CRYPTIC SPECIES IN *GEMMULOBORSONIA* (GASTROPODA: CONOIDEA)

N. PUILLANDRE[1], C. CRUAUD[2] AND YU. I. KANTOR[3]

[1]*UMR 7138, Museum National d'Histoire Naturelle, Departement Systematique et Evolution, CP26, 57 rue Cuvier, 75231 Paris Cedex 05, France;*
[2]*GENOSCOPE, Centre National de Séquençage, 2 rue Gaston Crémieux, CP 5706, 91057 Evry Cedex, France; and*
[3]*A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences, Leninski Prosp. 33, Moscow 119071, Russia*

(Received 17 December 2008; accepted 19 May 2009)

ABSTRACT

During a broad molecular taxonomic and phylogenetic survey of the gastropod superfamily Conoidea, 80 specimens of several species of the genus *Gemmuloborsonia* were sequenced for the cytochrome *c* oxidase subunit I gene. The genus, originally established for fossil species from the Plio-Pleistocene of the Philippines, now includes living species from bathyal depths of the Indo-Pacific Oceans. The molecular data demonstrated the presence of five separate entities, while only four 'morphospecies' could be isolated by visual examination. The two largest groups, representing separate species from the molecular data, were impossible to distinguish with certainty using shell or anatomical characters. To examine shell morphology in more detail the shape of the last whorl was analysed by Fourier analysis, and the Fourier coordinates were used in canonical variate analysis. The majority of the specimens were separated into two groups, but 21.6% of the specimens were impossible to distinguish by morphological characters. One of these two forms was attributed to the known species *Gemmuloborsonia moosai* Sysoev & Bouchet, 1996, while the other is described as a new species *Gemmuloborsonia clandestina*. *Bathytoma colorata* Sysoev & Bouchet, 2001 is transferred to *Gemmuloborsonia* on the basis of molecular analysis and radular morphology. Another species, represented in our material by a single specimen, remains undescribed.

INTRODUCTION

The taxonomy of shell-bearing molluscs was, and continues to be, largely based on shell characters. For more than 250 years of scientific malacology shell characters have proved to be effective species-level identifiers, especially when the protoconch is also included. Similar characters have been employed for the exceptionally good fossil record of molluscs. Moreover, reliance on shell characters is also justified by the fact that many species have simply never been collected alive; for example, during intensive surveys of coral reef sites 28% of the species were not collected alive (Bouchet *et al*., 2002). For the overwhelming majority of described species of molluscs the primary name-bearing types are shells, these usually being the only part of the animal preserved in collections. This leaves conchological characters as the major if not the only source of evidence for taxonomic decisions.

The conventional approach to documenting molluscan diversity or revisionary taxonomy is to sort material to morphospecies on the basis of shell characters, with subsequent testing of taxonomic hypotheses with all available data, such as anatomy, biogeographic information and, more recently, with molecular analyses. The final stage of any taxonomic decision in malacology is the critical reevaluation of the shell characters in order to identify and formalize reliable discriminating features, and this requires the estimation and evaluation of the intraspecific variability of the shell.

Convergence and homoplasy render shell characters much less reliable predictors of relationships at higher taxonomic levels (family, genus) within the gastropod superfamily Conoidea (=Toxoglossa). Sometimes the incongruence between the shell and internal anatomy is startling and species with very similar shells may be very distantly related. For instance, shells of *Toxicochlespira* Sysoev & Kantor, 1990 (Conidae) strongly resemble representatives of *Cochlespira* Conrad, 1865 (Turridae) (Sysoev & Kantor, 1990); shells of *Strictispira* McLean, 1971 (Strictispiridae), are hardly distinguishable from those of many species of *Crassispira* Swainson, 1840 (Turrridae, Crassispirinae) (Tippett, 2006); and the radula-less species *Cenodagreutes aethus* Smith, 1967 is said to be conchologically indistinguishable from the radulate *Raphitoma leufroyi* (Michaud, 1828) (both Conidae, Raphitominae) (Fretter & Graham, 1985).

Cryptic, or sibling, species are ubiquitous among marine animals and molluscs are no exception (see reviews by Knowlton, 1993, 2000). In reality, most recently discovered cryptic species of Gastropoda are forms with superficially similar shells that can usually be reliably distinguished by anatomical characters. A recent example is the discovery of two conchologically very similar pairs of species that were initially placed in the genus *Xenuroturris* Iredale, 1929, but differ markedly in radular morphology (Kantor *et al*., 2008).

Molecular techniques are now more frequently employed in taxonomic analysis and are revealing numerous cases of cryptic species in all groups, including molluscs (e.g. Williams & Reid, 2004; Collin, 2005; Reid *et al*., 2006; Duda *et al*., 2008; Malaquias & Reid, 2008). Molecular data are now routinely used in combination with shell and anatomical characters for taxonomic purposes and sometimes become the ultimate proof of the existence of separate species.

Cryptic species in molluscs pose significant nomenclatural problems since unambiguous assignment of older type specimens, which in molluscs are nearly always represented by the empty shell (sometimes even severely 'beach worn' and often without good locality data), to one of several forms may be extremely difficult or impossible.

During the course of a broad-scale taxonomic and phylogenetic survey of the superfamily Conoidea, several cases were found where molecular data conflicted with hypotheses based on conventional shell and sometimes even anatomical characters (Puillandre *et al*., 2008). A remarkable example is the

Correspondence: N. Puillandre; e-mail: puillandre@mnhn.fr

 doi:10.1093/mollus/eyp042

genus *Gemmuloborsonia* Shuto, 1989. The genus was established for four fossil species of Turridae from the Plio-Pleistocene of the Philippines, and Late Miocene of Indonesia and Italy (Shuto, 1989). Subsequently, five living species were described from bathyal waters of the Indo-Pacific by Sysoev & Bouchet (1996). *Gemmuloborsonia* was initially assigned to the subfamily Borsoniinae (=Clathurellinae *fide* Taylor *et al*., 1993), but transferred on the basis of radular characters to the Turrinae (Sysoev & Bouchet, 1996). However, recent molecular analyses do not support this result (Puillandre *et al*., 2008).

A molecular analysis of 80 specimens of *Gemmuloborsonia* from the central Indo-Pacific using the cytochrome *c* oxidase subunit I (COI) gene demonstrated the presence in our material of five separate entities, while only four 'morphospecies' could be isolated by visual examination. The two largest groups, representing separate species based on the molecular data, were initially impossible to distinguish by shell characters and it is not surprising that these forms avoided recognition even in the latest taxonomic revision of the genus (Sysoev & Bouchet, 1996). These discoveries led us to make a more detailed examination of the *Gemmuloborsonia* species complex, combining the molecular results with studies of the radula and multivariate analysis of shell form.

MATERIAL AND METHODS

Sampling

A total of 80 living specimens potentially belonging to the genus *Gemmuloborsonia* were collected between 2004 and 2007 in the Philippines, Solomon Islands and Coral Sea (Table 1). Specimens were preserved in 90% or 100% ethanol specifically for molecular analysis by clipping pieces of the head–foot from anaesthetized specimens, thus keeping the shell intact for morphological analyses. All material is deposited in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN).

In order to test the monophyly of the genus *Gemmuloborsonia*, we used as outgroups several species of Turrinae (*Lophiotoma albina* Lamarck, 1822; *Turris babylonia* Linnaeus, 1758; *Gemmula diomedea* E.A. Smith, 1894; *Lucerapex* sp.), and several species belonging to other subfamilies of Conoidea (*Clavus* sp., *Raphitoma* sp., *Conus orbignyi* Kilburn, 1975). Additionally, one specimen included in our samples was identified as *Bathytoma colorata* Sysoev & Bouchet, 2001, but this species is thought to belong to the genus *Gemmuloborsonia* (see Discussion). To test this hypothesis, we also included three specimens of the genus *Bathytoma* Harris & Burrows, 1891. A species of *Harpa* (Neogastropoda: Harpidae) was used as a distant outgroup.

Extraction and sequencing

DNA was extracted from a piece of foot, using 6100 Nucleic Acid Prepstation system (Applied Biosystems). A fragment of 658 bp of the COI mitochondrial gene was amplified using universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994). All PCRs were performed in a volume of 25 µl, containing 3 ng of DNA, 1× reaction buffer, 2.5 mM $MgCl_2$, 0.26 mM dNTP, 0.3 µM of each primer, 5% DMSO and 1.5 U of Q-Bio Taq (MPBiomedicals). Amplifications were performed according to Hebert *et al*. (2003). PCR products were purified and sequenced by a sequencing facility (Genoscope). In all cases, both directions were sequenced to confirm accuracy. For GenBank accession numbers see Table 1.

