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# Too familiar to be questioned? Revisiting the *Crassispira cerithina* species complex (Gastropoda: Conoidea: Pseudomelatomidae)

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## ABSTRACT

*Crassispira cerithina* (Anton, 1838) is a common shallow water conoidean gastropod species, broadly distributed throughout the Indo-West Pacific. It has a distinctive shell morphology and has been referred to in many publications. It is also the first species of its family to have been studied from the viewpoint of toxinology. However, our molecular phylogenetic analysis based on fragments of the COI and 28 S rRNA genes reveals the existence of two closely related distinct species, one of which is described as new (*C. scala* n. sp.). These two species are sympatric in several regions of the Indo-Pacific—in the Philippines, Papua New Guinea, Vanuatu and New Caledonia. They can be reliably distinguished by shell morphology and thus cannot be considered truly cryptic species. The radula is very similar in both species and does not permit species delimitation. A conchological reappraisal of further material similar to *C. cerithina* allows us to recognize two additional species, which are described as new (*C. procera* n. sp. from the Coral Sea and Philippines, and *C. aurea* n. sp. from Tahiti). These results demonstrate that even 'well-known' and seemingly well defined species may be species complexes and that molecular techniques should be routinely applied to confirm specimen identification, especially as part of resource-consuming studies, such as toxinology.

## INTRODUCTION

Neogastropods of the superfamily Conoidea are one of the most species-rich groups of marine Gastropoda. 'Turrids', a paraphyletic group previously designated as the family Turridae, encompasses more than 3,600 named living species (Tucker, 2004; WoRMS, 2016) with dozens of new species described every year. This number is just the tip of the iceberg (Bouchet, Lozouet & Sysoev, 2009) and molecular studies are continuously revealing species complexes (e.g. Fedosov & Puillandre, 2012; Fedosov & Stahlschmidt, 2014). Turrids are a taxonomically very challenging group with numerous examples of homoplasy in shell characters, making species delimitation and identification difficult using these characters alone. The sources of new turrid species are both new sampling in previously insufficiently studied areas (e.g. Fallon, 2016) and a refining of species boundaries with molecular techniques (Puillandre, Cruaud & Kantor, 2010).

Nevertheless there are a few 'iconic' turrid species with characteristic shell shape, sculpture and/or colour pattern that allow a seemingly easy and unambiguous identification. Because of this 'characteristic' morphology, their systematics often goes unchallenged. *Turris babylonia* (Linnaeus, 1758), the type species of the genus, is a good example; for 200 years it was considered an unambiguous, easily identifiable species, until it was demonstrated recently that it is a complex of two closely related species, both present in Linnaeus' original type series (Kilburn, Fedosov & Olivera, 2012). Similarly, the large and characteristic *Xenuroturris cingulifera* was shown to be a complex of two species, belonging to different genera, recognizable on both morphological and molecular grounds (Kantor *et al.*, 2008).

*Crassispira cerithina* (Anton, 1838) (family Pseudomelatomidae Morrison, 1965) is another such 'well-known' species, very common in shallow water in the Indo-Pacific, that is seemingly easily recognizable on the basis of its peculiar sculpture and coloration. It has been illustrated many times in publications (e.g. by Cernohorsky, 1978 and Springsteen & Leobrera, 1986, as *Turridrupa* in both cases; further references in synonymies below). Springsteen (1983) was puzzled by its variability and recognized two conchological forms, but nevertheless considered both forms as undoubtedly belonging to the same species. As it is fairly common in some areas, *C. cerithina* was the first in its family to have been studied from the viewpoint of toxinology (Cabang *et al.*, 2011; Gonzales & Saloma, 2014).

*Crassispira cerithina* attracted our attention several years ago during fieldwork in the Indo-Pacific, since two recognizable forms

with apparently stable differences in shell shape were present in our material, including specimens collected in syntopy. This led to more detailed studies, including molecular sequencing. Eventually we were able to confirm the existence of two closely related species, one of which is here described as new. Morphological screening of further dry material revealed the presence of two additional species similar to *C. cerithina*, which are also described here as new.

## MATERIAL AND METHODS

The material for this study was collected from various Indo-Pacific localities during a series of shallow-water expeditions to New Caledonia (Expedition Montrouzier—1993, Lagon—1984 to 1989, Corail 2—1988), Loyalty Islands (Atelier Lifou—2000), Philippines (Panglao 2004), Vanuatu (Santo 2006) and Papua New Guinea (Papua Niugini 2012, Kavieng 2014). Additional material originates from private collections, including the collection of P. Stahlschmidt. Unless otherwise stated, the material examined is stored in Muséum National d'Histoire Naturelle, Paris (MNHN).

Until 2012, live specimens for molecular analysis were anaesthetized with an isotonic solution of $MgCl_2$ and fixed in 96% ethanol. Specimens collected during later expeditions were processed with a microwave oven (Galindo *et al.*, 2014): the living molluscs in small volumes of sea water were exposed to microwaves for 7–12 s, depending on specimen size. Bodies were immediately removed from shells and dropped in 96% ethanol. Collection data for sequenced specimens are given in Supplementary Material Table S1.

DNA was extracted using the Epmotion 5075 robot (Eppendorf), following the manufacturers' recommendations. A fragment of the cytochrome oxidase subunit I (COI) and of the rRNA 28 S genes were amplified using the universal primers LCO1490/HCO2198 (Folmer *et al.*, 1994) and C2CONO (GAAAAGAACTTTGAAG AGAGAGT) / D3 (Ober, 2002). PCR reactions were performed in volumes of 25 µl, containing 3 ng DNA, 1X reaction buffer, 2.5 mM MgCl2, 0.26 mM dNTP, 0.3 mM of each primer, 5% DMSO and 1.5 units of Qbiogene Q-Bio Taq. For the COI fragment, amplification consisted of an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, followed by extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. The 28 S PCR reactions were performed in 20 µl reaction volumes, containing a final concentration of 1X SsoAdvanced Universal SYBR Green Supermix, 0.3 mM of primers and 0.5 µg/µl of BSA, plus 1 µl of DNA extract. The amplification thermal profiles consisted of an initial denaturation for 3 min at 94 °C, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR products were purified and sequenced by the Eurofins sequencing facility. Specimens are registered in the MNHN collection and sequences were deposited in the Barcode of Life Datasystem and GenBank (Supplementary Material Table S1).

All specimens were sorted to morphospecies before molecular analyses. Pairwise genetic distances were calculated using MEGA v. 6 (Tamura *et al.*, 2013). Trees based on COI and on 28 S were then constructed to test with both genes independently whether each morphospecies corresponded to a clade. *Crassispira quadrilirata* (E.A. Smith, 1882), a species of the same genus, and *Clavus canaliculari*s (Röding, 1798), a member of the Drilliidae, a family closely related to Pseudomelatomidae, were chosen as outgroups. Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference approaches, with respectively RaxML (Stamatakis, 2006) and MrBayes (Huelsenbeck, Ronquist & Hall, 2001). Both analyses were performed on the Cipres Science Gateway (http://www.phylo.org/portal2), using RAxML-HPC2 on XSEDE and MrBayes v. 3.2.2 on XSEDE. In all analyses the three codon positions of the COI gene were treated as independent partitions and the substitution model was set to GTR+G and GTR+I+G for the RAxML and MrBayes analyses, respectively; the parameters of the substitution model were evaluated independently for each partition during the analyses. Robustness of the nodes of the ML trees was assessed as bootstrap probability (BS) using 1,000 bootstraps. Each of the two runs of the BI analysis consisted of six Markov chains and 10,000,000 generations, with five chains, three swaps at each generation, a sampling frequency of one tree each 1,000 generations and a chain temperature set at 0.02. Convergence of each analysis was evaluated using Tracer v. 1.4.1 (Rambaut *et al.*, 2014) to check that all effective sample size values exceeded 200. Consensus trees were calculated after omitting the first 25% trees as burn-in. Nodal support was assessed as posterior probability (PP) in the BI analyses.

