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Cenozoic evolution of Muricidae (Mollusca, Neogastropoda) in the Southern Ocean, with the description of a new subfamily

Andrea Barco, Stefano Schiaparelli, Roland Houart & Marco Oliverio

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Gastropods are among the most studied group in Antarctica, and taxa with an advanced status of systematic knowledge can be used as a model to study how oceanographic and climatic patterns shaped Recent faunal assemblages. Within the ongoing study of the muricid phylogeny, we have analysed molecular and morphological data from species traditionally ascribed to the muricid subfamily Trophoninae. Particularly, the availability of specimens collected in the Southern Ocean and surrounding basins allowed to demonstrate as the genera *Pagodula*, *Xymenopsis*, *Xymene* and *Trophonella*, which are traditionally classified in the Trophoninae, actually belong to a distinct lineage, for which the new subfamily Pagodulinae is herein introduced. We propose and discuss a possible framework for the origin and radiation of Antarctic muricids.

Corresponding Author: *Andrea Barco, Dipartimento di Biologia e Biotecnologie "Charles Darwin", Università di Roma "La Sapienza", Viale dell'Università 32, I-00185 Rome, Italy.* E-mail: *andrea.barco@uniroma1.it*

Stefano Schiaparelli, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (Di. S. T. A. V.), Università di Genova, C.so Europa 26, I-16132 Genova, Italy. E-mail: stefano.schiaparelli@unige.it

Roland Houart, Institut Royal des Sciences Naturelles de Belgique, Rue Vautier 29, B-1000 Bruxelles, Belgium. E-mail: roland.houart@gmail.com

Marco Oliverio, Dipartimento di Biologia e Biotecnologie "Charles Darwin", Università di Roma "La Sapienza", Viale dell'Università 32, I-00185 Rome, Italy. E-mail: marco.oliverio@uniroma1.it

Introduction

The Southern Ocean (here referred to the area comprised within the Antarctic Polar Front) is one of the best-defined biogeographical regions for its widely recognized isolation and high level of endemism (Griffiths *et al.* 2011). Current Antarctic marine features represent a physiological and ecological challenge to many taxa, and many common benthic groups from tropical to temperate regions are not represented in the Recent Antarctic marine fauna (Aronson & Blake 2001; Clarke & Johnston 2003).

The last 50 million years included the most influential climatic, tectonic and oceanographic modifications that shaped the Southern Ocean fauna: the sharp cooling of the climate and the onset of glacial-interglacial cycles (Lear *et al.* 2000), the geographical isolation of the continent from South America and Australia (Lawver & Gahagan 2003) and the installation of the Antarctic Circumpolar Current (Lawver & Gahagan 2003). Brandt *et al.* (2007) proposed that the distribution and evolutionary history of species living in the Southern Ocean reflect these events, and a few recent studies provide evidence of such a strict relationship. Notable examples are the pattern of vicariance and adaptive radiation of notothenioid fishes (Near 2004), the multiple bathymetric diversification of isopods (Brandt *et al.* 2007) and the connection between Antarctic and deep-sea lineages of cephalopods (Strugnell *et al.* 2008).

Gastropods are among the most studied group in Antarctica (Clarke *et al.* 2007), and the current status of systematic knowledge of some of the lineages represented in the Southern Ocean allows us to use these organisms as a model to study how oceanographic and climatic patterns shaped Recent faunal assemblages.

The family Muricidae is a large group of marine snails known for their abundance on intertidal rocky shores and in marine benthic communities in general, where they play

an important ecological role as predators of other invertebrates (Ponder 1998 and references therein). Predation is mostly achieved by drilling the shell of the prey by the combined mechanical action of the radula and secretion of organic acid (Carriker 1961). Some species also represent an economically valuable source of food (Leiva & Castilla 2002), while others are pests for aquaculture because of drilling of edible bivalve species in farms (Buhle & Ruesink 2009).

Muricids are particularly common in tropical and temperate latitudes, where the group originated in the Late Cretaceous (Merle 1999; Merle *et al.* 2011). Muricids are relatively well represented among the benthic invertebrates of the Southern Ocean, where they are one of the most species-rich groups of molluscs. According to Linse *et al.* (2006), the richness centre of Antarctic Muricidae spans from the Weddell Sea to the Magellanic area through the Scotia Arc, although data from recent surveys used as evidence of Antarctic diversity hotspots are possibly biased by differential sampling efforts in the past (Griffiths *et al.* 2011). Almost all the muricids in the Southern Ocean are currently classified in the subfamily Trophoninae, which—as traditionally conceived—is one of the most species-rich among muricid subfamilies, including at least 27 genera and approximately 290 species (Houart in Appeltans *et al.* 2011). The subfamily is still used by malacologists, even though Radwin & D'Attilio (1976) drew attention to the heterogeneity of radular and shell characters among several of its taxa. This was endorsed by Pastorino (2002), who described major differences within the genus *Trophon* Montfort, 1810 from Patagonia and Antarctica, suggesting that both the genus and the subfamily might be polyphyletic.

In our recent molecular phylogeny of Muricidae (Barco *et al.* 2010), we provided evidence of the polyphyly of the Trophoninae as traditionally conceived. The Antarctic species *Trophon longstaffi* E.A. Smith, 1907 (therein misidentified as *Trophon shackletoni* Hedley, 1911) showed a greater affinity to the subfamily Haustrinae, a little group of species endemic to New Zealand and Australia, rather than to the Argentinian species *Trophon geversianus* (Pallas, 1774) (the type species of *Trophon*). Our finding has been supported by the introduction of the new genus *Trophonella* by Harasewych & Pastorino (2010). *Trophonella* accommodates some Antarctic species (including *T. lonstaffi*) that have marked anatomical differences from the type species of *Trophon*.

Many genera of Trophoninae are represented both in southern Argentina and New Zealand. Similarities between these groups were described by Ponder (1971, 1972) and Pastorino & Harasewych (2000), and used to formulate a first zoogeographical hypothesis for the evolution of the group (Beu *et al.* 1997). However, the general uncertainty about the phylogenetic relationships of this group hampered any attempt to reconstruct its evolutionary history, and a molecular framework is necessary to recognize natural groups and reconstruct their phylogeny and phylogeography.

In this paper, we propose and discuss a possible framework for the origin and radiation of Antarctic muricids, comparing our findings with similar scenarios from other groups. Within the ongoing re-definition of muricid phylogeny, we analyse molecular and morphological data from newly collected material from the Southern Ocean and surrounding basins, identifying a new subfamily of Muricidae that includes some genera traditionally classified in the Trophoninae.

Materials and methods

Taxon sampling

The sequences used in this work were to a large extent obtained during our previous survey of muricid phylogeny (Barco *et al.* 2010). The additional specimens included here are the representatives of species traditionally attributed to Trophoninae, in particular some of those inhabiting the southern hemisphere. Collection data, vouchers and accession numbers are listed in Table 1. For the molecular analyses, a piece of tissue was dissected from the foot of each specimen after collection, fixed in 100% ethanol and stored at −4 °C. Anatomical data were collected from some of the specimens used for the molecular analyses, as well as from other material reported in Table 1.

DNA amplification and sequencing

DNA extraction was performed after tissue digestion in proteinase K using a phenol chloroform protocol (Hillis *et al.* 1990) with slight modification as described by Oliverio & Mariottini (2001).

Fragments of the mitochondrial cytochrome oxidase I (COI) and 16S rRNA genes were amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994), and 16SA (Palumbi *et al.* 1991) and CGLeu^{UUR} (Hayashi 2005), respectively. Six domains of nuclear 28S rRNA were amplified using the primers LSU5' (Littlewood *et al.* 2000) and LSU1600R (Williams *et al.* 2003). Primer sequences are the same as reported by Barco *et al.* (2010), as well as the accession numbers (and voucher data) for previously published sequences. Accession numbers for the new sequences are reported in Table 1.

PCR amplifications were performed in 25 μL containing 2.5 μL of BIOLINE 10 × buffer, 0.5 μL of 10mM dNTPs mix, 0.4 μL of each primer (10 mM), 2.5–3 μl of 50 mM $MgCl_2$, 1 U of BIOLINE TaqPolymerase and 0.5–1 μL of genomic DNA. Amplification of 16S rRNA fragments yielded better results with the addition of 1μl of DMSO, which prevents the formation of secondary structures in

Table 1 Voucher number, collection localities, accession numbers of unpublished sequences included in the analysis and morphological data of Muricidae

