Revised version: September 22nd, 2016

Phylogenetic relationships of Mediterranean and North-East
Atlantic Cantharidinae and notes on Stomatellinae
(Vetigastropoda: Trochidae)

Juan E. Uribe¹, Suzanne T. Williams², José Templado¹, Barbara Buge³ and Rafael
Zardoya^{1*}

¹Museo Nacional de Ciencias Naturales (MNCN-CSIC), José Gutiérrez
Abascal 2, 28006, Madrid, Spain

²Department of Life Sciences, Natural History Museum, Cromwell Rd, London
SW7 5BD, UK

³Muséum National d'Histoire Naturelle, Direction des Collections, 55, rue de
Buffon, 75005 Paris, France

*Corresponding author: rafaz@mncn.csic.es

ABSTRACT

The subfamily Cantharidinae Gray, 1857 (Trochoidea: Trochidae) includes 23 recognized genera and over 200 known living species. These marine top shell snails are microphagous grazers that generally live in shallow rocky shores and in macroalgae and seagrass beds of sub-tropical and temperate waters from the Central and Western Indo-Pacific biogeographic regions to the Mediterranean Sea and the Eastern Atlantic Ocean. Recent molecular phylogenetic studies revising the family Trochidae supported the monophyly of the subfamily Cantharidinae and its sister group relationship to the subfamily Stomatellinae. These studies and others has thus far mostly focused on Indo-Pacific members of the subfamily Cantharidinae whereas here, we investigated phylogenetic relationships among their counterparts from the Mediterranean Sea and the North-eastern (NE) Atlantic Ocean including 33 species of genera *Gibbula*, *Jujubinus*, *Phorcus*, *Clelandella*, and *Callumbonella*. The Mediterranean and NE Atlantic taxa were supplemented with 30 Indo-Pacific Cantharidinae species plus 19 members of the sister group subfamily Stomatellinae. Phylogenetic trees were constructed using Bayesian inference and maximum likelihood with two datasets comprised of partial sequences of four or six mitochondrial (*cox1*, *rrnL*, *rrnS*, and *cob*) and nuclear (28S *rRNA* and *histone H3*) genes. A clade comprised of all Mediterranean and NE Atlantic taxa was recovered with high support, but its sister group among the Indo-Pacific lineages could not be determined with confidence (although the assignment of “*Trochus*” *kotschy* to *Priotrochus* could be rejected). Within the Mediterranean and NE Atlantic clade, genera *Phorcus* and *Jujubinus* were recovered as reciprocally monophyletic, and the deep-sea genera *Clelandella* and *Callumbonella* were placed with high support as sister to *Jujubinus*. However, the genus *Gibbula* as currently defined was not monophyletic and constituent species were divided into three major

clades and two independent lineages. Phylogenetic relationships among *Phorcus*, *Jujubinus* (plus *Clelandella* and *Callumbonella*), and the different clades of *Gibbula* were not fully resolved but received higher support in the phylogenetic analyses based on six genes. A first approach to resolve phylogenetic relationships within Stomatellinae was conducted showing that the diversity of the subfamily is highly underestimated at present, and that *Calliotrochus* is possibly a member of this subfamily. A chronogram was reconstructed using an uncorrelated relaxed lognormal molecular clock and the origin of the Mediterranean and NE Atlantic clade was dated right after the *Azolla* phase in the Middle Eocene about 48 million years ago whereas diversification of major clades (genera) followed the eastern closure of the Tethys Ocean in the Middle Miocene about 14 million years ago.

Keywords: Cantharidinae, Stomatellinae, mitochondrial DNA, nuclear genes, molecular phylogeny, Tethys