Five radulae of *C. cerithina* and four of *C. scala* were prepared by standard methods (Kantor & Puillandre, 2012) and examined by a scanning electron microscope (TeScan TS5130MM) in the Institute of Ecology and Evolution of Russian Academy of Sciences.

### *Abbreviations*

| | |
|---|---|
| AL | Aperture length |
| LWL | Last whorl length |
| MNHN | Muséum National d'Histoire Naturelle, Paris |
| NHMUK | Natural History Museum, London |
| SL | Shell length |
| SNSD | Senckenberg Naturhistorische Sammlungen, Dresden |
| W | Shell width (diameter) perpendicular to SL. |

## RESULTS

Among the 42 specimens sequenced for the COI gene, two morphospecies were recognized. A representative set of specimens for each morphospecies was also sequenced for the 28 S gene (13 specimens). In both trees (see Fig. 1 for BI topologies), these two morphospecies corresponded to monophyletic groups, highly supported (PP > 0.94) in both analyses. Pairwise genetic distances for COI between the two clades were never less than 7%, while within-morphospecies genetic distances never exceed 2%, even between geographically distant localities. Remarkably, in Vanuatu and Papua New Guinea the species are sympatric and sometimes even collected during the same dive, reinforcing our hypotheses that the two morphospecies constitute reproductively isolated lineages.

Morphologically the two forms show stable differences (described below), leaving little doubt about their specific rank. One of these two species has strong morphological similarity to the types of *Crassispira cerithina* (Anton, 1838) (Fig. 2A, B) and this name can be confidently applied to it. The other form is new and is described as *C. scala* n. sp.

## SYSTEMATIC DESCRIPTIONS

Superfamily CONOIDEA Fleming, 1822

PSEUDOMELATOMIDAE Morrison, 1965

*Crassispira* Swainson, 1840

Type species: *Pleurotoma bottae* Valenciennes [in Kiener], 1839; by subsequent designation, ICZN, Opinion 754, 1965.

*Crassispira cerithina* (Anton, 1838)
(Figs 2, 3A, B, 4)

*Pleurotoma cerithina* Anton, 1838: 73, sp. 2504 (2 syntypes, SNSD, Moll3369, Fig. 2A, B; no locality specified)

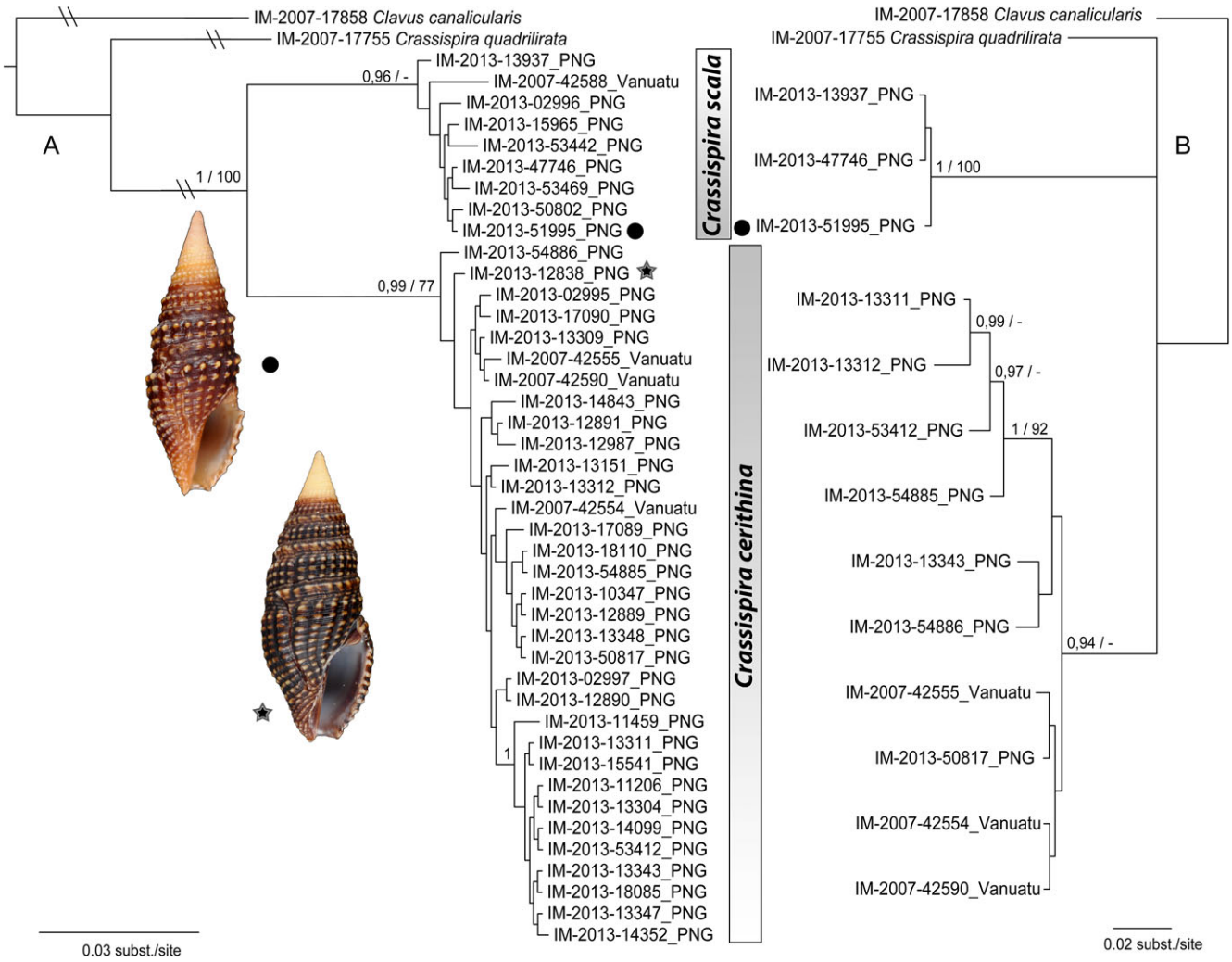


**Figure 1.** Molecular trees. **A.** COI. **B.** 28 S. *Crassispira scala* is represented by shell of holotype (marked by dot on trees), *C. cerithina* is represented by shell of MNHN IM-2013-12838 (marked by a star on COI tree). Support for nodes is given as PP/BS, where these exceed 0.90 and 75, respectively.

*Turridrupa cerithina*—Powell, 1967: 420, pl. 305, fig. 1. Cernohorsky, 1978: 151, pl. 54, fig. 4. Springsteen, 1983: 11, pl. 1 (not pl. 2). Okutani, 2000: 633, pl. 315, fig. 69.
*Inquisitor cerithina*—Kilburn, 1988: 267, figs 280, 281.
*Crassispira cerithina*—Olivera & Sysoev in Poppe, 2008: pl. 688, fig. 4 (not fig. 3).
*Pleurotoma digitale* Reeve, 1843: pl. 17, fig. 138 (3 syntypes NHMUK 1963802, fig. 2C; Island of Bureas [Burias], Philippines).

*Material examined*: Supplementary Material.

*Diagnosis*: Shell oval-fusiform with shallowly concave subsutural ramp, dense axial rows of pronounced differently coloured knobs.

