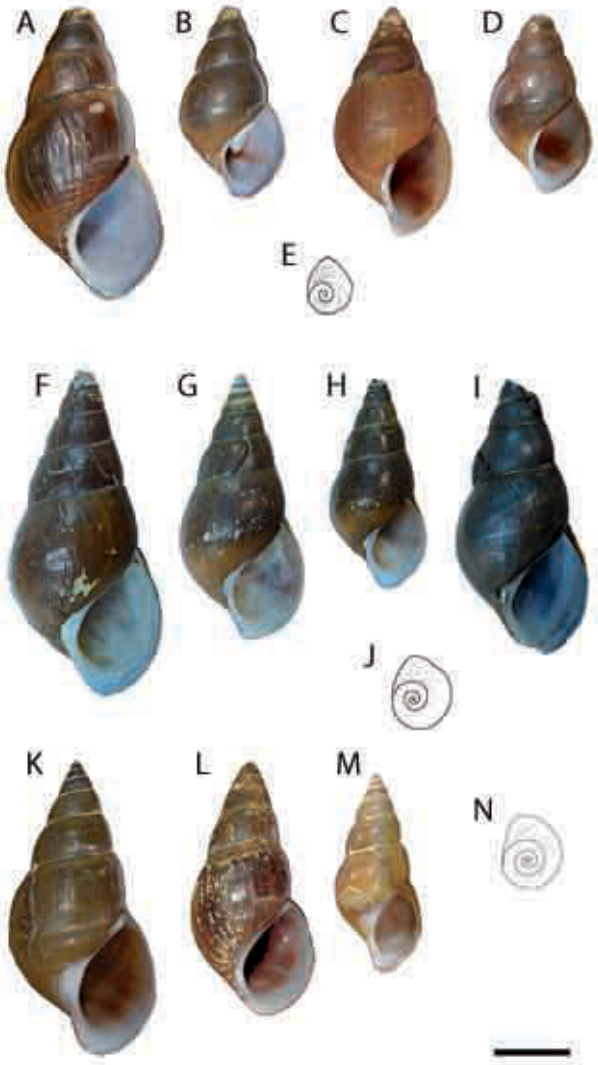

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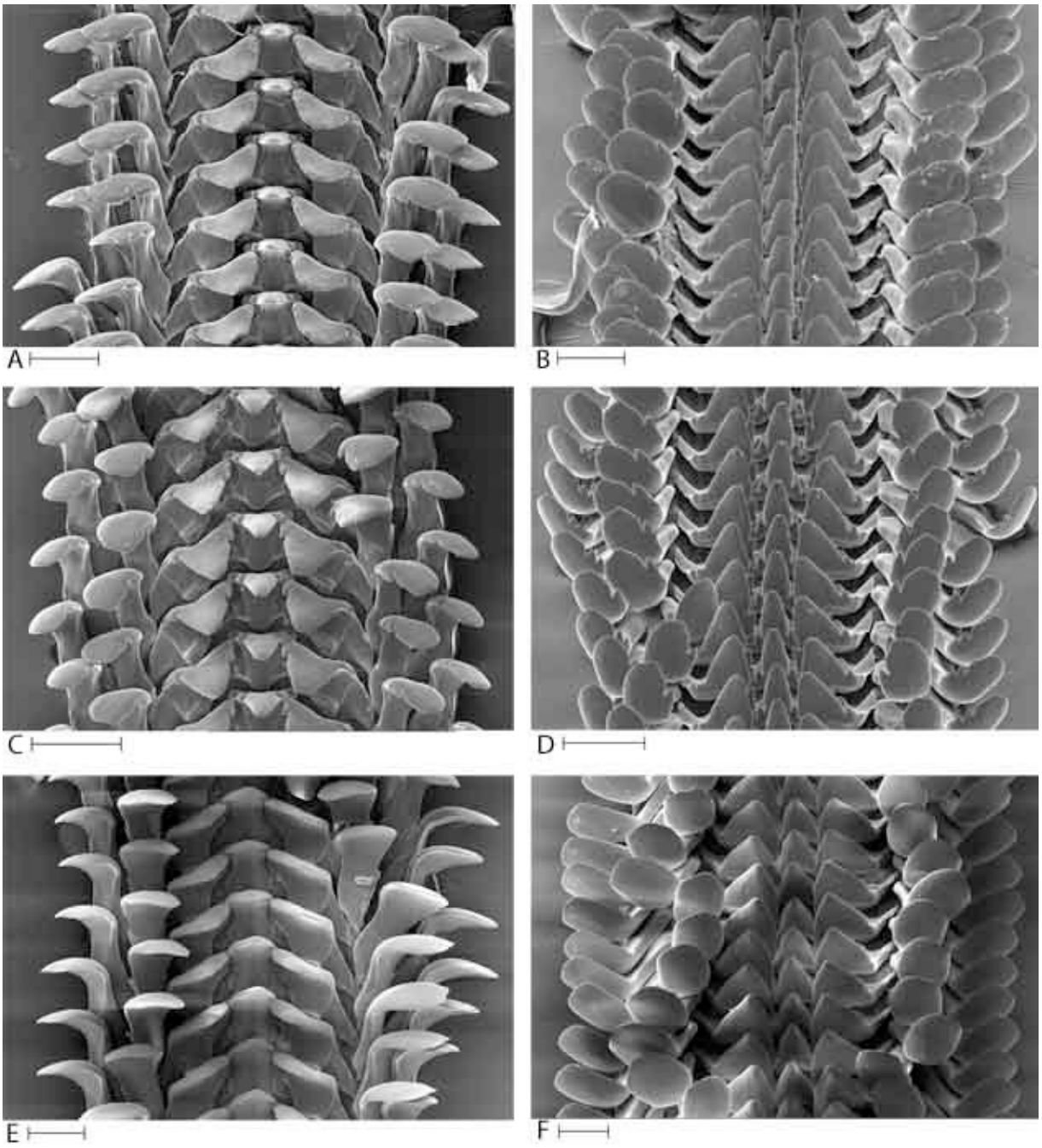