Phylogenetic analysis

COI sequences were manually aligned, because no ambiguous indels were found. Genetic distances (p-distances) between sequences were calculated using MEGA 3.1 (Kumar, Tamura & Nei, 2004). Phylogenetic reconstructions were conducted using Bayesian Analysis (BA), consisting of two Markov chains (10,000,000 generations each with a sampling frequency of one tree per 1,000 generations) run in four parallel analyses using MrBayes (Huelsenbeck, Ronquist & Hall, 2001). When the log-likelihood scores were found to stabilize, a consensus tree was calculated after omitting the first 25% trees as burn-in. Only the number of nucleotide substitution rates categories (6) was fixed, the other parameters of the substitution model being estimated during the BA.

Fourier analysis

Morphometric analyses were performed in an attempt to identify morphological differences between two groups recognized genetically but indistinguishable morphologically using traditional characters. The shape of the last whorl was analysed by Fourier analysis, as previously described by Puillandre *et al*. (2009).

Shells were placed horizontally, aperture up, and digitized at the same magnification using a macro stand, to reduce possible optical distortions. As the outer apertural lip of some shells was broken, this part of the whorl was not included in the analysis, as shown in Figure 1. Five landmarks were defined, corresponding to adapical and abapical margins of the peripheral keel on both sides of the shell, and to the tip of the siphonal canal (Fig. 1). The five landmarks, as well as the outlines, were digitized using TpsDig (Rohlf, 1996). The same starting point, corresponding to the first landmark, was always used. All pictures and outlines were taken by the same operator (N.P.).

Outlines were used as input for an EFA (Elliptic Fourier Analysis; Dommergues *et al*., 2003; Baylac & Friess, 2005). The five landmarks were used as control points to rotate the outlines into the same orientation. The images were then centred and normalized for size (using square roots of the surface). A visualization of Fourier reconstructions using different numbers of harmonics, compared to the original outline, was used to estimate that 40 harmonics were sufficient to reconstruct the outlines with high accuracy (Fig. 1).

The obtained Fourier coordinates were used in canonical variate analyses (CVAs), using two different grouping variables: (1) the genetic groups as defined by the molecular analysis and (2) the cruise of collection (Table 1). Visualizations of the outline deformations along the canonical axes were made using the procedure described by Monti *et al*. (2001). Assignation of a specimen to one or another genetic group was tested by a 'leave-one-out' cross-validation (1,000 bootstrap replicates). Several type specimens, not preserved in alcohol and thus not available for molecular analysis, were added to the CVAs and assigned successively to each of the genetic groups. All analyses were performed using specially devised MATLABv5.2 functions implemented by Michel Baylac.

Institutional abbreviations: MNHN, Muséum National d'Histoire Naturelle, Paris; NM, Natal Museum, Pietermaritzburg, South Africa; NMNZ, National Museum of New Zealand, Wellington; PPPO-LIPI, Pusat Penelitian dan Pengembangan Oseanologi LIPI, Jakarta, Indonesia; ZMMSU, Zoological Museum of Moscow State University, Moscow.

RESULTS

Eighty specimens were sequenced for COI, resulting in a 658-bp fragment. The Bayesian tree supports the monophyly of *Gemmuloborsonia* [posterior probability (PP) = 0.99], and shows five different groups, numbered from 1 to 5 in Figure 2. Each group includes from 1 to 49 specimens. Groups 3–5, each

Table 1. Identification number (MNHN ID), cruise, station, species identification, percentage of assignation obtained with the CVA for the two groups identified as *Gemmuloborsonia moosai* (percentage provided only when the specimens were assigned to the wrong group) and BOLD (Barcode Of Life Database) and GenBank numbers are given for each specimen.

ID	BOLD	GenBank	Cruise	Species	% wrong group assignation
17849	CONO192-08	EU015658	EBISCO (Chesterfield Islands)	*colorata*	
41918	CONO841-08	FJ462616	Salomon 3 (Solomon Islands)	sp.	
41919	CONO597-08	FJ462589	Norfolk 2 (Norfolk ridge)	*neocaledonica*	
41920	CONO598-08	FJ462588	Norfolk 2 (Norfolk ridge)	*neocaledonica*	
41921	CONO599-08	FJ462587	Norfolk 2 (Norfolk ridge)	*neocaledonica*	
41922	CONO758-08	FJ462590	Salomon 2 (Solomon Islands)	*moosai*	
41923	CONO780-08	FJ462593	Salomon 2 (Solomon Islands)	*moosai*	
41924	CONO822-08	FJ462602	Salomon 3 (Solomon Islands)	*moosai*	
41925	CONO811-08	FJ462595	Salomon 2 (Solomon Islands)	*moosai*	
41926	CONO798-08	FJ462594	Salomon 2 (Solomon Islands)	*moosai*	0.733
41927	CONO812-08	FJ462596	Salomon 3 (Solomon Islands)	*moosai*	
41928	CONO844-08	FJ462619	Salomon 3 (Solomon Islands)	*moosai*	
41929	CONO534-08	FJ462557	Aurora 07 (Philippines)	*clandestina*	
41930	CONO560-08	FJ462583	Aurora 07 (Philippines)	*clandestina*	
41931	CONO559-08	FJ462582	Aurora 07 (Philippines)	*clandestina*	
41932	CONO555-08	FJ462578	Aurora 07 (Philippines)	*clandestina*	0.7025
41933	CONO556-08	FJ462579	Aurora 07 (Philippines)	*clandestina*	
41934	CONO557-08	FJ462580	Aurora 07 (Philippines)	*clandestina*	
41935	CONO558-08	FJ462581	Aurora 07 (Philippines)	*clandestina*	
41936	CONO547-08	FJ462570	Aurora 07 (Philippines)	*clandestina*	0.6995
41937	CONO542-08	FJ462565	Aurora 07 (Philippines)	*clandestina*	
41938	CONO548-08	FJ462571	Aurora 07 (Philippines)	*clandestina*	
41939	CONO549-08	FJ462572	Aurora 07 (Philippines)	*clandestina*	
41940	CONO550-08	FJ462573	Aurora 07 (Philippines)	*clandestina*	
41941	CONO537-08	FJ462560	Aurora 07 (Philippines)	*clandestina*	
41942	CONO546-08	FJ462569	Aurora 07 (Philippines)	*clandestina*	
41943	CONO554-08	FJ462577	Aurora 07 (Philippines)	*clandestina*	
41944	CONO540-08	FJ462563	Aurora 07 (Philippines)	*clandestina*	0.5562
41945	CONO538-08	FJ462561	Aurora 07 (Philippines)	*clandestina*	0.7968
41946	CONO536-08	FJ462559	Aurora 07 (Philippines)	*clandestina*	0.9879
41947	CONO539-08	FJ462562	Aurora 07 (Philippines)	*clandestina*	0.9565
41948	CONO553-08	FJ462576	Aurora 07 (Philippines)	*clandestina*	0.6838
41949	CONO541-08	FJ462564	Aurora 07 (Philippines)	*clandestina*	
41950	CONO535-08	FJ462558	Aurora 07 (Philippines)	*clandestina*	
41951	CONO552-08	FJ462575	Aurora 07 (Philippines)	*clandestina*	
41952	CONO545-08	FJ462568	Aurora 07 (Philippines)	*clandestina*	
41953	CONO544-08	FJ462567	Aurora 07 (Philippines)	*clandestina*	
41954	CONO813-08	FJ462597	Salomon 3 (Solomon Islands)	*moosai*	
41955	CONO814-08	FJ462598	Salomon 3 (Solomon Islands)	*moosai*	
41956	CONO815-08	FJ462599	Salomon 3 (Solomon Islands)	*moosai*	
41957	CONO816-08	FJ462600	Salomon 3 (Solomon Islands)	*moosai*	
41958	CONO817-08	FJ462601	Salomon 3 (Solomon Islands)	*moosai*	
41959	CONO824-08	FJ462603	Salomon 3 (Solomon Islands)	*moosai*	
41960	CONO825-08	FJ462604	Salomon 3 (Solomon Islands)	*moosai*	
41961	CONO826-08	FJ462605	Salomon 3 (Solomon Islands)	*moosai*	0.8906
41962	CONO829-08	FJ462606	Salomon 3 (Solomon Islands)	*moosai*	0.9887
41963	CONO830-08	FJ462607	Salomon 3 (Solomon Islands)	*moosai*	0.6045
41964	CONO831-08	FJ462608	Salomon 3 (Solomon Islands)	*moosai*	
41965	CONO832-08	FJ462609	Salomon 3 (Solomon Islands)	*moosai*	
41966	CONO833-08	FJ462610	Salomon 3 (Solomon Islands)	*moosai*	
41967	CONO834-08	FJ462611	Salomon 3 (Solomon Islands)	*moosai*	
41968	CONO835-08	FJ462612	Salomon 3 (Solomon Islands)	*moosai*	
41969	CONO836-08	FJ462613	Salomon 3 (Solomon Islands)	*moosai*	
41970	CONO837-08	FJ462614	Salomon 3 (Solomon Islands)	*moosai*	
41971	CONO840-08	FJ462615	Salomon 3 (Solomon Islands)	*moosai*	

