

Molecular data provide new insights on the phylogeny of the Conoidea (Neogastropoda)

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ABSTRACT

The superfamily Conoidea is one of the most speciose groups of marine molluscs, with almost 700 genera and 10,000 living species. Previous classifications were based on morphological and anatomical characters, but clades and phylogenetic relationships were not well assessed. Information provided by one mitochondrial (COI) and three nuclear (28S, 18S, and H3) genes were used to infer the phylogeny of this group. Data were obtained from more than 100 specimens, belonging to 54 genera, collected during recent cruises in the western Pacific (Philippines, Vanuatu, Norfolk Ridge, and Chesterfield and Solomon Islands). Analyses were performed on each gene independently as well as for a data matrix where all genes were concatenated, using several methods (ML, Parsimony, Bayesian). Some families and subfamilies among Conoidea correspond to well-supported clades uniformly recovered with all genes and all methods, but others appear to be polyphyletic. Several bathyal and abyssal genera are also shown to be polyphyletic. Our results also point out some new phylogenetic relationships at the family, subfamily, and genus levels.

Additional keywords: 18S rDNA, 28S rDNA, classification, COI gene, Conoidea, Conidae, H3 gene, molecular phylogeny, Toxoglossa, Turridae, western Pacific

INTRODUCTION

The superfamily Conoidea, or Toxoglossa, is one of the most prolific groups of marine molluscs, both in genera,

with almost 700, and species, with perhaps 10,000 recent and fossil species (Bouchet, 1990). The genus *Conus* alone includes more than 500 species, making it the most speciose genus of marine animals (Kohn, 1990; Duda and Kohn, 2005). The monophyly of the group, characterized by a venom apparatus (Taylor et al., 1993), is not questioned, but the classification within Conoidea still remains problematic. Subdivisions within Toxoglossa and relationships between them are not well-defined, mostly because of the huge morphological and anatomical variation encountered.

During most of the 19th and 20th centuries, classifications (e.g., Fischer, 1887; Cossmann, 1896; Hedley, 1922; Thiele, 1929; Wenz, 1938–1944) were based on characters of the shell and of the radula, and Powell (1942, 1966) later gave emphasis on characters of the protoconch. All these authors traditionally recognized three families of Recent Conoidea: (1) Conidae, only containing the genus *Conus*, (2) Terebridae containing species with acuminate shells without a siphonal canal, and (3) Turridae, including the remainder, i.e., the vast majority of the group. Powell's (1942, 1966) subdivision of the Turridae in nine subfamilies was the basis for turrid classifications in the latter half of the 20th century. Subsequent authors diverged on the number of subfamilies they recognized, mostly splitting one subfamily into several (McLean, 1971; Kilburn, 1983, 1985, 1986, 1988, 1991, 1992, 1995). Taylor et al. (1993) extensively used anatomical characters, in addition to radulae, to

propose an entirely novel classification with six families (Conidae, Turridae, Terebridae, Drilliidae, Pseudomelatomidae, and Strictispiridae). The most important changes introduced in their classification were that Conidae was by then enlarged beyond Coninae (*Conus*) to include five subfamilies previously placed in Turridae, and that the newly restricted Turridae included five additional subfamilies. Bouchet and Rocroi's (2005) recent review of gastropod classification essentially retained Taylor's classification with updates based mainly on Rosenberg (1998) and Medinskaya and Sysoev (2003). We use "Turridae sensu lato" to designate all Conoidea except *Conus* and Terebridae (i.e., Turridae sensu Powell (1966) and most 20th century authors) and "Turridae sensu stricto" to designate the family as restricted by Taylor et al. (1993), while "Conidae" designates the expanded family after Taylor et al. (1993).

Although *Conus* itself has been subjected to intensive molecular studies (e.g., Duda and Kohn, 2005), the phylogeny of the broader Conoidea has not yet been addressed based on molecular characters. The present paper, which expands on our earlier work (Puillandre et al., 2008), presents the first molecular phylogeny based on one mitochondrial and three nuclear genes of the crown clade of the Caenogastropoda. It provides insights at several taxonomic levels (generic, subfamilial, and familial) and offers re-evaluations of the adequacy of previous classifications.

MATERIALS AND METHODS

MATERIALS: A total of 108 specimens of Conoidea were used for molecular analyses, representing 54 valid generic names (Table 1). Eight specimens, noted *cf.*, could not be attributed with certainty to a genus. Specimens of Terebridae and *Conus* were identified to species level. Specimens were sampled during several cruises from 2004 to 2006 in the southwestern Pacific. Living specimens were anesthetized, a piece of tissue was cut from the head-foot, and fixed in 95% ethanol. Shells were kept intact for identification. A specimen of a species of *Nassaria* and a specimen of a species of *Cancellopolia*, both in the neogastropod family Buccinidae, closely related to Conoidea (Harasewych et al., 1997; Colgan et al., 2007), were used as outgroups. *Littorina littorea* (Linnaeus, 1758), belonging in the non-neogastropod family Littorinidae, was used as a third outgroup, with sequences taken from GenBank (GenBank accession numbers: AJ622946.1, Q279985.1, AJ488712.1 and DQ093507.1). Outgroups were chosen to form a non-monophyletic group, as recommended by Darlu and Tassy (1993). All vouchers are kept in MNHN.