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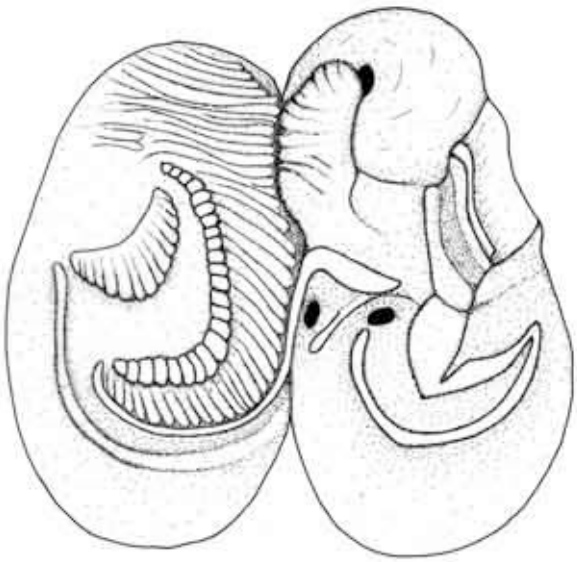

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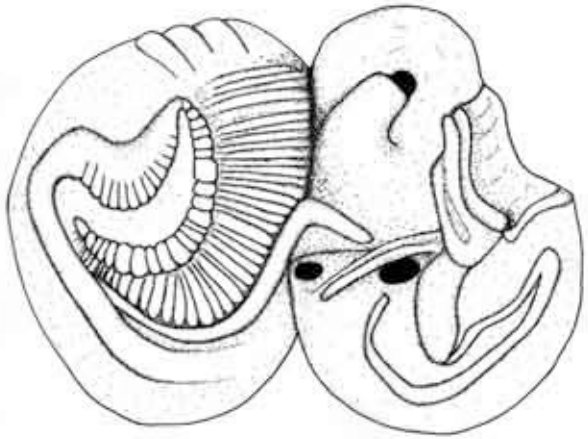

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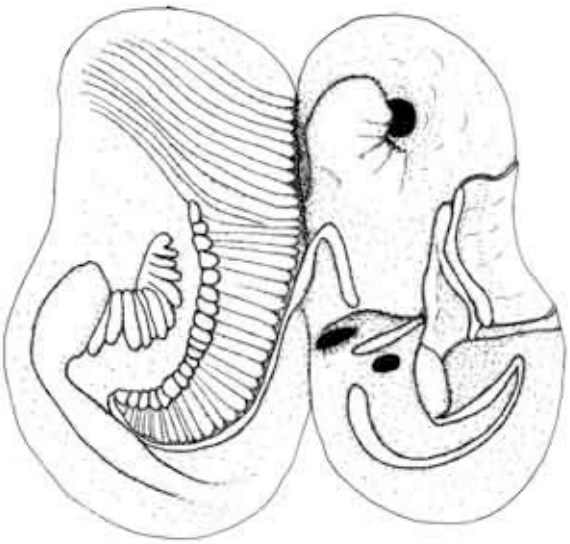

