



A NEW LINEAGE OF CONOIDEA (GASTROPODA:
NEOGASTROPODA) REVEALED BY MORPHOLOGICAL AND
MOLECULAR DATA

YURI I. KANTOR¹, ELLEN E. STRONG² AND NICOLAS PUILLANDRE^{3,4}

¹*A.N. Severtzov Institute of Ecology and Evolution of Russian Academy of Sciences, Leninski Pros. 33, Moscow 119071, Russia;*

²*National Museum of Natural History, PO Box 37012, Washington, DC 20013-7012, USA;*

³*Museum National d'Histoire Naturelle, Département Systematique et Evolution, UMR 7138, 43, Rue Cuvier, 75231 Paris, France; and*

⁴*Atheris Laboratories, Case Postale 314, CH-1233 Bernex-Geneva, Switzerland*

Correspondence: Y.I. Kantor; e-mail: kantor@malaco-sevin.msk.ru

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ABSTRACT

The hyperdiverse group of venomous Conoidea has eluded attempts to construct a robust and stable classification owing to the absence of a robust and stable phylogenetic framework. New molecular data have greatly enhanced our understanding of conoidean evolution, allowing the construction of a new family-level classification. This expanding framework has also allowed the discovery of several independent lineages that merit recognition at familial rank. One of these, based on seven specimens collected over more than 20 years from deep waters off New Caledonia, represents a unique, monotypic lineage closely related to Mitromorphidae, which we here name as the new family Bouchetispiridae. This new lineage bears a unique combination of teleoconch, protoconch and anatomical characters previously unknown within the Conoidea, including a translucent, fusiform shell with sculpture of strong axial ribs crossed by spiral cords, a multispiral protoconch of only 2.5 whorls with punctate sculpture, hypodermic marginal teeth and a multilayered venom bulb with two layers of muscle separated by connective tissue. This lineage may represent the sole survivor of a previously more diverse clade, or is simply one of many unique taxa that have arisen among the isolated sea mounts off New Caledonia.

INTRODUCTION

The Conoidea are a hyperdiverse group of venomous marine gastropods, representing one of six currently recognized superfamilies within the Neogastropoda. Owing to their exceptional levels of species richness and high levels of homoplasy among features of the shell and anterior alimentary system, the Conoidea have consistently defied attempts to construct a stable classification. These efforts have been thwarted primarily by the absence of a robust phylogenetic framework. In addition to the well-defined and universally recognized Conidae and Terebridae, the heterogeneous assortment of taxa relegated to the Turridae *s. l.* (or ‘turrids’), an acknowledged polyphyletic taxon (Taylor, Kantor & Sysoev, 1993; Puillandre *et al.*, 2008; Tucker & Tenorio, 2009), has posed a particularly daunting challenge. The ‘turrids’ include over 360 Recent valid genera and subgenera and 4,000 named living species (Tucker, 2004), while an estimated 80% of species are yet to be described (Bouchet, Lozouet & Sysoev, 2009). This assemblage remains one of the most intimidating targets in marine malacology, despite their important role in ecosystems and importance in current and future toxicological research (Cabang *et al.*, 2011).

The routine use of molecular data in systematic malacology has contributed enormously towards clarifying the phylogeny and taxonomy of Conoidea, including ‘turrids’. A recently published molecular phylogeny of Conoidea (Puillandre *et al.*, 2011) and corresponding operational classification (Bouchet *et al.*, 2011) were notable breakthroughs. The addition of many new taxa compared to the previous molecular phylogeny (Puillandre *et al.*, 2008) greatly enhanced our knowledge of evolutionary trends within the superfamily. The resulting classification includes 15 families, 13 of which were previously classified as ‘turrids’. Most lineages elevated to family rank had already been defined and recognized as families or subfamilies in previous classifications, such as the one proposed by Taylor *et al.* (1993) based on anatomical and conchological characters. However, some of the taxa recognized by Taylor *et al.* (1993) were merged with other families (e.g. Pseudomelatomidae *sensu* Taylor *et al.*, 1993, merged with Crassispirinae) and the content of many higher taxa was changed by the transfer of numerous genera. One new lineage, the family Horaiclavidae, was identified solely with the insight of molecular data.

When available, molecular data have been particularly useful in resolving the affinities of taxa characterized by

unusual combinations of morphological characters and/or reduction and loss of diagnostic features of the alimentary system. For example, *Toxicochlespira* Sysoev & Kantor, 1990, characterized by an extremely carinated shell similar to *Cochlespira* Conrad, 1865, but possessing hypodermic marginal radular teeth very different from the nonhypodermic, duplex radular teeth of known Cochlespiridae, could not be confidently assigned to any suprageneric taxon (Sysoev & Kantor, 1990). Molecular data unequivocally placed it in Mangeliidae. The latter have very different shell, but similar hypodermic marginal radular teeth. Likewise, the genus *Zemacies* Finlay, 1926 was considered an aberrant genus (lacking radula and venom apparatus) constituting its own subfamily Zemaciinae Sysoev, 2003 within the Turridae (sensu Taylor *et al.*, 1993) that normally possess nonhypodermic radulae. Molecular data demonstrated that *Bathytoma* (Borsoniidae), which possesses a hypodermic radula, is its sister group. However, with the present state of coverage of the molecular dataset (only 87 of 360 genera included), many genera can be only tentatively assigned to family, mostly on the basis of radular characters or sometimes on conchological characters alone (Bouchet *et al.*, 2011). It is clear that among the multitudes of unknown and inadequately described species the affinities of many are waiting to be resolved and undoubtedly many hidden independent lineages remain to be discovered (Bouchet *et al.*, 2009; Puillandre *et al.*, 2011).

Recently, several specimens in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN) from deep waters off New Caledonia were recognized as representing an unusual species of unclear affinity. Conchological characters indicated that it was a neogastropod and examination of the radula demonstrated a position among the Conoidea. More precisely, owing to the presence of hypodermic marginal radular teeth, it could be placed among the Conidae sensu Taylor *et al.* (1993) that united all groups of turrids with hypodermic marginal radular teeth. However, the affinity of this unusual species to any one of the currently defined families was unclear. Several live-collected specimens have allowed morphological and molecular analyses to be carried out, allowing its phylogenetic affinities to be assessed. These data indicate that it represents an independent lineage that cannot be accommodated in any currently recognized taxon of Conoidea.