Continued

Table 1. *Continued*

ID	BOLD	GenBank	Cruise	Species	% wrong group assignation
41972	CONO842-08	FJ462617	Salomon 3 (Solomon Islands)	*moosai*	
41973	CONO843-08	FJ462618	Salomon 3 (Solomon Islands)	*moosai*	
41974	CONO846-08	FJ462620	Salomon 3 (Solomon Islands)	*moosai*	
41975	CONO847-08	FJ462621	Salomon 3 (Solomon Islands)	*moosai*	
41976	CONO848-08	FJ462622	Salomon 3 (Solomon Islands)	*moosai*	0.9973
41977	CONO849-08	FJ462623	Salomon 3 (Solomon Islands)	*moosai*	0.9068
41978	CONO850-08	FJ462624	Salomon 3 (Solomon Islands)	*moosai*	0.9572
41979	CONO851-08	FJ462625	Salomon 3 (Solomon Islands)	*moosai*	
41980	CONO853-08	FJ462626	Salomon 3 (Solomon Islands)	*moosai*	
41981	CONO854-08	FJ462627	Salomon 3 (Solomon Islands)	*moosai*	
41982	CONO855-08	FJ462628	Salomon 3 (Solomon Islands)	*moosai*	
41983	CONO856-08	FJ462629	Salomon 3 (Solomon Islands)	*moosai*	
41984	CONO857-08	FJ462630	Salomon 3 (Solomon Islands)	*moosai*	
41985	CONO858-08	FJ462631	Salomon 3 (Solomon Islands)	*moosai*	
41986	CONO859-08	FJ462632	Salomon 3 (Solomon Islands)	*moosai*	
41987	CONO860-08	FJ462633	Salomon 3 (Solomon Islands)	*moosai*	
41988	CONO861-08	FJ462634	Salomon 3 (Solomon Islands)	*moosai*	
41989	CONO862-08	FJ462635	Salomon 3 (Solomon Islands)	*moosai*	
41990	CONO762-08	FJ462592	Salomon 2 (Solomon Islands)	*moosai*	
41991	CONO759-08	FJ462591	Salomon 2 (Solomon Islands)	*moosai*	
41992	CONO551-08	FJ462574	Aurora 07 (Philippines)	*moosai*	
41993	CONO543-08	FJ462566	Aurora 07 (Philippines)	*moosai*	0.8149
41994	CONO571-08	FJ462584	EBISCO (Chesterfield Islands)	*neocaledonica*	
41995	CONO580-08	FJ462585	EBISCO (Chesterfield Islands)	*moosai*	0.9999
41996	CONO581-08	FJ462586	EBISCO (Chesterfield Islands)	*moosai*	
17700	CONO147-08	EU015643	Vanuatu	*Bathytoma* sp.	
17754	CONO226-08	EU015677	Philippines	*Turris babylonia*	
17756	CONO481-08	EU127882	Vanuatu	*Lophiotoma albina*	
17865	CONO242-08	EU015687	Philippines	*Bathytoma tippetti*	
17890	CONO279-08	EU015713	Philippines	*Raphitoma* sp.	
17902	CONO225-08	EU015676	Philippines	*Clavus* sp.	
17921	CONO296-08	EU015721	Philippines	*Conus orbignyi*	
17929	CONO363-08	EU015742	Solomon Islands	*Bathytoma* sp.	
40569		EU685626	Vanuatu	Harpidae, *Harpa* sp.	
40813	FRANZ270-08	EU820609	Philippines	*Gemmula diomedea*	
42305	CONO570-08	FJ462636	Chesterfield Islands	*Lucerapex* sp.	

including several specimens, are well supported ($PP > 0.95$). Mean genetic distance between groups ranges from 5.6% (between Groups 1 and 3) to 10% (between Groups 1 and 2).

The high genetic distances found between the different groups within *Gemmuloborsonia* are generally interpreted as interspecific distances (see e.g. Hebert *et al.*, 2003; Smith, Fisher & Hebert, 2005; more specifically for molluscs: Mikkelsen, Schander & Willassen, 2007; Malaquias & Reid, 2008; Puillandre *et al.*, 2009). Furthermore, all the groups that include several specimens are reciprocally monophyletic. These two findings suggest that these five entities certainly correspond to different species (Samadi & Barberousse, 2006).

Taxonomic position of studied forms

Group 1: This group is represented by a single specimen identified as *Gemmuloborsonia* sp. (Fig. 3F).

Group 2: Group 2 consists of a single adult specimen from New Caledonia, identified as *Gemmuloborsonia colorata* (Sysoev & Bouchet, 2001) comb. nov. (Fig. 3D–E).

Group 4: This contains four specimens collected off New Caledonia and in all respects similar to *Gemmuloborsonia neocaledonica* Sysoev & Bouchet, 1996 (Fig. 3G).

Groups 3 and 5: These are the most numerous, containing 25 and 49 specimens, respectively. In general shell shape and in multispiral protoconch both groups resemble *Gemmuloborsonia moosai* Sysoev & Bouchet, 1996. The similarity between the two forms is striking; nevertheless the molecular data suggested the presence of two separate species. Both groups are reciprocally monophyletic, and the average genetic distance between them is 6.2%. Moreover, both forms were sympatric in a single dredge haul (400–500 m) from the Philippines. Studies of the radulae (Fig. 4C–D and E–F) revealed no significant differences, nor did standard measurements of the shell.

A Fourier analysis was performed on the 74 specimens included in genetic groups 3 and 5. Using CVA, the two genetic groups are separated along the axis, although not completely (Fig. 5). Among 74 specimens, 16 are not assigned to the correct genetic group (Table 1). Using the collection locality (cruise) as a discriminant variable (Fig. 6), specimens collected during the Salomon 2 cruise are separated from the

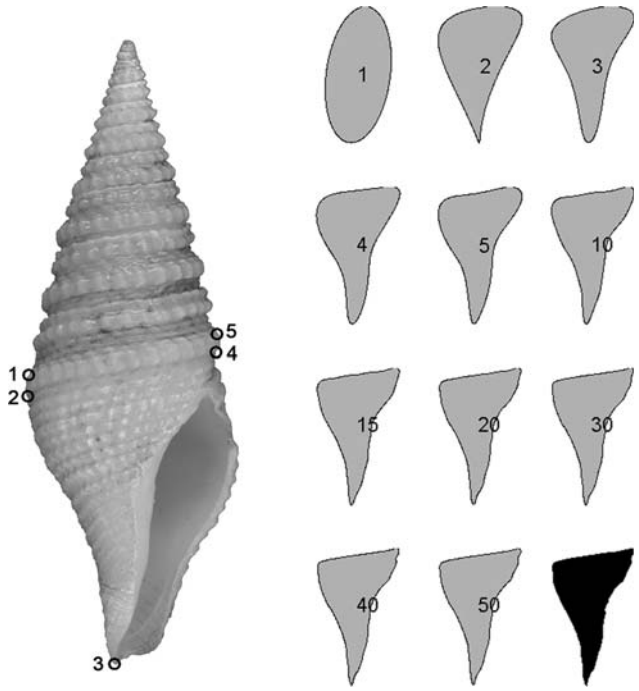

Figure 1. Outline reconstructions with increasing number of harmonics indicated within outlines. In black, original outline. The five landmarks are represented on the shell picture.

others on the first axis (representing 48.07% of the variance). On the second axis (39.44% of the variance), specimens collected during Aurora 07 (Philippines) and Salomon 3 (Solomon Islands) expeditions are discriminated, but the two groups overlap. Results of the CVA obtained both using genetic groups or collection locality as discriminant variable are similar, because genetic group 3 is exclusively from the Philippines, and genetic group 5 is exclusively from the Solomon Islands and Chesterfield Plateau, except for two specimens collected in the Philippines. The axes discriminating genetic groups 3 and 5 (Fig. 5) and specimens from the Solomon Islands and Philippines (Fig. 6) show a contrast between forms with stouter shells with lower last whorls and those forms with more elongated shells and taller last whorls.

The presence of two forms poses the question as to the applicability of names. One form is broadly distributed from the Coral Sea to Solomon Islands and Philippines, while the other is found only in the Philippines. The multivariate analysis demonstrated that the holotype of *Gemuloborsonia moosai* falls within Group 5 (Table 2). Thus, the name *moosai* can be attributed to Group 5.

SYSTEMATIC DESCRIPTIONS

Gemmuloborsonia sp.

(Fig. 3F)

Material examined: Solomon Islands (stn 2850), one live specimen, shell length 25.3 mm (MNHN 41918).

Remarks: The single specimen examined was an immature female. The radula was studied (Fig. 4B) and is typical for the genus. It consists of only 42 rows of teeth, 1.75 mm in length [0.20 of aperture length (AL)]. The marginal teeth are duplex, about 110 μm long (1.25% of AL), with the central formation (Kantor, 2006) nearly rectangular, formed of fused central and lateral teeth. The cusp (=central tooth) is rather weak and narrow.