			Accession numbers			Morphological data	
Species	Voucher No.	Locality	16S	COI	28S	Radula	Penis
Scabrotrophon inspiratus	MNHN 200915279	Papua New Guinea, West of New Hanover Island. Biopapua st. CP3653, 02.13°S, 150.23°E. 680–700 m depth	HE804822	JX033992			
Scabrotrophon inspiratus	MNHN 200915285	Papua New Guinea, off Madang. Biopapua st. CP3708, 4.58°S, 145.50°E, 502–529 m depth	HE804823	JX033991			
Scabrotrophon inspiratus	MNHN 200915286	Papua New Guinea, off Madang. Biopapua st. CP3708, 4.58°S, 145.50°E, 502–529 m depth	HE804824	JX033990			
*Pagodula echinata**	MNHN 200915463	Ibero-Moroccan gulf. Balgim st. CP98, 34.29°N, 7.42°W, 1747 m depth	HE804825				
*Pagodula echinata**	MNHN 200915465	Ibero-Moroccan gulf. Balgim st. CP65, 35.26°N, 8.00°W, 1805 m depth	HE804826				
*Pagodula echinata**	BAU 00918	Italy, Tyrrhenian Sea, Tuscan Archipelago				Fig. 3A,B	
Pagodula eos	NIWA 30162	New Zealand, Chatham Rise. RV Tangaroa, 42.655°S, 177.214°E, 1377–1402 m depth	HE804815	HE804834	HE804829		
Pagodula lata	NMNZ M284123	New Zealand, Challenger Plateau. RV Tangaroa, 36.913°S, 167.531°E, 1211–1216 m depth		HE804835	HE804830		
Pagodula lata	NMNZ M284040	New Zealand, Challenger Plateau. RV Tangaroa, 38.582°S, 167.149°E, 974 m depth	HE804816	HE804836			
Pagodula maxwelli	NMNZ M284122	New Zealand, Challenger Plateau. RV Tangaroa, 37.274°S, 169.667°E, 1713–1773 m depth		HE804837	HE804831		
Pagodula cf. *cuspidarioides*	NIWA 38174b	Antarctica, Ross Sea. Tangaroa 2008 st. 206, 68.1208°S, 179,248°E, 879 m depth		HM887932		Fig. 3C,D	
Pagodula cf. *cuspidarioides*	NIWA 38174a	Antarctica, Ross Sea. Tangaroa 2008 st. 206, 68.1208°S, 179,247°E, 879 m depth		HM887946			
Pagodula cf. *cuspidarioides*	NIWA 3361	Antarctica, Ross Sea. Tangaroa 2008 st. 218, 67.7233°S, 179,712°E, 1145 m depth		HM887934		Fig. 3E,F	Fig. 5C
Trophonella echinolamellata	MNA 2713	Antarctica, Bellingshausen Sea, Skua shelter. 65.15°S, 64.16°W, diving depth	HE804819	JX110857	HE804832		
Trophonella longstaffi	NIWA 38623b	Antarctica, Ross Sea. Tangaroa 2008 st. 249, 67.4122°S, 179,942°E, 300 m depth		HM431868		Fig. 4A	Fig 5A
Trophonella longstaffi	MNA42	Antarctica, Ross Sea, Tethys Bay. XVIII PNRA Expedition 74.41°S, 164.05°E, 10 m depth	HE804821	JX110858	HE804833	Fig. 4B	
*Trophonella scotiana**	MNA4	Antarctica, Ross Sea. Tangaroa 2004 st. 39, 71.755°S, 171,1475°E, 250 m depth	HE804820	JX110861		Fig. 3I	
Trophonella shackletoni	MNA 2	Antarctica, Ross Sea. Tangaroa 2004 st. 35, 71.7675°S, 171,1092°E, 282 m depth					Fig. 5B
Trophonella shackletoni	MNA 5	Antarctica, Ross Sea. Tangaroa 2004 st. 33, 71.7547°S, 171,417°E, 282 m depth	HE804817	JX110860		Fig. 3G,H	
Trophonella shackletoni	MNA 40	Antarctica, Ross Sea, Tethys Bay. XVIII PNRA Expedition 78.00°S, 164.00°E, 18 m depth	HE804818	JX110859			
*Trophonopsis muricatus**	RH	Italy, Adriatic Sea, off Chioggia				Fig. 4C	
Xymene pulcherrimus	NMNZ M284120	New Zealand, Challenger Plateau. RV Tangaroa, 38.024°S, 168.447°E, 570–575 m depth	HE804814	HE804838	HE804828		
*Xymenopsis muriciformis**	BAU 00913	Chile, Tierra del Fuego, Pueblo Porvenir	HE804813	JX033993	HE804827		

Institutional abbreviations: BAU, Department of Biology and Biotechnology "Charles Darwin", Rome (Italy); NMNZ, Museum of New Zealand Te Papa Tongarewa, Wellington (New Zealand); MNA, Museo Nazionale dell'Antartide, Genova (Italy); MNHN, Muséum National d'Histoire Naturelle, Paris (France); NIWA, National Institute of Water and Atmospheric Research, Auckland (New Zealand). RH, Roland Houart, personal collection. Type species of genera are marked with an asterisk.

single-stranded DNAs. Segments were amplified with an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 40 s and extension at 72 °C for 50 s. These cycles were followed by extension at 72 °C for 10 min.

PCR products were purified with the ExoSAP protocol. A mix of 2 μL containing 20 U/μL Exonuclease I (New England Biolabs, Ipswich, MA, USA) and 1 U/μL Shrimp Alkaline Phosphatase (Roche, Basel, Switzerland) was used to purify 5 μL of PCR product. Fragments were

sequenced by Macrogen Inc. (Seoul, South Korea) using the same PCR primers in BigDye terminator cycling conditions (Applied Biosystems, Carlsbad, CA, USA). Reacted products were purified using ethanol precipitation and run using an automatic sequencer AB3730XL (Applied Biosystems).

Sequences analysis

Forward and reverse sequences were assembled and reciprocally edited with Sequencher (v. 4.1.4; Gene Codes Corporation Ann Arbor, MI). Fragments of the ribosomal genes were aligned with MAFFT (Katoh *et al.* 2002) using the Q-INS-i algorithm (Katoh & Toh 2008), which accounts for secondary structures. Alignments were optimized manually and assembled to the COI matrix using BioEdit (Hall 1999). Highly variable regions with ambiguous alignment were identified and excluded with Gblocks (v. 0.91b, Castresana 2000) using the same settings as in Barco *et al.* (2010), which were identified as those optimizing the extrapolation of phylogenetic signal from our alignment. However, different Gblocks settings were tested on our data without affecting the recovered topologies. Remaining indels were coded in a separate matrix at the end of the alignment as binary characters (presence/absence of the indel across the sequences) according to the method of Simmons & Ochoterena (2000) implemented in FastGap (v. 1.2, Borchsenius 2009).

In the analysis, we also used the 12S sequences used in Barco *et al.* (2010). The unavailable sequences were coded as missing data.

Substitutional saturation was evaluated plotting transitions (Ts) and transversions (Tv) uncorrected *p*-distances against global *p*-distances. A linear regression was constructed on each saturation plot, and its significance tested calculating Parson's correlation index (*r*) with PaSt (Hammer *et al.* 2001). Base composition of nucleotide sequences and chi-square statistics to evaluate nucleotide homogeneity were calculated with PAUP* (v. 4.0b10, Swofford 2002). Codon usage was investigated using the effective number of codons value (ENC, Wright 1990) as implemented in DNAsp (v. 5, Librado & Rozas 2009).

Phylogenetic analysis

To maximize the extrapolation of the phylogenetic signal, we tested several types of schemes for partitioning our complete alignment. We used seven different partitioning schemes (Table 2), each analysed using the best-fitting evolutionary model proposed by the Akaike information criterion (AIC, Akaike 1973) implemented in MrModeltest (v. 2.3, Nylander 2004). The binary matrix coding for the indels was analysed using a continuous-time Markov model, which allows the trait to change from its present state in any given moment to any other state over infinitesimally small intervals of time (Lewis 2001).

Table 2 Partition schemes used in the phylogenetic analysis.

Partition name	Number of partitions	Genes partitions
MUR01	2	All genes; all gaps
MUR02	3	Ribosomal; all gaps; COI
MUR03	4	Mitochondrial; mitochondrial gaps; nuclear; nuclear gaps
MUR04	5	12S + 16S; 12S gaps + 16S gaps; COI; 28S; 28S gaps;
MUR05	7	12S; 12S gaps; 16S; 16Sgaps; 28S; 28S gaps; COI
MUR06	8	12S; 12S gaps; 16S; 16Sgaps; 28S; 28S gaps; COI 1st + 2nd pos; COI 3rd pos
MUR07	9	12S; 12S gaps; 16S; 16Sgaps; 28S; 28S gaps; COI 1st pos; COI 2nd pos; COI 3rd pos

We performed a Bayesian analysis for all the partition schemes running two MC^3 algorithms with four chains each for 20^7 generations (40^7 generations for MUR06 and MUR07) using MrBayes (v. 3.1.2; Huelsenbeck & Ronquist 2001). Each parameter in the evolutionary model (nucleotide frequencies, substitution rates, proportion of invariable sites and α parameter of Γ distributions) was estimated independently for each partition.

Convergences of the chains were evaluated by plotting values of standard deviation of average split frequencies sampled every 1000 generations, and with the potential scale of reduction factor (PRSF, Gelman & Rubin 1992). All the topologies sampled before convergence were excluded in the computation of the consensus tree. With the command *sump*, we obtained the harmonic mean of estimated marginal likelihoods (EML), which was used to address the choice among different partitioning schemes.

We applied the AIC and the Bayes factor (Kass & Raftery 1995) to test for the best partitioning scheme. AIC was calculated following Huelsenbeck *et al.* (2004), Posada & Buckley (2004) and Strugnell *et al.* (2005) as $AIC = -2EML + 2K$, where K represents the number of free parameters in the topology (number of branches) and in the substitution model (nucleotide frequencies, substitution rates, gamma shape parameter and proportion of invariable sites for each partition). Bayes factors were calculated as $2\ln B_{ij} = 2(\ln EML_i - \ln EML_j)$, where B_{ij} is the Bayes factor measuring the strength of the *i*th hypothesis on the *j*th hypothesis. Bayes factors were interpreted according to Kass & Raftery (1995) and Brandley *et al.* (2005). Nodes in the phylogenetic trees were considered 'highly' supported with posterior probability (PP) values >96% and 'moderately' supported when posterior probability was between 90% and 95%. Lower values were considered as indicating weak support.

Molecular clock analysis

An ultrametric tree was obtained with BEAST (v. 1.6.1, Drummond & Rambaut 2007) using the same alignment used in the phylogenetic analysis. The tree was calibrated using three divergence points based on fossil data. To obtain a realistic assessment of the uncertainty associated with the fossil record, we used exponentially distributed calibration prior (Ho & Phillips 2009). We set the fossil age as upper bound (setoff) of the prior distribution, and the mean value was selected to include 95% of the distribution within upper and lower bounds.

Fossil calibrations are detailed as follows:

1. Muricidae vs. outgroup (*Buccinum undatum* Linnaeus, 1758 *and Conus judaceus* Bergh, 1895). This point was calibrated on the earliest known species unquestionably attributed to Muricidae, the fossil *Paziella* (*Flexopteron*) *cretacea* (Garvie, 1991) from the Late Cretaceous of Texas (Maastrichtian, 70.6 Mya). Earlier specimens have been attributed tentatively to the Muricidae, but their correct classification is still unclear (Merle 1999). However, even including those specimens, muricids were certainly not present before the Albian (Lower Cretaceous, 112 Mya), which was set as the lower bound.
2. Coralliophilinae vs. Ergalataxinae. The Middle Eocene *Coralliophila* (*Timotia*) *aldrichi* (Cossmann, 1903) is the earliest known species of Coralliophilinae (Clairbonian of Mississippi and Louisiana, approx. 40 Mya; Dockery 1980). The lower bound was defined at 65.5 Mya, in agreement with the estimate that the diversification of the muricid subfamilies probably occurred during the Paleocene and Eocene (Oliverio 2008).
3. Typhinae vs. sister group (*Timbellus fulgens* + *Vitularia* spp.). We calibrated this node according to the fossil record of Typhinae, which date the first certain appearance of the subfamily to the Middle Eocene based on the occurrence of *Typhis tubifer* (Bruguière, 1792). Consequently, the upper and lower bounds of the prior distribution were constrained as for the Coralliophilinae.

Morphological data

Radulae and external penis morphology of some specimens were examined (see Table 1). Radulae of adult specimens were obtained via dissection of the proboscis, cleaned in liquid bleach, washed with warm distilled water and dried at room temperature. Penes of male specimens were dissected from the body, dehydrated in 100% ethanol, soaked overnight in hexamethyldisilazane (HMDS) and dried at room temperature under a hood. Both radulae and penis samples were sputter-coated with a 50-nm layer of gold/palladium and examined under a Leica/LEO Stereoscan S440 scanning electron microscope at the University of Genova (Department of Chemistry and Industrial Chemistry, Laboratory of Electronic Microscopy).

