

THE DRAGON TAMED? A MOLECULAR PHYLOGENY OF THE CONOIDEA (GASTROPODA)

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ABSTRACT

The superfamily Conoidea constitutes one of the most diverse and taxonomically challenging groups among marine molluscs. Classifications based on shell or radular characters are highly contradictory and disputed. Whereas the monophyly of the Conidae and Terebridae has not been challenged, the other constituents of the superfamily are placed in a 'trash' group, the turrids, the non-monophyly of which has been demonstrated by anatomical and molecular evidence. We present here a new molecular phylogeny based on a total of 102 conoidean genera (87 'turrids', 5 cones and 10 terebrids) and three mitochondrial genes [cytochrome oxidase I (COI), 12S rRNA and 16S rRNA]. The resulting tree recognizes 14 clades. When the Conidae (*Conus s.l.*) and Terebridae are ranked as families for consistency of usage, the 'turrids' must be split into 12 families of comparable rank. A new genus-level classification of the Conoidea is published in an accompanying paper.

INTRODUCTION

Taxonomy, including phylogeny, is often considered as the cornerstone of the biological building, providing a foundation indispensable for ecological, biochemical or physiological analyses. However, such studies cannot wait indefinitely for a fully resolved evolutionary hypothesis, and studies are conducted frequently on groups in which the limits of the taxa and their relationships are not clearly delineated. This is particularly true for the hyperdiverse superfamily Conoidea (Bouchet, Lozouet & Sysoev, 2009), the crown clade of the caenogastropods that includes the famous genus *Conus*. There is an abundant literature dealing with the venoms and toxinology of the Conoidea, or Toxoglossa (e.g. Imperial *et al.*, 2003, 2007; Lopez-Vera *et al.*, 2004; Watkins, Hillyard & Olivera, 2006; Heralde *et al.*, 2008; Rojas *et al.*, 2008), and several toxin-derived compounds are currently applied (or should be soon) for medical purposes (see Olivera, 2006 and references therein). The classification of Taylor, Kantor & Sysoev (1993; and in slightly modified form in Bouchet & Rocroi, 2005) has already abolished the restricted *Conus*-centred view that prevailed before and triggered an extension of toxinological research previously confined to *Conus* to other conoidean taxa. With the promise of new drug discoveries, biochemists now call for a clear phylogenetic context for this group (Biggs *et al.*, 2010), particularly within the genus *Conus*, but also in the promising Terebridae and 'turrids'.

Of the constituent taxa that form the Conoidea, Conidae *s.s.* (= *Conus s.l.*) and the Terebridae have been shown to be monophyletic (Espiritu *et al.*, 2001; Duda & Kohn, 2005; Holford *et al.*, 2009). The third component, the 'Turridae', basically defined as conoideans that are neither terebrids nor members of *Conus*, has since been understood to be non-monophyletic, although several discrete subgroups of 'turrids'

have also been shown to be well-supported clades (e.g. Heralde *et al.*, 2007, 2010). With over 360 Recent valid genera and subgenera and 4,000 named living species (Tucker, 2004), the 'turrids' *sensu lato* have resisted several decades of attempts of organization by morphologists and they form an intimidating Gordian knot in conoidean systematics.

A first molecular approach to the phylogeny of Conoidea was attempted by Puillandre *et al.* (2008). Their results broadly supported the Taylor, Kantor & Sysoev (1993) classification, especially the monophyly of an extended family Conidae including the genus *Conus* ('a conoidean genus amongst others'); they also showed a close relationship between the Terebridae and the Turridae *s. s.* and the polyphyly of several subfamilies. However, although this initial attempt included 57 genera, several nominal families and subfamilies were not represented in the analysis and many relationships were weakly supported. In this new study, 102 genera are included, almost doubling the previous dataset, and representatives of families and subfamilies missing in earlier analyses have been added. Two other mitochondrial genes have been sequenced (12S and 16S) in addition to Cytochrome Oxidase I (COI). This extended taxonomic and sequence dataset results in a more robust phylogeny of the Conoidea and serves as the basis for a new classification published in conjunction with the present paper (Bouchet *et al.*, 2011).

MATERIAL

Sampling

Most specimens (Table 1) were collected during cruises organized between 2004 and 2007 in the West Pacific by the Muséum National d'Histoire Naturelle (MNHN) and Institut de Recherche pour le Développement (IRD): (1) *Philippines*.

Table 1. List of specimens

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	BOLD ID	GenBank numbers		
						COI	16S	12S
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, 637–650m	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
	Borsoniidae gen. 1	sp.	MNHN IM200717911	Panglao 2005, CP2333, 9°38.20'N, 123°43.50'E, 584–596m	CONO290-08	EU015718	HQ401665	HQ401596
	<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, 95–128m	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, 123–135m	CONO255-08	EU015697	HQ401710	HQ401648
	<i>Typhlomangelia</i> (cf.)	sp.	MNHN IM200717931	Salomon 2, CP2269, 7°45.10'S, 156°56.30'E, 768–890m	CONO366-08	EU015744	HQ401716	HQ401653
Clathurellidae	<i>Zemacies</i>	<i>excelsa</i> Sysoev & Bouchet, 2001	MNHN IM200911056	Musorstom 4, DW226, 22°47'S, 167°22'E, 395m	CONO992-10		HQ401720	HQ401658
	<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, 4–12m	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, 97–120m	CONO228-08	EU015679	HQ401698	HQ401634
Clavatulidae	<i>Clavatula</i>	<i>xanteni</i> Nolf & Verstraeten, 2006	MNHN IM200717829	Angola, AF1, 8°78'0"S, 13°23'0"E, 40–60m	CONO001-08	EU015637	HQ401667	HQ401600
	<i>Gemmuloborsonia</i>	<i>colorata</i> (Sysoev & Bouchet, 2001)	MNHN IM200717849	EBISCO, DW2619, 20°06'0"S, 160°23'0"E, 490–550m	CONO192-08	EU015658	HQ401679	HQ401613
	<i>Perrona</i>	<i>subspirata</i> (von Martens, 1902)	MNHN IM200717833	Angola, AF10, 15°14'0"S, 12°29'0"E, 50m	CONO005-08	EU015641		HQ401639
	<i>Pusionella</i>	<i>compacta</i> Strebel, 1914	MNHN IM200717830	Angola, AF3, 10°51'0"S, 14°23'0"E, 5–10m	CONO002-08	EU015638	HQ401702	HQ401641
	<i>Turricula</i>	<i>nelliae</i> (E. Smith, 1877)	NHMUK MOEA 20100551	Danang, Vietnam	CONUK001-11	JF276924	JF276945	JF276966
Cochlespiridae	<i>Cochlespira</i>	<i>pulchella</i> (Schepman, 1913)	MNHN IM200717920	Panglao 2005, CP2340, 9°29.40'N, 123°44.40'E, 271–318m	CONO295-08	EU015720	HQ401669	HQ401602
	<i>Sibogasyrinx</i>	sp.	MNHN IM200717701	BOA1, CP2432, 14°59.70'S, 166°55.00'E, 630–705m	CONO153-08	EU015646	HQ401705	HQ401643
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, 150–163m	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, 235–271m	CONO513-08	EU015751	HQ401672	HQ401605
	<i>Profundiconus</i> <i>Taranteconus</i>	<i>teramachii</i> (Kuroda, 1956) <i>chiangi</i> Azuma, 1972		Philippines Philippines		JF300171 GU134381.1	JF300175 GU134361	JF300173 GU134364.1