The specimen lacks a protoconch and is characterized by very prominent, bulging subsutural fold and peripheral keel.

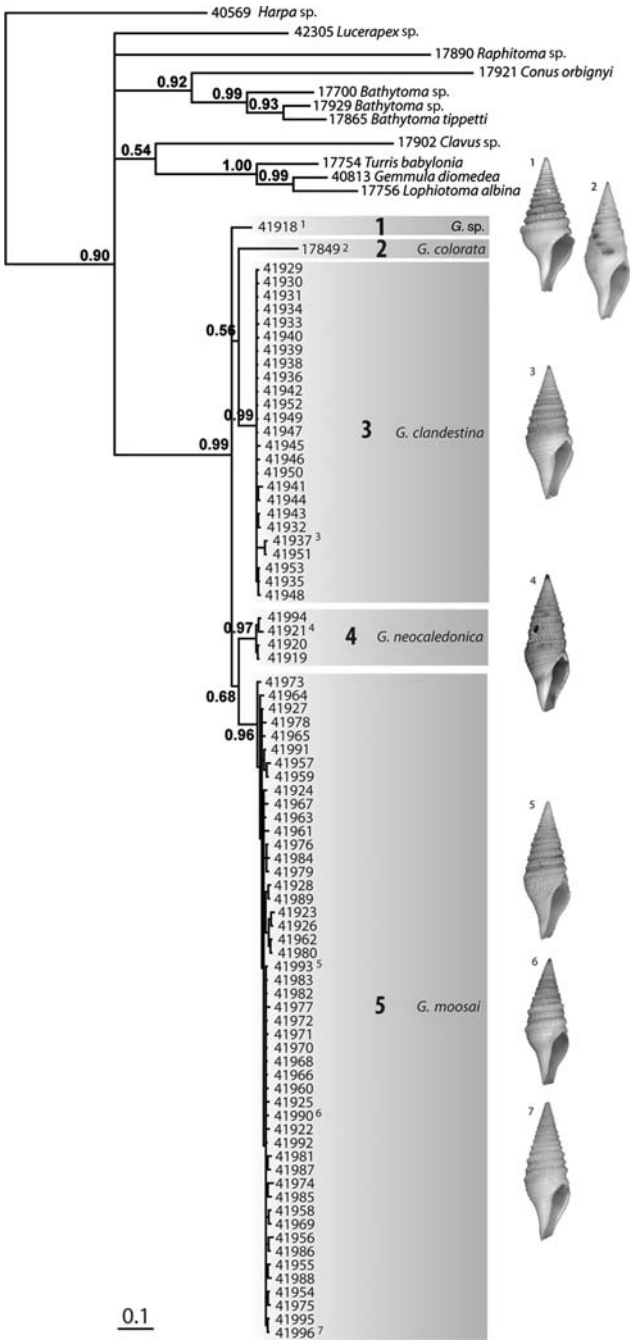

Figure 2. Bayesian tree obtained with the COI gene. Posterior probabilities (above 0.5) are given for each node. Groups are numbered from top downwards from 1 to 5. For each group and for each cruise within a group, one shell is illustrated (numbered from 1 to 7).

Nevertheless, in nearly all studied species of *Gemmuloborsonia* the degree of prominence of fold and keel decreases with age and therefore it is difficult to predict the definitive shell form. Therefore, despite the fact that it is rather distinct from other species we refrain from description of a new species until additional fully grown specimens become available.

Gemmuloborsonia colorata (Sysoev & Bouchet, 2001) comb. nov.

(Fig. 3A–E)

Bathytoma colorata Sysoev & Bouchet, 2001: 294, 296, figs 97–98 (Vanuatu, NE of Tanna, 19°22′S, 169°26′E, 408–410 m,

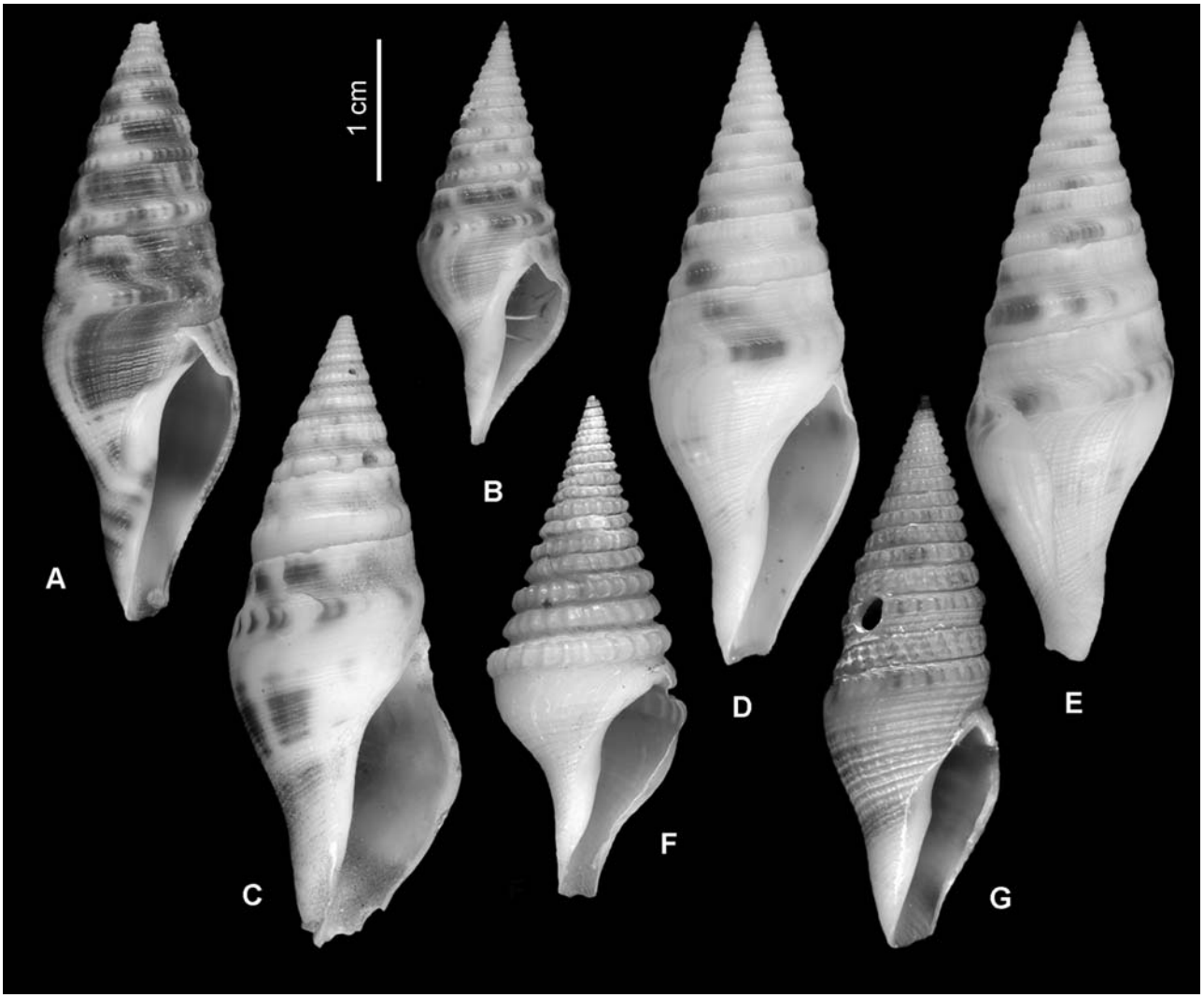

Figure 3. Examined species of *Gemmuloborsonia*. **A–E.** *G. colorata* (Sysoev & Bouchet, 2001). **A.** Holotype, MNHN, shell length 42.5 mm. **B.** Specimen from New Caledonia, Norfolk Ridge, MNHN, shell length 30.0 mm. **C.** Specimen from French Polynesia, E off Rapa, MNHN, shell length 44.7 mm. **D.** Specimen from New Caledonia, Lansdowne Bank, MNHN 17849, shell length 45.5 mm. **F.** Undescribed species, Solomon Islands (stn 2850), MNHN 41918, shell length 25.8 mm. **G.** *G. neocaledonica* Sysoev & Bouchet, 1996, specimen from New Caledonia, Norfolk Ridge (stn 2097), MNHN 41921, shell length 25.2 mm. **A–E,** shells at the same scale; **F, G,** not to scale.

Expedition MUSORSTOM 8, stn CP982; holotype and paratype MNHN).

Material examined: Type material; Lansdowne Bank, 20°06′S, 160°23′E, New Caledonia, 490–550 m (Expedition EBISCO, stn DW 2619, 20 October 2005), one specimen sequenced (MNHN 17849).