Results

Sequence analyses

The data set was based on three mitochondrial and one nuclear gene, for a total of 3373 aligned nucleotide sites. A matrix of 158 standard binary characters was added to the alignment, coding for indels in all the ribosomal genes. Saturation plots (additional material) were constructed for each ribosomal gene and for 1st, 2nd and 3rd codon position of COI. None of the plots shows evidence of saturation, and the linear regressions were in all cases highly significant; therefore, all the sites were considered to provide a phylogenetic signal useful for the analyses. The chi-square statistic showed no significant heterogeneity in the distribution of bases, and the sequences of COI showed no evidence for codon bias.

Phylogenetic analysis

Evaluating the best partition model, we found that AIC and BF favoured MUR05 and MUR06, respectively (Table 3). The difference between the two partition schemes is that in MUR06, the 3rd COI codon position is separated from the 1st and 2nd. The topologies resulting from these two analyses do not differ significantly; how-

Table 3 Results of the Akaike Information Criterion (AIC) and Bayes Factors (BF) tests for the selection of the appropriate partition scheme in the bayesian analysis

					BF						
Partition scheme	EML	No. of partitions	K	AIC	MUR01	MUR 02	MUR 03	MUR 04	MUR 05	MUR 06	MUR 07
MUR01	−64164.87	2	397	129124	–	492.22	927.48	−11617.6	2426.54	2874.68	2716.58
MUR02	−63918.76	3	642	129122		–	435.26	−12109.8	1934.32	2382.46	2224.36
MUR03	−63701.13	4	860	129122			–	−12545.1	1499.06	1947.2	1789.1
MUR04	−69973.67	5	1125	142197				–	14044.14	14492.28	14334.18
MUR05	−62951.6	7	1652	129207					–	448.14	290.04
MUR06	−62727.53	8	1936	129327						–	−158.1
MUR07	−62806.58	9	2250	130113							–

Underlined values represent the partitions with best outcome for the two tests (see text).

ever, AIC and BF differ in the evaluation of the partition model. BF does not account for parameter numbers, so the outcome of this test is not affected by the number of partitions and models used for the analysis. However, it has been shown by Nylander *et al.* (2004) that the most complex model is generally favoured. On the other hand, an AIC accounts for both likelihood and the number of parameters, introducing a penalization as the complexity of the models and the number of partitions increases.

No major differences were found in the two topologies, but we preferred the outcome of AIC because of the problems encountered in reaching the convergence for the most complex partition schemes (MUR06 and MUR07), a probable consequence of over-parameterization (Nylander *et al.* 2004). It is noteworthy that the partitioning scheme MUR04 showed the worst performance among all schemes according to both AIC and BF. In this model, the mitochondrial ribosomal genes were considered as a single marker with a unique substitution model, an interpretation that apparently biased the extrapolation of phylogenetic information from our data set.

The topology in Figure 1 (see also Fig. 6 for details) represents the consensus of 16 000 trees sampled after chain convergence. In our tree, the Trophoninae are polyphyletic, and the species are divided into two clades, both with high posterior probabilities. The first clade (PP = 98) includes the type genus *Trophon* (with its type species *T. geversianus*) and species of *Leptotrophon*, *Nipponotrophon* and *Scabrotrophon*.

The other species are grouped in a highly supported clade (PP = 100), which is the sister-group of the Haustrinae. The position of the Magellanic *Xymenopsis muriciformis* at the base of this clade is highly supported (PP = 100), while the support for the separation of the other taxa into two subclades is moderate (PP = 94). The first subclade includes *Xymene* and the Antarctic species of *Trophon* and *Trophonella*; the latter are all grouped together with high support (PP = 100). The second clade comprises species included in the genus *Pagodula* and the specimens of *Trophon* cf. *cuspidarioides* (hereafter, *Pagodula* cf. *cuspidarioides*) from the Ross seamounts with high support (PP = 100). This analysis did not recover the monophyly of the genus *Pagodula*, which was instead obtained in the molecular clock analysis.

Molecular clock analysis

Divergence times for each taxon estimated by BEAST are shown in Figure 2. The topology of the ultrametric tree agreed with that obtained by MrBayes for all the clades with high support, the only exception being the position of *Thais nodosa*. The chronogram resolved the basal node of Trophoninae and the monophyly of *Pagodula*, which in the Bayesian tree were represented as polytomies. The inferred divergence times of the subfamilies are reported in Table 4. Of major interest is the origin of the new *Trophon*-like muricid clade, estimated in our analysis at *c.* 40 Mya. Notably, the Coralliophilinae origin is around 70 Mya, earlier than the lower bound of 65 Mya defined as a prior.

Morphological data

The radula of *Pagodula echinata* (Figure 3A,B), as described here and in the previous works (Houart 2001; Marshall & Houart 2011), shows a typical muricine shape with the rachidian tooth bearing a major triangular central cusp, two small intermediate denticles on each side and two lateral cusps slightly smaller than the central one. The rachidian base is subrectangular, broad, anteriorly concave and has no marginal cusps. Lateral teeth are sickle shaped and approximately the same size as the central tooth. The radula of the specimens identified as *Pagodula* cf. *cuspidarioides* (Figure 3C,F) also has a typical muricine shape, the central and lateral cusps of the rachidian are triangular and have approximately the same dimensions and the intermediate cusps are small and separated from the major cusps. The rachidian base is wide and rectangular, and the marginal area bears no marginal cusps. The lateral teeth are sickle shaped and of the same dimension as the central tooth. The radula of *Trophon shackletoni* (Figure 3G,H) has a muricine appearance; the central and lateral cusps of the central tooth are triangular, but the central ones are larger than the lateral ones. A pair of independent, small, pointed intermediate denticles is present between the central and lateral cusps. The rachidian base is subrectangular with narrow marginal areas and has no marginal cusps. The lateral teeth are sickle shaped, and their dimensions are approximately the same as the central tooth. The radula of *Trophon longstaffi* (Figure 4A,B) and *Trophonella scotiana* (Figure 3I) shows no major differences from that of *T. shackletoni*, apart from the different size of the intermediate denticles which, however, in muricids, changes between young and adult specimens (Herbert *et al.* 2007). The radula of *Trophonopsis muricatus* (Figure 4C) is also muricine-like, with triangular central and lateral cusps and pointed intermediate denticles. The lower marginal area is slightly rounded where it is in contact with the upper marginal area of the preceding tooth, but no marginal cusps are developed.

The penis of *Trophon longstaffi* (Figure 5A) is generally large with respect to the body size, wide, with a terminal conical papilla emerging from a concavity. This feature gives the appearance of a collar around the papilla at the tip of the penis. The morphology of the penis of *Trophon shackletoni* (Figure 5B) and *Pagodula* cf. *cuspidarioides* (Figure 5C) is similar to that of *T. longstaffi*, with a well-defined collar around the terminal papilla.