Conorbidae	<i>Benthofascis</i>	<i>lozoueti</i> Sysoev & Bouchet, 2001	MNHN IM200742331	Norfolk 2, DW2147, 22°50'S, 167°16'E, 496m	CONO602-08	HQ401574		HQ401593
Drilliidae	<i>Agladrillia</i>	<i>pudica</i> (Hinds, 1843)	NHMUK MOEA 20100543	Gulf of Panama, JTD-00-51, 08°36.41'N, 79°09.70'W, 73m	CONUK002-11	JF276925	JF276946	JF276967
	<i>Calliclava</i>	sp.	NHMUK MOEA 20100546	Gulf of Panama, JTD-00-47, 08°31.83'N, 79°05.09'W, 21m	CONUK003-11	JF276926	JF276947	JF276968
	<i>Cerodrillia</i>	<i>cybele</i> (Pilsbry & Lowe, 1932)	NHMUK MOEA 20100548	Gulf of Panama, JTD-00-18, 08°19.50'N, 78°47.71'W, 25–32m	CONUK004-11	JF276927	JF276948	JF276969
	<i>Clathrodrillia</i>	<i>walteri</i> (M. Smith, 1946)	NHMUK MOEA 20100549	Gulf of Panama, JTD-00-46, 08°31.37'N, 79°05.79'W, 24–25m	CONUK005-11	JF276928	JF276949	JF276970
	<i>Clavus</i>	<i>canalicularis</i> (Roeding, 1798)	MNHN IM200717858	Panglao 2004, S12, 9°29.40'N, 123°56.00'E, 6–8m	CONO229-08	EU015680	HQ401668	HQ401601
	<i>Conopleura</i>	<i>striata</i> Hinds, 1844	MNHN IM200717889	Panglao 2004, T41, 9°29.70'N, 123°50.20'E, 110–112m	CONO278-08	EU015712	HQ401671	HQ401604
	<i>Cruziturrucula</i>	<i>arcuata</i> (Reeve, 1843)	NHMUK MOEA 20100541	Gulf of Panama, JTD-00–34, 08°26.24'N, 79°09.14'W, 66–68m	CONUK006-11	JF276929	JF276950	JF276971
	<i>Drillia</i>	<i>acapulcana</i> (Lowe, 1935)	NHMUK MOEA 20110067	Gulf of Panama, JTD-00-57 08°40.18'N, 79°02.76'W, 15-26m	CONUK007-11	JF276930	JF276951	JF276972
	<i>Fusiturrucula</i>	<i>enae</i> (Bartsch, 1934)	INVEMAR MOL-1929	Colombia, E-73, 09°57.53'N, 79°07.71'W, 268–270m	CONUK008-11	JF276931	JF276952	JF276973
	<i>Imaclava</i>	<i>unimaculata</i> (Sowerby I, 1834)	NHMUK MOEA 20100527	Gulf of Panama, JTD-00-46, 08°31.37'N, 79°05.79'W, 24–25m	CONUK009-11	JF276932	JF276953	JF276974
	<i>Iredalea</i>	<i>pupoidea</i> (H. Adams, 1872)	MNHN IM200742556	Santo 2006, DB25, 15°37.7'S, 167°11.3'E, 10m	CONO873-08	HQ401578	HQ401687	HQ401623
		<i>Splendrillia</i>	sp.	MNHN IM200717847	EBISCO, DW2617, 20°06'0'S, 160°22'0'E, 427–505m	CONO190-08	EU015655	HQ401706
Horaiclavidae	<i>Anacithara</i>	<i>lita</i> (Mellvill & Standen, 1896)	MNHN IM200742614	Santo 2006, DS99, 15°32'S, 167°17'E, 100–105m	CONO931-08	HQ401571	HQ401659	HQ401588
	<i>Anguloclavus</i>	sp.	MNHN IM200717908	Panglao 2005, CP2332, 9°38.80'N, 123°45.90'E, 396–418m	CONO288-08	EU015717		HQ401589
	<i>Carinapex</i>	<i>minutissima</i> (Garrett, 1873)	MNHN IM200717868	Panglao 2004, B19, 9°29.40'N, 123°56.00'E, 17m	CONO246-08	EU015690		HQ401597
	<i>Ceritoturris</i>	<i>pupiformis</i> (Smith, 1884)	MNHN IM200717888	Panglao 2004, T36, 9°29.30'N, 123°51.50'E, 95–128m	CONO276-08	EU015711		HQ401598
	Horaiclavidae gen. 1	sp. (juvenile)	MNHN IM200742501	Salomon 2, CP2219, 7°58'S, 157°34'E, 650–836m	CONO775-08	HQ401577		HQ401620
	<i>Horaiclavus</i>	<i>splendidus</i> (A. Adams, 1867)	MNHN IM200717840	EBISCO, DW2631, 21°03'0'S, 160°44'0'E, 372–404m	CONO183-08	EU015649		HQ401621
	<i>Paradrillia</i>	sp. (juvenile)	MNHN IM200742475	Panglao 2005, CP2396, 9°36'N, 123°42'E, 609–673m	CONO746-08	HQ401583		HQ401638

Continued

Table 1. *Continued*

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	BOLD ID	GenBank numbers		
						COI	16S	12S
Mangeliidae	<i>Anticlinura</i>	sp.	MNHN IM200742513	Salomon 2, CP2182, 8°47'S, 159°38'E, 762–1060m	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, 618–641m	CONO148-08	EU015644	HQ401663	HQ401594
	<i>Eucithara</i>	cf. <i>coronata</i> (Hinds, 1843)	MNHN IM200717900	Panglao 2004, B8, 9°37.10'N, 123°46.10'E, 3m	CONO223-08	EU015674		HQ401609
	<i>Heterocithara</i>	sp.	MNHN IM200717884	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110m	CONO271-08	EU015706	HQ401685	HQ401619
	Mangeliidae gen. 1	sp.	MNHN IM200717874	Panglao 2004, T26, 9°43.30'N, 123°48.80'E, 123–135m	CONO254-08	EU015696	HQ401694	HQ401630
	Mangeliidae gen. 2	sp.	MNHN IM200717872	Panglao 2004, S26, 9°41.50'N, 123°51.00'E, 21m	CONO252-08	EU015694	HQ401695	HQ401631
	<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, 508–522m	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, 11m	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, 3m	CONO221-08	EU015672	HQ401697	HQ401633
Pseudomelatomidae	<i>Carinodrillia</i>	<i>dichroa</i> Pilsbry & Lowe, 1932	NHMUK MOEA 20100530	Gulf of Panama, JTD-00-18, 08°19.50'N, 78°47.71'W, 25–32m	CONUK010-11	JF276933	JF276954	JF276975
	<i>Cheungbeia</i>	<i>robusta</i> (Hinds, 1839)	NHMUK MOEA 20100556	Off southern Hong Kong, South China Sea, Sta. 70	CONUK011-11	JF276934	JF276955	JF276976
	<i>Comitas</i>	sp.	MNHN IM200717918	Panglao 2005, CP2388, 9°26.90'N, 123°34.50'E, 762–786m	CONO336-08	EU015733	HQ401670	HQ401603
	<i>Crassispira</i>	<i>quadrilirata</i> (E.A.Smith, 1882)	MNHN IM200717755	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110m	CONO272-08	EU015707	HQ401673	HQ401606
	<i>Funa</i>	<i>incerta</i> (Smith, 1877)	NHMUK MOEA 20100553	Off southern Hong Kong, South China Sea, Sta. 70	CONUK012-11	JF276935	JF276956	JF276977
	<i>Hindsiclava</i>	<i>alesidota</i> (Dall, 1889)	NHMUK MOEA 20100524	Lower Florida Keys, JTD-01-15, 24°33.47'N, 81°07.72'W, 117–148m	CONUK013-11	JF276936	JF276957	JF276978
	<i>Inquisitor</i>	sp.	MNHN IM200717851	EBISCO, DW2625, 20°050'S, 160°190'E, 627–741m	CONO194-08	EU015660	HQ401686	HQ401622
	<i>Knefastia</i>	<i>tuberculifera</i> (Broderip & Sowerby, 1829)	NHMUK MOEA 20100533	Gulf of Panama, JTD-00-18, 08°19.50'N, 78°47.71'W, 25–32m	CONUK014-11	JF276937	JF276958	JF276979
	<i>Leucosyrinx</i>	sp.	MNHN IM200717846	EBISCO, CP2600, 19°380'S, 158°460'E, 603–630m	CONO189-08	EU015655	HQ401690	HQ401626