MATERIAL AND METHODS

Specimens were obtained during a series of cruises between 1985 and 2008 in New Caledonia organized by the MNHN and Institut de Recherche pour le Développement (IRD). The specimen MNHN IM200735029, collected alive and used for the molecular analyses, was sampled during the Norfolk 2 expedition (PI Sarah Samadi), on board the R/V *Alis* deployed from Nouméa by the IRD.

Internal anatomy

The dried body was rehydrated in water and the buccal mass excised and dissolved in dilute bleach. Individual teeth were mounted on a glass cover slip, which was glued to a stub using a carbon adhesive tab. Teeth were examined with a Philips XL-30 Environmental Scanning Electron Microscope at the National Museum of Natural History in Washington, DC, USA.

Serial histological sections of the headfoot were prepared from paratypes MNHN 24504; tissues were embedded in paraplast, sectioned at 6 µm and stained with haematoxylin and eosin-phloxine.

DNA extraction, amplification and sequencing

DNA was extracted from foot tissue of the specimen MNHN IM200735029 using the 6100 Nucleic Acid PrepStation system (Applied Biosystems), following the manufacturer's recommendations. Fragments of the mitochondrial genes 12S rRNA, 16S rRNA and cytochrome oxidase subunit I (COI) were amplified using universal primers 12S1/12SB (Simon, Franke & Martin, 1991; Palumbi, 1996), 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer *et al.*, 1994), respectively. All PCRs were performed in 25 µl, containing 3 ng of DNA, 1 × reaction buffer, 2.5 mM MgCl₂, 0.26 mM dNTP, 0.3 mM each primer, 5% DMSO and 1.5 U of Qbiogene Q-Bio Taq. 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 for 30 s at 54°C, 52 and 50°C for 12S, 16S and COI genes respectively, and extension at 72°C for 1 min. The final extension was at 72°C for 5 min. PCR products were purified and sequenced by a sequencing facility (Eurofins). All genes were sequenced in both directions for increased accuracy. Voucher information and sequences have been deposited in BOLD (CONO1099-10) and in GenBank (JN640292, JN662500, JN662502).

Phylogenetic analyses

Phylogenetic trees were constructed using a subset of taxa analysed in Puillandre *et al.* (2011) (Table 1). As the radula and anatomy of MNHN 24504 indicate that it belongs to the clade of Conoidea with hypodermic radulae (as defined in the molecular analysis by Puillandre *et al.*, 2011), representatives of this clade were included preferentially, while only a few taxa from the clade with nonhypodermic radulae were selected as closely related outgroups.

Sequences were manually (COI) or automatically (16S and 12S) aligned using Muscle online (<http://www.ebi.ac.uk/Tools/msa/muscle/>). Phylogenetic analyses were first performed on each gene separately to check for inconsistency between trees. As trees were mostly congruent, all genes were concatenated in a single dataset. Bayesian Analyses were performed running two parallel analyses in MrBayes (Huelsenbeck, Ronquist & Hall, 2001), each consisting of eight Markov chains of 20,000,000 generations, with a sampling frequency of one tree from each thousand generations. The number of swaps was set to 5 and the chain temperature to 0.02. A different substitution model (each with six substitution categories, a gamma-distributed rate variation across sites approximated in four discrete categories and a proportion of invariable sites) was applied to each of the five unlinked partitions, corresponding to the 12S gene, the 16S gene and each codon position of COI. Convergence of each analysis was evaluated using Tracer v. 1.4.1 (Rambaut & Drummond, 2007), and analyses were terminated when ESS values were all superior to 200. A consensus tree was then calculated after omitting the first 25% trees as burn-in.

RESULTS

Phylogenetic analyses of each molecular partition independently (results not shown), and when analysed together (Fig. 1), robustly support the new species as the sister taxon to the family Mitromorphidae. The latter is a conchologically well-circumscribed assemblage, uniting taxa with stout, coniform to biconical shells. The new species, with its fusiform, translucent and rather fragile shell, cannot be easily accommodated in the current concept of Mitromorphidae. Consequently, we here recognize a new monotypic family of Conoidea for this unique lineage.

Table 1. List of specimens analysed.