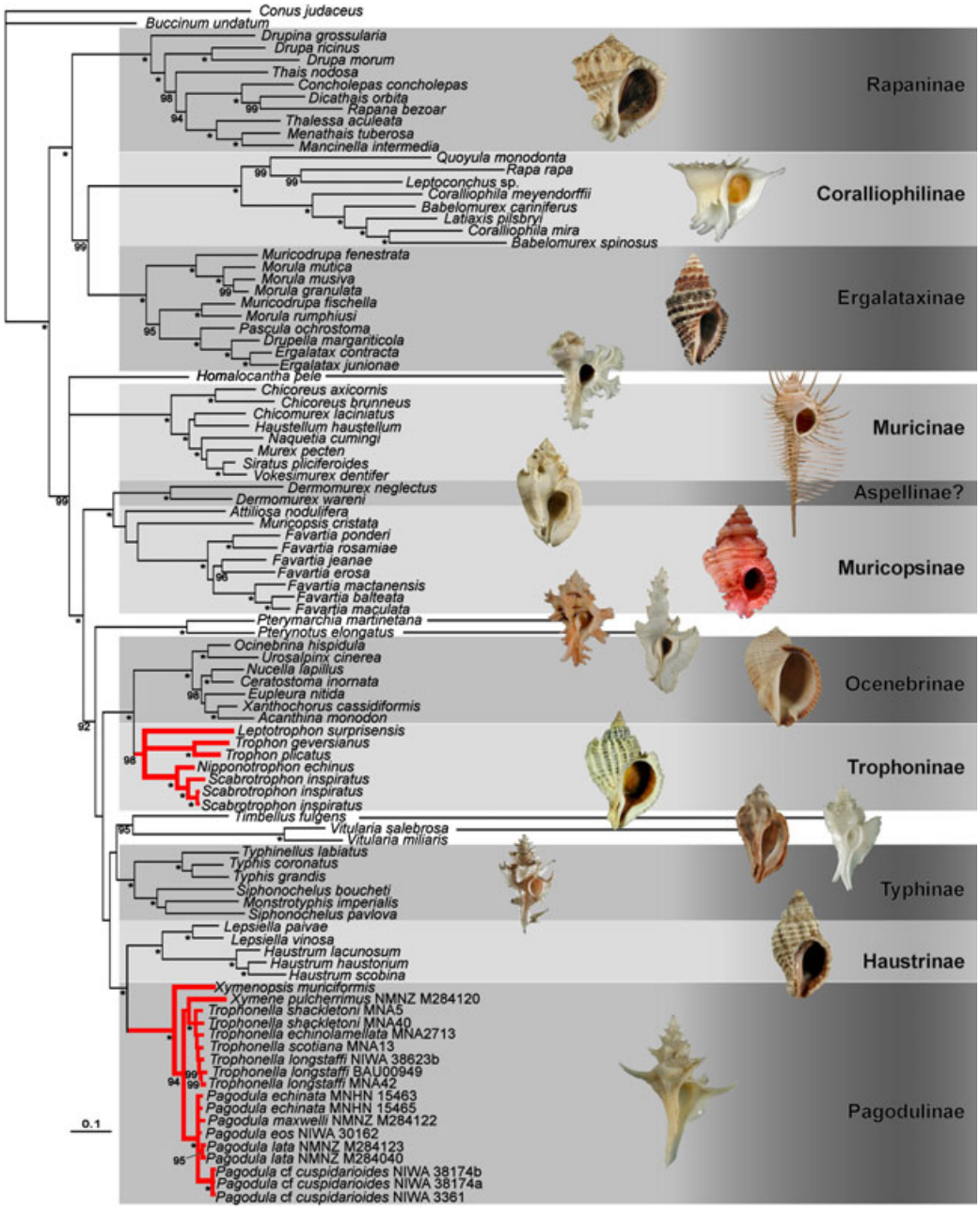

Fig. 1 Tree topology obtained by a Bayesian analysis (10^7 generations, trees sampled every 1000 generations) of the combined dataset (12S, 16S, COI, 28S) partitioned according to the scheme MUR05 (see Table 2). A conservative burn-in at 4×10^6 generations (4000 topologies) was selected. Bayesian posterior probability values from 16000 trees are reported only for moderate to high support (P > 90%). Trophoninae are highlighted in red, and Pagodulinae are highlighted in blue. Generic allocations of species reflect the taxonomic changes proposed in this study. Representative shells of each subfamily and unassigned lineages are illustrated (shells not to scale), from top to bottom and from left to rigth: *Rapana bezoar* (Linnaeus, 1767), Japan 62.5 mm; *Latiaxis pilsbryi* Hirase, 1908, Philippines, 32.2 mm; *Drupella margariticola* (Broderip, 1833),Moluccas, 26.5 mm; *Homalocantha pele* (Pilsbry, 1918), Japan, 40.2 mm; *Murex pecten* Lightfoot, 1786, Philippines, 130 mm; *Dermomurex (Takia) neglectus* (Habe & Kosuge, 1971), Philippines, 22.9 mm; *Favartia maculata* (Reeve, 1845), Philippines, 16.7 mm; *Pterymarchia martinetana* (Röding, 1798), Guam, 23.4 mm; *Pterynotus elongatus* (Lightfoot, 1786), Philippines, 83.4 mm; *Acanthina monodon* (Pallas, 1774), Patagonia, 48.9 mm; *Trophon geversianus* (Pallas, 1774), Patagonia, 48.2 mm; *Vitularia salebrosa* (King, 1832), W. Mexico, 68.6 mm; *Timbellus fulgens* (Houart, 1988), New Caledonia, 22.9 mm; *Monstrotyphis imperialis* (Keen & Campbell, 1964), New Caledonia, 14.8 mm; *Lepsiella vinosa* (Lamarck, 1822), South Australia, 19.7 mm; *Pagodula echinata* Italy, 22.4 mm.

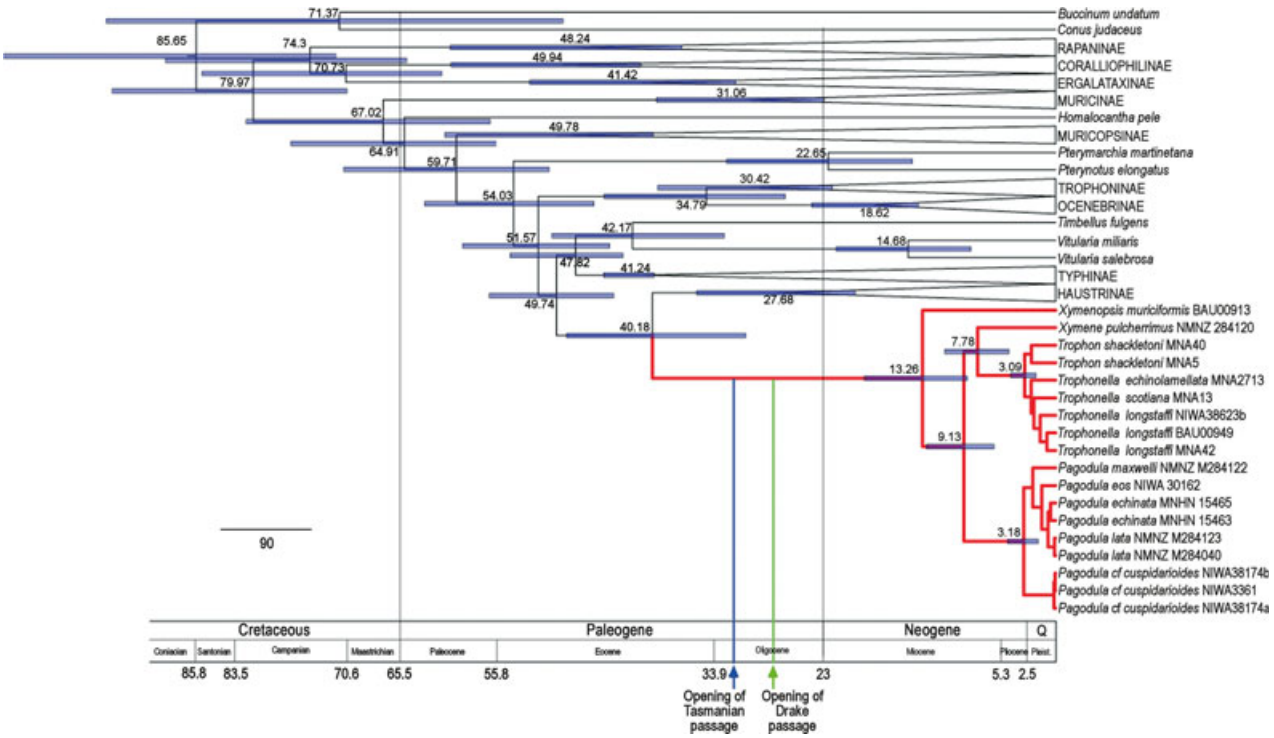

Fig. 2 Chronogram for the muricid radiation. Ultrametric tree obtained with BEAST. Standard error for the estimated ages of the nodes is reported as a blue bar on the figure. Pagodulinae are highlighted.

Table 4 Estimated node ages for the Muricidae

Node	Node age (median, Ma)	95% HPD (Ma)
TMRCA Muricidae	85.65	71.74–104.86
TMRCA Rapaninae	74.30	64.68–88.71
TMRCA Coralliophilinae/Ergalataxinae	70.73	61.19–85.07
TMRCA Muricinae	67.02	56.35–80.65
TMRCA Muricopsinae (including *Dermomurex* and *Attiliosa*)	49.78	50.47–70.92
TMRCA Trophoninae/Ocenebrinae	34.79	26.93–44.99
TMRCA Typhinae	41.24	43.11–54.37
TMRCA Pagodulinae/Haustrinae	40.18	30.89–48.73
Xymenopsis vs. ingroup	13.26	8.8–19.08
Xymene/*Trophonella* vs. '*Pagodula*' cf. *cuspidarioides*	9.13	6.14–12.87
Xymene vs. *Trophonella*	7.78	4.67–11.05
Radiation of *Trophonella*	3.19	1.97–4.48
TMRCA '*Pagodula*' cf. *cuspidarioides*	3.17	1.73–4.8

Confidence intervals for the estimated ages are represented by the 95% Highest Posterior Density interval, the shortest interval in parameter space that contains 95% of the posterior probability.

Discussion and systematics

Phylogenetic analysis

This is the first molecular study published to date on the subfamily Trophoninae as currently defined. It is based on nucleotide sequences of 12 species representing eight genera of the 28 commonly ascribed to this subfamily, including several Antarctic taxa for which no molecular data were available previously.

The topology obtained in this study confirmed our previous results (Barco *et al.* 2010) and showed even higher support values for most of the clades. The polyphyly of the Trophoninae, as traditionally conceived, was confirmed by a clear separation into two clades; one was the sister-group of the Ocenebrinae, and included the genera *Trophon* (with its type species *T. geversianus*), *Leptotrophon*, *Nipponotrophon* and *Scabrotrophon*; the other included taxa with a *Trophon*-like shell morphology (representing the genera *Xymenopsis*, *Xymene*, *Trophonella* and *Pagodula*) clustered together in a highly supported clade as the sister-group of the Haustrinae. This result confirms our previous finding for the phylogenetic position of *Trophonella longstaffi* (Barco *et al.* 2010) and the cladistic analysis of Tan (2003), which recovered a separation between *T. geversianus* and the genera *Xymene* and *Paratrophon*. According to the molecular and morphological data described in this work and to the previously available information cited below, this previously unrecognized lineage of muricids deserves, in our opinion, the rank of subfamily.

We provide here a new framework for the classification of the genus *Trophon* and the traditionally related