	<i>Otitoma</i>	sp.	MNHN IM200717905	Panglao 2005, CP2348, 9°29.60'N, 123°52.50'E, 196–216m	CONO301-08	EU015722		HQ401636
	<i>Pilsbryspira</i>	<i>jayana</i> (C. B. Adams, 1850)	USNM 857830	Carrie Bow Cay, Belize, intertidal	CONUK015-11	JF276938	JF276959	JF276980
	<i>Pseudomelatoma</i>	<i>moesta</i> (Carpenter, 1865)		California			JF300174	JF300172
	<i>Ptychobela</i>	<i>suturalis</i> (Gray, 1838)	NHMUK MOEA 20100560	Off southern Hong Kong, South China Sea, Sta. 71	CONUK016-11	JF276939	JF276960	JF276981
	<i>Pyrgospira</i>	<i>aenone</i> (Dall, 1919)	NHMUK MOEA 20100539	Gulf of Panama, JTD-00-18, 08°19.50'N, 78°47.71'W, 25–32m	CONUK021-11	JF276944	JF276965	JF276986
	<i>Tiariturris</i>	<i>spectabilis</i> Berry, 1958	NHMUK MOEA 20100540	Gulf of Panama, JTD-00-34, 08°26.24'N, 79°09.14'W, 66–68m	CONUK017-11	JF276940	JF276961	JF276982
	<i>Zonulispira</i>	sp.	NHMUK MOEA 20100536	Gulf of Panama, JTD-00-18, 08°19.50'N, 78°47.71'W, 25–32m	CONUK018-11	JF276941	JF276962	JF276983
Raphitomidae	<i>Daphnella</i>	sp.	MNHN IM200717927	Salomon 2, CP2260, 8°03.50'S, 156°54.50'E, 399–427m	CONO360-08	EU015740	HQ401674	HQ401607
	<i>Eucyclotoma</i>	<i>cymatodes</i> (Hervier, 1899)	MNHN IM200717903	Panglao 2004, S12, 9°29.40'N, 123°56.00'E, 6–8m	CONO227-08	EU015678	HQ401676	HQ401610
	<i>Glyphostomoides</i>	sp.	MNHN IM200717892	Panglao 2004, T39, 9°30.10'N, 123°50.40'E, 100–138m	CONO281-08	EU015715	HQ401681	
	<i>Gymnobela</i>	sp.	MNHN IM200717841	EBISCO, CP2648, 21°320'S, 162°300'E, 750–458m	CONO184-08	EU015650	HQ401682	HQ401616
	<i>Hemilienardia</i>	<i>calcicincta</i> (Melvill & Standen, 1895)	MNHN IM200717861	Panglao 2004, B14, 9°38.50'N, 123°49.20'E, 2–4m	CONO232-08	EU015683	HQ401684	HQ401618
	<i>Kermia</i>	<i>aureotincta</i> (Hervier, 1897)	MNHN IM200717878	Panglao 2004, B25, 9°29.40'N, 123°56.10'E, 16m	CONO259-08	EU015700	HQ401688	HQ401624
	<i>Pleurotomella</i>	sp.	MNHN IM200717848	EBISCO, DW2625, 20°050'S, 160°190'E, 627–741m	CONO191-08	EU015657	HQ401701	HQ401640
	<i>Raphitoma</i>	<i>rubroapicata</i> (E. A. Smith, 1885)	MNHN IM200717890	Panglao 2004, L49, 9°36.50'N, 123°45.30'E, 90m	CONO279-08	EU015713	HQ401703	HQ401642
	<i>Rimosodaphnella</i>	sp.	MNHN IM200717836	BOA1, CP2462, 16°37.50'S, 167°57.40'E, 618–641m	CONO150-08	EU015645	HQ401704	
	<i>Taranis</i>	sp.	MNHN IM200742296	Aurora 07, CP2749, 15°57'N, 121°50'E, 743m	CONO561-08	HQ401584	HQ401707	HQ401645
	<i>Teretiopsis</i>	cf. <i>hyalina</i> Sysoev & Bouchet, 2001	MNHN IM200717845	EBISCO, CP2651, 21°290'S, 162°360'E, 883–957m	CONO188-08	EU015654	HQ401708	HQ401646
	<i>Thatcheria</i>	<i>mirabilis</i> (Angas, 1877)	MNHN IM200717924	Salomon 2, CP2184, 8°16.90'S, 159°59.70'E, 464–523m	CONO349-08	EU015736	FJ868138	HQ401647
	<i>Tritonoturris</i> (cf.)	sp.	MNHN IM200717891	Panglao 2004, T39, 9°30.10'N, 123°50.40'E, 100–138m	CONO280-08	EU015714	HQ401712	
	<i>Veprecula</i>	sp.	MNHN IM200717883	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110m	CONO270-08	EU015705	HQ401717	HQ401654