*Description*: Shell (Fig. 2): maximum SL 24 mm (syntype of *P. digitale*). Ovate-claviform (W/SL 0.37–0.51, mean = 0.41, $n$ = 12), with high spire (LWL/SL 0.55–0.62, mean = 0.59, $n$ = 12), aperture narrow; short, obliquely truncated base. Teleoconch with 7 or more whorls, suture shallow, whorls angulated. Protoconch strongly eroded or decollated in available specimens, of about 2.5 smooth whorls, conical-mamillate, boundary with teleoconch indistinct. Teleoconch whorls with weak shoulder. Subsutural ramp weakly concave or nearly flat, separated from rest of whorl by more or less deep groove, bordered abapically by row of pronounced oval knobs, equally pronounced on all whorls. Knobs formed by low arcuate axial ribs intersecting with spiral cords. Ribs 24–27 on last and 27–28 on penultimate whorls. On subsutural ramp, a medium-sized smooth cord sometimes present (in large specimens or on lower spire whorls) immediately below suture, followed by row of distinct knobs. Two more smaller cords present on ramp, sometimes forming smaller knobs at intersection with axial ribs, sometimes nearly smooth. Below subsutural ramp 15–17 rows of knobs gradually diminishing in size. On penultimate whorl usually 3 rows of knobs present below ramp, rarely 4, on upper whorls 2. Entire shell covered by distinct, narrow, spiral riblets, 2 to 4 between rows of knobs, additionally very thin striation can cover riblets and major spiral cords, although not visible or weak on knobs. Distinct varix close behind outer lip. Spiral cords more pronounced when crossing varix, knobs either much weaker or absent. Aperture narrowly oblong-oval, greatest width at about posterior third, tapering gradually to wide, terminally shallowly-notched siphonal canal. Outer lip thickened in adults, weakly convex, crenulated. Anal sinus nearly symmetrical, U-shaped, occupying lower part of subsutural ramp. Strong parietal pad bordering sinus. Columella nearly straight, covered in adults by thick narrow callus. Background colour dark or very dark brown, with light orange upper spire whorls. Knobs dark orange to dark yellow. Aperture brown to light violet inside.
Operculum: oval leaf-shaped, dark brown, with terminal nucleus.

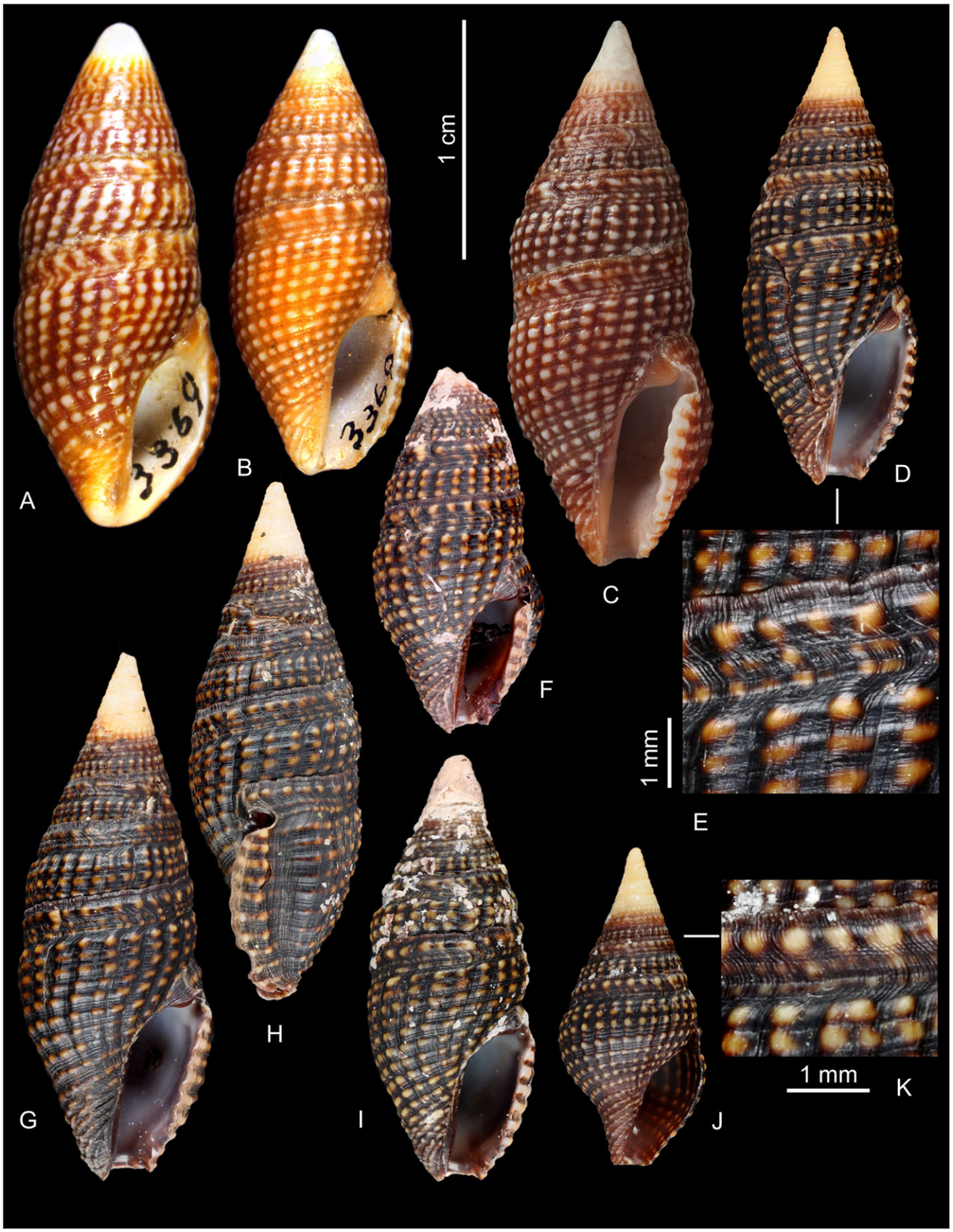


**Figure 2.** Shells of *Crassispira cerithina*. **A, B.** Syntypes of *Pleurotoma cerithina*, (Anton, 1838) SNSD Moll3369 (photo K. Schniebs). **A.** SL 21.2 mm. **B.** SL 18.6 mm. **C.** Syntype of *Pleurotoma digitale* (Reeve, 1843), NHMUK 1963802, SL 22.9 mm (photo K. Webb). **D, E.** MNHN IM-2013-12838, SL 19.1 mm. **E.** Enlarged area of subsutural ramp. **F.** MNHN IM-2013-14099, SL 15.4 mm. **G, H.** MNHN IM-2013-18110, SL 21.7 mm. **I.** MNHN IM-2013-13151, SL 17.8 mm. **J, K.** MNHN IM-2013-14843, SL 13.2 mm.