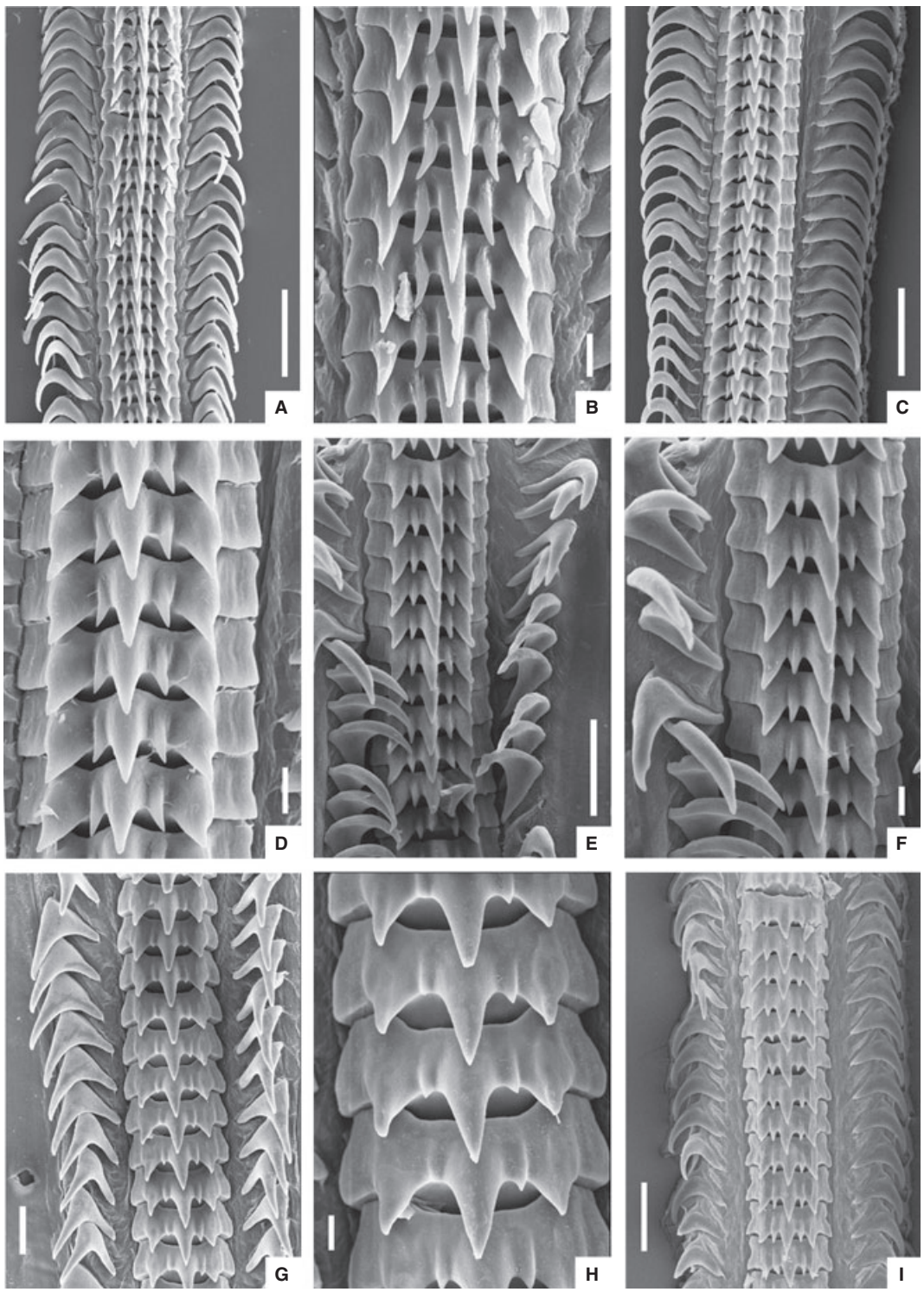

Fig. 3 Radulae of Pagodulinae. A, B: *Pagodula echinata* BAU918 (Tyrrhenian Sea); C, D: '*Pagodula*' cf. *cuspidarioides*, MNASS103 (Ross Sea, Antarctica); E, F: '*Pagodula*' cf. *cuspidarioides*, MNASS105 (Ross Sea, Antarctica); G, H: *Trophonella shackletoni*, MNA10 (Ross Sea, Antarctica); I: *Trophonella scotiana*, MNA4 = MNA13 (Ross Sea, Antarctica). Scale bars: 50 μm (A, C, G, I), 10 μm (B, D, E, F, H).

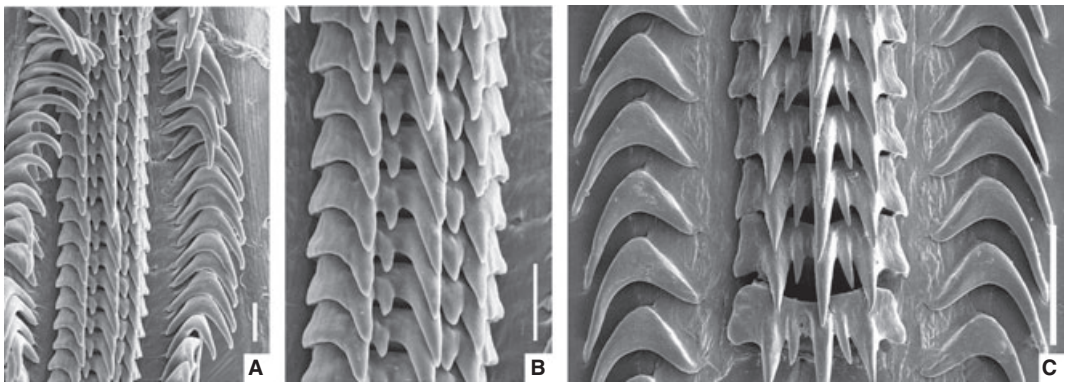

Fig. 4 Radulae of Pagodulinae. A: *Trophonella longstaffi* (NIWA 38623b, Ross Sea, Antarctica); B: *Trophonella longstaffi* (MNA42, Ross Sea, Antarctica); C: *Trophonopsis muricatus* (Chioggia, Italy).

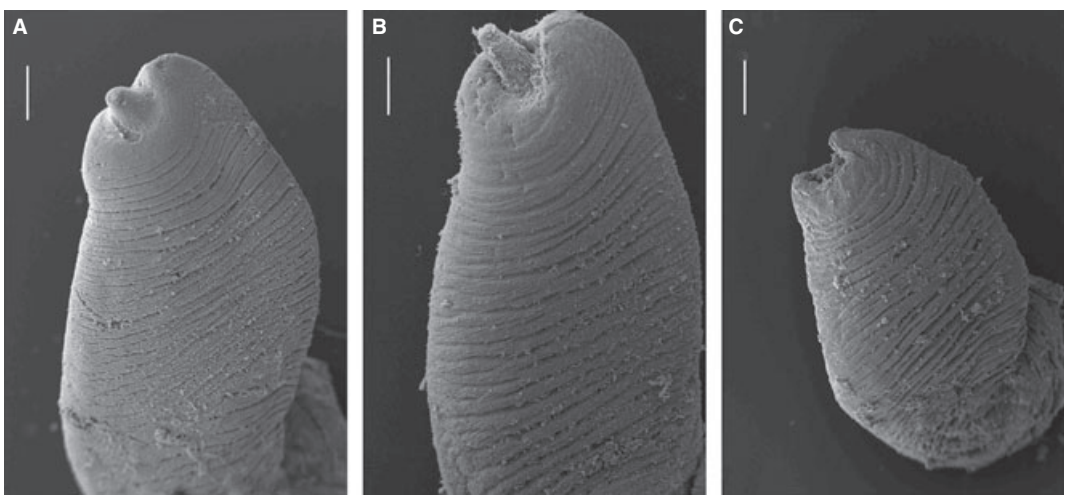

Fig. 5 Penes of Pagodulinae. A: *Trophonella longstaffi* (NIWA 38623b, Ross Sea, Antarctica). B: *Trophonella shackletoni* (MNA2, Ross Sea, Antarctica). C: *Pagodula* cf. *cuspidarioides* (NIWA 3361, Ross Sea, Antarctica).

genera within the ongoing revision of muricid systematics. We propose below a revised classification of several of the genera previously included in the Trophoninae, restrict the scope of the Trophoninae s.s., describe a new subfamily for *Pagodula* and related genera, provide further support to the recently introduced genus *Trophonella* and, more generally, provide evidence for the importance of radular morphology in supporting the definition of suprageneric groups of muricids. In the following scheme, the inclusion of each genus in one or the other of the two subfamilies is supported (as indicated in brackets) by [M] molecular data, [R] radular characters and [A] anatomical features. A complete list of the currently accepted trophonine-like genera is reported in the Appendix 1, with their proposed subfamilial allocation when possible (and the source of the molecular, radular and anatomical data supporting familial placement), and an *incertae sedis* placement for the others. We stress again that shell similarity must be interpreted with great caution. Although in most cases shell characters have no higher homoplasy than anatomical ones (Shander & Sundberg 2001), many shells of *Trophon*-like taxa are very convergent, producing a similar 'pagodiform morphology' (Merle *et al.* 2011: 158) that can be severely misleading.

Systematics

Superfamily Muricoidea Rafinesque, 1815
Family Muricidae Rafinesque, 1815

Subfamily Trophoninae Cossmann, 1903.
Type genus: *Trophon* Montfort, 1810 [type species *Murex magellanicus* Gmelin, 1791 = *Buccinum geversianum* Pallas, 1774; Recent, southern South America].

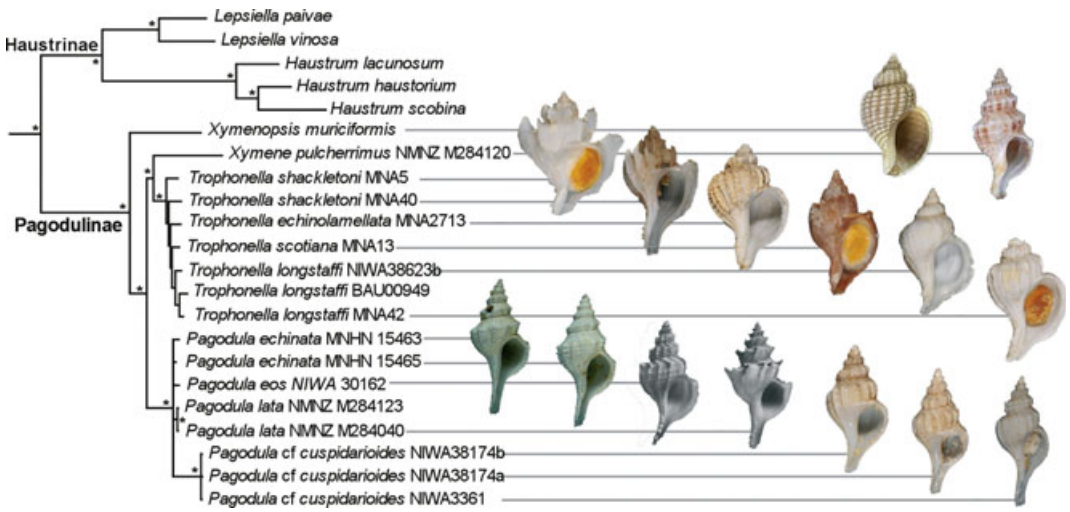

Fig. 6. Detail of the Pagodulinae phylogeny (after the topology of Fig. 1) with voucher shells (of sequenced specimens).

Included genera (see Appendix 1).

Trophon Montfort, 1810. [M, R, A]
Leptotrophon Houart, 1995. [M, R]
Nipponotrophon Kuroda & Habe, 1971. [M, R]
Scabrotrophon McLean, 1996. [M]
Coronium Simone, 1996. [R]

Diagnosis. Shell small to large for the family, fusiform, inflated, most taxa with a distinct shoulder, sculptured with short to broadly flaring axial lamellae. Radula ocenebrine-like; rachidian teeth with five major cusps, one central cusp with a smaller lateral cusp on each side, and two marginal cusps. Penis simple, without terminal papilla and collar.

Description. Shell small to medium sized fusiform, narrow to broad, with conical spire of variable height. Most taxa white, some species with coloured shells, yellowish to red or brown. Protoconch paucispiral or multispiral, 0.5–3 whorls in Recent species, all with non-planktotrophic development, devoid of obvious sculpture, or with pitted apex, fine spiral threads and axial growth lines. Teleoconch with round to angulate and well-defined shoulder, which develops shoulder spines in some species. Axial sculpture of ribs or high lamellae of variable strength, present in most species. Spiral sculpture of low, rounded cords present in some species. Aperture rounded, ovate to broadly round, outer lip thickened in very few taxa. Siphonal canal short to long, spineless, open for the entire length in all taxa, twisted in some. Radula with rachidian tooth and two sickle-shaped lateral teeth. Rachidian with rectangular-rounded basal plate, a long triangular central cusp, two shorter lateral cusps, and two marginal cusps. intermediate denticles present in most taxa, partially fused with the internal margin of the lateral cusps in some. Penis simple, without terminal papilla and collar.