Continued

Table 1. *Continued*

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	BOLD ID	GenBank numbers			
						COI	16S	12S	
Terebridae	<i>Cinguloterebra</i>	<i>cf. fenestrata</i> (Hinds, 1844)	MNHN IM200716735	Panglao 2005, CP2395, 9°36.2'N, 123°43.8'E, 382–434m	CONO340-08	EU015735	EU685670	EU685379	
	<i>Clathroterebra</i>	<i>fortunei</i> (Deshayes, 1857)	MNHN IM200730401	Panglao 2005, CP2331, 9°39.2'N, 123°47.5'E, 255–268m	CONO284-08	EU685526	EU685663	EU685371	
	<i>Euterebra</i>	<i>tristis</i> (Deshayes, 1859)	MNZ	New-Zealand				HQ401677	HQ401611
	<i>Hastula</i>	<i>strigilata</i> (Linnaeus, 1758)	MNHN IM200730420	Santo 2006, VM24, 15°35.2'S, 167°59.4'E, intertidal	CONO466-08	EU685581	EU685726	EU685434	
	<i>Hastulopsis</i>	<i>amoena</i> (Deshayes, 1859)	MNHN IM200730480	Santo 2006, FR10, 15°36.9'S, 167°10.5'E, 6–33m	CONO444-08	EU685559	EU685701	EU685410	
	<i>Myurella</i>	<i>kilburni</i> (Burch, 1965)	MNHN IM200730459	Panglao 2004, S18, 9°35.7'N, 123°44.4'E, 0–2m	CONO238-08	EU685511	EU685647	EU685355	
	<i>Oxymeris</i>	<i>maculatus</i> (Linnaeus, 1758)	MNHN IM200730371	Santo 2006, NR5, 15°28.7'S, 167°15.2'E, 19m	CONO406-08	EU685559	HQ401700	HQ401636	
	<i>Strioterebrum</i>	<i>plumbea</i> (Quoy & Gaimard, 1833)	MNHN IM200730558	Santo 2006, ED5, 15°31'S, 167°09'E, intertidal	CONO455-08	EU685570	EU685712	EU685421	
	<i>Terebra</i>	<i>textilis</i> Hinds, 1844	MNHN IM200717938	Santo 2006, LD28, 15°35.40'S, 166°58.70'E, 3–8m	CONO509-08	EU015750	EU685771	EU685478	
	<i>Terenolla</i>	<i>pygmaea</i> (Hinds, 1844)	MNHN IM200730449	Panglao 2004, S2, 9°37.4'N, 123°54.5'E, 4–5m	CONO213-08	EU685505	EU685641	EU685350	
Turridae	<i>Gemmula</i>	<i>rarimaculata</i> Kuroda & Oyama, 1971	MNHN IM200717838	EBISCO, DW2533, 22°180'S, 159°280'E, 360–370m	CONO199-08	EU015664	HQ401678	HQ401612	
	<i>Iotyrrix</i>	<i>cingulifera</i> (Lamarck, 1822)	MNHN IM200717685	Santo 2006, FS84, 15°33.6'S, 167°16.6', 8–9m	CONO515-08	EU127881	EU685780	EU685490	
	<i>Lophiotoma</i>	<i>acuta</i> (Perry, 1811)	MNHN IM200717860	Panglao 2004, R44, 9°33.30'N, 123°43.90'E, 2m	CONO231-08	EU015682	HQ401691	HQ401627	
	<i>Lucerapex</i>	<i>cf. casearia</i> (Hedley & Petterd, 1906)	MNHN IM200742448	Panglao 2005, CP2363, 9°06'N, 123°25'E, 437–439m	CONO719-08	HQ401581	HQ401693	HQ401629	
	<i>Polystira</i>	<i>albida</i> (Perry, 1811)	NHMUK MOEA 20110066	S. of Bahia Honda Key, Florida Keys, 24°34.24'N; 81°16.64'W, 30–34m	CONUK019-11	JF276942	JF276963	JF276984	
	<i>Ptychosyrinx</i>	<i>carynae</i> (Haas, 1949)	USNM 832922	North Atlantic, 38°0.14'N, 70°29.28'W, 3188–5300m	CONUK020-11	JF276943	JF276964	JF276985	
	<i>Turridrupa</i>	<i>cf. armillata</i> (Reeve, 1845)	MNHN IM200717850	EBISCO, DW2607, 19°330'S, 158°400'E, 400–413m	CONO193-08	EU015659	HQ401713	HQ401650	
	<i>Turris</i>	<i>babylonia</i> (Linnaeus, 1758)	MNHN IM200717754	Panglao 2004, R42, 9°37.10'N, 123°52.60'E, 8–22m	CONO226-08	EU015677	HQ401715	HQ401652	
<i>Xenuroturris</i>	<i>legitima</i> Iredale, 1929	MNHN IM200717684	Santo 2006, DR87, 15°38.5'S, 167°15.1'E, 13m	CONO489-08	EU127878		HQ401657		

Outgroups	<i>Belomitra</i>	sp.	MNHN IM200911057	Salomon 2, CP2184, 8°17'S, 160°00'E, 464–523m	HQ401573	HQ401662	HQ401592
	<i>Harpa</i>	<i>kajiyamai</i> Habe & Kosuge, 1970	MNHN IM200740569	Santo 2006, EP22, 15°37.3'S, 167°05.8'E, 78–91m	EU685626	HQ401683	HQ401617
	<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
	<i>Turritatirus</i>	<i>turritus</i> (Gmelin, 1791)	MNHN IM200911059	Santo 2006, FBS2, 15°42.7'S, 167°15.1'E, 7m	HQ401585	HQ401714	HQ401651
	<i>Vexillum</i>	<i>costatum</i> (Gmelin, 1791)	MNHN IM200911058	Santo 2006, DR64, 15°27.6'S, 167°14.3'E, 6–35m	HQ401586	HQ401718	HQ401655
	<i>Xenophora</i>	<i>solarioides</i> (Reeve, 1845)	MNHN IM200911061	Santo 2006, AT55, 15°36.2'S, 167°02.5'E, 80–82m	HQ401587	HQ401719	HQ401656

The PANGLAO 2004 Marine Biodiversity Project was a joint project between University of San Carlos, Cebu City (USC; co-PI Danilo Largo) and MNHN (co-PI Philippe Bouchet), and the follow-up PANGLAO 2005 cruise on board M/V *DA-BFAR* associating the USC, MNHN (co-PI Philippe Bouchet) and the Philippines Bureau of Fisheries and Aquatic Research (BFAR; co-PI Ludivina Labe); the AURORA 2007 cruise also on board M/V *DA-BFAR* associated the National Museum of the Philippines (NMP, co-PI Marivene Manuel), MNHN (co-PI Philippe Bouchet) and BFAR. (2) *Vanuatu*. Material from the MNHN-IRD-PNI Santo 2006 expedition, and from the BOA 1 expedition. (3) *New Caledonia, Coral Sea and Solomon Islands*. MUSORSTOM 4, EBISCO and SALOMON 1, 2 and 3 cruises. Specimens from tropical America were mostly dredged from the Gulf of Panama in 2000 by Jonathan Todd on a cruise by the Smithsonian Tropical Research Institute R/V *Urraca* (lead scientist Harilaos Lessios) and from the Lower Florida Keys in 2001 by Timothy Rawlings and Jonathan Todd on a cruise by the Florida Institute of Oceanography R/V *Bellows* (lead scientist Timothy Collins).

Living specimens were anaesthetized using MgCl₂, a piece of tissue was cut from the head-foot and fixed in 95% ethanol (MNHN) or the shell was cracked and the entire animal preserved in 95% ethanol, with heart or foot tissue later dissected out for processing (NHMUK). Additional specimens were collected by Rüdiger Bieler and Paula Mikkelsen as part of their ongoing research on molluscs of the Florida Keys, Serge Gofas (when in Angola with SNEAP), Jon-Arne Snelli (Trondhjem Biologiske Stasjon; *Oenopota*), Bruce Marshall (Museum of New Zealand Te Papa Tongarewa; *Euterebra tristis*), David Reid and John Taylor (Natural History Museum, London), Brian Morton (then University of Hong Kong), and Baldomero Olivera (University of Utah). Most specimens are vouchered in MNHN, Paris and NHM, London (NHMUK), single lots are deposited in the collections of the FLMNH (Gainesville, FL), FMNH (Chicago, IL) and INVEMAR (Colombia). MNHN specimens are recorded in the Barcode of Life Data Systems (BOLD; Table 1).