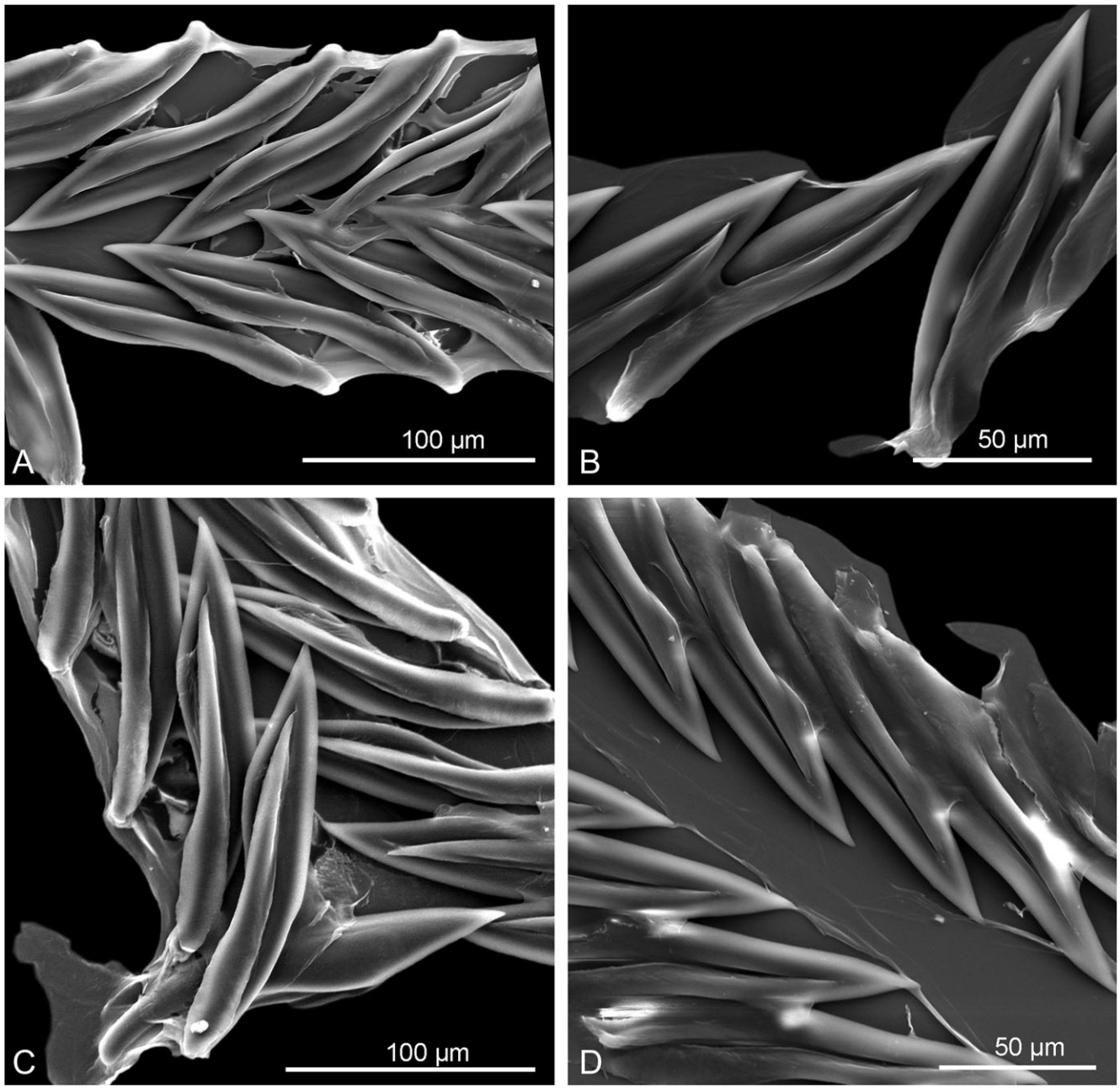


**Figure 3.** Radulae of *Crassispira cerithina* and *C. scala*. **A, B.** *C. cerithina*. **A.** MNHN IM-2013-13151 (shell Fig. 2I). **B.** MNHN IM-2013-13343. **C, D.** *C. scala* n. sp. **C.** Holotype, MNHN IM-2013-51995, Wonad I., Madang Lagoon, Papua New Guinea (shell Fig. 4A–D). **D.** MNHN IM-2013-53443.

Radula (Fig. 3A, B) of 24–35 rows of teeth, 1.1–2.1 mm long (19–26% of AL), consisting of duplex marginal teeth. Marginal teeth with shoe-shaped, pointed major limb, broadest at 1/3 of their length, gradually narrowing outwards. Dorsally limb forming rather deep, groove-like socket, in which anterior part of accessory limb inserts. Accessory limb long, 70–80% of total tooth length, gradually broadening outwards. Tooth length 110–165 µm (1.7–2.0% of AL).

*Distribution and habitat*: *Crassispira cerithina* is known from Sri Lanka, Philippines, Papua New Guinea, Vanuatu, Queensland, Coral Sea, New Caledonia and the Loyalty Islands (see Supplementary Material for records). It is found intertidally to 40 m (Fig. 4).

*Remarks*: This species is highly variable in shell shape, especially regarding the relative width (W/SL ratio). Juveniles differ markedly in shell shape from adults due to their more constricted shell base, leading to a much more convex appearance of the last whorl, and in their relatively broader shell (W/SL = 0.51 *vs* 0.37–0.41 in adults). Shells are usually dark (but the syntypes of *P. cerithina* are very pale, nearly orange, probably a consequence of their being worn and faded). The syntypes of *P. cerithina* (Figs 2A, B) very much resemble the syntypes of *P. digitale* (Fig. 5), leaving little doubt that they are conspecific.

*Crassipira scala* n. sp.
(Figs 3C, D, 4, 5)

*Turridrupa cerithina*—Springsteen, 1983: 11, pl. 2 (not pl. 1). Springsteen & Leobrera, 1986: 272, pl. 78, fig. 1. (Both not *P. cerithina* Anton, 1838).