1. Introduction

With more than 600 species grouped in about 60 genera (Gofas, 2015), the family Trochidae Rafinesque, 1815 is one of the largest within the highly diverse superfamily Trochoidea (Gastropoda: Vetigastropoda) (Hickman and McLean, 1990; Williams et al., 2008, 2010; Williams, 2012). Members of the family are ecologically and economically important, and commonly known as top shells due to their characteristic spiral, conical, and internally nacreous shell. Top shells are unspecialized grazers that typically feed by rasping algae and small organisms from rocky or vegetal surfaces (Hawkins et al., 1989). Although cosmopolitan in distribution and adapted to different marine environments, they radiated preferentially on tropical intertidal rocky shores in the West Pacific and Indian Oceans (Williams et al., 2010). The phylogenetic relationships of the family (and superfamily) have traditionally been highly contentious due to high levels of homoplasy in anatomical, radular and shell characters (Hickman and McLean, 1990), which is also evident in recent molecular phylogenies (Kano, 2008; Williams et al., 2008, 2010; Williams, 2012). In their seminal work on the systematics of trochoidean gastropods, Hickman and McLean (1990) assigned the different genera within Trochidae to 13 subfamilies (some of which were further subdivided into tribes) based on morphology. Some of the subfamilies were later raised to the familial status (Bouchet et al., 2005). However, recently reconstructed molecular trees have prompted for a thorough taxonomic revision of the family. Some taxa traditionally thought to belong in Trochidae were excluded and provisionally placed in Turbinidae or Seguenzioidea (Williams and Ozawa, 2006; Kano, 2008; Williams et al., 2008), whereas some subfamilies were redefined (Williams et al., 2010). Currently, Trochidae is divided into ten subfamilies according to the new classification for Trochoidea proposed by Williams (2012): Trochinae Rafinesque, 1815; Umboniinae H. Adams &

A. Adams, 1854; Stomatellinae Gray, 1840; Cantharidinae Gray 1857; Monodontinae Gray, 1857; Halistylinae Keen, 1958 (pending molecular analysis); Kaiparathininae B. A. Marshall, 1993; Fossarininae Bandel, 2009; Chrysostomatinae Williams, Donald, Spencer and Nakano, 2010, and Alcyoninae Williams, Donald, Spencer and Nakano, 2010.

In this study, we focused on the subfamily Cantharidinae, which is known from the Upper Cretaceous (Hickman and McLean, 1990), and is widely distributed from the Central and Western Indo-Pacific regions to the Mediterranean Sea and the Eastern Atlantic Ocean (Williams et al., 2010). Members of Cantharidinae are found predominantly in sub-tropical or temperate waters, and are mostly microphagous grazers mainly associated with macroalgae and seagrass beds (Hickman and McLean, 1990; Hickman, 2005; Williams et al., 2010; Donald and Spencer, 2016). The taxonomic history of Cantharidinae has been particularly controversial. The group was considered a tribe (Cantharidini) within Trochinae by Hickman and McLean (1990), and was only recently raised to subfamilial rank (Williams et al., 2008), sister to the subfamily Stomatellinae (Herbert, 1998; Williams et al., 2008, 2010; Donald and Spencer, 2016). New definitions of Cantharidinae and Monodontinae (previously ‘Gibbulini’) resulted in the mutual transfer of many taxa between both subfamilies (Donald et al., 2005; Williams et al., 2008, 2010; Donald and Spencer, 2016). For instance, the genera *Oxystele*, *Osilinus*, *Gibbula* and *Diloma* traditionally included in Monodontinae now belong to Cantharidinae (Herbert, 1998; Donald et al., 2005; Williams et al., 2010). The subfamily Cantharidinae currently includes 23 genera and more than 200 species (Bouchet and Gofas, 2015) but the taxonomy of the group at the generic level is in constant revision and redefinition with numerous instances of polyphyly and synonymizations (e.g. *Osilinus* and *Phorcus*; Donald et al., 2012) due to

rampant levels of anatomical convergence and lack of reliable morphological data (Williams et al., 2010; Donald et al., 2016).