Ingroup

Our taxon strategy was to include as many unique conoidea genera as possible. As such we based our selection of OTUs on Puillandre *et al.* (2008), taking a single exemplar from each genus that had multiple representatives and augmenting this culled list with new additions from more recent expeditionary material, previously unrepresented taxa from tropical America and others from our expanded network of collaborators.

Outgroups

Within the Neogastropoda, the sister-group of Conoidea is the rachiglossate group of superfamilies (Oliverio & Modica, 2010). Consequently, we included in our analyses several outgroups from four families of Rachiglossa: Costellariidae (*Vexillum costatum* MNHN IM200911058), Harpidae (*Harpa kajiyamai* MNHN IM200740569), Buccinidae (*Belomitra* sp. MNHN IM200911057) and Fascioliariidae (*Turritatirus turritus* MNHN IM200911059). Two other distant outgroups were also included: *Xenophora solarioides* (MNHN IM200911061), Xenophoridae, Littorinimorpha) and *Laevistrombus guidoi* (MNHN IM200911060, Strombidae, Littorinimorpha).

Identification of the material

Proper identification of Conoidea taxa may be extremely challenging, as evidenced by name changes since the work of

Puillandre *et al.* (2008) recorded herein (see Table 1). The specimens of *Comus s.l.* were identified by Baldomero Olivera, and the Terebridae were identified by Yves Terryn. All the other specimens ('turrids') were identified by AS, YuIK, JAT (for tropical American taxa) and Rüdiger Bieler (*Pilsbryspira leucomya*). Richard N. Kilburn also participated in the identification of some problematic specimens. Eight specimens, noted 'cf.', were not confidently identified to a named species, but display shell characters similar to that species; 28 others, noted 'sp.', certainly correspond to new species. Among them, three are not confidently attributed to a genus ('cf. '), and four others certainly correspond to new genera (Borsoniidae gen. 1, Horaiclavidae gen. 1, Mangeliidae gen. 1 and Mangeliidae gen. 2). Outgroups were identified by Alain Robin, Jean-Claude Martin, Gilbert Jaux and Kurt Kreipl.

DNA extraction, amplification and sequencing

Specimens analysed in the MNHN, Paris, were extracted using 6100 Nucleic Acid Preperation system (Applied Biosystem) or Dneasy96 Tissue kit (Qiagen) for smaller specimens, following the manufacturer's recommendations. For the majority of tissue samples analysed at the NHM, DNA was extracted using Qiagen's DNeasy blood and tissue kit following the manufacturer's recommendations, with a final elution of extracted DNA in 50–100 µl of AE buffer.

At the MNHN, Paris (see Table 1), fragments of the mitochondrial genes 12S rRNA, 16S rRNA and COI were amplified using the universal primers 12S1/12S3 (Simon, Franke & Martin, 1991), 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer *et al.*, 1994), respectively. All polymerase chain reactions (PCRs) were performed in 25 µl, containing 3 ng of DNA, 1X reaction buffer, 2.5 mM MgCl₂, 0.26 mM dNTP, 0.3 mM each primer, 5% DMSO and 1.5 units of Qbiogene Q-Bio Taq. Amplification consisted of an initial denaturation step at 94°C for 4 min, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 12S gene, and 52°C for 16S, followed by extension at 72°C for 1 min. The final extension was at 72°C for 5 min. The amplification of COI genes were performed similarly, except there were two annealing cycles: the first repeated five times at an annealing temperature of 45°C, and the second repeated 30 times at 50°C as described in Hebert *et al.* (2003). PCR products were purified and sequenced by a sequencing facility (Genoscope). All genes were sequenced in both directions for increased accuracy. Sequences were deposited in GenBank (Table 1). COI sequences were also deposited in BOLD (Table 1).

At the NHM, London (see Table 1), the molecular procedures followed were slightly different. Primer pairs for 16S and COI were as described above, but the 12S gene region was amplified using 12SA (Palumbi, 1996)/12SR (5'-GCCATGATRCAAAAGGT-3') primers. In addition, where DNA extractions revealed badly degraded DNA, gene regions were amplified in two smaller but overlapping fragments (internal primers available upon request). For each amplification, genomic DNA was used as template in a 25–50-µl PCR consisting of 1.5–2.0 mM MgCl₂ buffer, 0.5 µM each primer, 0.2 mM each dNTP and 1.5–2 units of Taq DNA polymerase (Promega) depending on the reaction volume. The Taq DNA polymerase was added after the PCR mixture had undergone an initial 5 min denaturation step at 94°C followed by a 5 min hold at 4°C. PCR was performed under the following cycling parameters using an MJ Gradient thermocycler: denaturation at 94°C for 10 s, annealing at 48–55°C for 30–40 s (depending on primer pairs and DNA templates) and an extension at 72°C for 30–40 s, for a total of 38–40 cycles. The final cycle consisted of a 5 min-long extension. When single bands of the appropriate size resulted from

these amplifications, the PCR product was purified for sequencing using GeneClean III (Qbiogene). When supernumerary bands were present, the entire PCR product was run out on a 3% Nu-Sieve TAE agarose gel and the band of the correct size was excised under long-wavelength UV and purified following GeneClean III protocols. Sequencing of both strands for all amplified products was undertaken at the NHM.

Phylogenetic analyses

Sequences were manually (COI) or automatically (16S and 12S) aligned using ClustalW multiple alignment, implemented in BioEdit v. 7.0.5.3 (Hall, 1999). We used the RNAalifold webserver (<http://rna.tbi.univie.ac.at/cgi-bin/RNAalifold.cgi>) to predict a consensus secondary structure for the 12S and 16S genes and identify the loops and stems. The best model of evolution was selected for five different partitions: the three positions of the codon for the COI gene, the stems of the 12S and 16S genes and the loops of the 12S and 16S genes using Modelgenerator v. 85 (Keane *et al.*, 2006) under the Hierarchical Likelihood Ratio Tests (with four discrete gamma categories).

Phylogenetic analyses were first performed on each gene separately to check for inconsistency between trees. Since trees were mostly congruent, all genes were concatenated in a single dataset. Maximum likelihood analyses (MLAs) were performed using RAXML 7.0.4 (Stamatakis, 2006), with a GAMMAI model for each gene (when analysed independently) and each of the five partitions (when genes were analysed in a concatenated dataset). The best tree was identified among 20 runs. Robustness of the nodes was assessed with a bootstrap analysis (200 replicates with 10 runs each). The node supports were summarized on the best tree among the 20 search replicates using RAXML.