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	BOLD ID	GenBank numbers		
						COI	16S	12S
Bouchetispiridae	<i>Bouchetispira</i>	<i>vitrea</i> n. sp.	MNHN IM200735029	Norfolk 2, DW2112, 23°43'S 22°50'S, 168°18'E, 640–1434 m	CONO1099-10	JN640292	JN662500	JN662502
Borsoniidae	<i>Bathytoma</i>	<i>neocaledonica</i> Puillandre <i>et al.</i> , 2010	MNHN IM200717857	EBISCO, CP2551, 21°06'0"S, 158°35'0"E, 637–650 m	CONO187-08	EU015653	HQ401661	HQ401591
	<i>Borsonia</i>	sp.	MNHN IM200717932	Salomon 2, CP2197, 8°24.40'S, 159°22.50'E, 897–1057	CONO350-08	EU015737	HQ401664	HQ401595
	<i>Genota</i>	<i>mitriformis</i> (Wood, 1828)	MNHN IM200742293	Angola, AF7, Pta. Das Lagostas	CONO531-08	HQ401576	HQ401680	HQ401614
	<i>Microdrillia</i>	cf. <i>optima</i> (Thiele, 1925)	MNHN IM200717887	Panglao 2004, T36, 9°29.30'N, 123°51.50'E, 95–128 m	CONO275-08	EU015710	HQ401696	HQ401632
	<i>Tomopleura</i>	<i>reevii</i> (C. B. Adams, 1850)	MNHN IM200717875	Panglao 2004, T26, 9°43.30'N, 123°48.80'E, 123–135 m	CONO255-08	EU015697	HQ401710	HQ401648
Clathurellidae	<i>Clathurella</i>	<i>nigrotincta</i> (Montrouzier, 1872)	MNHN IM200742607	Santo 2006, VM53, 15°31'S, 167°09'E, intertidal	CONO924-08	HQ401575	HQ401666	HQ401599
	<i>Etrema</i>	cf. <i>tenera</i> (Hedley, 1899)	MNHN IM200717869	Panglao 2004, S21, 9°41.70'N, 123°50.90'E, 4–12 m	CONO249-08	EU015691	HQ401675	HQ401608
	<i>Nannodiella</i>	<i>ravella</i> (Hedley, 1922)	MNHN IM200717904	Panglao 2004, T9, 9°33.5'N, 123°49.50'E, 97–120 m	CONO228-08	EU015679	HQ401698	HQ401634
Conidae	<i>Californiconus</i>	<i>californicus</i> (Hinds, 1844)		Monterey, California		FJ868112.1	AF036534.1	FJ868044.1
	<i>Conasprella</i>	<i>pagoda</i> (Kiener, 1845)	MNHN IM200717914	Panglao 2005, CP2380, 8°41.30'N, 123°17.80'E, 150–163 m	CONO313-08	EU015729	FJ868151	FJ868136
	<i>Conus</i>	<i>consors</i> Sowerby I, 1833	MNHN IM200717939	Santo 2006, AT87, 15°32.10'S, 167°16.10'E, 235–271 m	CONO513-08	EU015751	HQ401672	HQ401605
Conorbidae	<i>Profundiconus</i>	<i>teramachii</i> (Kuroda, 1956)		Philippines		JF300171	JF300175	JF300173
	<i>Benthofascis</i>	<i>lozoueti</i> Sysoev & Bouchet, 2001	MNHN IM200742331	Norfolk 2, DW2147, 22°50'S, 167°16'E, 496 m	CONO602-08	HQ401574		HQ401593
Mangeliidae	<i>Anticlinura</i>	sp.	MNHN IM200742513	Salomon 2, CP2182, 8°47'S, 159°38'E, 762–1060 m	CONO788-08	HQ401572	HQ401660	HQ401590
	<i>Benthomangelia</i>	cf. <i>trophonoidea</i> (Schepman, 1913)	MNHN IM200717835	BOA1, CP2462, 16°37.50'S, 167°57.40'E, 618–641 m	CONO148-08	EU015644	HQ401663	HQ401594
	<i>Heterocithara</i>	sp.	MNHN IM200717884	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110 m	CONO271-08	EU015706	HQ401685	HQ401619
	<i>Oenopota</i>	sp.	MNHN IM200742325	Hornsund, Svalbard, 1184-2001,	CONO593-08	HQ401582	HQ401699	HQ401635
	<i>Toxicochlespira</i>	<i>pagoda</i> Sysoev & Kantor, 1990	MNHN IM200717925	Salomon 2, CP2227, 6 37'20"S, 156°12.70'E, 508–522 m	CONO354-08	EU015738	HQ401711	HQ401649
Mitromorphidae	<i>Lovellona</i>	<i>atramentosa</i> (Reeve, 1849)	MNHN IM200742552	Santo 2006, NR8, 15°35.7'S, 167°07.4'E, 11 m	CONO869-08	HQ401580	HQ401692	HQ401628
	<i>Mitromorpha</i>	<i>metula</i> (Hinds, 1843)	MNHN IM200717898	Panglao 2004, B8, 9°37.10'N, 123°46.10'E, 3 m	CONO221-08	EU015672	HQ401697	HQ401633

Raphitomidae	<i>Hemilienardia</i>	<i>calcicincta</i> (Melvill & Standen, 1895)	MNHN IM200717861	Panglao 2004, B14, 9°38.50'N, 123°49.20'E, 2–4 m	CONO232-08	EU015683	HQ401684	HQ401618
	<i>Pleurotomella</i>	sp.	MNHN IM200717848	EBISCO, DW2625, 20°050'S, 160°190'E, 627–741 m	CONO191-08	EU015657	HQ401701	HQ401640
	<i>Kermia</i>	<i>aureotincta</i> (Hervier, 1897)	MNHN IM200717878	Panglao 2004, B25, 9°29.40'N, 123°56.10'E, 16 m	CONO259-08	EU015700	HQ401688	HQ401624
	<i>Raphitoma</i>	<i>rubroapicata</i> (E. A. Smith, 1885)	MNHN IM200717890	Panglao 2004, L49, 9°36.50'N, 123°45.30'E, 90 m	CONO279-08	EU015713	HQ401703	HQ401642
	<i>Taranis</i>	sp.	MNHN IM200742296	Aurora 07, CP2749, 15°57'N, 121°50'E, 743 m	CONO561-08	HQ401584	HQ401707	HQ401645
	<i>Thatcheria</i>	<i>mirabilis</i> (Angas, 1877)	MNHN IM200717924	Salomon 2, CP2184, 8°16.90'S, 159°59.70'E, 464–523 m	CONO349-08	EU015736	FJ868138	HQ401647
Cochlespiridae (O)	<i>Cochlespira</i>	<i>pulchella</i> (Schepman, 1913)	MNHN IM200717920	Panglao 2005, CP2340, 9°29.40'N, 123°44.40'E, 271–318 m	CONO295-08	EU015720	HQ401669	HQ401602
Terebridae (O)	<i>Terebra</i>	<i>textilis</i> Hinds, 1844	MNHN IM200717938	Santo 2006, LD28, 15°35.40'S, 166°58.70'E, 3–8 m	CONO509-08	EU015750	EU685771	EU685478
Turridae (O)	<i>Turris</i>	<i>babylonia</i> (Linnaeus, 1758)	MNHN IM200717754	Panglao 2004, R42, 9°37.10'N, 123°52.60'E, 8–22 m	CONO226-08	EU015677	HQ401715	HQ401652
Harpidae (O)	<i>Harpa</i>	<i>kajiyamai</i> Habe & Kosuge, 1970	MNHN IM200740569	Santo 2006, EP22, 15°37.3'S, 167°05.8E, 78–91 m		EU685626	HQ401683	HQ401617
Strombidae (O)	<i>Laevistrombus</i>	<i>guidoi</i> (Man in't Veld & De Turck, 1998)	MNHN IM200911060	Santo 2006, LR3, 15°35.8'S, 167°06.1'E		HQ401579	HQ401689	HQ401625

Outgroup taxa indicated by (O).