The most extensive phylogenetic analysis of Cantharidinae to date (Williams et al., 2010) included representatives of 15 genera but was mostly focused on Indo-Pacific species. In that study (Williams et al., 2010), concatenated partial sequences from four genes (28S rRNA, *rrnL*, *rrnS*, and *cox1*) were analyzed using Bayesian Inference as implemented in MrBayes but were insufficient to resolve basal relationships within the Cantharidinae clade. Instead, BEAST analysis with incorporation of an uncorrelated relaxed, lognormal clock resulted in moderate support for *Kanekotrochus* as sister to all remaining Cantharidinae (Williams et al., 2010). *Oxystele* was recovered as sister to a clade including the New Zealand genera *Cantharidus* and *Micrelenchus* (plus the genus *Prothalotia* and two unidentified species from Vanuatu and the Solomon islands) and a group of seven Mediterranean and North-eastern (NE) Atlantic species, although with poor support (Williams et al., 2010). Recently, Donald and Spencer (2016) have presented an extensive molecular study of 18 New Zealand and temperate Australian Cantharidinae species based on partial sequence data of three mitochondrial genes (*rrnL*, *rrnS* and *cox1*) and one nuclear gene (28S rRNA). The genera *Cantharidus*, *Micrelenchus* and *Prothalotia* were redefined, the new genus *Roseaplagis* was erected, and the Australian genus *Cantharidella* was removed from the Cantharidinae to the subfamily Trochinae (Donald and Spencer, 2016). In the reconstructed phylogeny based on the concatenated data set, New Zealand endemics were not monophyletic as *Roseaplagis* was recovered in a clade together with *Prothalotia*, *Oxystele*, and Mediterranean and NE Atlantic species, although with low support (Donald and Spencer, 2016). Due to limited taxon sampling, phylogenetic relationships within the Mediterranean and NE Atlantic clade have not been rigorously assessed, and remain

virtually unstudied using molecular methods.

Three genera are currently recognized in the Mediterranean Sea and NE Atlantic Ocean: *Gibbula*, which was tentatively shown to be polyphyletic (Williams et al., 2010) but nominally includes around 55 species (according to Gofas and Bouchet, 2015a), most of them from the European coasts; *Jujubinus*, which comprises about 34 species (Gofas and Bouchet, 2015b), most of them from the Mediterranean Sea, and *Phorcus*, which has nine species (Donald et al., 2012), all of them from European and Macaronesian coasts. In addition, the deep-sea genus *Clelandella* was placed close to genus *Jujubinus* by Cretella et al. (1990). This genus was considered monotypic until Gofas (2005) described four new species from different Macaronesian seamounts and Vilvens et al. (2011) described another one from off Western Sahara.

Top shells of the genus *Phorcus* live in intertidal rocky shores, whereas species of the genus *Jujubinus* inhabit subtidal bottoms down to about 80 m, mainly associated with marine vegetation, where they feed on the periphyton. In contrast, species of the genus *Gibbula* are widespread, occurring in a variety of habitats, from intertidal and subtidal rocky shores to marine vegetation and detritic sublittoral bottoms, and form a guild of mainly microphagous herbivores (Templado, 2011). Finally, *Clelandella* species are adapted to life in deep detritic bottoms.

Here, we extend previous phylogenetic works on Cantharidinae (Williams et al., 2010; Donald and Spencer, 2016) by focusing on Mediterranean and NE Atlantic genera. We amplified and sequenced fragments of four mitochondrial (*cox1*, *rrnL*, *rrnS*, and *cob*) and two nuclear (28S *rRNA* and *histone H3*) genes for 47 specimens representing 29 species (16 of them type species of their respective genera). We included in the phylogenetic analyses the deep-water genus *Callumbonella*, of uncertain taxonomic placement, in order to determine its evolutionary origin within the subfamily.

The newly determined sequences were aligned to orthologous sequences available in GenBank from 13 previously studied specimens corresponding to 12 Mediterranean and NE Atlantic species. We also included in the phylogenetic analyses, newly determined and previously published (Williams et al., 2010; Donald et al., 2012) orthologous sequences from 14 and 23 specimens, respectively, corresponding to 30 Indo-Pacific Cantharidinae species. We aimed to test the monophyly of each of the studied genera, and whether the origin of the Mediterranean and NE Atlantic fauna was connected with the eastern closure of the Tethys Sea in the Late Miocene (14 million years ago, MYA).