SEQUENCING: DNA was extracted from a piece of foot, using 6100 Nucleic Acid Preparation system (Applied Biosystem) or DNeasy[®] 96 Tissue kit (Qiagen) for smaller specimens. A fragment of 658 bp of Cytochrome Oxidase I (COI) mitochondrial gene was amplified using the universal primers LCO1490 and HCO2198 developed by Folmer et al. (1994). Three nuclear gene frag-

ments were also analyzed: (1) 900 bp of the rDNA 28S gene, involving D1, D2 and D3 domains (Hassouma et al., 1984), using the primers C1 and D3 (Jovelin and Justine, 2001); (2) 328 bp of the H3 gene using the primers H3aF and H3aR (Okusu et al., 2003); (3) 1770 bp of the 18S gene using three pairs of primers: 1F and 5R, 3F and B1, A2 and 9R (Giribet et al., 1996; Okusu et al., 2003). All PCR reactions were performed in 25 μ l, containing 3 ng of DNA, 1X reaction buffer, 2.5 mM MgCl₂, 0.26 mM dNTP, 0.3 μ M of each primer, 5% DMSO and 1.5 units of Q-Bio Taq (Qbiogene) for all genes. Amplifications consisted of an initial denaturation step at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 28S gene and first and third fragment of 18S gene, and 53°C for H3 gene and second fragment of 18S gene for 40 sec and extension at 72°C for 1 min. The final extension was at 72°C for 10 min. Thermocycles used for COI gene were described in Hebert et al. (2003). PCR products were purified and sequenced by the Genoscope (Genbank accession numbers: EU015417-EU015558).

PHYLOGENETIC ANALYSES: COI and H3 genes were manually aligned whereas 28S and 18S genes were automatically aligned using ClustalW multiple alignments implemented in BioEdit version 7.0.5.3 (Hall, 1999). Nucleotide substitution models were selected for each gene separately and for each combined dataset using the program Modeltest (Posada and Crandall, 2001), in conjunction with PAUP 4.0b10 (Swofford, 2002). Analyses were conducted using three different approaches. A heuristic Maximum Parsimony (MP) search was executed with 100 Random Taxon-Addition (RA), Tree-Bisection and Reconnection (TBR) branch-swapping, all sites equally weighted and indels treated as fifth states, using PAUP 4.0b10 (Swofford, 2002). Maximum Likelihood (ML) heuristic search was conducted with 100 replicates with TBR branch-swapping using PhyML 2.4.4 (Guindon and Gascuel, 2003). Robustness of the nodes was assessed using nonparametric bootstrapping (Felsenstein, 1985) with 100 bootstraps replicates for MP analysis and 1000 for ML analysis, TBR branch-swapping and 100 RA replicates. Bayesian Analysis (BA) consisted of six Markov chains (8000000 generations each with a sampling frequency of one tree each hundred generations) run in two parallel analyses using Mr. Bayes (Huelsenbeck et al., 2001). For the treatment of combined data using BA, the data were separated into four different partitions corresponding to the four genes analyzed, each following the best fitting model of substitution estimated for each gene.

PHYLOGENY AND CLASSIFICATION: Because of the instability of the taxonomy of the group, currently accepted synonymies cannot be taken for certain and must be re-evaluated. Our taxon sampling includes several genera for as many as possible of the subfamilies proposed in the literature (Table 2). From a nomenclatural perspective, only the occurrence of the type genus of a family-group name in a clade allows for an unequivocal application of this name to that clade. For example, the

Table 1. Specimens of Conoidea used in this study. Identification number (ID) and cruise of collection are given for each specimen. Specimens are identified to genus level, except *Conus* and Terebridae which are identified at species level. A cross indicates that the specimen was successfully sequenced for the gene. Allocation to clades A, B, C and 1 to 21, as defined by the molecular analysis, is given for each taxon.