Remarks: This species was described in the genus *Bathytoma* (Conidae: Borsoniinae) on the basis of three empty shells from Vanuatu. Due to the lack of data on protoconch and radula the authors did not attribute it to any recognized subgenus of *Bathytoma*. Later, additional material was collected off New Caledonia (Norfolk Ridge), in the Coral Sea (Chesterfield Plateau) and French Polynesia.

The specimen analysed (shell length 45.5 mm) (Fig. 3D, E) was collected alive from the Lansdowne Bank. Conchologically it is very similar to the holotype (Fig. 3A), but differs in the much paler coloration. Similarly coloured specimens have been found in French Polynesia (Fig. 3C), with intermediate ones from New Caledonia (Fig. 3B). The protoconch appears typical of the subfamily Turrinae of Turridae. It is dark brown, multispiral, formed of 3.25 whorls, diameter 790 μm. It consists of *c*. 1.75 smooth whorls of protoconch I and 1.5 whorls of protoconch II covered with arcuate strongly prosocline ribs not reaching the suture below. In general shape, size and ornamentation it is very similar to the protoconchs illustrated and described for *Gemmuloborsonia neocaledonica* Sysoev & Bouchet, 1996 and *G. moosai* Sysoev & Bouchet, 1996.

The radula of *Gemmuloborsonia colorata* (Fig. 4A) is typical for subfamily Turrinae and is extremely similar to that of other examined species of *Gemmuloborsonia*. It is formed of about 60 rows of teeth, 22 immature, 3.75 mm in length (0.23 of AL). Marginal teeth are duplex, about 210 μm long (0.013 of AL). The central formation is rather short, and strongly notched anteriorly. The cusp is strong and curved in profile.

The molecular analysis groups the species unambiguously within *Gemmuloborsonia* (Fig. 2).

The species is widely distributed in the Indo-Pacific from French Polynesia to Vanuatu, New Caledonia and westward to

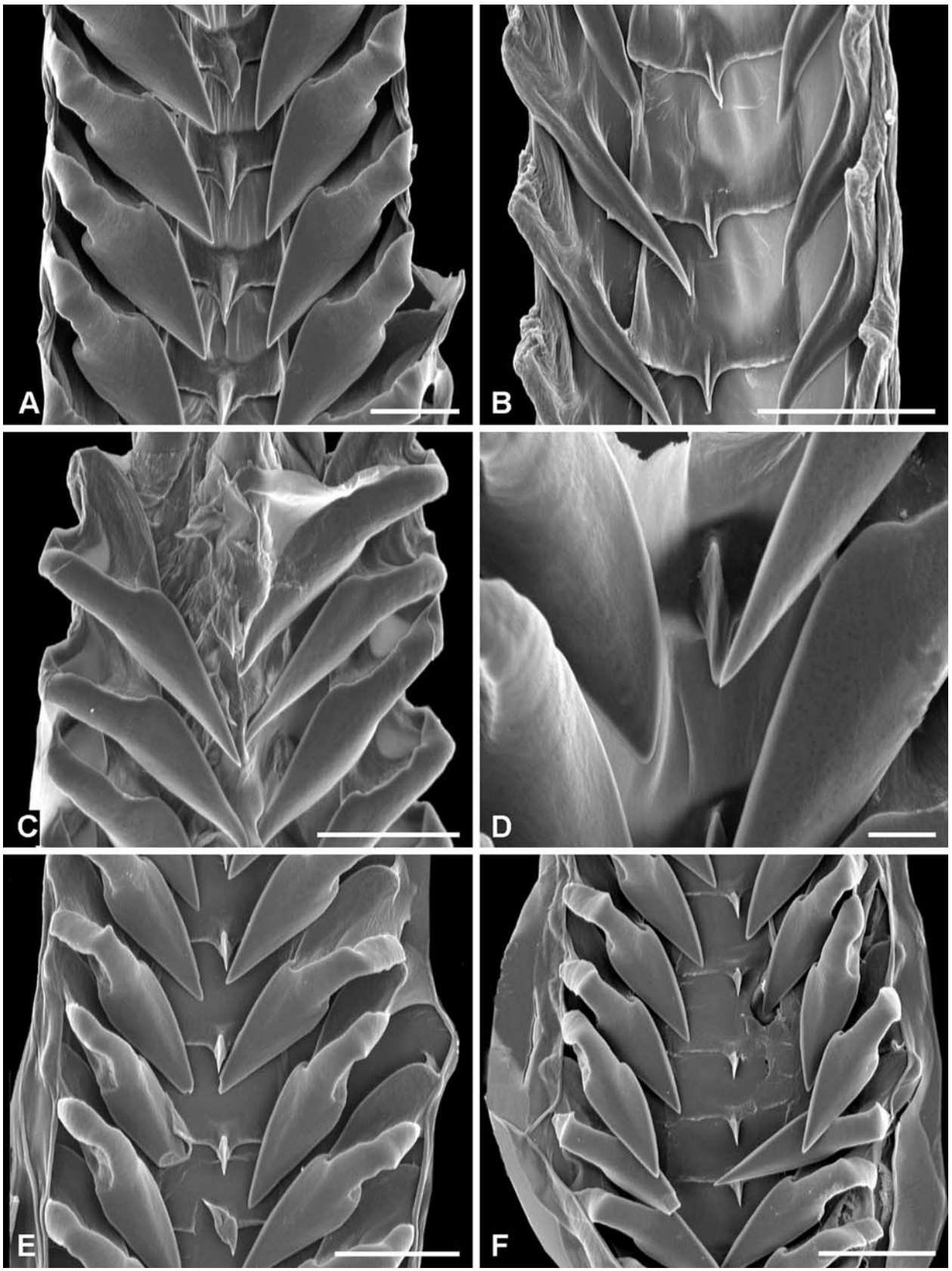

Figure 4. Radula of examined *Gemmuloborsonia* species. **A.** *G. colorata* (Sysoev & Bouchet, 2001), MNHN 17849, shell see Fig. 3D–E. **B.** Undescribed species, Solomon Islands (stn 2850), MNHN 41918, shell see Fig. 3F. **C, D.** *G. moosai* Sysoev & Bouchet, 1996, Philippines, Aurora 07, stn CP2658, MNHN 41993, shell see Fig. 7D. **E, F.** *G. clandestina* sp. nov. **E.** Paratype, MNHN 41952, shell see Fig. 8E. **F.** Paratype, MNHN 41943, shell length 18.7 mm. Scale bars 50 μm, for D – 10 μm.

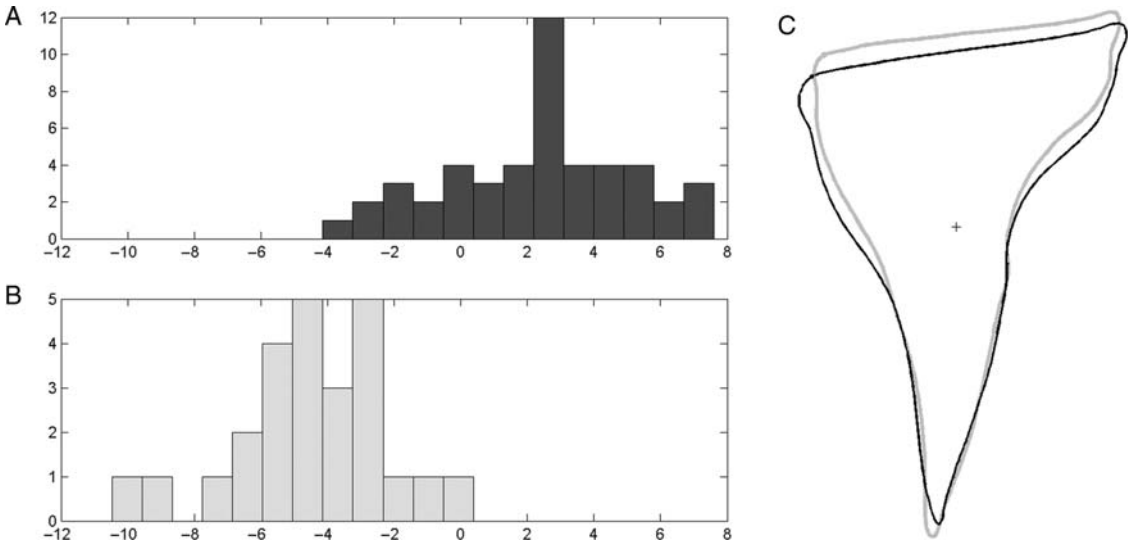

Figure 5. CVA for the two groups identified as *Gemmuloborsonia moosai*, using genetic groups as grouping variable. **A.** Genetic group 1. **B.** Genetic group 2. **C.** Superimposed outlines for minimum (grey line) and maximum (black line) projections onto the axis are represented.