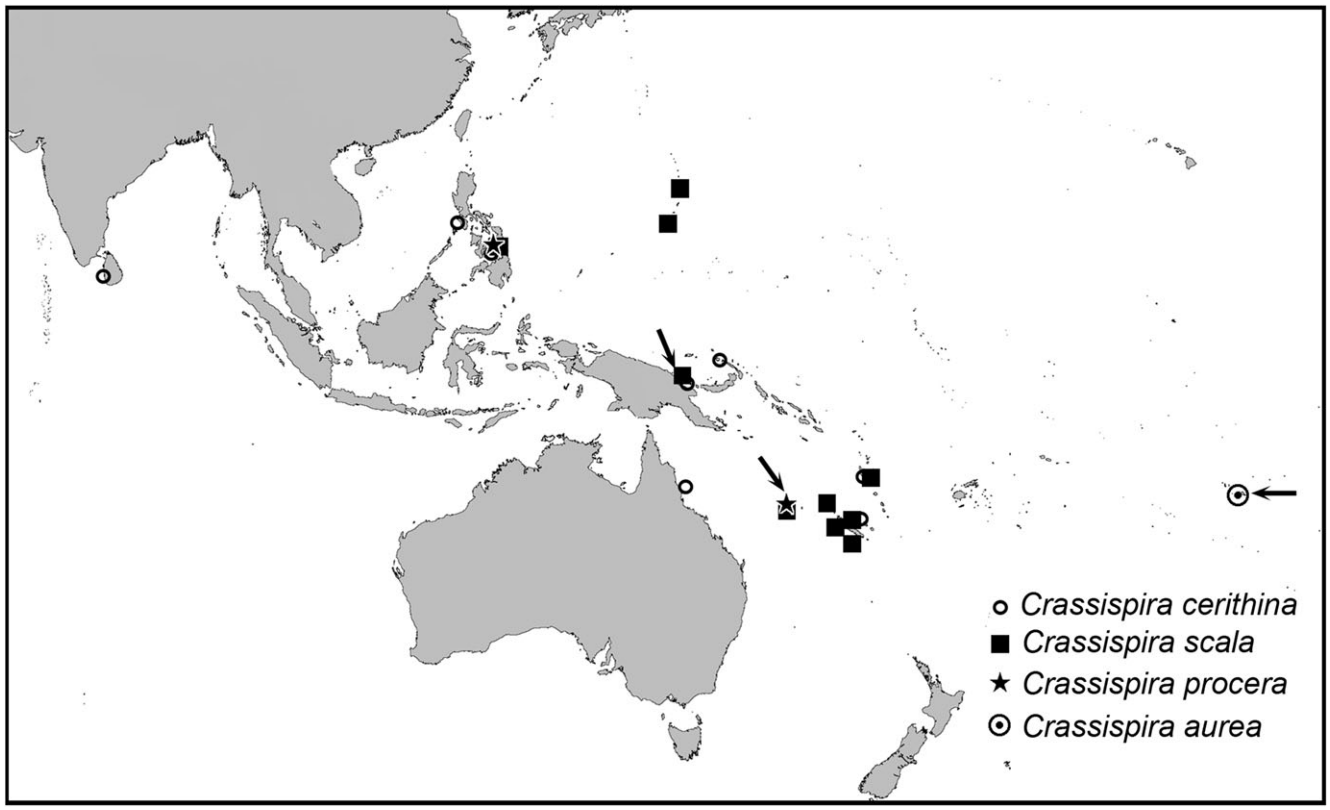


**Figure 4.** Distribution of *Crassispira* species. Type localities of corresponding species are marked by arrows.

*Crassispira cerithina*—Olivera & Sysoev in Poppe, 2008: pl. 688, fig. 3 (not fig. 4). Cabang *et al.*, 2011: fig. 1 (left and middle shells, referred to as light brown and brown variants). (Both not *P. cerithina* Anton, 1838).

*Types*: Holotype MNHN IM-2013-51995, female; N Wonad I., Madang Lagoon, Papua New Guinea, 05°07.1′S, 145°49.4′E (Papua Niugini stn PR159), 15 m depth.

*ZooBank registration*: urn:lsid:zoobank.org:act:E10207FC-73DA-40C3-AA03-D206F8377897

*Material examined*: Supplementary Material.

*Etymology*: *scala*—a ladder (Latin), reflecting the turreted teleoconch shape, similar to winding stairs, used as noun in apposition.

*Diagnosis*: Shell claviform with deeply concave subsutural ramp and a pronounced row of knobs on the shoulder.

*Description (holotype)*: Shell (Fig. 5A–D): narrowly claviform (SL/W 0.35), with high spire (LWL/SL 0.55), aperture rather narrow; short, obliquely truncated base. Teleoconch whorls approximately 7.5, suture shallow, whorls angulated. Protoconch strongly eroded, of 3+ whorls, conical-mamillate, boundary with teleoconch indistinct. Teleoconch whorls with strong shoulder. Subsutural ramp distinctly concave, with row of well developed oval knobs, more pronounced on last 3 whorls. Knobs formed by low, nearly straight, axial ribs intersecting spiral cords. Ribs 20 on last and penultimate whorls. Below row of major knobs one more row of much weaker knobs, similar in shape, followed by shoulder with a row of strongest rounded knobs (Fig. 5D). Similar, gradually diminishing rows of knobs situated towards canal, 12 in addition to shoulder row. On penultimate whorl 3 rows of knobs, on upper whorls 2. Axial sculpture of rather weak, slightly curved ribs, accentuated by rows of knobs. Between knobs in interspaces between axial ribs, very weak, slightly raised rounded cords, very indistinct spiral riblets can be additional present. Distinct varix close behind outer lip. Spiral cords more pronounced when crossing varix, knobs either much weaker or absent. Aperture narrowly oblong-oval, greatest width at about posterior third, tapering gradually to wide, terminally shallowly-notched siphonal canal. Outer lip slightly thickened, weakly convex, with traces of spiral cords and knobs visible from inside. Anal sinus nearly symmetrical, U-shaped, occupying lower part of subsutural ramp and adjoining shoulder. Strong parietal pad bordering sinus. Columella nearly straight, covered by narrow thick callus. Background colour chocolate, with light orange upper part of spire and lighter base. Knobs orange. Aperture orange inside.
Operculum: oval leaf-shaped, dark brown, with terminal nucleus.
Measurements: SL 18.1, AL 7.1, LWL 10.0, W 6.2 mm.
Radula (Fig. 3C): of about 30 rows of teeth, 1.7 mm long (24% of AL), consisting of duplex marginal teeth. Marginal teeth with shoe-shaped pointed major limb, broadest at 1/3 of its length, gradually narrowing towards outer edge. Dorsally limb forming a deep groove-like socket, in which anterior part of accessory limb inserts. Accessory limb long, 85% of total tooth length, gradually broadening towards outer end. Tooth length 175 μm (2.5% of AL).

*Distribution and habitat*: The Philippines, Papua New Guinea, New Caledonia, Vanuatu and Micronesia, alive from intertidal to 40 m, shells down to 100 m (Fig. 4).

*Remarks*: *Crassispira scala* is variable in shell shape, with adult specimens more slender (mean W/SL 0.39, $n = 7$, in juveniles reaching 0.48, Fig. 5L, in adults as low as 0.34–0.35, Figs 5A, H) and with higher spire (mean BWL/SL is 0.56, $n = 7$, in juveniles reaching 0.61, Fig. 5L, in adults as low as 0.49, Fig. 5H). It is also variable in coloration, specimens with lighter background than holotype exist (e.g. Fig. 5H, L). Specimens from New Caledonia seem generally


**Figure 5.** Shells of *Crassispira scala* n. sp. **A–D.** Holotype, MNHN IM-2013-51995, N Wonad I., Madang Lagoon, Papua New Guinea, SL 18.1 mm. **D.** Enlarged area of subsutural ramp. **E.** MNHN IM-2007-42588, SL 16.4 mm. **F, G.** MNHN IM-2013-02996, SL 13.9 mm, **G.** Area of subsutural ramp. **H.** New Caledonia, Expedition Montrouzier, st. 1310, SL 25.9 mm. **I.** MNHN IM-2013-53469, SL 17.7 mm. **J.** MNHN IM-2013-53442, SL 17.5 mm. **K, L.** Lifou Expedition stn 1422, Lifu, Loyalty Islands. **K.** SL 17.9 mm. **L.** SL 10.7 mm. **M.** Barracuda Point, Guam, Colln P. Stahlschmidt, SL 23.9 mm.