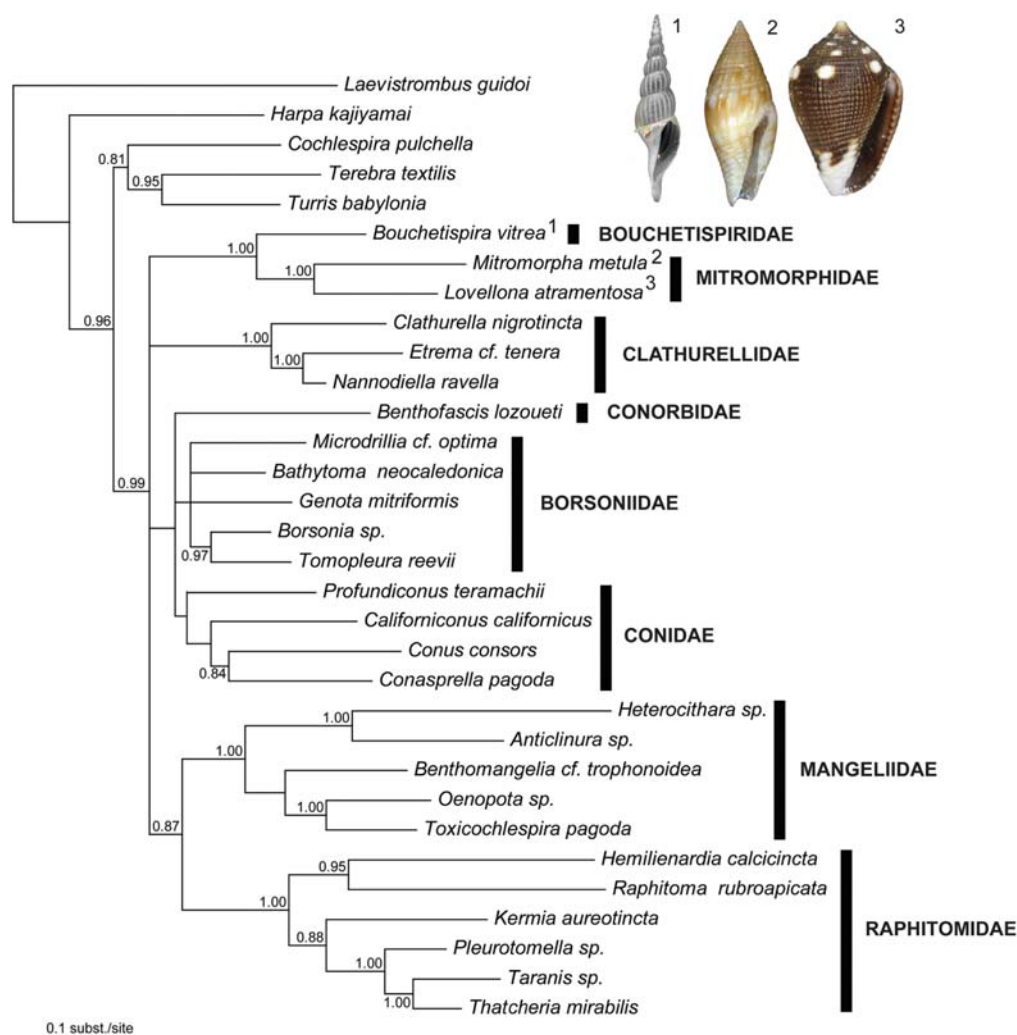


Figure 1. Bayesian phylogenetic tree obtained with the COI, 12S and 16S genes. Posterior probabilities >0.80 are indicated for each node. Shells of Bouchetispiridae and Mitromorphidae are illustrated for comparison.

Clade NEOGASTROPODA Wenz, 1938

Superfamily CONOIDEA Fleming, 1822

Bouchetispiridae new family

Type genus: *Bouchetispira* new genus

Diagnosis: Shell medium in size, reaching roughly 30 mm in adult shell length (SL), elongate fusiform, thin, translucent, with elevated spire and short, poorly differentiated siphonal canal. Whorls evenly rounded, subsutural ramp absent. Axial sculpture of numerous thin orthocline ribs. Spiral sculpture well developed, of numerous densely set cords. Anal sinus on teleoconch whorls not pronounced. Protoconch of about 2.5 whorls, with finely punctate ornament and several strongly sinuous growth lines at transition to teleoconch. Operculum absent. Radula of hypodermic marginal teeth; teeth small, straight, with swollen, solid base and very small lateral basal opening. Venom gland bulb multilayered.

Remarks: There are no described species of Conoidea with a shell comparable to that of the new species, although it superficially appears allied to some Raphitomidae. For example, a thin, transparent shell with sculpture consisting of strong axial

ribs crossed by spiral cords is not uncommon in *Rimosodaphnella* Cossmann, 1915 and *Pleurotomella* Verrill, 1873 (see Bouchet *et al.*, 2011: fig. 9C, J). The new family differs from all known Raphitomidae in the multilayered muscular bulb of the venom gland, which is always single-layered in Raphitomidae (Kantor & Taylor, 2002). In addition, most members of the Raphitomidae possess a characteristic multispiral protoconch with spiral striae on protoconch I and diagonally cancellate sculpture on protoconch II.

The family shares with its sister group, Mitromorphidae, absence of the operculum and similar morphology of the marginal radular teeth. It differs clearly in teleoconch morphology, as described above, and in protoconch morphology, which is multispiral with smooth whorls in Mitromorphidae (Bouchet *et al.*, 2011: fig. 23I).

This combination of shell shape, teleoconch sculpture, morphology of the protoconch and anterior alimentary system anatomy is thus far unknown among conoideans (see also Discussion).

Bouchetispira new genus

Type species: *Bouchetispira vitrea* n. sp. (here designated).