Remarks. The subfamily Trophoninae is a group of predominantly temperate–cold water species distributed mainly in high latitudes, but also occurring in tropical waters at great depths. All Recent species we examined and unequivocally assign to this subfamily have lecithotrophic (probably entirely intracapsular) development.

Although many authors have pointed out that the traditional classification of this group includes genera with obvious morphological discrepancies (Radwin & D'Attilio 1976; Kool 1993a; Pastorino 2002), and the subfamily is polyphyletic in both the cladistic analyses of Harasewych (1984) and Tan (2003), no data were available previously on which to base an alternative classification. The present molecular data provide the basis to include *Trophon geversianus* (type species of *Trophon*) and species of *Leptotrophon*, *Nipponotrophon* and *Scabrotrophon* in the one clade. These also appear to share morphological characters of the radula (e.g. Egorov 1993; Houart 1995a,b; Pastorino 2005). On the basis of the radular characters illustrated by Pastorino *et al.* (2007) and Houart & Sellanes (2010), *Coronium* Simone, 1996 also should be included in the Trophoninae as redefined here.

The presence of marginal cusps and the partial fusion of the intermediate denticles with the internal margin of the lateral cusps are characters found in the radula of several genera of Trophoninae and Ocenebrinae, in agreement with the close relationship between these two subfamilies.

Subfamily Pagodulinae new subfamily.
Type genus: *Pagodula* Monterosato, 1884: 116
[type species by subsequent designation (under ICZN Art. 70.3, by Houart & Sellanes 2006: 59): *Fusus echinatus* Kiener, 1840; Recent, western Mediterranean and north-eastern Atlantic].

Included genera (see Appendix 1).
Pagodula Monterosato, 1884. [M, R]
Paratrophon Finlay, 1927. [R, A]
Trophonella Harasewych & Pastorino 2010. [M, R, A]
Trophonopsis Bucquoy and Dautzenberg, 1882. [R]
Xymene Iredale, 1915. [M, R, A]
?= *Lenitrophon* Finlay, 1926.
?= *Axymene* Finlay, 1926.
?= *Xymenella* Finlay, 1926.
?= *Zeatrophon* Finlay, 1926.
Xymenopsis Powell, 1951. [M, R, A]

Diagnosis. Shell small to medium sized for the family, fusiform, inflated, with or without a distinct shoulder, sculptured with short to broadly flaring axial lamellae. Radula muricine-like; rachidian teeth with three major cusps, a larger central cusp with a smaller lateral cusp on each side; two intermediate denticles independent from central and lateral cusps; marginal cusps absent. Penis with terminal conical papilla and surrounding collar.

Description. Shell small to medium sized, fusiform, narrow to broad, with conical spire of variable height. Most taxa white, some species with yellow to pale brown shells. Protoconch multispiral with up to three evenly convex whorls in species with planktotrophic development, with or without fine spiral threads on first 0.5–1 whorl. Protoconch paucispiral, of 0.5–2 whorls, in species with non-planktotrophic development, in most taxa pitted or sculptured with fine, irregular spiral threads. Teleoconch with round to angulate and well-defined shoulder, which develops spines in some species. Axial sculpture present in most taxa, in the form of variably developed ribs or broad lamellae. Spiral cords present in most taxa, low and rounded. Aperture rounded, ovate to broadly round, outer and inner lips thickened in some. Siphonal canal short to long, spineless, open for the entire length in all taxa, twisted in some. Whole radula small, thin, extending beyond rear of buccal mass. Rachidian teeth with subrectangular to rectangular-rounded, weakly recurved basal plate and smooth, broad, marginal surfaces lacking marginal cusps. Central cusp long to moderately long, triangular; flanked by a single, small to nearly obsolete lateral cusp on each side, cusp short, robust, smooth; two small to obsolete intermediate denticles independent from central and lateral cusps. Lateral teeth sickle shaped, of moderate size, basal plate shorter and narrower than in rachidian teeth, with single scythe-like cusp along outer edge that is slightly longer than the basal plate.

Remarks. The new subfamily Pagodulinae is established here for a strongly supported clade sister to the Haustrinae in our molecular phylogeny, comprising the genera *Pagodula*, *Trophonella*, *Xymene*, *Xymenopsis* and *Trophonopsis* for its radular morphological affinity with these genera. If, as suggested by Beu (2011), the various synonyms of *Xymene* actually represent distinct lineages (see below), then we have proven the inclusion in Pagodulinae of *Zeatrophon* (‘*Xymene*’ *pulcherrimus*), while the position of *Xymene* s.s. and of the other lineages should be conclusively tested by the analysis of the relevant type species. However, given their evident morphological affinity, it is very likely that most of those New Zealand genera resembling *Xymene* (Beu 2011) belong to the Pagodulinae.

Pagodulinae are similar to the Trophoninae in gross shell morphology, which caused the Trophoninae traditionally to be conceived as comprising a polyphyletic assemblage. Members of the two subfamilies are more clearly distinguishable by radular features, Pagodulinae having a muricine-like radula without marginal cusps and with independent intermediate denticles, whereas the Trophoninae have an ocenebrine-like radula with marginal cusps and the partial fusion of the intermediate denticles with the internal margin of the lateral cusps. Other apparently diagnostic characters may be represented in the external penis morphology by the presence of a terminal papilla and collar, and the globular shape of the accessory salivary glands, as previously described for *Paratrophon quoyi* (Reeve, 1846), *Pagodula veronicae* (Pastorino, 1999) and *Xymenopsis muriciformis* (King, 1833) by Tan (2003), Pastorino (1999) and Pastorino & Harasewych (2000), respectively. It is also possible that further phylogenetically useful characters will be detected in the anatomy of the foregut and of the female reproductive tract (Kool 1993b; Tan 2003), regrettably still unknown for many muricid lineages. Recently, based on the similarities in shell and radular morphology highlighted by Marshall & Houart (1995), Merle *et al.* (2011) considered *Pagodula* as a subgenus of *Poirieria* and included them in the Muricinae. Instead, molecular data clearly indicate that *Pagodula* is a derived member of the Muricidae, belonging to a clade sister to the Haustrinae, the muricine-like radula possibly representing plesiomorphy. On the other hand, the similarity to *Poirieria* raises the possibility that this genus, very rare outside New Zealand, but with a long paleontological history in New Zealand, also possibly belongs in the Pagodulinae. This is a hypothesis to test when suitable live-collected material becomes available. Marshall & Houart (2011) listed 47 described living species of *Pagodula* ranging

through all latitudes and pointed out their particularly variable bathymetry (depth range, 70–3259 m); most species are found in deep seas, especially on seamounts and ridges, but some species are reported from shallower water.

The description of the genus *Trophonopsis* is mostly based on Mediterranean and northern Europe species (see e.g. Kantor & Sysoev 2006), but the intrageneric classification is currently doubtful (Houart 2001). The type species, however, show a clear radular similarity to *Pagodula*.

The genus *Boreotrophon* P. Fischer, 1884 as currently conceived is possibly polyphyletic, as species with different radulae are included in it. For instance *B. gaidenkoi* Houart, 1995 has an ocenebrine-type radula (Houart 1995b), indicating trophonine affinity. However, the radula of the type species (*Murex clathratus* Linnaeus, 1767) as illustrated by Houart (2001) is clearly of the muricine type, similar to *Pagodula*. A thorough revision of the species ascribed to *Boreotrophon* is needed to asses their actual relationship.

Most Antarctic muricids were formerly placed in the genus *Trophon*, but Harasewych & Pastorino (2010) recently erected the genus *Trophonella* for *Trophon scotianus* and four other species on the basis of notable differences in the shell, radula, accessory salivary gland and external penis morphology. Our data clearly indicate that *Trophonella* is to be classified into the new subfamily Pagodulinae. In this study, the Antarctic species *Trophon shackletoni*, *Trophonella echinolamellata* and *Trophon longstaffi* form a well-supported clade with *Trophonella scotiana* (Figure 1), and the similarities of these species in radular and external penis morphology (Figures 3–5) support this close relationship. We propose that these species be referred to the genus *Trophonella* which, therefore, includes at least eight taxa living exclusively in Antarctic waters.

Powell (1951) described *Trophon cuspidarioides* from South Georgia (see also Zelaya 2005), and since its description, the species has been recorded only once, off Peter I Island, in the Bellingshausen Sea at 410 m (Aldea Venegas & Troncoso 2010). This species likely belongs to the genus *Trophonella*, as suggested by the radula depicted by Powell (1951: fig. 89). However, the specimens in our data set, although resembling *T. cuspidarioides* in shell characters, were determined from their phylogeny (Figure 1) to be closer to *Pagodula* than to *Trophonella*. For this reason, we refer to these specimens as *Pagodula* cf. *cuspidarioides*. Our specimens were collected on the Scott seamounts (north of the Ross Sea, see Table 1). Seamount faunas have been considered for a long time to be a 'cradle' of endemic species. However, both increased sampling and genetic data now indicate that seamount faunas are not so unique, many being broadly similar to that of the adjacent continental margins. Therefore, although we argue that these specimens represent an isolated entity, we refrain from describing a new species based on this material, pending a more comprehensive revision of the Pagodulinae with wider taxon sampling.

Xymene is a Neozelanic group of muricids including 13 described living species inhabiting intertidal to deep waters. The genera *Axymene*, *Lenitrophon*, *Xymenella* and *Zeatrophon* were described by Finlay (1926) to discriminate among different *Trophon*-like forms of from New Zealand. Although Ponder (1972) tentatively synonymized those genera with *Xymene* for the absence of convincing morphological distinctions, Beu (2011) rather convincingly proposed their resurrection. This group (which may comprise at least five distinct lineages: A. Beu pers. comm.) appeared in the Late Eocene (~42.5 Mya, Beu & Maxwell 1990) and is the most ancient group among those so far included in the new subfamily Pagodulinae. Notably, the genus was present also in Southern South America in the Early Miocene (Del Rio 2004), suggesting a later reduction in range because of climate cooling (see below). Among the other genera described by Finlay (1926) to separate Neozelanic species previously classified as *Trophon* are *Comptella*, *Terefundus* and *Minortrophon*. These groups may belong to the Pagodulinae as likely does *Paratrophon*, which has been suggested to be closely related to *Xymene ambiguus* (type species of *Zeatrophon* Finlay, 1926) in the phylogenetic analysis of anatomical data (Tan 2003). Additionally, some of them (e.g. *Terefundus*) might prove to be polyphyletic assemblages, and therefore, the entire *Xymene*-like group deserves a more thorough study (A. Beu, pers. comm.).