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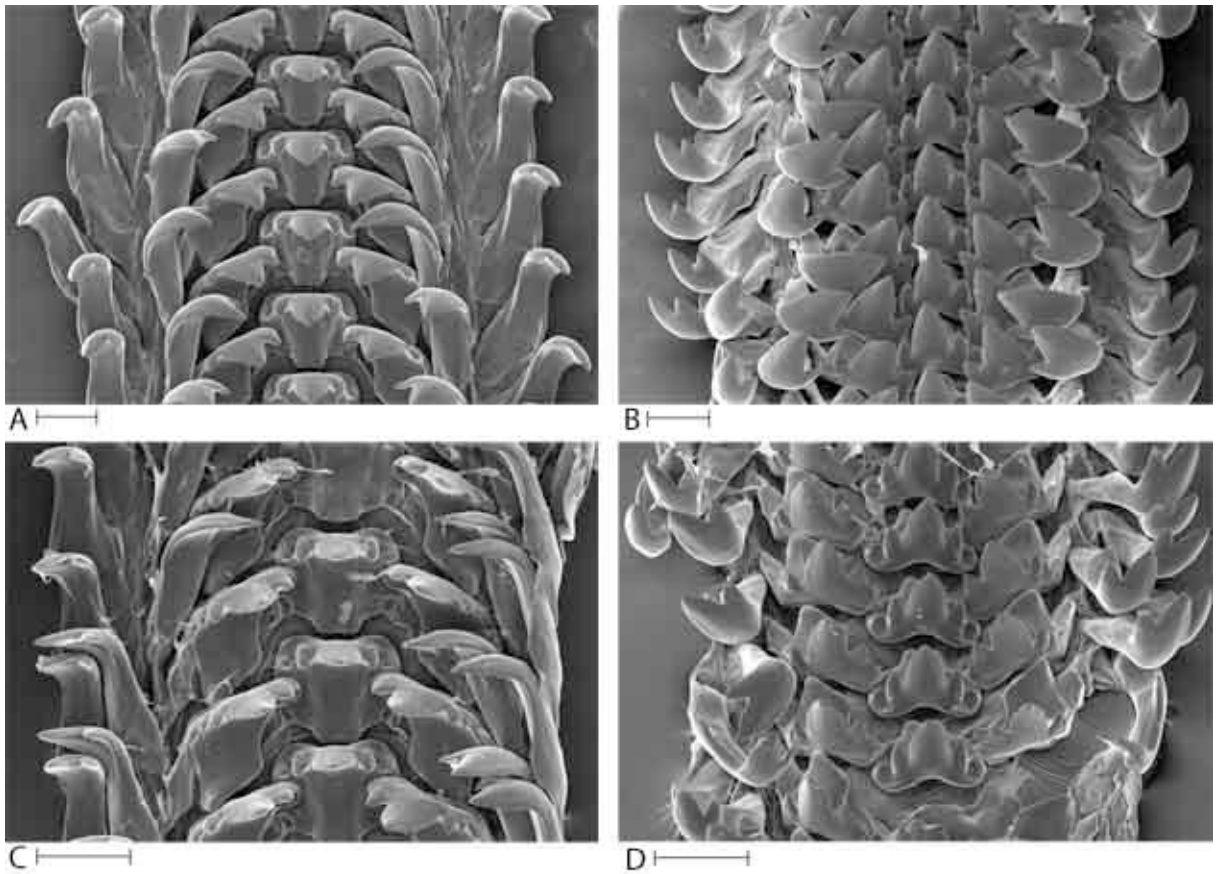

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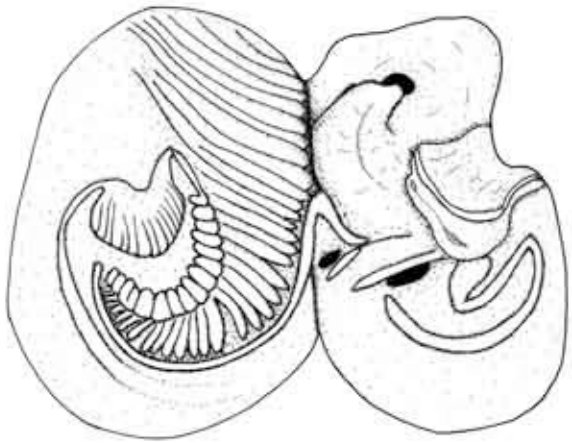