ID	Cruise	Genus (or species) identification	COI	28S	18S	H3	Clades
17700	BOA 1	<i>Bathytoma</i> Harris and Burrows, 1891	×	×	×	×	20 B
17701	BOA 1	<i>Leucosyrinx</i> Dall, 1889	×	×	×	×	9 A
17702	BOA 1	<i>Leucosyrinx</i> Dall, 1889	×	×	×	×	9 A
17754	Panglao 2004	<i>Turris</i> Röding, 1798	×	×	×	×	5 A
17755	Panglao 2004	<i>Crassispira</i> Swainson, 1840	×	×	×	×	2, C A
17835	BOA 1	<i>Benthomangelia</i> Thiele, 1925	×	×	×	×	17 B
17836	BOA 1	<i>Rimosodaplnella</i> Cossmann, 1915	×	×	×	×	10 B
17837	EBISCO	<i>Inquisitor</i> Hedley, 1918	×	×	×	×	2, C A
17838	EBISCO	<i>Gemmula</i> Weinkauff, 1875	×	×	×	×	5 A
17839	EBISCO	<i>Borsonia</i> Bellardi, 1839	×	×	×	×	16 B
17840	EBISCO	<i>Horaielavus</i> Oyama, 1954	×	×	×	×	7 A
17841	EBISCO	<i>Gymnobela</i> Verrill, 1884	×	×	×	×	10 B
17842	EBISCO	<i>Coehlespira</i> Conrad, 1865	×	×	×	×	8 A
17843	EBISCO	<i>Fina</i> Kilburn, 1988	×	×	×	×	2, C A
17844	EBISCO	<i>Gymnobela</i> Verrill, 1884	×	×	×	×	10 B
17845	EBISCO	<i>Teretopsis</i> Kantor and Sysoev, 1989	×	×	×	×	10 B
17846	EBISCO	<i>Leucosyrinx</i> Dall, 1889	×	×	×	×	3, C A
17847	EBISCO	<i>Splendrillia</i> Hedley, 1922	×	×	×	×	1, C A
17848	EBISCO	<i>Pleurotomella</i> Verrill, 1873	×	×	×	×	10 B
17849	EBISCO	<i>cf. Gemmuloborsonia</i> Shuto, 1989	×	×	×	×	A
17850	EBISCO	<i>Turridrupa</i> Hedley, 1922	×	×	×	×	5 A
17851	EBISCO	<i>Inquisitor</i> Hedley, 1918	×	×	×	×	2, C A
17852	EBISCO	<i>Gemmula</i> Weinkauff, 1875	×	×	×	×	5 A
17853	EBISCO	<i>Heteroturris</i> Powell, 1967	×	×	×	×	18 B
17855	Norfolk 2	<i>Benthofoseis</i> Iredale, 1936	×	×	×	×	B
17857	EBISCO	<i>Bathytoma</i> Harris and Burrows, 1891	×	×	×	×	20 B
17858	Panglao 2004	<i>Clavus</i> Moufort, 1810	×	×	×	×	1, C A
17859	Panglao 2004	<i>Turridrupa</i> Hedley, 1922	×	×	×	×	5 A
17860	Panglao 2004	<i>Lophitoma</i> Casey, 1904	×	×	×	×	5 B
17861	Panglao 2004	<i>Kermia</i> Oliver, 1915	×	×	×	×	10 B
17862	Panglao 2004	<i>Gemmula</i> Weinkauff, 1875	×	×	×	×	5 A
17863	Panglao 2004	<i>Macteola</i> Hedley, 1918	×	×	×	×	11 B
17864	Panglao 2004	<i>cf. Guraleus</i> Hedley, 1918	×	×	×	×	11 B
17865	Panglao 2004	<i>Bathytoma</i> Harris and Burrows, 1891	×	×	×	×	20 B
17866	Panglao 2004	<i>Mangelia</i> Risso, 1826	×	×	×	×	11 B
17867	Panglao 2004	<i>Borsonia</i> Bellardi, 1839	×	×	×	×	16 B
17868	Panglao 2004	<i>Anacithara</i> Hedley, 1922	×	×	×	×	7 A
17869	Panglao 2004	<i>Etrona</i> Hedley, 1918	×	×	×	×	12 B
17870	Panglao 2004	<i>Otitoma</i> Jousseaume, 1898	×	×	×	×	2, C A
17871	Panglao 2004	<i>Kermia</i> Oliver, 1915	×	×	×	×	10 B
17872	Panglao 2004	<i>Macteola</i> Hedley, 1918	×	×	×	×	11 B
17873	Panglao 2004	<i>Guraleus</i> Hedley, 1918	×	×	×	×	11 B
17874	Panglao 2004	<i>Guraleus</i> Hedley, 1918	×	×	×	×	11 B
17875	Panglao 2004	<i>Tomopleura</i> Casey, 1924	×	×	×	×	14 B
17876	Panglao 2004	<i>Lienardia</i> Jousseaume, 1928	×	×	×	×	12 B
17877	Panglao 2004	<i>Mitromorpha</i> Carpenter, 1865	×	×	×	×	13 B
17878	Panglao 2004	<i>Kermia</i> Oliver, 1915	×	×	×	×	10 B
17879	Panglao 2004	<i>Inquisitor</i> Hedley, 1918	×	×	×	×	2, C A
17880	Panglao 2004	<i>Kermia</i> Oliver, 1915	×	×	×	×	10 B
17881	Panglao 2004	<i>Daplnella</i> Hinds, 1844	×	×	×	×	10 B
17882	Panglao 2004	<i>Raphitoma</i> Bellardi, 1848	×	×	×	×	10 B
17883	Panglao 2004	<i>Vepraea</i> Melvill, 1917	×	×	×	×	10 B
17884	Panglao 2004	<i>Leiocithara</i> Hedley, 1922	×	×	×	×	11 B
17885	Panglao 2004	<i>Ceritoturris</i> Dall, 1924	×	×	×	×	7 A
17886	Panglao 2004	<i>Splendrillia</i> Hedley, 1922	×	×	×	×	1, C A
17887	Panglao 2004	<i>Microdrillia</i> Casey, 1903	×	×	×	×	18 B
17888	Panglao 2004	<i>Ceritoturris</i> Dall, 1924	×	×	×	×	7 A
17889	Panglao 2004	<i>Conopleura</i> Hinds, 1844	×	×	×	×	1, C A