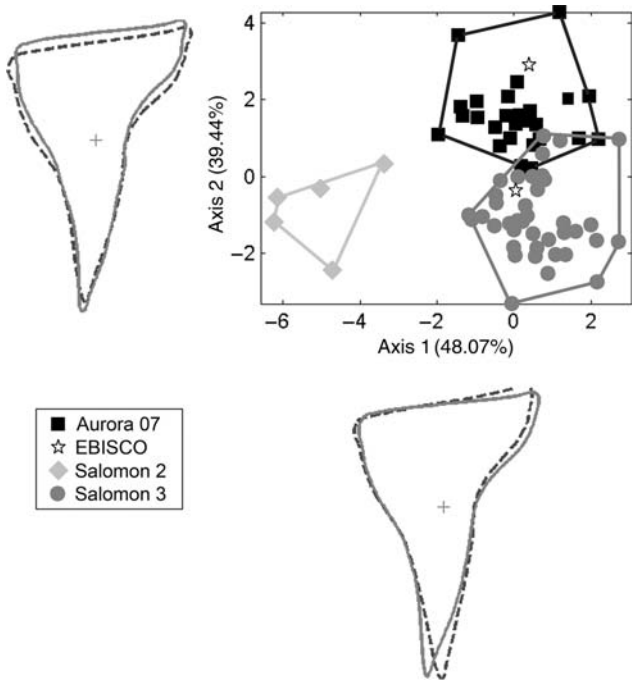

Figure 6. CVA for the two groups identified as *Gemmuloborsonia moosai*, using cruise of collection as grouping variable. Superimposed outlines for minimum (dotted line) and maximum (black line) projections onto the two principal axes are represented.

Madagascar and Reunion. It is illustrated as *Lucerapex indagarotis* (Finlay, 1927) on the website http://vieoceane.free.fr/, dedicated to the molluscs of Reunion Island.

Gemmuloborsonia neocaledonica Sysoev & Bouchet, 1996
(Fig. 3G)

Gemmuloborsonia neocaledonica Sysoev & Bouchet, 1996: 76–78, figs 1, 2A, D, 3A–D (Southern New Caledonia, 24°40′S, 168°38′E, 650 m, Expedition CHALCAL 2, stn DW74; holotype and nine paratypes MNHN, one paratype ZMMSU, one paratype NM, one paratype NMNZ).

Table 2. Assignation of the holotype and the two paratypes of *Gemmuloborsonia moosai* obtained with CVA.

	Locality	*G. moosai*	*G. clandestina*
Holotype	Indonesia	0.982	0.018
Paratype stn CP78	Philippines	0.0003	0.9997
Paratype stn CP118	Philippines	0.0823	0.9177

Material examined: Holotype and nine paratypes in MNHN; see also Table 1.

Remarks: Our specimens match the types and were collected in close proximity to the type locality at similar depths.

The species is distributed in New Caledonia, Loyalty Islands and the southern New Hebrides arc, at depths of 420–550 m.

Gemmuloborsonia moosai Sysoev & Bouchet, 1996
(Fig. 7A–H)

Gemmuloborsonia moosai Sysoev & Bouchet, 1996: 82, 84–85, figs 2C, E, 5A–G (E of Palau Jamdena I., Indonesia, 08°20′S, 132°11′E, 405–399 m, Expedition KARUBAR, stn CP59; holotype and 48 paratypes MNHN, 2 paratypes PPPO-LIPI, 2 paratypes NM, 2 paratypes ZMMSU).

Material examined: Holotype and 48 paratypes in MNHN; see also Table 1.

Remarks: Sysoev & Bouchet (1996) remarked on the high variability of the shell characters, including sculpture, shell outline and relative height of the last whorl. The holotype is one of the most slender specimens examined. Some of the paratypes from Indonesia match well with our specimens collected around the Solomon Islands at similar depths.

The species is extremely similar to *Gemmuloborsonia clandestina* sp. nov. and cannot be distinguished visually. The Fourier analysis of the shell outline showed that in general the specimens of *Gemmuloborsonia moosai* have slightly narrower and slightly taller last whorls. These differences are easily obscured by the shell sculpture. In addition, there is overlap in characters and not all of specimens can be distinguished by the morphometric analysis.

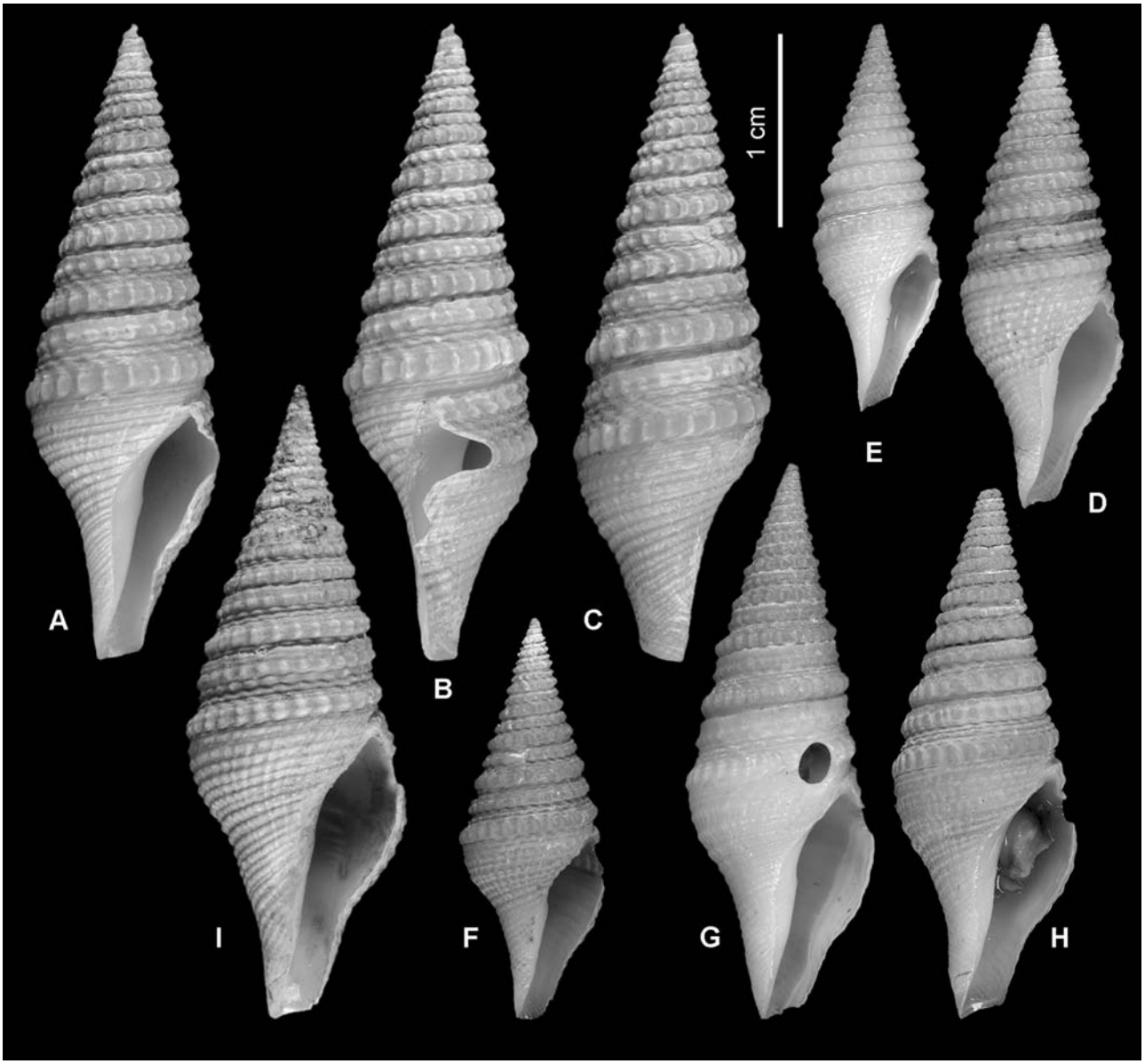

Figure 7. A–H. *Gemmuloborsonia moosai* Sysoev & Bouchet, 1996. **A–C.** Holotype, MNHN, shell length 32.4 mm. **D.** Philippines, Aurora 07, stn CP2658, MNHN 41993, shell length 25.2 mm. **E.** Coral Sea, EBISCO, DW2546, MNHN 41996, shell length 20.1 mm. **F.** Philippines, Aurora 07, stn CP2660, MNHN 41992, shell length 20.9 mm. **G.** Solomon Islands, Salomon 2, stn 2177, MNHN 41922, shell length 28.6 mm. See radula on Fig. 4C, D. **H.** Solomon Islands, Salomon 3, stn 2857, MNHN 41927, shell length 27.4 mm. **I.** *Gemmuloborsonia clandestina* sp. nov. (paratype of *G. moosai*) shell length 32.2 mm.

The two species co-occur in the Philippines and were found in a single dredge haul (stn CP2658). In contrast to the usual phenomenon of character displacement in the zone of overlap between closely related species, these *Gemmuloborsonia* that co-occurred were very similar. Most of the specimens from this station, which were molecularly identified as *G. clandestina* new species, were placed in the wrong group (that is *G. moosai*) by CVA (Table 1).