Figure 2. Shells of *Bouchetispira vitrea* n. sp. **A, B.** Holotype, MNHN 24503 (IM200735029), SL 25.1 mm. **C–E.** Paratype 1, MNHN 24504, SL 21.0 mm. **F, G.** Paratype 2, MNHN 24504, SL 26.3 mm. **H, I.** Banc Zorro, stn DW 2073, 25°24'S, 168°19'E, 609 m, SL 21.2 mm. **J, K.** Norfolk Ridge, Banc Kaimon Manu, stn DW 2091, 24°45'S, 168°06'E, 600–896 m (protoconch and radula examined), SL 14.9 mm. All shells to same scale.

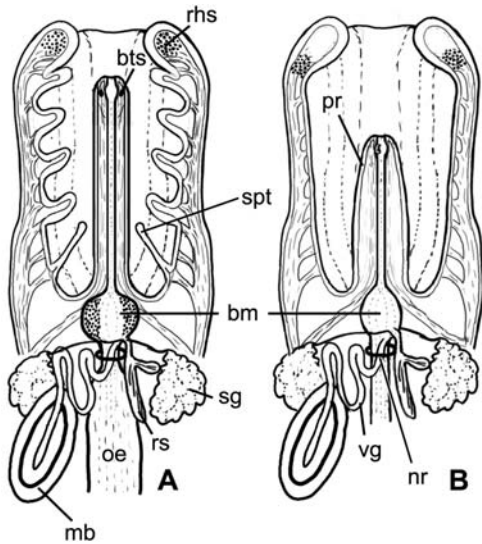


Figure 3. Diagram of foregut morphology. **A.** *Bouchetispira vitrea* n. sp. **B.** *Mitromorpha metula* (Hinds, 1843). Abbreviations: bm, buccal mass; bts, buccal tube sphincter; mb, muscular bulb of venom gland; nr, circumoesophageal nerve ring; oe, oesophagus; pr, proboscis; rhs, rhynchostomal sphincter; rs, radular sac; sg, salivary gland; spt, septum; vg, venom gland. Not to scale.

Etymology: The genus is named in honour of Philippe Bouchet, leader of malacological studies at the MNHN, who has invested many years of his life in leading research on molluscs of the tropical Pacific.

Diagnosis: As for family.

Distribution: Thus far, known only from the New Caledonia region.

***Bouchetispira vitrea* new species**

(Figs 2, 3A, 4A–D, 5)

Etymology: *Vitrum* (Latin), glass; the name reflects the glassy appearance of the shell.

Type material: MNHN 24503 (holotype, sequenced specimen IM200735029); MNHN 24504 (two live-collected paratypes, 23°10'S, 167°10'E, 680 m, BIOCAL, stn DW 33). Type locality: Norfolk Ridge, southern New Caledonia, 23°44'S, 168°18'E, 640–1434 m (NORFOLK 2, stn DW 2112).

Material examined: Norfolk Ridge, New Caledonia: NORFOLK 2: Banc Zorro, stn DW 2073, 25°24'S, 168°19'E, 609 m (one dead shell); Banc Kaimon Manu, stn DW 2091, 24°45'S, 168°06'E, 600–896 m (one live). Loyalty Ridge, New Caledonia: TERRASES: stn DW 3031, 22°39'S, 168°58'E, 720 m (two dead shells).

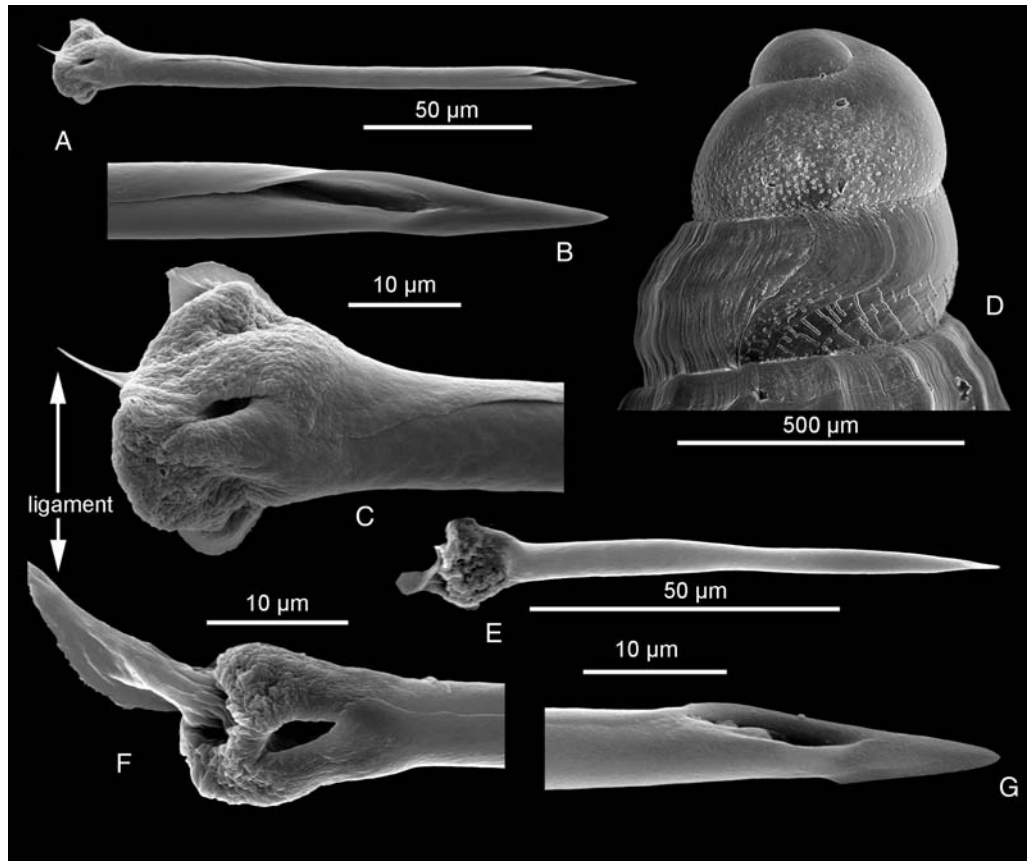


Figure 4. A–D. Radulae and protoconch of *Bouchetispira vitrea* n. sp. A–C. Separate marginal tooth, Norfolk Ridge, Banc Kaimon Manu, stnDW 2091, 24°45'S, 168°06'E, 600–896 m. B. Enlarged tooth tip. C. Enlarged tooth base. D. Protoconch of same specimen. E–G. Separate marginal tooth of *Anaritmia* sp. (Mitromorphidae), MNHN, Philippines, PANGLAO 2004, stn S5, 09°37.1'N, 123°46.1'E, 2–4 m, SL 6.9 mm.