(Continued)

Table 1. (Continued)

ID	Cruise	Genus (or species) identification	COI	28S	18S	H3	Clades
17890	Panglao 2004	<i>Raphitoma</i> Bellardi, 1848	×	×	×	×	10 B
17891	Panglao 2004	<i>cf. Tritonoturris</i> Dall, 1924	×	×	×	×	10 B
17892	Panglao 2004	<i>cf. Glyphostomoides</i> Shuto, 1983	×	×	×	×	10 B
17893	Panglao 2004	<i>cf. Mitromorpha</i> Carpenter, 1865	×	×	×	×	13 B
17894	Panglao 2004	<i>Lienardia</i> Jousseau, 1928	×	×	×	×	12 B
17895	Panglao 2004	<i>Inquisitor</i> Hedley, 1918	×	×	×	×	2, C A
17896	Panglao 2004	<i>Eucithara</i> Fischer, 1883	×	×	×	×	11 B
17897	Panglao 2004	<i>Lienardia</i> Jousseau, 1928	×	×	×	×	12 B
17898	Panglao 2004	<i>Mitromorpha</i> Carpenter, 1865	×	×	×	×	13 B
17899	Panglao 2004	<i>Eucithara</i> Fischer, 1883	×	×	×	×	11 B
17900	Panglao 2004	<i>Eucithara</i> Fischer, 1883	×	×	×	×	11 B
17901	Panglao 2004	<i>Anarithma</i> Iredale, 1916	×	×	×	×	13 B
17902	Panglao 2004	<i>Clavus</i> Monfort, 1810	×	×	×	×	1, C A
17903	Panglao 2004	<i>Eucyclotoma</i> Boettger, 1895	×	×	×	×	10 B
17904	Panglao 2004	<i>cf. Nannodiella</i> Dall, 1919	×	×	×	×	12 B
17905	Panglao 2005	<i>Otitoma</i> Jousseau, 1898	×	×	×	×	2, C A
17906	Panglao 2005	<i>Ptychobela</i> Thiele, 1925	×	×	×	×	2, C A
17907	Panglao 2005	<i>Gemmula</i> Weinkauff, 1875	×	×	×	×	5 A
17908	Panglao 2005	<i>Iwaoa</i> Kuroda, 1953	×	×	×	×	7 A
17909	Panglao 2005	<i>Cinguloterebra cf. fujitai</i> Kuroda and Habe, 1952	×	×	×	×	6 A
17910	Panglao 2005	<i>Tomopleura</i> Casey, 1924	×	×	×	×	14 B
17911	Panglao 2005	<i>cf. Heteroturris</i> Powell, 1967	×	×	×	×	18 B
17912	Panglao 2005	<i>Conus praececellens</i> Adams, 1854	×	×	×	×	19 B
17913	Panglao 2005	<i>Conus sulcatus</i> Hwass in Bruguière, 1792	×	×	×	×	19 B
17914	Panglao 2005	<i>Conus sulcatus</i> Hwass in Bruguière, 1792	×	×	×	×	21 B
17915	Panglao 2005	<i>Toxicochlespira</i> Sysoev and Kantor, 1990	×	×	×	×	17 B
17916	Panglao 2005	<i>Comitas</i> Finlay, 1926	×	×	×	×	4, C A
17917	Panglao 2005	<i>Terebra polygyrata</i> Deshayes, 1859	×	×	×	×	6 A
17918	Panglao 2005	<i>Comitas</i> Finlay, 1926	×	×	×	×	4, C A
17919	Panglao 2005	<i>Cochlespira</i> Conrad, 1865	×	×	×		8 A
17920	Panglao 2005	<i>Cochlespira</i> Conrad, 1865	×	×	×		8 A
17921	Panglao 2005	<i>Conus orbigny</i> Kilburn, 1975	×	×	×	×	21 B
17922	Panglao 2005	<i>Conus wakayamaensis</i> Kuroda, 1956	×	×	×	×	21 B
17923	Panglao 2005	<i>Cinguloterebra cf. fenestrata</i> Hinds, 1844	×	×	×	×	6 A
17924	Salomon 2	<i>Thatcheria</i> Angas, 1877	×	×	×	×	10 B
17925	Salomon 2	<i>Toxicochlespira</i> Sysoev and Kantor, 1990	×	×	×	×	17 B
17926	Salomon 2	<i>Borsonia</i> Bellardi, 1839	×	×	×	×	15 B
17927	Salomon 2	<i>Daphnella</i> Hinds, 1844	×	×	×	×	10 B
17928	Salomon 2	<i>Comitas</i> Finlay, 1926	×	×	×	×	3, C A
17929	Salomon 2	<i>Bathytoma</i> Harris and Burrows, 1891	×	×	×	×	20 B
17930	Salomon 2	<i>Benthomangelia</i> Thiele, 1925	×	×	×	×	17 B
17931	Salomon 2	<i>cf. Typliomangelia</i> Sars, 1878	×	×	×	×	18 B
17932	Salomon 2	<i>Borsonia</i> Bellardi, 1839	×	×	×	×	15 B
17933	Salomon 2	<i>Comitas</i> Finlay, 1926	×	×	×	×	3, C A
17934	Salomon 2	<i>Borsonia</i> Bellardi, 1839	×	×	×	×	16 B
17935	Salomon 2	<i>Inquisitor</i> Hedley, 1918	×	×	×	×	2, C A
17936	Santo 2006	<i>Conus generalis</i> Linne, 1758	×	×	×	×	19 B
17937	Santo 2006	<i>Conus gauguini</i> Richard and Salvat, 1973	×	×	×	×	19 B
17938	Santo 2006	<i>Terebra textilis</i> Hinds, 1844	×	×	×	×	6 A
17939	Santo 2006	<i>Conus consors</i> Sowerby, 1833	×	×	×	×	19 B
17854	Norfolk 2	<i>Nassaria</i> , Buccinidae	×	×	×	×	
17856	Norfolk 2	<i>Cancellipollia</i> , Buccinidae	×	×	×	×	
GenBank		<i>Littorina</i> , Littorinidae	×	×	×	×	