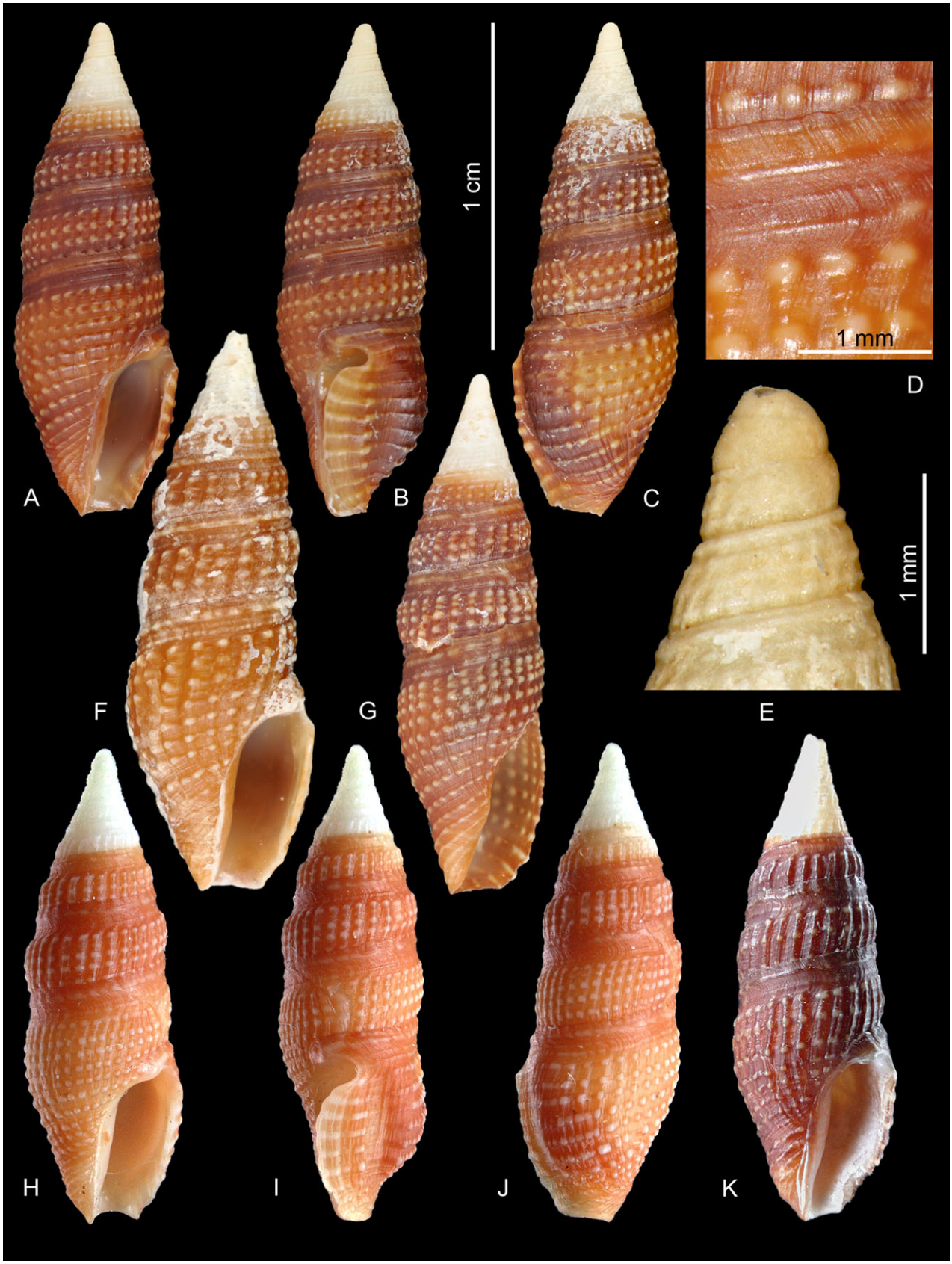


**Figure 6.** Shells of *Crassispira procera* n. sp. **A–E.** Holotype, MNHN IM-2000-31327, Chesterfield Plateau, Coral Sea, Corail 2, stn DW144, SL 15.0 mm. **D.** Enlarged area of subsutural ramp. **E.** Protoconch. **F.** Paratype, MNHN IM-2000-31704, Coral Sea, Corail 2, stn DW122, 19°28.2′S, 158°17.1′E, SL 17.1 mm. **G.** Paratype, MNHN IM-2000-31705, Coral Sea, CORAIL 2, stn DW160, 19°46′S, 158°23′E, SL 15.9 mm. **H–J.** Momo Beach, Panglao I., Philippines, 9°36.5′N, 123°45.3′E, SL 14.7 mm. **K.** Lumun-lumun, Panglao I., Colln P. Stahlschmidt, SL 14. 8 mm.

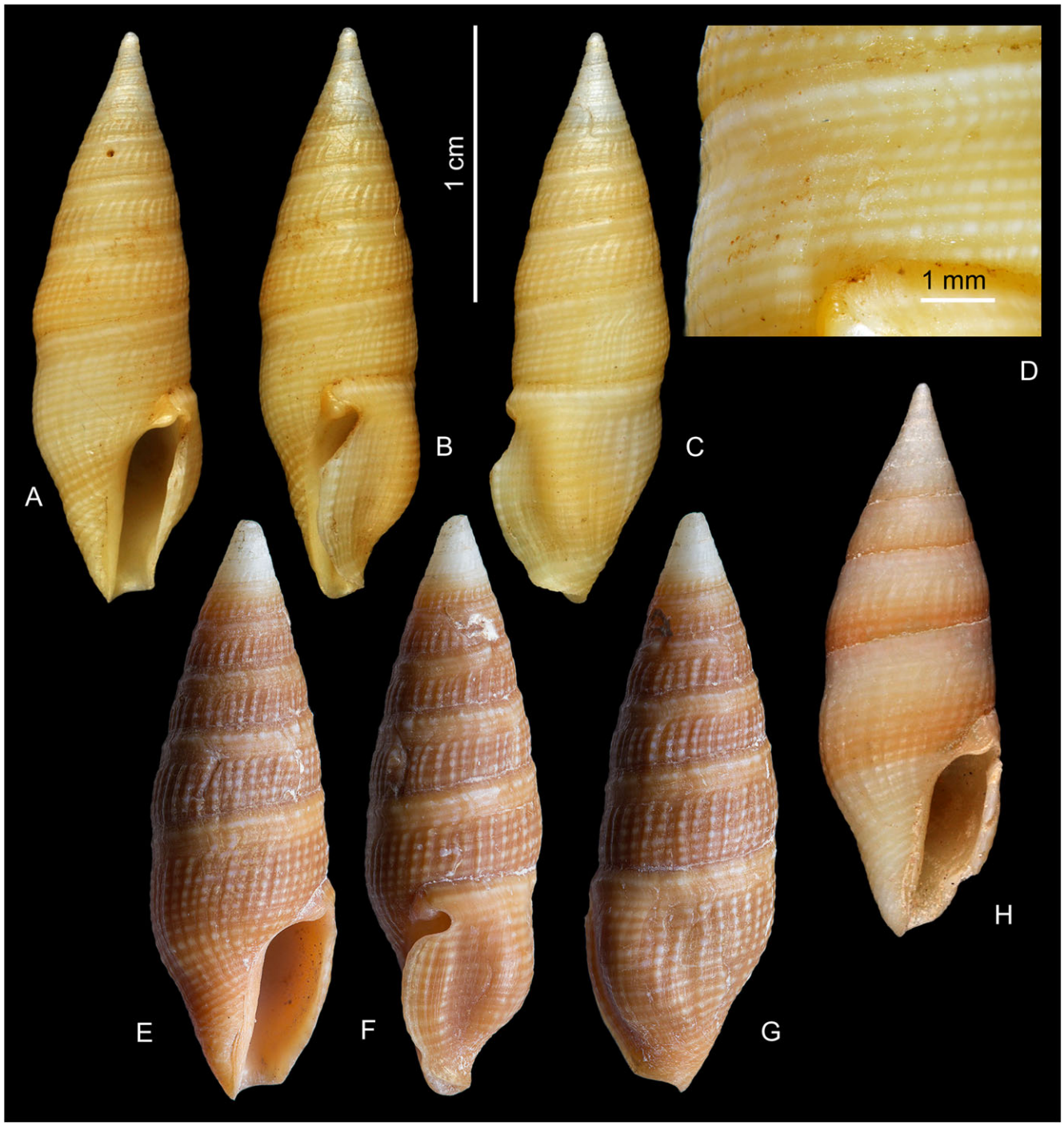


**Figure 7.** Shells of *Crassispira aurea* n. sp. **A–D.** Holotype, SL 20.9 mm, Punaauia, Tahiti, MNHN IM-2000-31328. **D.** Enlarged area of subsutural ramp. **E–G.** Paratype, Colln P. Stahlschmidt, Tahiti, SL 21.0 mm. **H.** Paratype, Colln P. Stahlschmidt, Tahiti, SL 20.2 mm.

to be paler, sometimes with dark orange background colour. Some specimens (Fig. 5M) can be very dark, similar to *C. cerithina*. The number of rows of knobs on the last whorl is variable, seemingly increasing in larger specimens, for instance in a specimen with SL 25.9 mm (Fig. 5H) the number of rows of knobs rises to 16, while the number of axial ribs on the last whorl can reach 25 (*cf.* 20 in holotype).