Bayesian analyses (BAs) were performed running two parallel analyses in MrBayes (Huelsenbeck, Ronquist & Hall, 2001), consisting each of eight Markov chains of 100,000,000 generations each with a sampling frequency of one tree each thousand generations. The number of swaps was set to 5, and the chain temperature at 0.02. Similar to the MLA, one different 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 for each gene and partition. For the concatenated dataset analysis, the five previously defined partitions were unlinked. Parameters of each model were estimated during the analysis. 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. We considered a clade to be 'moderately supported' if it had bootstrap support values (B) between 75 and 89 and posterior probability (PP) between 0.95 and 0.97, and 'highly supported' when B ≥ 90 and PP ≥ 0.98.

Nomenclature

Although the names *Conoidea* and *Toxoglossa* are used interchangeably in the taxonomic literature, we have avoided the name *Toxoglossa* because (i) it is not typified and cannot be used for a family-group name and (ii) many of the included taxa do not have a toxoglossate radula. In Results and Discussion, we refer to the accompanying paper by Bouchet *et al.* (2011) for the names applied to the clades recovered by the analysis.

RESULTS

DNA sequences

The majority (>85%) of the 108 taxa (102 ingroups and six outgroups) were sequenced successfully for all three fragments: 105 for the COI gene, 97 for 16S and 105 for 12S. Failure to recover a particular sequence could be linked to the preservation quality of some specimens (e.g. *Zemacies*, presumably preserved first in formalin, and then in ethanol) or to specific mutations in the primer region (e.g. all but one Horaiclavidae have not been successfully sequenced for the 16S gene). After alignment, the COI, 16S and 12S genes were, respectively, 658, 543 and 632 bp long. The best model of evolution was determined for eight different partitions: each of the three genes (COI, 12S and 16S) and each of the five partitions defined for the combined analyses (COI-1, COI-2, COI-3, stems and loops). Only the second and third position partitions of the COI gene deviated from GTR + I + G model (Table 2).

Phylogenetic analyses

Since independent analyses of each gene provided trees with a lower resolution than the phylogeny obtained with the combined dataset, only the latter is presented and discussed hereafter (Fig. 1). The 10 best trees obtained with MLA were topologically identical and highly similar to the topology obtained with BA. The only incongruencies between methods concerned intra-familial relationships and the position of *Benthofascis*, nested within Borsoniidae (BA) or sister-group of the Borsoniidae (MLA). No incongruencies were strongly supported one way or another.

Both neogastropods (B/PP = 100/1) (*Belomitra*, *Turrilaturis*, *Vexillum*, *Harpa* and Conoidea) and Conoidea (90/1) are highly supported as clades. Conoidea are further divided in two main lineages (98/1 and 89/1). These two lineages were also recovered in the previous molecular analysis of the group (Clades A and B in Puillandre *et al.*, 2008), and approximately correspond to the family Conidae *s. l.* (Clade B) and to the association of the families Turridae, Terebridae, Drilliidae and Pseudomelatomidae (Clade A) *sensu* Taylor *et al.* (1993). At the next level of phylogenetic resolution, 11 mutually exclusive clades are well supported (>84/1) which broadly correspond to previously recognized suprageneric rankings within Conoidea. In Clade A *sensu* Puillandre *et al.* (2008), five taxa (*Cruziturricula*, *Fusiturricula*, *Leucosyrinx*, *Gemmuloborsonia* and *Lucerapex*) are not confidently assigned within the six well-supported, reciprocally monophyletic groups. In Clade B, a grade of poorly supported lineages generally associated with Conidae *s. s.* was recovered in addition to four well-supported clades. This grade generally corresponds to the polytomy recovered by Puillandre *et al.* (2008) (Fig. 1).

DISCUSSION

Based on the resulting phylogeny and in order to maintain nomenclatural consistency, 14 clades are recognized as Conoidean families herein (Fig. 1) (Bouchet *et al.*, 2011). We have chosen to raise these clades to familial ranks in order to maintain equivalent status with the two best-known clades in common usage (Conidae and Terebridae). The majority of these clades are strongly supported and provide a foundation for future taxonomic work in the group. All but two (Conorbidae and Mangeliidae) are shown to contain the type genus. Seven of these clades directly correspond to numbered clades from Puillandre *et al.* (2008). However, many of the para- or polyphyletic subfamilial clades from Puillandre *et al.*

Table 2. The best model of evolution for each gene or partition as determined using Hierarchical Likelihood Ratio Tests in Modelgenerator v. 85 (Keane *et al.*, 2006).

	Best model	l	α
COI	GTR + I + G	0.60	0.43
16S	GTR + I + G	0.32	0.38
12S	GTR + I + G	0.16	0.49
COI-1	GTR + I + G	0.01	0.68
COI-2	GTR + G		0.15
COI-3	HKY + I + G	0.68	0.13
Stems	GTR + I + G	0.41	0.53
Loops	GTR + I + G	0.06	0.82

(2008) have either been separated out or merged into a single family. Table 3 compares our current classification with Puillandre *et al.*'s numbered clades. A brief discussion of each of the 14 family-level clade follows.

Conidae

We recover a single clade of 'cones' with both MLA and BA, but this was only strongly supported by the BA (B = 36, PP = 0.97). The family Conidae corresponds traditionally to a single genus, *Conus*, but there have been numerous attempts to split it into different genera. The latest one (Tucker & Tenorio, 2009) recognized more than 80 genera, of which five (*Profundiconus*, *Californiconus*, *Conasprella*, *Conus* and *Taranteconus*) are included in our analysis. Tucker & Tenorio (2009) included them in three different families (Conidae for *Conus*, Conilithidae for *Profundiconus*, *Californiconus* and *Conasprella*, and Taranteconidae for *Taranteconus*). *Taranteconus chiangi* was recently discussed by Watkins *et al.* (2010), who concluded that *Taranteconus* should be included in the 'large major clade' (*sensu* Duda & Kohn, 2005) of *Conus*. This result is congruent with ours (actually, the same specimen of *T. chiangi* was used by both Watkins *et al.*, 2010 and the present study). In our tree, the Conilithidae would be paraphyletic, as they include *Conus* and *Taranteconus*. Our results are also congruent with previously published molecular phylogenies (e.g. Espiritu *et al.*, 2001; Duda & Kohn, 2005). It is also mostly congruent with the classification proposed by Tucker & Tenorio (2009), mainly based on radula characters, except for the family Conilithidae, undoubtedly paraphyletic in our tree. Other discrepancies between molecular trees and the Tucker and Tenorio classification do not correspond to incompatibilities but rather to opinions regarding the ranks. A detailed classification of the Conidae ('cone snails') is currently in preparation (C. Meyer, in prep.).

Clathurellidae

Several genera traditionally placed in the subfamily Clathurellinae are here included in other families (see Borsoniidae and Mitromorphidae). Only the genera *Nannodiella* and *Etrema* are placed together with the type-genus *Clathurella* in a highly supported clade (100/1).

Conorbidae

This family is represented by only one species, *Benthofascis lozoueti*, which corresponds to a lineage distinct from all the other families.