clade containing the genus *Raphitoma* can unambiguously carry the name Raphitominae. However, many type genera are not represented in our taxon sampling and some of our molecular clades do not include a type genus. In such cases, we have relied on the traditional

allocation of non-type genera to a subfamily to link clade and name. For example, a clade containing three genera classically classified in the family Drilliidae (Taylor et al., 1993; Tippet and Tucker, 1995) can carry the name Drilliidae, even though *Drillia* itself is not part of our taxon

sampling. However, this approach does not lead to an unequivocal application of names when genera (or subfamilies) as traditionally construed prove to be non-monophyletic; in that case, only the type species (or the type genus) is the legitimate bearer of the name.

RESULTS

Almost all specimens were sequenced for the four genes (see details in Table 1). Saturation analyses for the two protein-coding genes revealed that the COI gene was highly saturated at the third codon position; accordingly, we used only the first and second positions in the phylogenetic analyses. Independent analyses of each of the four genes provided very poorly resolved trees, with few well-supported clades (results not shown). Since no incongruency was revealed among the single gene analyses, we constructed a combined dataset comprising the data of the four gene fragments resulting in a sequence length of 3428 bp, including 108 ingroups.

The Conoidea were found to be monophyletic, although not strongly supported (MP and ML bootstraps respectively: 65 and 79, Posterior Probabilities PP: 1). Within the Conoidea, two clades could be distinguished: clade A (MP bootstraps: 58, ML bootstraps: 68, PP: 0.73) and clade B (MP bootstraps: 28, ML bootstraps: 52, PP: 1). Within the clade A, the clade C is found strongly supported with ML bootstraps (91) and PP (1). Analysis of the combined datasets allowed the definition of 21 higher level clades, each of them strongly supported: MP and ML bootstraps > 80 and PP > 0.99 (Mason-Gamer and Kellogg, 1996; Zander, 2004). They included from one to 12 genera each (Figure 1, Table 2). Clades were numbered according to their position in the tree. Clades 1 to 9 are included in clade A, and among them clades 1 to 4 are included in clade C. Clades 10 to 21 are included in clade B.