The sculpture and shape of the subsutural ramp are also variable. In some specimens, the knobs of the subsutural cord can be very weak, nearly obsolete on part of the whorl, but in other parts are always present. Similarly, the smaller knobs on the adjacent cord can also become obsolete on some parts of the whorls, with the cord appearing nearly smooth. Sometimes, in addition to regular cords on the subsutural ramp, an additional thinner cord can be present immediately above the sutural row. Knobs can also be present only on the upper subsutural cord.

Based on examination of shells of different sizes, the varix on the last whorl is formed once at the end of shell growth and is present in specimens with an immature lip. The parietal pad bordering the anal sinus is not pronounced in immature shells.

The largest specimen examined has a SL of 28.1 mm (New Caledonia, Lagon stn DW553).

*Crassispira scala* has been confused with *C. cerithina*, but can be reliably distinguished from it by its much more pronounced and deeply concave subsutural ramp and a pronounced row of knobs on the shoulder, producing a turreted shell outline, and in the lower number of knobs. In *C. cerithina* the subsutural ramp is not distinctly concave and therefore the shell has a more regular oval outline, with a weak constriction at the abapical border of the subsutural ramp. The shells of *C. scala* have a generally paler colour (sometimes uniformly orange) and have an orange aperture (*vs* light violet or brown in *C. cerithina*). In *C. scala*, the shell base is always paler than the remaining part of the last whorl, whereas the last whorl is of uniform colour in *C. cerithina*.

The relative abundance of *C. scala* and *C. cerithina* varies in different localities. In New Caledonia, *C. scala* constitutes the majority of the specimens in the complex, with very few *C. cerithina* and, although globally sympatric, they are in fact never recorded at the same spot. In Santo (Vanuatu), the two species are uncommon and occur in similar proportions. In Papua New Guinea, *C. cerithina* outnumbers *C. scala*, while in the Philippines the reverse is true. Although the two species were occasionally collected during the same dive, they may live in different microhabitats and their syntopy is a matter of geographical scale.

*Crassispira procera* n. sp.
(Figs 4, 6)

*Types*: Holotype MNHN IM-2000-31327, Chesterfield Plateau, Coral Sea, 19°27.7′S, 158°23.3′E (Corail 2 stn DW144), 50 m depth. Paratypes: MNHN IM-2000-31704, Chesterfield Plateau, Coral Sea, 19°28.2′S, 158°17.1′E (Corail 2 stn DW122), 32 m depth (1 dd;, Fig. 6F); MNHN IM-2000-31705, Chesterfield Plateau, Coral Sea, 19°46′S, 158°23′E, (Corail 2 stn DW160), 35–41 m depth (1 dd, Fig. 6G).

*ZooBank registration*: urn:lsid:zoobank.org:act:173CC455-4465-4F48-B61D-B483AE38F31D

*Other material examined*: Supplementary Material.

*Etymology*: *procerus, -a, -um*—tall, slender (Latin adjective), reflecting the narrowly elongate shell form.

*Diagnosis*: Shell narrowly fusiform with very shallow subsutural ramp without defined knobs.

*Description (holotype)*: *Shell* (Fig. 6A–E): narrowly fusiform (SL/W 0.31), with high spire (LWL/SL 0.50), narrow aperture and short, obliquely truncated base. Teleoconch whorls *c.* 7.5, suture shallow, whorls weakly angulated. Protoconch eroded, nonplanktotrophic, of slightly less than 2 whorls, mamillate, with large nucleus, boundary with teleoconch indistinct; exposed height and diameter about 0.8 mm. Teleoconch whorls weakly convex, with shallowly concave subsutural ramp and rounded shoulder angulation. Subsutural ramp very weakly concave, with 4 uneven spiral cords, second abapical strongest, sometimes forming indistinct oval lighter knobs, smooth on other parts of shell. Rest of shell covered by spiral rows of knobs, formed by very low, nearly straight, slightly prosocline axial ribs intersecting spiral cords; knobs of even size, absent on lowermost spiral cords on canal. On last whorl 25 axial ribs; 30 on penultimate whorl. On last whorl 9 spiral rows of knobs; 6 smooth cords on shell base and canal; 4 rows of knobs on penultimate whorl; 3 rows on upper spire whorls. Between some rows of knobs indistinct irregularly spaced riblets and sometimes microscopic striation. Distinct varix close behind outer lip. Spiral cords when crossing varix either with indistinct knobs or smooth. Aperture narrowly oblong-oval, greatest width at about half, tapering gradually to wide, terminally unnotched siphonal canal. Outer lip slightly thickened, weakly convex, crenulated within. Anal sinus nearly symmetrical, U-shaped, occupying lower part of subsutural ramp and adjoining shoulder. Strong parietal pad bordering sinus. Columella very weakly convex, nearly straight, covered by narrow thick callus. Background colour dark orange, with very light yellow upper part of spire. Subsutural ramp darker, of same shade. Knobs orange. Aperture light yellow inside, with orange outer lip.
Measurements (holotype): SL 15.0, AL 6.4, LWL 8.0, W 5.1 mm (holotype).
Animal: unknown.

*Distribution and habitat*: Coral Sea and the Philippines, shells in 32–80 m (Fig. 4).

*Remarks*: *Crassispira procera* is only slightly variable in terms of shell shape and sculpture. In one specimen there are eight spiral rows of knobs below the subsutural ramp on the last whorl, in another nine, as in the holotype. The largest specimen reaches 17.1 mm (upper protoconch whorls eroded) (Fig. 6F).

Based on the examination of shells of different sizes, the varix on last whorl is formed once at the end of shell growth. The parietal pad bordering the anal sinus is not pronounced in an immature shell (Fig. 6G).

*Crassispira procera* can be distinguished from *C. cerithina* by its much more slender and paler shell and by the absence of defined knobs on the subsutural ramp, and from *C. scala* by the non-angulated whorl profile, the very shallow subsutural ramp, the much smaller and less defined knobs, as well as the absence of rows of knobs on the subsutural ramp. For the differences with *Crassispira aurea* n. sp. see Description of the latter species.

*Crassispira aurea* n. sp.
(Figs 4, 7)

*Type material*: Holotype MNHN IM-2000-31328, Tahiti, in about 35 m depth. Paratypes: Tahiti, shallow water (2 dd), Colln Stahlschmidt; Tahiti, Pueu, 25 m (1 dd), Colln Marty.

*ZooBank registration*: urn:lsid:zoobank.org:act:CC68EB8A-FF99-40E7-8810-DEAF1523D7CA

*Etymology*: *aureus, -a, -um*—golden (Latin adjective), reflecting the colouration of the shell.

*Diagnosis*: Shell narrowly fusiform with very shallowly concave poorly differentiated subsutural ramp and sculpture of weak nodules.

*Description (holotype)*: Shell (Fig. 7A–D): narrowly fusiform (W/SL 0.28), with high spire (LWL/SL 0.51), narrow aperture and short, obliquely truncated base. Teleoconch whorls *c.* 8, suture very shallow. Protoconch eroded, nonplanktotrophic, of slightly less than 2 whorls, mamillate, with large nucleus, boundary with teleoconch indistinct, marked by appearance of postlarval sculpture; exposed height 0.85 mm, diameter about 0.8 mm. Teleoconch whorls weakly convex, with shallowly concave, nearly straight subsutural ramp poorly differentiated from abapical part of whorl, on last and penultimate whorls with three cords unequal in size of which adapical is strongest. On upper teleoconch whorls 2 rather indistinct adapical cords. Rest of shell covered by spiral cords about equal in size, slightly thickened where intersecting weak axial folds and forming low nodules. Around 33 axial ribs on last whorl (indistinct on anteriormost part close to aperture), 36 on penultimate; 23 spiral cords on last whorl; 11 on shell base and canal. On penultimate whorl 7 spiral cords, diminishing in number adapically from 5 to 3 rows on upper spire whorls. Between some rows of knobs indistinct, irregularly spaced riblets and sometimes microscopic striation. Distinct varix close behind outer lip. Spiral cords very low when crossing varix, without distinct nodules.