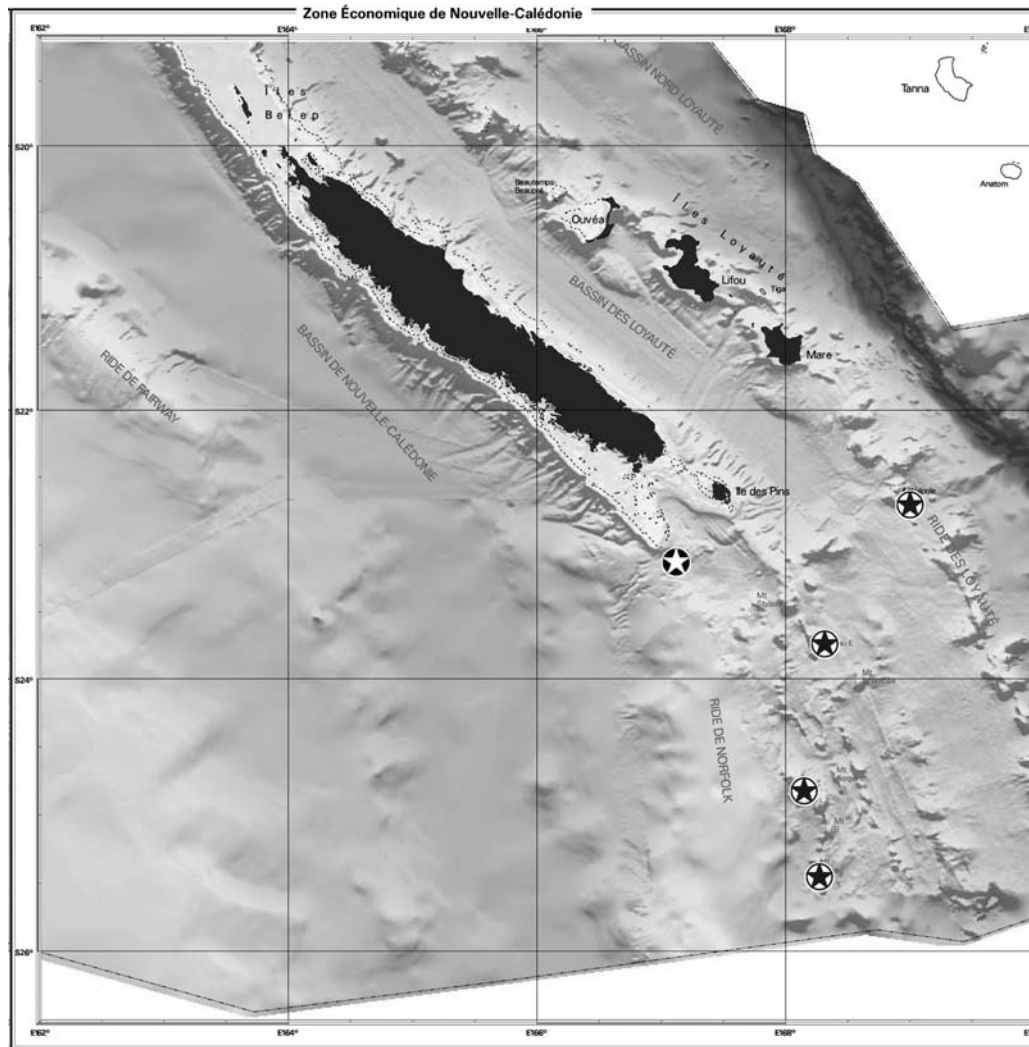


Figure 5. Distribution of *Bouchetispira vitrea* n. sp. White star, type locality; black stars, examined material.

Shell. Description of holotype (Fig. 2A–B): SL 25.1 mm, last whorl length 14.0 mm; impossible to measure aperture length (AL) and shell diameter due to incomplete outer aperture lip. Shell fusiform, very thin, translucent, fragile, medium-sized, glossy, with attenuated base and long, narrow, slightly twisted siphonal canal. Protoconch multispiral, partially broken. Teleoconch of 9+ convex rounded whorls separated by deeply impressed suture. Sculpture of narrow but solid axial ribs and thin but distinct spiral cords. Axial ribs slightly arcuate, proscyrt on early teleoconch whorls and orthocline on penultimate and last whorls. First teleoconch whorl with 15 axial ribs, penultimate whorl with 22 ribs. Outer lip broken, only part of last whorl remaining. Interstices between ribs two to three times wider than ribs. Ribs extending from suture to suture on spire whorls and to canal. Anal sinus very shallow, subsutural, pronounced on upper teleoconch whorls and absent on lower ones. Narrow, distinct rounded spiral cords cover entire shell, separated by interstices 0.5–2 times wider than cords. Seventeen cords on penultimate whorl and 40 on last whorl, 8 of which on canal. Last whorl comprising 0.56 of SL. Aperture narrow-ovate, inner lip smooth, convex along parietal part and nearly straight along columellar part. Lip covered with thin, off-white, glossy callus. Outer lip thin, evenly rounded, concave at transition to canal.

Only one intact protoconch examined (shell in Fig. 2J, K), multispiral, globose, comprising roughly 2.5 whorls (Fig. 4D). Ornament on initial two whorls finely punctate, with thin, irregular, opisthocline ribblets on lower part of last three-quarter whorl. Diameter 570 μm , height 570 μm . Transition from protoconch to teleoconch marked by three strongly sinuous growth lines.