The genus *Xymenopsis* was introduced by Powell (1951) for a group of muricids restricted to the Magellanic province, with differences in shell and radular morphology from typical *Trophon*. In particular, the author pointed out that the protoconch was similar to that of *Zeatrophon*, and the sculpture to *Xymene*, both from New Zealand. Such similarity was confirmed by Ponder (1972) (who, however, considered *Zeatrophon* a synonym of *Xymene*) and by Pastorino & Harasewych (2000). The latter authors revised the group, restricting the number of living species to four and compared the radulae of *Xymenopsis* and *Xymene plebeius* (the type species of *Xymene*). Although positioned at the base of our tree, the first fossil record of *Xymenopsis* is dated to the Late Miocene (Brunet 1997), more recently than *Xymene* (Early Miocene: Beu *et al.* 1997). As suggested by Pastorino & Harasewych (2000), *Xymenopsis* possibly represents a radiation endemic to the Magellanic region from ancestors of *Xymene* and *Xymenella* (see also below).

The Pagodulinae and their evolution in Antarctic waters

The recognized high degree of endemism in Antarctic marine invertebrate and fish faunas (Arntz *et al.* 1997;

Clarke & Johnston 2003) is the product of a long period of evolution in relative isolation (Clarke & Crame 1989).

After the Gondwana break-up (~150 Mya), the Antarctic continent moved south and has occupied a polar position since the early Cretaceous (~120 Mya) (Barrett 1999). Isotopic ($\delta^{18}O$) and Mg/Ca paleothermometry data from benthic foraminiferans both indicate a deep-sea temperature decrease of 12 °C in the last 50 Mya, with four main cooling phases (in the early Middle Eocene, the Late Eocene through Early Oligocene, the late Middle Miocene and the Plio-Pleistocene). Observed benthic faunal turnovers correspond to these same periods (Lear *et al.* 2000; Zachos *et al.* 2001).

Well-recognized key events in shaping Antarctic oceanographic patterns and determining the isolation of the continent were the opening of the Tasmanian and Drake passages, at about 32 and 31 Mya (Lawver & Gahagan 2003; Livermore *et al.* 2005), respectively. These seaways allowed the establishment of the Antarctic Circumpolar Current (ACC) since the early Oligocene (Lawver & Gahagan 2003), which, however, become an important factor in the ongoing cooling process and a major one in determining the isolation of Antarctic waters by preventing the supply of pelagic propagules (to and from Antarctica) only later, with the beginning of the Miocene (~23 Mya) (Rack 1993). The overall result of these dramatic climatic events was the extinction of several taxa, followed by faunal migrations in the deep or, in some cases, possible survival in refugia during glacial maxima which, together, shaped the Antarctic marine fauna to the present-day composition (Clarke 2003; Thatje *et al.* 2005; Clarke & Crame 2010).

According to our estimate, the origin of the Pagodulinae occurred around 40 Mya, not later than the Late Eocene, when the new subfamily separated from the Haustrinae lineage. This node is consistent with the initial cooling of Antarctica and the Late Eocene/Early Oligocene transition in the benthic foraminiferal faunas and the marked extinction of planktonic taxa that occurred in parallel with the 5 °C temperature drop and the establishment of thermohaline circulation (Lear *et al.* 2000).

At this stage, connections between New Zealand, western Antarctica and South America were possible thanks to the existence of a reasonably strong current flowing southward along eastern Australia and possibly north along the west coast of South America (Lawver & Gahagan 2003).

The fossil record of muricids is consistent with this scenario, because the oldest species of *Xymene* are reported in the Late Eocene of New Zealand (Beu & Maxwell 1990) and appeared in Patagonia in the Late Oligocene/Early Miocene (Beu *et al.* 1997; Del Rio 2004; Beu 2009). In Antarctica, two *Xymene* species were reported from the Lower Tertiary fossil record of the La Meseta formation (Seymour Island, western Antarctic Peninsula) (Stilwell & Zinsmeister, 1992): *Xymene marincovichi* Stilwell & Zinsmeister, 1992 and *Xymene lamesetaensis* Stilwell & Zinsmeister, 1992; ascribed, respectively, to the Ypresian to Lutetian (55.8–40.4 Mya) and to the Late/Upper Eocene (37.2–33.9 Mya). However, these two records recently have been re-interpreted by Beu (2009), who moved the two taxa to different genera and families: *X. marincovichi* to the genus *Trichosirius* (Capulidae) and *X. lamesetaensis* to the genus *Prosipho* (Buccinidae *sensu lato*). This reassignment, therefore, excludes the presence of *Xymene*-like gastropods in Antarctica in the Eocene.

Our data indicate that this New Zealand and Patagonian widely dispersed *Xymene* clade fragmented into subclades around 13 Mya coincident with the Middle Miocene abrupt cooling phase, the strongest of all, when Antarctic water temperatures dropped close to zero (Lear *et al.* 2000) and the production of Antarctic Bottom Waters (AABW) increased considerably (Lawver & Gahagan 2003; Maldonado *et al.* 2003). These events seem to have isolated a *Xymene*-derived, relictual clade in the Magellanic region, the genus *Xymenopsis* Powell, 1951; which today numbers only four extant species (see above). Almost the same dating (14.5 ± 0.5 Mya) was indicated for the divergence time of a magellanic fish species, the Patagonian toothfish (*Dissosticus eleginoides*), from its Antarctic counterpart, the Antarctic toothfish (*Dissosticus mawsoni*) (Near 2004).

The remaining clades comprising *Xymene* + *Trophonella* + *Pagodula*, according to our estimates, diverged around 9 Mya, followed by another split at 8 Mya between the NZ genus *Xymene* and the Antarctic *Trophonella*. Although more recent, these Late Miocene divergences occurred when both an enhanced ACC and strong production of AABW were still in place, therefore in an oceanographic setting not that different from the Middle Miocene one (Lawver & Gahagan 2003).

The '*Xymene* clade', represented in our study by '*Xymene*' *pulcherrimus*, but probably comprised of at least five genera in New Zealand, is now a Neozelanic endemic group of genera, which likely represents the end-product of the evolution of the originally more widespread Eocene genus *Xymene*. The divergence at 8 Mya definitively separated this *Xymene* clade from the Antarctic endemic genus *Trophonella*.

It is evident from our molecular data that *Trophonella* underwent a radiation in Antarctica during the early Pliocene (~3 Mya). However, it is not clear whether this taxon evolved *in situ*, from a *Xymene*-like ancestor that survived the cooling phases, or it colonized Antarctica only later, after muricids had become extinct during Eocene or

Middle Miocene cooling phases. Although the two options are theoretically both equally valid, we consider the latter the more probable, because of the existence of other similar examples of colonization from other invertebrate species, almost simultaneous with that of *Trophonella*, and because of the occurrence of major ecological shifts in the ecology of some Antarctic pectinids during the Pliocene. There is a growing body of evidence that the thermohaline circulation (the 'thermohaline expressway', Strugnell *et al.* 2008) has been a powerful engine for the dispersal of propagules in and out of Antarctica, especially during the strongest phases of AABW production (Strugnell *et al.* 2011). This mechanism could explain the appearance of the *Trophonella* clade during the Pliocene through 'polar emergence'. This hypothesis finds a good support in the timing of emergence of another Antarctic invertebrate clade, that is, the octopuses belonging to the *Bentoctopus rigbyae* species complex, whose estimated 'emergence' time (Strugnell *et al.* 2011) almost overlaps that of *Trophonella*. Both these events of 'polar emergence' overlap with periods of prolonged or extreme warmth, with water temperatures about 5.6 °C above present, which affected the Antarctic marine biota between 4 and 3.5 Mya (Escutia *et al.* 2009) and may have brought propagules of several invertebrate species from outside Antarctica.

A detailed reinterpretation of the fossil record may provide conclusive evidence to solve the problem of the origin of *Trophonella*. However, very few data about post-Miocene and pre-Pliocene fossil records of muricids are available (see below) particularly because of the poor late Neogene fossil record of Antarctica, making it very difficult, if not impossible, to reconstruct the evolution of this group before 5.3 Mya. Moreover, the now-recognized polyphyly of the genus '*Trophon*', whose members in the southern hemisphere belong to two distinct subfamilies, makes unequivocal attribution of a fossil shell (especially if abraded) to one of the two groups difficult, as shell morphological characters may be misleading and subject to convergence, only the radula being informative.

Our molecular results indicate that, at least for Antarctica, all Pliocene records of '*Trophon*' should instead be referred to *Trophonella*. Here, fossil species belonging to '*Trophon*' have been reported for the Late Holocene (Taylor Formation, McMurdo Region, as *T. longstaffi*) (Buckeridge 1989), the Early Pleistocene (1.8–0.8 Mya, CRP-1 drillhole, Cape Roberts Project (Taviani *et al.* 1998) and the Late Pliocene (Cockburn Island Formation, James Ross Basin, as *Trophon* sp.) (Stilwell 2002).

More difficult is the interpretation of two older records of Antarctic fossil '*Trophon*-like' species. The first, *Trophon*. cf. *disparoides* Wilckens, 1912 was reported (Birkenmajer 1987) to occur in the Polonez Cove Formation (King George Island, South Shetland Islands, Antarctica). This site was initially regarded as Pliocene (Gazdzicki & Pugaczewska 1984), but further studies showed it to be middle to late Oligocene (Birkenmajer 1987; Troedson & Smellie 2002; Quaglio *et al.* 2008). An even older record is attributed to Beu (2009), who gave a new interpretation of the Early to Late Eocene molluscan fauna of La Meseta Formation (Seymour Island, Antarctic Peninsula) illustrated by Stilwell & Zinsmeister (1992). Beu (2009) placed the species *Coelobassus radwini*, initially placed by Stilwell & Zinsmeister (1992) in their new genus *Coelobassus* (described in the same paper), in the genus *Trophon*. However, this record is based on a severely abraded specimen of a *Trophon*-like gastropod and as Beu (2009, note 68, p. 217) himself suggested an analysis of shell mineralogy would be necessary to confirm the identity of this taxon. This new generic placement has therefore to be considered provisional, although *Coelobassus* is considered a possible synonym of '*Trophon*' (Beu 2009), and Merle *et al.* (2011: 502) included *Coelobassus* as a subgenus of *Poirieria*, its position remains provisional. Both pre-Pliocene records of *Trophon*-like gastropods are inconclusive, and the possibility that both are related to the Trophoninae and not to Pagodulinae remains open.

Also difficult is the interpretation of records from outside Antarctica, such as the Neozelanic late Neogene species *T. munitus* (Marwick, 1934), tentatively placed in *Trophon* by Beu (2009), and to all the Cenozoic records of species still living in the southern South American fauna (e.g. Griffin & Pastorino 2005; Pastorino 2005), which, on a geographical basis, more likely represent lineages of Trophoninae rather than of Pagodulinae.