All representatives of a genus clustered together in one of the 22 clades, except for representatives of *Borsonia*, *Comitas*, *Conus*, and *Lucosyrinx*. The representatives of *Borsonia* and *Conus* split respectively in clades 15–16 and 19–21, each including only specimens from a single genus. The relationships between the two clades were not resolved and thus the monophyly of each of these genera cannot be rejected. Conversely, the monophyly of genera *Lucosyrinx* and *Comitas* (clades 3, 4 and 9) can be rejected, since representatives of the two genera clustered in the clade 4.

DISCUSSION

CLASSIFICATION OF THE CONOIDEA: Although not strongly supported, our analysis suggests that the superfamily Conoidea is monophyletic. However, the Conoidea and two outgroups used here (*Cancellopollia* and *Nassaria*) both belong in the Neogastropoda, a group for which the phylogeny is not well resolved (Harascewicz et al., 1997; Colgan et al., 2007), and the monophyly observed

here could thus be an artifact due to under-sampling within Neogastropoda. Within Conoidea, the large amount of diversity included in our dataset allows us to discuss the current classification at genus, subfamily, and family levels.

Accuracy of Taxonomic Delineations at the Genus Level:

The genus is the lowest level for which we can discuss taxonomic delineations since most of our specimens are not identified at species level. Among the 54 genera identified in our dataset, monophyly can be rejected for only two of them (*Lucosyrinx* and *Comitas*), which indicates that in most cases shell morphology is an appropriate predictor of generic allocations. Two further genera (*Borsonia* and *Conus*) are found to be diphyletic, but the position of the two defined clades is unresolved and thus monophyly cannot be excluded.

Position of the Genera within the Subfamilies:

Our analysis confirms many previous assignments of genera to subfamilies as in Taylor et al. (1993) and subsequent refinements of their classification (Table 2). However several results do not confirm established classifications. For example, the genus *Otitoma*, tentatively retained by in the Mangeliinae by Kilburn (2004), who acted based on shell characters, is here allocated to the Crassispirinae.

Robustness of Subfamilial Delineations:

We found discrepancies between our phylogeny and previous classifications at the subfamily level. Thus, crassispirine genera are present in two clades (2 and 7), one of them (clade 2) containing the type genus. The polyphyly of this subfamily is supported by the existence of clade C, which includes clade 2, but excludes clade 7. Given that the relationships between clade 7 and others clades within clade A are not resolved, it is inconclusive whether clade 7 must be ranked as its own subfamily or whether it must be grouped together with another existing subfamily. Similarly, the subfamily Cochlespirinae as currently construed appears polyphyletic. In three cases (Mangeliinae, Coninae, Clathurellinae), polyphyly is possible but not demonstrated because of a general lack of support for deeper nodes in clade B.

Robustness of Familial Delineations:

Finally, our results also permit a discussion of family classification within Conoidea. Taylor et al.'s (1993) anatomical study suggested a closer relationship of Clathurellinae, Conorbinae, Mangeliinae, Oenopotinae, and Raphitomininae to *Conus* than to other members of the family Turridae sensu lato and their extension of Conidae included these turrid subfamilies. In our study, clade B, although weakly supported, corresponds to Taylor et al.'s (1993) family Conidae.

Our study also revealed another weakly supported deep clade (clade A) that includes genera classified by Taylor et al. (1993) in three different families: Drilliidae, Terebridae and Turridae sensu stricto (consisting of Clavatulinae, Cochlespirinae, Crassispirinae, Turrinae and Zonulspirinae). Genera of the family Drilliidae