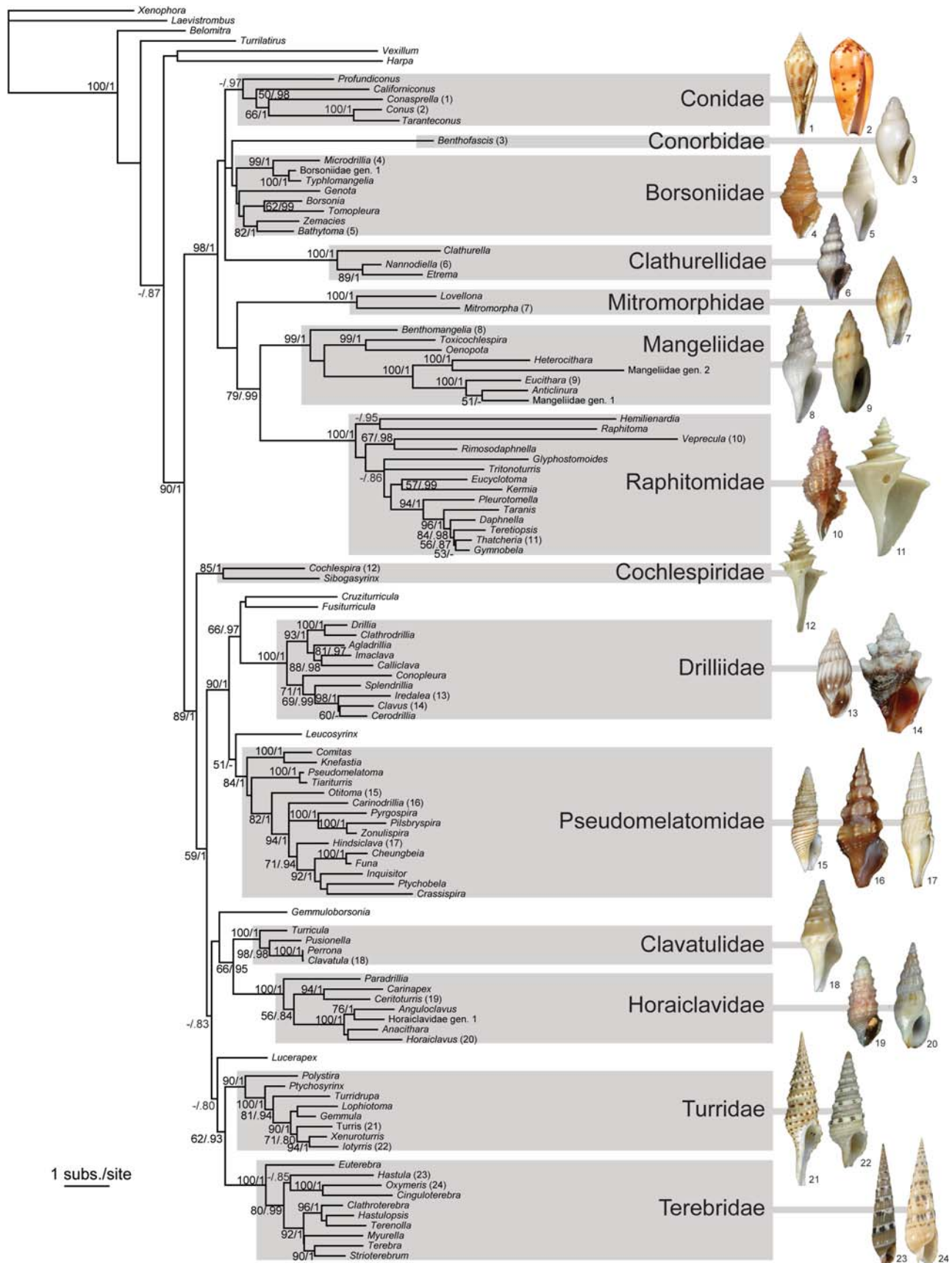


Figure 1. Best MLA tree obtained with the COI, 16S and 12S genes. Bootstrap values (B: when >50%) and posterior probabilities (PP: when >0.8) are indicated for each node. Grey boxes correspond to the families defined in the classification of the Conoidea (Bouchet et al., 2011). Some representative shells are illustrated for each family (the links between names in the tree and pictures of the shell are provided by the numbers in parentheses).

Table 3. Comparison between families defined in the present study and numbered clades in Puillandre *et al.* (2008).

Proposed classification	Puillandre <i>et al.</i> (2008) clades
Cochlespiridae	Cochlespirinae 8 and 9
Drilliidae	Drilliidae 1
Pseudomelatomidae	Crassispirinae 2, 'Cochlespirinae' 3(?) and 4
Clavatulidae	New
Horaiclavidae	'Crassispirinae' 7
Turridae	Turrinae 5
Terebridae	Terebridae 6
Conidae	Coninae 19 and 21
Conorbidae	Conorbinae
Borsoniidae	Clathurellinae 14, 15, 16, 18 and 20
Clathurellidae	Clathurellinae 12
Mitromorphidae	Clathurellinae 13
Mangeliidae	Mangeliinae 11 and 17
Raphitomidae	Raphitominiae 10

Borsoniidae

This family is the only clade we recognize as a family that is not strongly supported. In fact, it is only recovered as a monophyletic group in the ML analyses. Most of the eight genera placed in this group, except *Zemacies* and *Genota*, were previously placed in an informal 'borsoniid' group within the subfamily Clathurellinae (Taylor *et al.*, 1993). We thus retain this family in the classification, as molecular data do not reject this hypothesis. Further research will be needed to test the monophyly of this family, either by adding more taxa or more genes.

The four families Conidae, Clathurellidae, Conorbidae and Borsoniidae correspond to a poorly supported clade (B = 36, PP = 0.65) sister to the remaining members of Clade B *sensu* Puillandre *et al.* (2008).

Mitromorphidae

Mitromorphidae is a highly supported clade (100/1) represented by two genera, *Mitromorpha* and *Lovellona*. This family was also previously recognized as an informal group within the Clathurellinae by Taylor *et al.* (1993).

Mangeliidae

Mangeliidae is the only family for which the Recent type-genus is not included. Eight different genera are included in this highly supported clade (99/1). *Benthomangelia* is the sister group of all the other Mangeliidae. The remaining genera are mainly separated into two clades: *Toxicochlespira* and *Oenopota* (99/1) and all others (100/1). *Oenopota* was traditionally placed in a separate taxon, Oenopotinae, but is here included within the Mangeliidae.

Raphitomidae

This highly supported clade (100/1) is characterized by highly divergent lineages (*Hemilienardia*, *Raphitoma*, *Veprecula*) associated with shorter branches. Only one clade within the Raphitomidae (*Pleurotomella*, *Taranis*, *Daphnella*, *Teretiopsis*, *Thatcheria* and *Gymnobela*) is supported (94/1). These genera mostly include deep water species. Raphitomidae and Mangeliidae are supported as sister-families (79/0.99).

Cochlespiridae

Cochlespiridae include the two genera *Cochlespira* and *Sibogasyrinx* (formerly *Leucosyrinx* 17701 from Puillandre *et al.*, 2008, see Bouchet *et al.*, 2011). The Cochlespiridae is the sister group to the remaining families from Drilliidae to Terebridae. While well supported (85/1), the clade merges two subclades from Puillandre *et al.* (2008) that were formerly polyphyletic (clades 8 and 9), albeit not strongly supported (<50/<0.89) in the previous analysis. The addition of the two mitochondrial genes (12S and 16S) significantly increases resolution of the topology in this region.