We examined the radulae of both species, including those of sympatric specimens (Fig. 4C–F). No significant differences of specific value could be found. The radula of *G. moosai* (Fig. 4C, D) is typical for the genus. The marginal teeth are duplex, about 145 μm long (0.0184 of AL), while the central formation is rather short and notched anteriorly. The cusp is strong and curved in profile.

Shell morphometry of the two paratypes of *G. moosai* from the Philippines revealed that they belong to Group 3 (*G. clandestina*) (Table 2).

There is a possibility that the Indonesian population of *G. moosai* (i.e. from the type locality) represents yet another species, separate from Groups 3 and 5. In this case we are dealing with three species, but until material suitably preserved for molecular analysis becomes available from the type locality of *G. moosai*, we prefer to use this name for specimens in Group 5.

Gemmuloborsonia moosai is distributed off the Tanimbar Islands (Banda and Arafura Seas), Indonesia, Philippines (our material) and Solomon Islands (our material). The species was also thought to inhabit the Mozambique Channel, although the specimens from this area differ significantly in

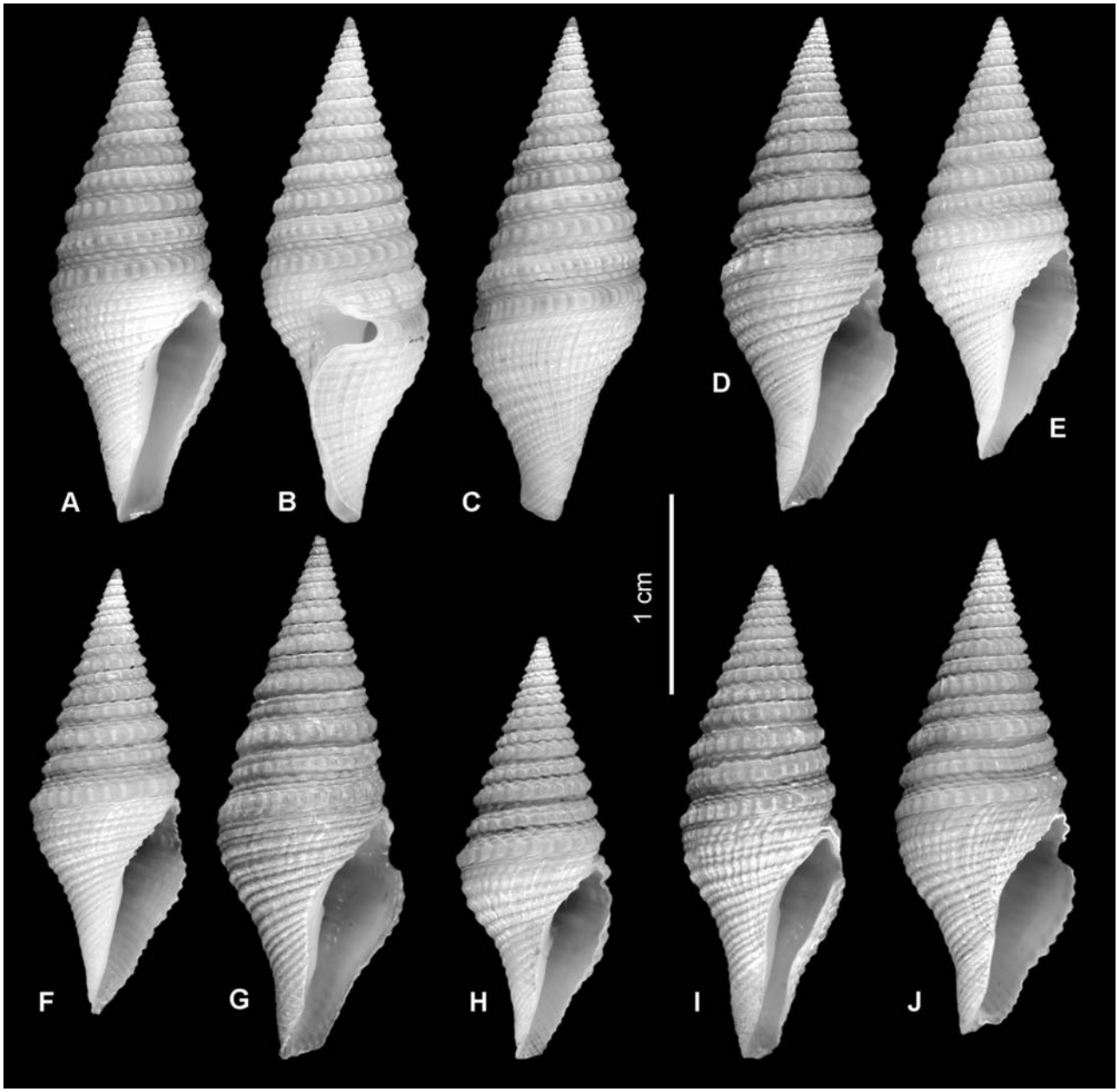

Figure 8. *Gemmuloborsonia clandestina* sp. nov. **A–C.** Holotype, MNHN 41937. **D–J.** Paratypes, stn CP2658. **D.** Shell length 24.9 mm, MNHN 41949. **E.** Shell length 22.4 mm, MNHN 41952. **F.** Shell length 22.3 mm, MNHN 41953. **G.** Shell length 26.3 mm, MNHN 41950. **H.** Shell length 21.4 mm, MNHN 41948. **I.** Shell length 24.8 mm, MNHN 41947. **J.** Shell length 24.8 mm, MNHN 41946. **H–J** were erroneously attributed to *G. moosai* with Fourier analysis (see text).

having a rather different shell outline, in particular the much taller last whorl. They are much more different from the specimens from Indonesia and Solomons than those of *G. clandestina*. We consider that the Mozambique Channel should be excluded from the distributional range area of this species, and that these specimens perhaps constitute a different taxon.

***Gemmuloborsonia clandestina* new species**
(Fig. 8)

Types: Holotype: MNHN 41937; 13 paratypes: MNHN 41941–41953.

Type locality: E of Luzon Island, Philippines, Philippine Sea, 15°58′N, 121°49.1′E, 422–431 m (Expedition Aurora, stn CP2658).

Material examined: Type material; E of Luzon Island, Philippines, 16°00.9′N, 121°51.2′E, 342–348 m (Expedition Aurora, stn CP2657), one live (MNHN 41929); 15°56.4′N, 121°48.9′E, 460–480 m (Expedition Aurora, stn CP2659), one live (MNHN 41936); 15°52.2′N, 121°48.8′E, 506–542 m (Expedition Aurora, stn CP2660), three live (MNHN 41938–41940); 15°01.4′N, 121°44.8′E, 431–493 m (Expedition Aurora, stn CP2673), one live (MNHN 41932); 15°04.1′N, 121°41.1′E, 368–442 m (Expedition Aurora, stn CP2707), three live (MNHN 41933–41935); 15°19′N, 121°33.9′E, 300–318 m (Expedition Aurora, stn CP2727), one live (MNHN 41931); 15°58.1′N, 121°49.2′E, 418–456 m (Expedition Aurora, stn CP2744), one live (MNHN 41930); Philippines, 13°49′N, 120°28′E, 441–550 m (Expedition MUSORSTOM 2, stn CP78), two dead (paratypes of *Gemmuloborsonia moosai* Sysoev &

Bouchet, 1996); Philippines, 11°58′N, 121°06′E, 448–466 m (Expedition MUSORSTOM 3, stn CP118), one dead (paratype of *Gemmuloborsonia moosai* Sysoev & Bouchet, 1996).

Etymology: *clandestinus* – Latin, hidden, concealed, with reference to the extreme similarity of the species to *G. moosai*.

Description (holotype): Shell elongate-biconic, strong, medium-sized, slightly glossy, covered by thin light yellowish smooth periostracum. Protoconch eroded, brown, multispiral, of *c.* 2.6 whorls, diameter 670 μm. Transition from protoconch to teleoconch clearly marked by change in colour. Teleoconch of 9.75 low whorls separated by shallow-channelled suture. Whorls bear well-developed subsutural fold and peripheral keel. Subsutural fold appears on first teleoconch whorls and is narrower than keel, although its relative width progressively enlarges with shell growth. Subsutural fold covered by rounded blunt gemmules, which occupy whole fold on early whorls, but on last and most of penultimate whorl are confined to narrowing cord in middle of fold which becomes more flat on last whorl; there are 39 gemmules on last whorl and 28 on penultimate whorl. On seventh teleoconch whorl the cord at upper edge of subsutural fold appears and over the extent of one whorl this cord is split in two, which become more convex and well developed on last whorl. Peripheral keel bears longitudinally elongate gemmules that are arcuate on last and penultimate whorls; there are 36 gemmules on last whorl and 28 on penultimate whorl. Interspace between subsutural fold and peripheral keel is very narrow and smooth on upper 5 teleoconch whorls; later there appears an initially narrow cord, becoming progressively broader and more pronounced. On last whorl the interspace between fold and keel is broad with three narrow but distinct spiral cords. Body whorl occupies 0.61 of shell length. Periphery of whorl below keel, shell base and canal are covered by narrow granulated cords slightly differing in width. There are in total 22 such cords, with interspaces not exceeding cord width. Aperture is narrow and its width slowly decreases to a broad and obliquely truncated canal. Inner lip smooth and convex in its parietal part and nearly straight in columellar part; covered with thin off-white glossy callus. Weak columellar pleat encircles columellar obliquely. Outer lip projects strongly forward below anal sinus; sinus is deep, U-shaped, slightly adapically directed.