Gross anatomy. Headfoot of paratype (shell in Fig. 2C–E): Head swollen, bulbous, on distinct neck. Tentacles short, stout, conical, widely separated. Small eyes present at outer tentacle bases. Foot tapering posteriorly, with transversely folded sole, operculum absent. Small venom gland and relatively large muscular bulb seen through body wall by transparency.

Due to poor preservation, only gross anatomical observations were possible using histological sections (Fig. 3A). Proboscis rather long, folded within rhynchodaeum. Rhynchodaeum strongly folded, apparently forming septum surrounding proboscis base. Buccal mass short, with thick muscular walls, situated outside contracted proboscis immediately in front of nerve ring. Oesophagus muscular, expanding greatly after passing through nerve ring. Venom gland of uniform histology along entire length. Muscular bulb of venom gland thick-walled, composed of two layers of longitudinal muscle fibres; muscle layers of unequal but similar thickness, separated by

thin, distinct layer of connective tissue. Salivary gland(s?) medium-sized, acinous.

Radula examined in one specimen (shell in Fig. 2J, K), of hypodermic marginal teeth. Teeth small (roughly 170 μm in length, or 2.8% of AL and 1% of SL) (Fig. 4A–C), straight, with swollen base. Tooth canal opening laterally on tooth tip; apical opening large, oval. Basal opening very small, oval, lateral. No barbs or blade. Teeth connected to vestigial subradular membrane by narrow ligament.

Distribution (Fig. 5): Norfolk and Loyalty Ridges, live at 600–896 m. Holotype collected in a dredge haul recorded from an unusually large depth range (640–1434 m). Since it is not possible to specify the exact collecting depth, the greater depth is not confirmed.

Remarks: Paratypes and other studied specimens are very similar to holotype in sculpture. The outer aperture lip is easily broken, altering overall shell outline and producing a more slender appearance of the holotype.

DISCUSSION

The rapidly expanding molecular framework for Conoidea has provided many new insights and has fundamentally changed our understanding of conoidean relationships, classification and evolutionary dynamics. Conoidea are well known for a high rate of homoplasy among shell characters and their uncanny ability to impersonate many families of Neogastropoda. Accordingly, many species of various neogastropod families were originally described as conoideans, or ‘turrids’, including some *Exilia* (Ptychactractidae), *Belomitra* and *Antimitra* (Buccinidae) and *Daphnellopsis* (Muricidae) (for more examples, see Bouchet *et al.*, 2011: appendix 1). In addition, unrelated conoideans sometimes possess extremely similar shell (and sometimes radular) morphologies, rendering the higher placement of genera (and species) virtually impossible to establish with certainty. However, new molecular characters and the independent estimate of phylogeny that they provide (Puillandre *et al.*, 2011) have revealed many unexpected relationships, including one other new family (Horaiclavidae; Bouchet *et al.*, 2011), similarly representing a previously unrecognized independent lineage. Conchologically and in radular morphology, horaiclavids are most similar to members of the Pseudomelatomidae, but molecular data reveal them to be the sister group of the conchologically rather dissimilar Clavatulidae.

This new molecular framework has also contributed to the elevation of a number of conoidean taxa to family rank. With Bouchetispiridae, the number of families has more than doubled compared to previous classifications (Taylor *et al.*, 1993; Bouchet & Rocroi, 2005) and now includes 16 families. However, given that conoideans encompass more than 10,000 species, this result should come as no surprise. This is approximately the same species richness as in birds, a clade of similar age, which include more than 200 different families (wikipedia.org/wiki/List_of_birds). Of course, there are many reasons why the ranks of birds are so inflated that have nothing to do with age or diversity, but arguably the increasing number of families in Conoidea reflects the increased attention they are now receiving and the enhanced understanding of relationships this attention is providing. Moreover, a number of genera in the molecular analysis of Puillandre *et al.* (2011) could not be confidently attributed to any proposed family, including *Cruziturricula* Marks, 1951, *Fusiturricula* Woodring, 1928, *Leucosyrinx* Dall 1889, *Gemmuloborsonia* Shuto, 1989 and *Lucerapex* Iredale, 1936, each constituting its own branch. No new suprageneric taxa were proposed because: (1) the type

species of the corresponding genera were not available for sequencing, and/or (2) position of these branches was not robustly supported. In addition, there are a significant number of unstudied species (and genera) that possess similar shells, and it is highly likely that, with additional material, new taxa belonging to the same lineages will be found and relationships with other families more accurately defined. Consequently, the number of family-group taxa can be expected to increase in the future.

In the case of *Bouchetispira* the situation is different. The combination of shell, radula, anatomy and DNA of this new genus is so distinct and different from known conoideans that finding new genera and species belonging to this clade among the known fauna seems very improbable. Additionally, it was possible to establish clearly the phylogenetic position of *Bouchetispira*, as sister group of the family Mitromorphidae, only with the addition of molecular characters. This result is also supported by radular morphology, the two families sharing hypodermic marginal teeth with large swollen bases, a tooth canal with a small lateral basal opening and a rather large lateral oval apical opening, absence of barbs and presence of a ligament (Fig. 4E–G). The relative sizes of individual teeth are also similar. In *Bouchetispira* the marginal tooth length constitutes about 2.8% of AL and 1% of SL, while in three studied species of Mitromorphidae [*Lovellona atramentosa* (Reeve, 1849), *Mitromorpha metula* (Hinds, 1843) and *Anarithma* sp.] tooth length is 1.7–3.6% of AL and 1.3–1.9 of SL. Mitromorphidae, although poorly studied, are anatomically distinct from Bouchetispiridae (Fig. 3). The proboscis is relatively much shorter and broader and not folded. The rhynchostomal sphincter is small and shifted posteriorly, while it is anterior in *Bouchetispira*. Walls of the rhynchodaemum are smooth in *Mitromorpha* and strongly folded in *Bouchetispira*. The septum surrounding the proboscis base, as well as the small buccal tube sphincter, is present in the latter and absent in the former. In *Mitromorpha* instead of the sphincter there is a pad of epithelial cells in the anterior part of the buccal tube, to which the base of the marginal tooth adheres (Taylor *et al.*, 1993).