Drilliidae

With the addition of seven new genera, including the type genus (*Drillia*), 10 genera are now included in this well-supported clade (100/1). Several clades within the Drilliidae are also highly supported, such as the association of *Drillia* and *Clathrodrillia* (100/1) and *Iredalea*, *Clavus* and *Cerodrillia* (98/1).

Pseudomelatomidae

Nine new genera were added to those previously analysed, including *Pseudomelatoma*, the type genus of Pseudomelatomidae. These nine new genera plus the six already analysed form a well-supported clade (84/1). Past analyses characterized members of this group as Crassispirinae or even 'Cochlespirinae' clades (Table 3). However, this newly recovered clade contains both *Crassispira* and *Pseudomelatoma*, and Pseudomelatomidae has nomenclatural priority over Crassispiridae. Three genera are well supported (90/1) as members within a clade including Drilliidae and Pseudomelatomidae: *Cruziturricula*, *Fusiturricula* and *Leucosyrinx*. While *Cruziturricula* and *Fusiturricula* are shown with marginal support to share a more recent common ancestor with Drilliidae (66/0.97), they are sufficiently distinct from the crown group members of Drilliidae and possess significantly different anatomical features (Bouchet *et al.*, 2011) that these taxa are likely to comprise a new family. However, until either more taxa are sampled or more sequences generated, *Cruziturricula* and *Fusiturricula* are tentatively assigned to Drilliidae; similarly, *Leucosyrinx* is assigned to Pseudomelatomidae.

Clavatulidae

Four genera are included in this well-supported clade (100/1): *Turricula*, *Pusionella*, *Perrona* and *Clavatula*. The last two possess highly similar sequences for the three genes, suggesting that they could potentially be synonymized, or that at least a species of one genus must be transferred to the other. We choose to recognize this clade at the familial level because of strong ingroup support, a lack of the sister group support (66/0.95) and for consistency with previous classifications.

Horaiclavidae

This is a new family (Bouchet *et al.*, 2011) that mostly includes genera previously placed in the Drilliidae and Pseudomelatomidae. However, molecular data clearly show that the included genera are distinct from these families and correspond to a highly supported clade (100/1), currently arranged as sister to the Clavatulidae.

Turridae

The family Turridae is here restricted to a well-supported clade (90/1) that includes eight genera, including *Turris*, all of them being traditionally placed in the subfamily Turrinae.

Terebridae

This family also corresponds to a well-recognized and highly supported clade (100/1). The species *Euterebra tristis* (subfamily Pervicaciinae) is the sister group of all the other Terebridae. Our phylogeny is similar to that presented in Holford *et al.* (2009) and the same four main lineages are found: *Hastula*, *Oxymuris* (= *Acus*), *Cingloterebra*, and a clade that includes *Clathroterebra*, *Hastulopsis*, *Terenolla*, *Myurella*, *Terebra* and *Strioterebrum* (92/1).

Although not highly supported (66/0.95), the Clavatulidae and Horaiclavidae are sister groups, and Terebridae and Turridae are sister groups (62/0.93). These results leave two additional renegade genera among these four families: *Gemmuloborsonia* and *Lucerapex*. As with *Cruizturricula*, *Fusiturricula* and *Leucosyrinx*, confident placement of these lineages must await further sampling.

The addition of 50 new genera and two new genes to the molecular systematic analyses of Conoidea has significantly improved the phylogenetic resolution and allows us to subdivide the group with some confidence into 14 families. However, many internal nodes remain unresolved. This is particularly true for the relationships between the different families: apart from the two main clades defined within the Conoidea and the sister-clade relationships between Mangeliidae and Raphitomidae, and Drilliidae and Pseudomelatomidae, none of the other internal nodes are highly supported. This lack of resolution could be linked to the fact that most of conoidean lineages appeared in a very short period of time (late Cretaceous-Paleocene; Powell, 1966). Such rapid radiation does not facilitate the appearance of molecular apomorphies in each lineage (Whitfield & Lockhart, 2007). This problem is amplified when the concerned taxa are old, because the probability that apomorphies are lost by mutations will be higher. Clearly, the most controversial proposed systematic arrangement concerns the earliest diverging lineages of Clade B and particularly the proposed monophyly of Borsoniidae. Further research may determine that the Borsoniidae as described herein is polyphyletic or a paraphyletic grade. Several nuclear markers were previously used for the Conoidea (28S, H3, 18S – Puillandre *et al.*, 2008), but those results were similar to the ones obtained with the three mitochondrial genes in this portion of the phylogeny, and relationships were globally less resolved and supported. Indeed, this is why we choose to sequence only the mitochondrial genes for this new dataset, as they are broadly compatible and provide more variation and resolving power.

Future of conoidean systematics

Sequences from two additional mitochondrial genes and the inclusion of 50 new taxa increase the resolution of the phylogenetic tree and confirm several hypotheses not clearly supported in the first molecular phylogeny of Puillandre *et al.* (2008). In most phylogenetic analyses of higher rank gastropods (caenogastropods, neogastropods), conoideans are also found to be monophyletic (e.g. Oliverio & Modica, 2010), but relationships with other neogastropods are still unresolved (Colgan *et al.*, 2007; Cunha, Grande & Zardoya, 2009). In the present study, the addition of several outgroups in the Neogastropoda did not disrupt the monophyly of the Conoidea. However, since these analyses have not yet included representatives from all the

families of neogastropods, the relationship of the Conoidea to their sister taxon remains to be tested.

The way is now open to a new period of Conoidea classification; a new family-level framework is available that should promote stability. The accompanying paper by Bouchet *et al.* uses the systematic arrangements revealed in this analysis to assign unsampled genera to the 14 clades based on a reassessment of appropriate morphological features (anatomical and conchological). However, more work is needed to increase the resolution along the backbone of the Conoidean phylogeny in order to test character transformations and evolutionary dynamics throughout the group, particularly among the Borsoniidae and surrounding our five renegade genera. Additionally, the family Strictispiridae, recognized as valid in recent classifications (Taylor *et al.*, 1993; Bouchet & Rocroi, 2005; Tucker & Tenorio, 2009), is not represented in our dataset, and awaits further analyses. However, we feel that our taxon sampling is adequate for the discovery of the major clades (Bouchet *et al.*, 2011).

A molecular phylogeny provides support not only for a new classification but also a framework for other evolutionary studies. For example, analysing the evolution of morphological characters to determine character transformations in features of the protoconch, teleoconch or radula will necessitate reconstructing the ancestral states in a phylogenetic framework. Similarly, the evolution of ecological or anatomical characters can also be mapped on a phylogenetic tree. Holford *et al.* (2009) showed that the venom duct apparatus has been lost at least twice during the evolution of the family Terebridae. Other conoidean lineages have lost several anatomical features (Medinskaya & Sysoev, 2003; Fedosov, 2007; Fedosov & Kantor, 2008), including the venom apparatus, but whether each corresponds to an independent event remains to be demonstrated. Identifying which lineages are likely to possess toxins, mapping conotoxin superfamilies upon their discovery and the detection of different toxins in other lineages, are the main goals of the ‘concerted discovery strategy’ (Olivera, 2006; Puillandre & Holford, 2010). In this way, phylogeny becomes the guide for the discovery of potential new drugs.

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