Aperture narrow, nearly obliquely rectangular, not tapering to terminally unnotched, siphonal canal of similar width to aperture. Outer lip slightly thickened, weakly convex, smooth within. Anal sinus shallow, nearly symmetrical, U-shaped, occupying lower part of subsutural ramp. Strong parietal pad bordering sinus. Columella straight, covered by narrow thick callus. Background colour golden orange, upper part of spire slightly paler.
Measurements (holotype): SL 20.9, AL 7.9, LWL 10.6, W 6.0 mm.
Animal: unknown.

*Distribution and habitat*: Although we have only examined material from Tahiti (Fig. 4), *C. procera* is present in the Society, Tuamotus and Gambier archipelagoes (Tröndle & Boutet, 2009, as *C. cerithina*, J. Tröndlé pers. comm.) and might be endemic to the region; the depth was not exactly recorded except for the holotype (35 m).

*Remarks*: The species is rather stable in shell characters, the holotype being the lightest coloured specimen. The species reaches 21.0 mm in shell length.

*Crassispira aurea* is most similar to *C. procera*, but differs in having a more slender and larger shell, weaker sculpture, more closely spaced and more numerous axial ribs, and lighter shell coloration. The shell base is more constricted in *C. aurea*, the aperture is narrower and the whole subsutural ramp is less pronounced.

## DISCUSSION

The recognition of sibling species previously hidden under a single species name, is the frequent outcome of many recent taxonomic investigations on members of the Conoidea. Many if not most analyses of molecular diversity of species groups of Conoidea have revealed previously undetected species (e.g. Puillandre, Cruaud, & Kantor, 2010; Fedosov & Puillandre, 2012; Tenorio & Castellin, 2016). In most cases, molecular analyses—and in particular barcoding studies—point to previously unrecognized species, thus providing a basis for re-evaluation of interspecific and intraspecific variability. Often the newly revealed species can be distinguished based on morphological characters. In contrast, the detection of truly cryptic species that remain indistinguishable by morphological means is actually quite exceptional. One example is *Gemmuloborsonia clandestina* (Puillandre *et al.*, 2010), which was revealed by blind, intensive sequencing of representatives of this genus, and cannot be reliably distinguished by anatomical characters, radula and shell morphometry from a morphologically identical (although not even sister) species.

*Crassispira cerithina* and *C. scala* cannot be considered cryptic species, since their shells are reliably and relatively easily distinguishable. Indeed, morphological differences between the two species were detected before any DNA sequences were available. Nevertheless, the integrative taxonomic approach applied here, combining morphological and molecular data analysed through phenetic (morphological differences and genetic distances) and phylogenetic approaches, was crucial in confirming the shell-based hypotheses. After that the re-identification of material accumulated in MNHN before the molecular revolution did not pose any problem. It is in fact surprising that nobody previously suspected the existence of two species, especially since they are found sympatrically in the Philippines. Springsteen (1983) was the only one who recognized two 'structural forms' of what he considered as undoubtedly the same species, of which he regarded *C. cerithina* as the 'normal' form. Later *C. scala* was illustrated under the name *Turridrupa cerithina* by Springsteen & Leobrera (1986) and Poppe (2008: pl. 688, Figs 3, 4) depicted the two species side by side. Indeed, both species have a remarkable—and, among medium-sized turrids, unique—sculpture of spiral rows of knobs of contrasting colour; this peculiar sculpture probably disguised the differences in shell shape. It should also be noted that the two available toxinology studies of *C. cerithina* (Cabang *et al.*, 2011; Gonzales & Saloma, 2014) pooled venom glands of several specimens before RNA extraction. It is a common practice in toxinology to increase the amount of material used in order to facilitate subsequent molecular analyses. Cabang *et al.* (2011: 673, fig. 1) illustrated both *C. cerithina* and *C. scala*, and specified that "the dark brown variant, which was most abundant, was used in this work". This dark form corresponds to *C. cerithina*. It is unclear whether the results of Gonzales & Saloma (2014) were based on one or both species, in which case toxin diversity per species would be biased.

Besides stable shell differences, we were not able to find any characters in soft body morphology or operculum that support the distinction of the two species. The radula of several specimens of each species was examined and proved to be nearly identical in both tooth shape and relative size of marginal teeth. Very similar teeth are found in a number of Pseudomelatomidae, in additional *Crassispira* species, *Miraclathurella bicanalifera* and others (Kantor, Medinskaya & Taylor, 1997). Thus, in this and many other cases, the radular morphology of Pseudomelatomidae is of little taxonomic value at the species level.

The generic placement of the species treated herein remains ambiguous. *Crassispira cerithina* was previously classified either as a member of *Turridrupa* (Turridae) or *Inquisitor* (see synonymies above). Inclusion in *Turridrupa* is not supported either by morphological or molecular data. *Turridrupa* as a member of Turridae (confirmed by molecular phylogenetic analysis; Puillandre *et al.*, 2011) has a radula formed of marginal teeth and a well developed central formation ('central tooth'; see Kantor, 2006, for details), while radulae of *C. cerithina* and *C. scala* lack any central formation. In the tree presented here (as in a much larger dataset, unpublished results) the two species of *Crassipira* are nested within Pseudomelatomidae. In the unpublished *Atlas of turrids of New Caledonia* (manuscript in MNHN) R.N. Kilburn placed *C. cerithina* (together with several other species) in *Crassispira*, albeit with the following remark: "none of these are really genus *Crassispira* but belong to unnamed genera".

The type species of *Crassispira* is *Pleurotoma bottae* Valenciennes in Kiener, 1839, a species quite different in shell size, shape and sculpture. The holotype, stored in MNHN (IM-2000-22691, photo available online at: https://science.mnhn.fr/institution/mnhn/collection/im/item/2000-22691) was collected in Mexico, Mazatlan. *Pleurotoma bottae* was synonymized with *Crassispira incrassata* (G.B. Sowerby I, 1834) by McLean in Keen (1971) and the animal was studied anatomically by Kantor *et al.* (1997).

Although the sculpture and other shell characters of the species under consideration do merit description of a new genus, we refrain from this for several reasons. The generic classification of Pseudomelatomidae is already very complicated, sometimes confused and has not yet been adequately addressed using molecular phylogenetics. Furthermore, no sequences are available for *C. incrassata*, the type of the genus. Therefore, in the absence of more detailed studies and without the clarification of the validity of existing genera and subgenera, we consider that the description of one more genus would be premature.

Two of the species described here, *Crassispira aurea* and *C. procera*, are known only from empty shells. Although their validity as separate species is beyond doubt, their generic placement is equally arbitrary. We do not know with any certainty whether they represent members of the same complex as *C. cerithina* and *C. scala*, but this is our hypothesis.

Notwithstanding a recommendation to routinely implement molecular techniques prior to any detailed and resource-consuming studies such as toxinology, our results affirm that, once DNA characters have been used to test species hypotheses, shell characters remain in many cases a good guide for species-level recognition (Kantor *et al.*, 2008). In a parallel case, Lindsay & Valdés (2016) have shown that the facelinid nudibranch *Hermissenda crassicornis*, an important model organism in neuroscience, behavioural ecology, pharmacology and toxicology, is also a species complex, a result that potentially impacts the replicability of generations of studies.

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## SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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