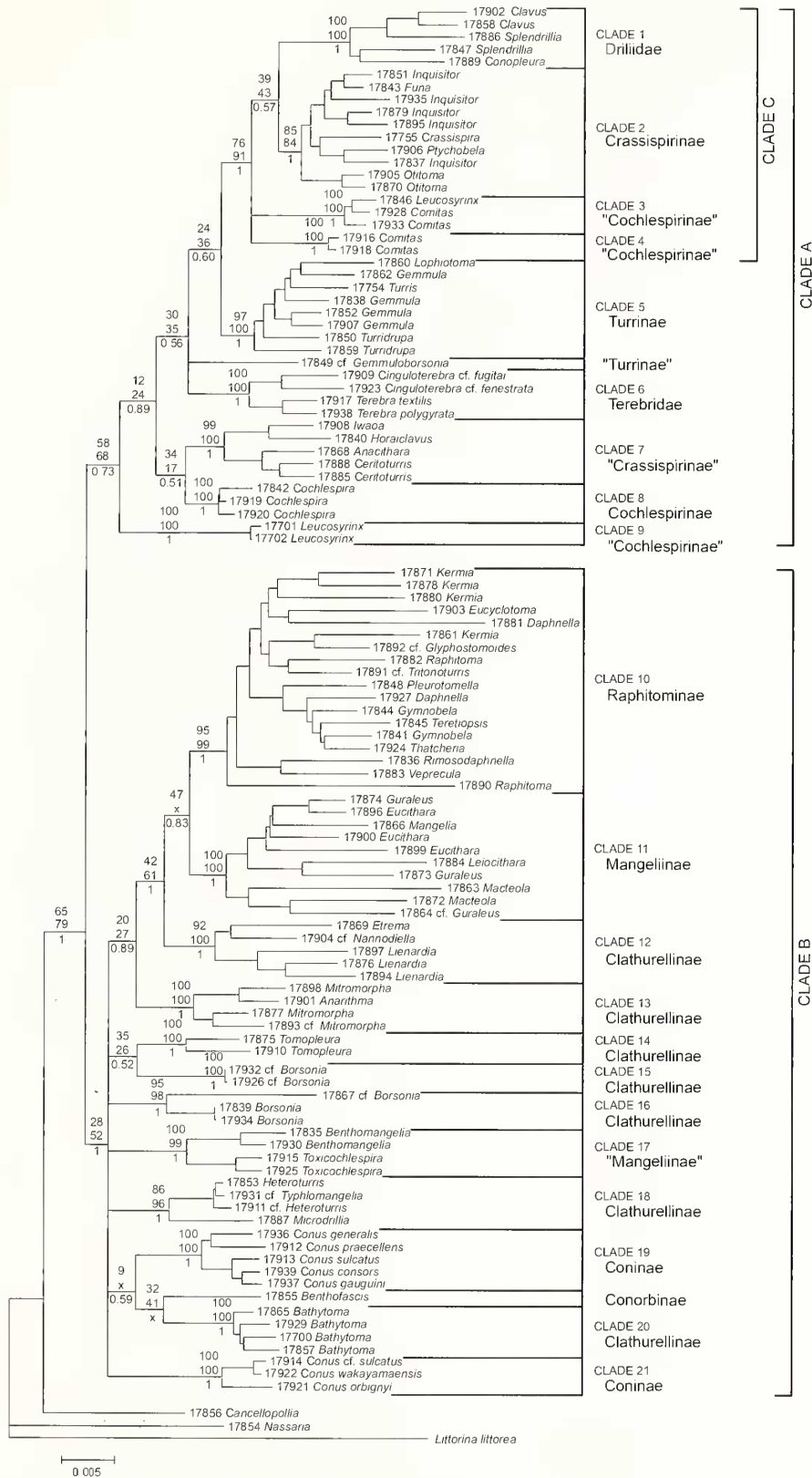


Figure 1. Consensus tree of MP, ML and BA. Nodes presented here were found with at least two of the three methods used. Top downwards, MP bootstraps, ML bootstraps and Posterior Probabilities are specified for each node. Support for intranodes of clades 1 to 21 are not presented.

Table 2. Current Conoidea classification and comparison with our results. Current Conoidea classification including genera used in the present study (based mainly on Taylor et al., 1993) and clades defined by the molecular phylogeny. Subfamilies are in bold, families in bold and capital.