If the scenario of an occurrence of *Trophonella* in Antarctica only in the Pliocene is corroborated by further analyses of fossils, there will also be important ecological considerations to take into account in the Cenozoic dynamics of community assembly in Antarctica.

In fact, although muricid predation is a very slow process at low temperatures (Harper & Peck 2003), this is one of the most important driving forces in shaping the structure of Antarctic benthic communities which, at present, have a 'Paleozoic' degree of organization, being characterized by the absence of 'durophagous' predators (Aronson & Blake 2001).

Our molecular reconstruction indicates the possibility that this kind of predation could have been absent in Antarctica for a long time, if the Middle Eocene muricids, responsible for the intense predation in the fossils of La Meseta formation (Kelley *et al.* 1997), became extinct after the initial phases of cooling. According to this scenario, muricid predation would have been reintroduced in Antarctica only during the Pliocene, with the radiation

of *Trophonella*. This possibility finds very good support in the change of lifestyle that occurred in the extinct Antarctic pectinid *Austrochlamys anderssoni* (Hennig, 1911), which switched from a byssate lifestyle to higher motility during the Pliocene, possibly as a response to gastropod predation as suggested by Jonkers (2000). Specimens of this thick-shelled pectinid in the Cockburn Island Formation (~3 Mya) all have been heavily predated by drilling muricids, and this species shows morphological characters typical of a bottom-reclining, non-swimming pectinid (Jonkers 2000), while in the Scallop Hill Formation (<2.58 Mya; Webb & Andreasen 1986), *A. anderssoni* seems to have acquired mobility and has no sign of predation (Jonkers 2000). In parallel to this process, other prey items, such as the barnacle *Bathylasma corolliforme* started to show boreholes because of drilling muricids, as the abundant fossil record of the Scallop Hill Formation testify (Jonkers 2000). It would seem, therefore, that major ecological shifts occurred during the Pliocene, possibly as a consequence of the radiation of *Trophonella*, leading to (i) the acquirement of a higher mobility by *A. anderssoni* (which eventually got extinct in Antarctica mostly because of cooling; Jonkers 1998) and the persistence until Recent of a single, capable of swimming, pectinid species, *Adamussium colbecki*; and (ii) to the targeting of other prey items by pagodulines, such as the small and slow-moving bivalves *Cyclocardia astartoides* and *Thracia meridionalis*, which also represent the current prey of Antarctic muricids (Jonkers 2000 and references therein).

The last clade, *Pagodula*, is widespread in deep-water basins around New Zealand and south-eastern Australia (Marshall & Houart 2011), the North Atlantic and Mediterranean Sea, as well as the Antarctic seamounts off the Ross Sea (with the subclade *Pagodula* cf. *cuspidarioides*). Our temporal reconstruction indicates that it escaped out of the Southern Ocean and radiated during the Pliocene (about 3 Mya), well after its divergence (9 Mya) from an ancestor shared with *Trophonella*. This radiation seems to coincide with the closure of the deep-water part of the Panamanian seaway (in Mid Pliocene time: see Beu), which led to a new increase in the thermohaline circulation (Keigwin 1982; Lawver & Gahagan 2003) following the warmer phase that occurred between 4 and 3.5 Mya (Escutia *et al.* 2009).

The migration out of the Antarctic continental shelf to the deep sea through 'submergence' following the thermohaline circulation may have represented a dispersal route for several Antarctic taxa that were able to colonize deep basins at higher latitudes, as demonstrated also for some octopuses (Strugnell *et al.* 2008). The mainly deep-water occurrence of *Pagodula* (see above) supports this hypothesis.

The *Pagodula* cf. *cuspidarioides* lineage is recorded only from the seamounts off the Ross Sea and, as indicated by our molecular clock dating, originated during the Pleistocene. At least 38 Pleistocene glacial-interglacial cycles (Pillans *et al.* 1998; Lisiecki & Raymo 2005; Naish *et al.* 2009) are known to have acted as a 'biodiversity pump' (Clarke & Crame 1989, 1992), causing migration and dispersal-driven speciation phenomena following the changes in habitat availability during ice-shelf extension and shrinkage, and adaptive radiations and vicariance-driven speciation when shelves become available again. *Pagodula* cf. *cuspidarioides* possibly belongs to a lineage that was 'forced' to migrate outside the continental shelf during glacial periods, and its current distribution within the ACC, but outside the Antarctic shelf (see above), may be interpreted by considering the possible role of refugia for the islands or seamounts where it has been found, as also was demonstrated for such other invertebrate groups as the stalked crinoids (Bowden *et al.* 2011).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Saturation plots of transitions (Ts) and transversions (Tv) uncorrected p-distances against global pdistances. A linear regression (red line) and its significance (r) is reported for each plot.

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Appendix 1 List of *Trophon*-like genera with their type species (by original designation unless differently specified) and their putative classification. Source of data (from the type species unless differently specified) for their subfamilial placement (A, anatomy; R, radula; M, molecular data).

Genus level taxon	Type species	Source of data
Trophoninae Cossmann, 1903		
Trophon Montfort, 1810	*Murex magellanicus* Gmelin, 1791 = *Buccinum geversianum* Pallas, 1774	[A, R] Pastorino (2002); [M] Barco *et al.* (2010)
=*Polyplex* Perry, 1810	*Polyplex bulbosa* Perry, 1811 (ICZN Opinion 911-c) = *Buccinum geversianum* Pallas, 1774	
=*Muricidea* Swainson, 1840	*Murex magellanicus* Chemnitz, 1780 (*nomen nudum*) = *Murex magellanicus* Gmelin, 1791 = *Buccinum geversianum* Pallas, 1774	
=*Stramonitrophon* Powell 1951	*Trophon laciniatus* Martyn, 1789 (*nomen nudum*) = *Murex plicatus* (Lightfoot, 1786)	

Appendix 1 (Continued)

Genus level taxon	Type species	Source of data
Coronium Simone, 1996	*Columbarium coronatum* Penna-Neme & Leme, 1978	[R] Pastorino *et al.* (2007); Houart & Sellanes (2010)
Leptotrophon Houart, 1995	*Leptotrophon caroae* Houart, 1995	[R] Houart (1995a); [M] This work
Nipponotrophon Kuroda & Habe, 1971	*Boreotrophon echinus* Dall, 1918	[R] Golikov & Sirenko (1992); *Nipponotrophon magnifica* (Golikov & Sirenko (1992)); [M] This work
Scabrotrophon McLean, 1996	*Trophon maltzani* Kobelt and Küster, 1878	[R] Houart (2010); [M] This work
Pagodulinae new subfamily		
Pagodula Monterosato, 1884	*Fusus echinatus* Kiener, 1840 by subsequent designation (Houart & Sellanes 2006)	[A] Pastorino (1999); *Pagodula veronicae* (Pastorino, 1999) [R] Bouchet & Warén (1985);
=*Pinon* de Gregorio, 1885	*Murex vaginatus* De Cristofori & Jan, 1832	Houart (2001); Marshall & Houart (2011); This
=*Enixotrophon* Iredale, 1929	*Trophon carduelis* Watson, 1883	work [M] This work
Boreotrophon P. Fischer, 1884	*Murex clathratus* Linnaeus, 1767 by monotypy	[R] Houart (2001)
Paratrophon Finlay, 1926	*Polytropa cheesemani* Hutton, 1882	[A] Tan (2003): *P. quoyi* (Reeve, 1846)
Trophonella Harasewych & Pastorino, 2010	*Trophonella rugosolamellata* Harasewych & Pastorino, 2010;	[A, R] Harasewych & Pastorino (2010); [M] This work: *T.* spp.
Trophonopsis Bucquoy & Dautzenberg, 1882	*Murex muricatus* Montagu, 1803	[R] Bouchet & Warén (1985); Houart (1995a);
=*Chalmon* de Gregorio, 1885	*Murex muricatus* Montagu, 1803	this work
Xymene Iredale, 1915	*Fusus plebeius* Hutton, 1873	[M] This work: *"Xymene" pulcherrimus*
?= *Lenitrophon* Finlay, 1926	*Trophon convexus* Suter, 1909	
?= *Axymene* Finlay, 1926	*Axymene turbator* Finlay, 1926;	
?= *Xymenella* Finlay, 1926	*Trophon pusillus* Suter, 1907	
?= *Zeatrophon* Finlay, 1926	*Fusus ambiguus* Philippi, 1844	
Xymenopsis Powell, 1951	*Fusus liratus* Gould, 1849 = *Buccinum muriciformis* King, 1832[1]	[A, R] Pastorino & Harasewych (2000)
Incertae sedis		
Abyssotrophon Egorov, 1993	*Abyssotrophon ruthenicus* Egorov, 1993	
Afritrophon Tomlin, 1947	*Trophon kowieensis* Sowerby, G.B. III, 1901	
Anatrophon Iredale, 1929	*Trophon sarmentosus* Hedley & May, 1908	
Benthoxystus Iredale, 1929	*Trophon columnarius* Hedley & May, 1908	
Comptella Finlay, 1926	*Trophon* (*Kalydon*) *curta* Murdoch, 1905	
Conchatalos Houart, 1995	*Trophon lacrima* Houart, 1991	
Enatimene Iredale, 1929	*Trophon simplex* Hedley, 1903	
Fuegotrophon Powell, 1951	*Fusus crispus* Gould, 1849 = *Murex pallidus* Broderip, 1833	
Gemixystus Iredale, 1929	*Trophon laminatus* Petterd, 1884	
= *Apixystus* Iredale, 1929	*Trophonopsis stimuleus* Hedley, C., 1907	
Houartiella Smriglio, Mariottini & Bonfitto, 1997	*Houartiella alboranensis* Smriglio, Mariottini and Bonfitto, 1997	
Litozamia Iredale, 1929	*Peristernia rudolphi* Brazier, 1894	
?= *Benthoxystus Iredale, 1929*	*Trophon columnarius* Hedley and May, 1908	
Minortrophon Finlay, 1926	*Daphnella crassilirata* Suter, 1908	
Nodulotrophon Habe & Ito, 1965	*Trophon dalli* Kobelt, 1878[2] = *Trophon coronatus* H. & A. Adams, 1864	
Terefundus Finlay, 1926	*Trophon crispulatus* Suter, 1908	
Tromina Dall, 1918	*Fusus unicarinatus* Philippi, 1868[3] = *Tromina dispectata* Dell, 1990	
Xenotrophon Iredale, 1929	*Trophon euschema* Iredale, 1929	

[1]See Coan *et al.* (2011) for the authorship of this taxon.
[2]Unnecessary new name pro *muriciformis* Dall, 1877.
[3]Not *Fusus unicarinatus* Deshayes, 1835.