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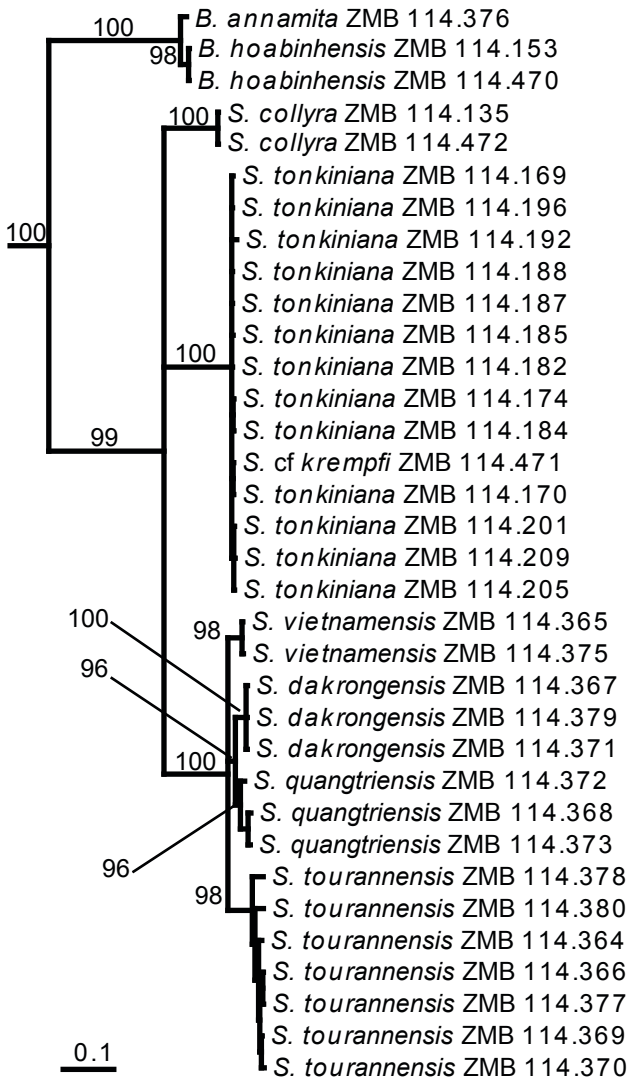
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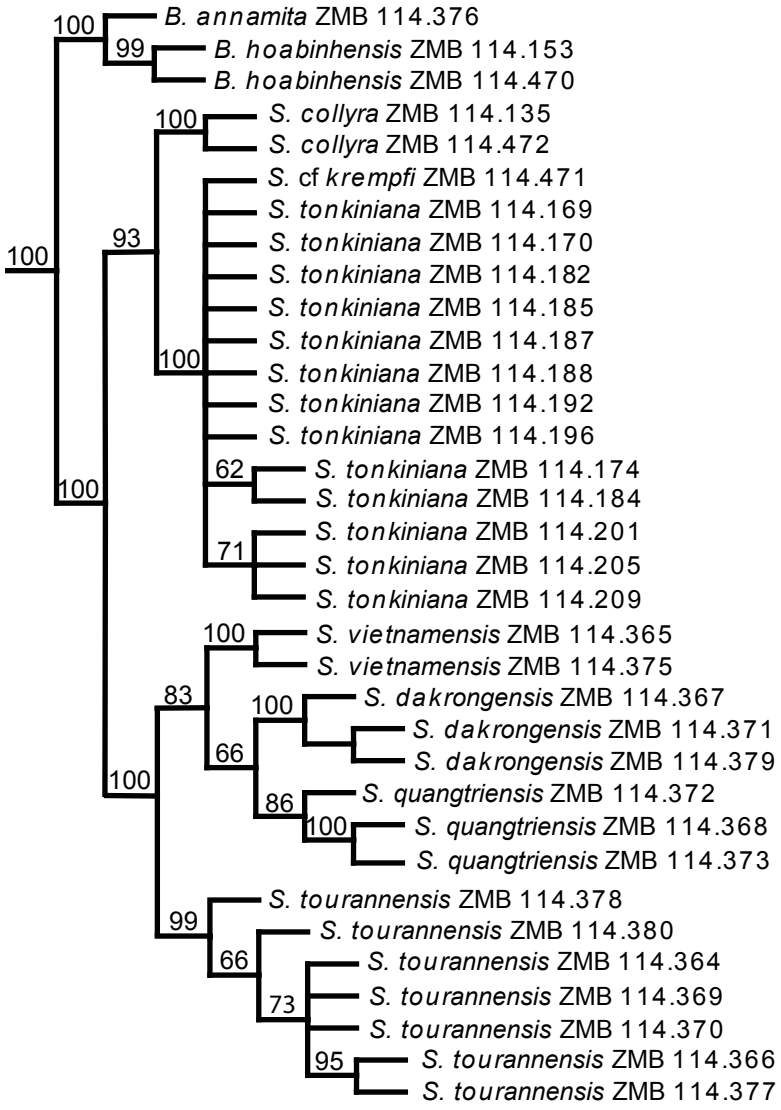
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