	<u>Current System</u>	<u>Molecular Phylogeny</u>		
TURRIDAE sensu stricto	DRILLIIDAE <i>Clavus</i> <i>Conopleura</i> <i>Splendrilla</i>	Clade 1 <i>Clavus</i> <i>Conopleura</i> <i>Splendrilla</i>	DRILLIIDAE	
	Crassispirinae <i>Anacithara</i> <i>Horaiclavus</i> <i>Ceritoturris</i> <i>Inquisitor</i> <i>Crassispira</i> <i>Iwaoa</i> <i>Funa</i> <i>Ptychobela</i>	Clade 2 <i>Crassispira</i> <i>Funa</i> <i>Inquisitor</i> <i>Otitoma</i> <i>Ptychobela</i>	Clade 7 <i>Anacithara</i> <i>Ceritoturris</i> <i>Horaiclavus</i> <i>Iwaoa</i>	Crassispirinae
	Cochlespirinae <i>Cochlespira</i> <i>Comitas</i> <i>Leucosyrinx</i>	Clade 3 & 4 <i>Comitas</i> Clade 3 <i>Leucosyrinx</i>	Clade 8 <i>Cochlespira</i> Clade 9 <i>Leucosyrinx</i>	Cochlespirinae
	Turrinae <i>Gemmula</i> <i>Gemmuloborsonia</i> <i>Lophiotoma</i> <i>Turris</i> <i>Turridrupa</i>	Clade 5 <i>Gemmula</i> <i>Lophiotoma</i> <i>Turris</i> <i>Turridrupa</i>	<i>Gemmuloborsonia</i>	Turrinae
	TEREBRIDAE <i>Cinguloterebra</i> <i>Terebra</i>	Clade 6 <i>Cinguloterebra</i> <i>Terebra</i>		TEREBRIDAE
TURRIDAE sensu stricto	Zonulispirinae			
	Zemaciinae			
	PSEUDOMELATOMIDAE			
	STRICTISPIRIDAE			
	CLAVATULIDAE			
CONIDAE	Raphitominae <i>Daphnella</i> <i>Raphitoma</i> <i>Eucyclotoma</i> <i>Rimosodaphnella</i> <i>Glyphostomoides</i> <i>Teretiopsis</i> <i>Gymnobela</i> <i>Thatcheria</i> <i>Kermia</i> <i>Tritonoturris</i> <i>Pleurotomella</i> <i>Veprecula</i>	Clade 10 <i>Daphnella</i> <i>Raphitoma</i> <i>Eucyclotoma</i> <i>Rimosodaphnella</i> <i>Glyphostomoides</i> <i>Gymnobela</i> <i>Thatcheria</i> <i>Kermia</i> <i>Tritonoturris</i> <i>Pleurotomella</i> <i>Veprecula</i>		Raphitominae
	Mangeliinae <i>Benthomangelia</i> <i>Macteola</i> <i>Eucithara</i> <i>Mangelia</i> <i>Guraleus</i> <i>Otitoma</i> <i>Leiocithara</i> <i>Toxicochlespira</i> <i>Lienardia</i>	Clade 11 <i>Eucithara</i> <i>Guraleus</i> <i>Leiocithara</i> <i>Macteola</i> <i>Mangelia</i>	Clade 17 <i>Benthomangelia</i> <i>Toxicochlespira</i>	Mangeliinae
	Clathurellinae Borsoniid: <i>Borsonia</i> <i>Typhlomangelia</i> Mitromorphid: <i>Anarithma</i> <i>Mitromorpha</i> Bathytomid: <i>Bathytoma</i> Clathurellid: <i>Erema</i> ? <i>Nannodiella</i> Tomopleurid: <i>Heteroturris</i> <i>Microdrillia</i> <i>Tomopleura</i>	Clade 12 <i>Lienardia</i> <i>Erema</i> <i>Nannodiella</i> Clade 13 <i>Mitromorpha</i> <i>Anarithma</i> Clade 14 <i>Tomopleura</i> Clade 15 & 16 <i>Borsonia</i>	Clade 18 <i>Heteroturris</i> <i>Microdrillia</i> <i>Typhlomangelia</i> Clade 20 <i>Bathytoma</i>	Clathurellinae
	Conorbinae <i>Benthofascis</i>	<i>Benthofascis</i>		Conorbinae
	Coninae <i>Conus</i>	Clade 19 <i>Conus</i>		Coninae
	Oenopotinae	Clade 21 <i>Conus</i>		

A
TURRIDAE
+ DRILLIIDAE
+ TEREBRIDAE ?

B
CONIDAE

(clade 1) are included in clade C. This well-supported clade also contains taxa of the family Turridae sensu stricto (Crassispirinae and *Comitas*), and excludes the other taxa of the family Turridae "sensu stricto". Consequently, Turridae sensu stricto are not monophyletic. Furthermore, according to Kantor (2006), the radula of Drilliidae is not fundamentally different from that of Turridae sensu stricto. Both our molecular data and this morphological evidence suggest that Drilliidae should be subsumed as a subfamily within the Turridae sensu stricto.

Within clade A, the monophyly of the family Terebridae is supported but its relationships with other clades of Turridae sensu stricto is not resolved. However, this result suggests that Terebridae are closely related to Turridae sensu stricto, as already proposed by Cossmann (1896), and Powell (1942; 1966).

TOWARD A STABILIZED SYSTEM FOR CONOIDEA: The taxonomic sampling used here allows for an estimate of molecular variability within clades at each level: several genera are included in each subfamily, several subfamilies are included in each family, and most of the families defined by Taylor et al. (1993) are present. However, even with a dataset of 54 genera, covering most of the previously recognized families and subfamilies of Conoidea, the present study only brings preliminary results. At genus level, these 54 genera represent only 16% of the 340 Recent genera described. It is clear that the shell-based current taxonomic definition of many genera will not stand after molecular testing. At subfamily and family levels, although a large part of the conoidean diversity is represented in this study, some families and subfamilies are not part of our taxon sampling. The highly divergent clades found here in several subfamilies as previously defined demonstrate the need for further research, which could better circumscribe subfamilies already known and probably formally name new subfamilies and/or tribes. Finally new relationships are suggested at the family level. As a remake of the *Conus* story, it now appears that the long recognized family Terebridae does not stand alone apart from the rest of the Conoidea, but could be the sister-group or even part of the Turridae sensu stricto.

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