Nothing in the shell suggests any relationship to Mitromorphidae (Fig. 1). As discussed above, based only on teleoconch morphology, a relationship with Raphitomidae (Bouchet *et al.*, 2011: fig. 9) would be more easily accommodated. However, the protoconch in the latter is typically multi-spiral and characteristically diagonally cancellate. In *Bouchetispira* the wall of the muscular bulb of the venom gland consists of two layers of muscles separated by a connective tissue layer, while in all studied Raphitomidae it always has a single layer of muscle fibres and no connective tissue (Kantor & Taylor, 2002). Overall, the studied anatomical features of the new species appear to be plesiomorphic, lacking distinctive characteristics even in the foregut; similar foregut arrangements are found in many conoideans with hypodermic teeth. Once again, this result highlights the difficulty in reliably assessing higher taxonomic affinities of Conoidea when only morphological characters are available.

Thus far, this lineage is represented only by a single species found on seamounts or banks of the Norfolk Ridge and in a single locality on the Loyalty Ridge. The species is rare in collections, represented by only seven specimens collected during several expeditions over the course of more than 20 years. It has only been obtained in dredge hauls, indicating it is associated with rugged terrain and/or hard substrates, which are difficult to sample.

It is difficult to speculate on the origin of this unique and distinctive conoidean lineage restricted to such a small geographic area of the Pacific Ocean. Two hypotheses can be proposed. It could represent the sole survivor of an ancient

radiation. Alternatively, it is known that the fauna of sea mounts is highly endemic (e.g. Richer de Forges, Koslow & Poore, 2000), and the Norfolk Ridge in particular is known to harbour an especially diverse and spectacular assortment of taxa. This new lineage could represent one of many that have arisen in this unique, complex and isolated habitat, which has evidently promoted the divergence of many such lineages found nowhere else. Therefore it is still possible that additional closely related species and/or genera are waiting to be discovered in these underexplored, rugged, submerged mountain habitats around New Caledonia. It is also possible that related species will be found in other parts of the Pacific that will change our perception of distribution and evolution of this unique lineage.

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REFERENCES

- BOUCHET, P., KANTOR, YU.I., SYSOEV, A. & PUILLANDRE, N. 2011. New operational classification of the Conoidea (Gastropoda). *Journal of Molluscan Studies*, **77**: 273–308.
- BOUCHET, P., LOZOUET, P. & SYSOEV, A.V. 2009. An inordinate fondness for turrids. *Deep-Sea Research II*, **56**: 1724–1731.
- BOUCHET, P. & ROCROI, J.-P. 2005. Classification and nomenclator of gastropod families. *Malacologia*, **47**: 1–397.
- CABANG, A.P., IMPERIAL, J.S., GAJEWIAK, J., WATKINS, M., CORNELI, P.S., OLIVERA, B.M. & CONCEPCION, G.P. 2011. Characterization of a venom peptide from a crassispirid gastropod. *Toxicon*, **58**: 672–680.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.
- HUELSENBECK, J.P., RONQUIST, F. & HALL, B. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, **17**: 754–755.
- KANTOR, YU.I. & TAYLOR, J.D. 2002. Foregut anatomy and relationships of raphitomine gastropods (Gastropoda: Conoidea: Raphitominae). In: *Systematics, Phylogeny and Biology of the Neogastropoda* (M. Oliverio & R. Chemello, eds). *Bollettino Malacologico, Supplement 5*: 161–174.
- PALUMBI, S. 1996. Nucleic acids II: the polymerase chain reaction. In: *Molecular systematics* (D. Hillis, C. Moritz & B.K. Mable, eds), pp. 205–247. Sinauer Associates, Sunderland, MA.
- PUILLANDRE, N., KANTOR, YU.I., SYSOEV, A., COULOUX, A., MEYER, C., RAWLINGS, T., TODD, J.A. & BOUCHET, P. 2011. The dragon tamed? A molecular phylogeny of the Conoidea (Gastropoda). *Journal of Molluscan Studies*, **77**: 259–272.
- PUILLANDRE, N., SAMADI, S., BOISSELIER, M.-C., SYSOEV, A.V., KANTOR, Y.I., CRUAUD, C., COULOUX, A. & BOUCHET, P. 2008. Starting to unravel the toxoglossan knot: molecular phylogeny of the "turrids" (Neogastropoda: Conoidea). *Molecular Phylogenetics and Evolution*, **47**: 1122–1134.
- RAMBAUT, A. & DRUMMOND, A.J. 2007. Tracer v1.4. Available from <http://beast.bio.ed.ac.uk/Tracer>.
- RICHER DE FORGES, B., KOSLOW, J.A. & POORE, G.C.B. 2000. Diversity and endemism of the benthic seamount fauna in the southwest Pacific. *Nature*, **405**: 944–947.
- SIMON, C., FRANKE, A. & MARTIN, A. 1991. The polymerase chain reaction: DNA extraction and amplification. In: *Molecular techniques in taxonomy* (G.M. Hewitt, A.W.B. Johnson & J.P.W. Young eds), pp. 329–355. Springer-Verlag, New York.
- SYSOEV, A.V. & KANTOR, YU.I. 1990. A new genus and species of "Cochlespira-like" turrids (Gastropoda, Toxoglossa, Turridae). *Apex*, **5**: 1–6.
- TAYLOR, J.D., KANTOR, YU.I. & SYSOEV, A.V. 1993. Foregut anatomy, feeding mechanisms, relationships and classification of Conoidea (=Toxoglossa) (Gastropoda). *Bulletin of the Natural History Museum, London (Zoology)*, **59**: 125–169.
- TUCKER, J.K. 2004. Catalogue of Recent and fossil turrids (Mollusca: Gastropoda). *Zootaxa*, **682**: 1–1295.
- TUCKER, J.K. & TENORIO, M.J. 2009. *Systematic classification of Recent and fossil conoidean gastropods*. Conchbooks, Hackenheim, Germany.