Shell length 25.3 mm, body whorl length 15.6 mm, aperture length 8.5 mm, canal length 3.0 mm and shell diameter 8.5 mm.

Remarks: Although there is clear conchological similarity among all specimens that are attributed to the new species on the basis of molecular data, *G. clandestina* is rather variable in terms of shell outline, especially in terms of shell slenderness. The type locality is remarkable in this respect, since the variability within the type series equals the maximal variability within the species (shell width/shell length ratio varies from 0.34 to 0.40, average 0.36 ± 0.01, $n = 24$).

The radula was examined in three specimens, including two paratypes (Fig. 4E, F). The radula is formed of about 48 (paratype MNHN 41943) to 60 rows of teeth (MNHN 41934), 12–20 nascent, 2.51–2.87 mm in length (0.32–0.34 of AL). The marginal teeth are duplex, about 105–137 μm long (1.75–1.37% of AL) and the central formation is rather short, with the anterior border indistinct and fused with the membrane. The cusp is strong and curved in profile.

The species is extremely similar conchologically to *G. moosai* and some specimens cannot be distinguished even by morphometric analysis. For discussion and comparison see Remarks on the previous species. The holotype of the new species is distinguished from the holotype of *G. moosai* in having a smaller and broader shell. Both species can be readily distinguished by COI sequences.

The species has so far been found only in the Philippines, at depths of 342–542 m.

DISCUSSION

The conventional practice of distinguishing species is to find the gaps in the morphological continuum. Therefore, until discrete differences (at least in some of the parameters) are found, entities are usually not considered as separate species. Prior to use of molecular techniques the status of allopatric forms was, in reality, decided arbitrarily.

In our analysis, two discrete entities, revealed by the DNA analysis, are not morphologically distinguishable. Before conducting time-consuming Fourier analysis we tried the more standard morphometric parameters that are operational for different groups of marine gastropods (Bouchet & Kantor, 2004; Kantor & Bouchet, 2007), but we were not able to delimit the species. Similarly, the radular morphology and gross anatomy did not reveal any significant differences. Finally, Fourier analysis allowed us to attribute most, but not all specimens to one of the two groups.

However, COI sequences clearly suggested that two different species (*Gemmuloborsonia moosai* and *Gemmuloborsonia clandestina*) were included in what was considered before as single '*G. moosai*'. Genetic distances between *G. moosai* and *G. clandestina* are similar to those found between others unquestioned species included in *Gemmuloborsonia* (Fig. 2). Although our conclusion is based on a single gene, it is unlikely that there is gene flow between these two species, because they co-occur sympatrically in the Philippines. We also sequenced a nuclear gene (28S rRNA; results not shown) to test if the genetic differences found in the mitochondrial marker were supported, thus reflecting the species boundaries (see e.g. Nichols, 2001; Funk & Omland, 2003), but all the specimens shared the same 28S sequence. A more variable nuclear marker would test the hypothesis proposed from the results using the COI gene.

The described situation with *G. moosai* and *G. clandestina* is presently very uncommon for molluscs. Although species are now commonly delimited using molecular data (e.g. Meyer & Paulay, 2005; Mikkelsen *et al.*, 2007; Campbell *et al.*, 2008), species limits are most of the time illustrated by morphological differences, known *a priori* or found *a posteriori*. Even when cryptic species are revealed by DNA surveys, morphological differences can usually be identified.

In several cases, morphologically indistinguishable entities have been recognized as separate species in molluscs. One is the recognition of two sister pairs of species of *Bulla* (Bullidae) (Malaquias & Reid, 2008). The other is a recognition of a cryptic speciation of the genus *Bostrycapulus* Olsson & Harbison, 1953 (Calyptraeidae) (Collin, 2005). In both cases the specific status was proved on the basis of molecular data for allopatric forms. Using COI sequences Gittenberger & Gittenberger (2006) demonstrated the existence of several morphologically indistinguishable parasitic species of the genus *Leptoconchus* (Coralliophilidae). In this case, sympatric species inhabited different species of the hosts, scleractinian corals of the family Fungiidae. The authors refrained from formal introduction of 14 recognized new species.

Our case is different from those mentioned above. Firstly, the unexpected diversity was found without any clue from conchology, anatomy or ecology. Secondly, comparison of syntopic specimens of *G. moosai* and *G. clandestina*, found in the same dredge haul, revealed that they are more similar to each other than the specimens of allopatric populations. Most of specimens from that station which molecularly were shown to belong to *G. clandestina* were placed in the wrong group (that is *G. moosai*) by CVA (Table 1). Usually the situation is the opposite and the

sympatric specimens of different species are easier to recognize than those from allopatric populations.

Although we were unable to demonstrate that these species are morphologically discrete, we feel that it is necessary formally to recognize two clades within what was previously considered to be *G. moosai*, as separate species, even if DNA constitutes the only tool to separate them. We agree with Collin (2005) that there is no theoretical reason to expect that mechanisms of speciation should always result in species that can be distinguished visually, especially for recent speciation events.

For the practical purpose of the discrimination of *G. moosai* from *G. clandestina*, only the specimens from the Philippines constitute a problem (as far as our sampling revealed). All of the specimens collected in the Solomon Islands and Chesterfield Plateau belonged to *G. moosai* and 92.6% of the specimens collected in the Philippines were *G. clandestina* (only 2.7% of the specimens were not correctly assigned). Morphological characters failed to distinguish 21.6% of specimens.

Although discriminating morphological (or anatomical) characters were not found in *Gemmuloborsonia* species, this does not mean that some discrete differences might not be identified, most probably by detailed anatomical study. At the same time such an intensive search may not prove to be operational. Empty shells cannot be identified with certainty (at least from Philippines). At present, even the examination of the radulae with the scanning electron microscope (not to mention serial histological sectioning) is probably more costly and labour intensive than molecular sequencing. Molecular analysis is becoming more and more a standard procedure with rapidly decreasing costs that can be performed by personnel without taxonomic expertise.

Current developments in malacology clearly demonstrate that the recognition of cryptic species, indistinguishable by shell characters and anatomy, is becoming a more common phenomenon. This highlights a general problem of traditional taxonomic malacology – the absence of a reliable link to the overwhelming majority of existing name-bearing types of molluscs, which are represented by material unsuitable for molecular sequencing. At present, when describing new species the preference when designating the types is given to well-preserved adult shells. It is advisable that in future preference should be given to specimens for which either a sequence exists, or at least of which the samples are suitable for future molecular analysis.

ACKNOWLEDGEMENTS

The material was collected during several deep-sea cruises of the Tropical Deep Sea Benthos programme as follows. Philippines: the AURORA 2007 cruise on board M/V *DA-BFAR* associated with the National Museum of the Philippines (NMP, co-PI Marivene Manuel), MNHN (co-PI Philippe Bouchet) and BFAR, made possible through a grant from the Lounsbery Foundation. Coral Sea, Norfolk Ridge and Solomon Islands: the EBISCO (PI Philippe Bouchet), SALOMON 1 (PI Bertrand Richer de Forges), SALOMON 2 (PI Philippe Bouchet), SALOMONBOA 3 (PI Sarah Samadi) and NORFOLK 2 (PI Sarah Samadi) cruises, on board R/V *Alis* deployed from Noumea by the Institut de Recherche pour le Developpement (IRD). Marie-Catherine Boisselier and Ellen Strong are thanked for their role in molecular sampling during these expeditions. This work was supported by the Consortium National de Recherche en Genomique and the Service de Systematique Moleculaire of the Museum National d'Histoire Naturelle (IFR 101). It is part of the agreement no. 2005/67 between the Genoscope and the Museum National d'Histoire Naturelle on the project 'Macrophylogeny of Life' directed by Guillaume Lecointre. We are also pleased to thank Michel Baylac and Allowen Evin (Plateforme Morphometrie) for their help in the morphological analysis. Philippe Bouchet helped a lot with his taxonomic expertise at all stages of the project. The work was done during a visiting curatorship of Yu.I.K. at MNHN and he expresses his thanks to Virginie Héros, Barbara Buge, Philippe Maestrati and Pierre Lozouet for assistance during his stay in Paris.

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