In order to properly reconstruct phylogenetic relationships within the subfamily Cantharidinae, we also included in the phylogenetic analyses an important representation of species from the subfamily Stomatellinae since most recent phylogenies suggest that this subfamily is the closest living sister group of Cantharidinae (Williams et al., 2008; Williams et al., 2010; Williams, 2012; Donald and Spencer, 2016). The Stomatellinae are distributed in the Indian and Pacific Oceans, in both temperate and tropical waters, and are typically adapted to intertidal and shallow water hard substrata. Their shells are auriform, oblong and small (<40mm), and the foot has a highly extensible metapodium that cannot be withdrawn into the shell. At present, five genera with 36 species are recognized (Gofas, 2009), although both numbers may be underestimated since the group has been barely studied (Williams et al., 2010; Herbert, 2015).

2. Materials and methods

2.1. Samples and DNA extraction

A full list of species from the subfamilies Cantharidinae and Stomatellinae analyzed in this study along with sampling localities, voucher specimens, and GenBank accession numbers is provided in Table 1. Some specimens belonged to unknown genera and species (see Supplementary Material 1 for photos). Photos of most of the specimens used in Williams et al. (2010) are publicly available on MorphoBank at <http://morphobank.geongrid.org/permalink/?P223>. Type species of the following genera (original name in brackets; Gofas, 2015) were included: *Agagus* Jousseaume, 1894 (Type: *A. agagus* Jousseaume, 1894); *Calliotrochus* Fischer, 1879 (Type: *Margarita marmorea* Pease, 1861); *Callumbonella* Thiele, 1924 (Type: *Trochus suturalis* Philippi, 1836); *Cantharidus* Montfort, 1810 (Type: *Limax opalus* Martyn, 1784); *Clelandella* Winckworth, 1932 (Type: *Trochus miliaris* Brocchi, 1814); *Gibbula* Risso, 1826 (Type: *Trochus magus* Linnaeus, 1758); *Jujubinus* Monterosato, 1884 (Type: *Trochus exasperatus* Pennant, 1777); *Kanekotrochus* Habe, 1958 (Type: *Zizyphinus infuscatus* Gould, 1861); *Oxystele* Philippi, 1847 (Type: *Trochus sinensis* Gmelin, 1791); *Phorcus* Risso, 1826 (Type: *Monodonta richardi* Payraudeau, 1826); *Pictodiloma* Habe, 1946 (Type: *Trochus suavis* Philippi, 1849); *Priotrochus* Fischer, 1879 (Type: *Trochus obscurus* Wood, 1828); *Roseaplagis* Donald & Spencer, 2016 (Type: *Cantharidis* [sic.] *rufozona* A. Adams); *Stomatia* Helbling, 1779 (Type: *Stomatia phymotis* Helbling, 1779); *Stomatolina* Iredale, 1927 (Type: *Stomatolina rufescens* Gray, J.E., 1847); and *Thalotia* Gray, 1847 (Type: *Monodonta conica* Gray, 1827). Tissues were stored in 100% ethanol and total genomic DNA was isolated from up to 30-60 mg of foot following a standard phenol-chloroform extraction (Sambrook et al., 1989).

2.2. PCR amplification and sequencing

We carried out standard PCR reactions using universal primers to amplify partial regions of mitochondrial genes *coxI* (Folmer et al., 1994), *rrnL* (Palumbi et al., 1991), *rrnS* (Oliverio and Mariottini, 2001), *cob* (F-151 5'-GTGGRGCNACYGTWATYACTAA-3' and R-270 5'-AANAGGAARTAYCAYTCNGGYTG-3' modified from Merritt et al. 1998) as well as two nuclear genes, *histone H3* (H3MF 5'-ATGGCTCGTACCAAGCAGACTGG-3 and H3MR 5'-TGGATGTCCTTGGGCATGATTGTTAC-3' modified from Colgan et al. 1998), and 28S *rRNA* (see Williams et al., 2010 and references therein) (Table 1). Each PCR reaction contained 1x buffer, 1.5 mM MgCl₂, 0.05 mM dNTPs, 0.2 mM each primer, 20-100 ng of template DNA, 1 unit of Taq DNA polymerase 5PRIME (Hamburg, Germany), and sterilized distilled water to 25 µl. The following cycling scheme was applied: a denaturing step at 94° C for 60 s; 45 cycles of denaturation at 94° C for 30 s, annealing at different temperatures depending on the gene (44° C for *coxI*, 50°C for *rrnL*, 62° C for *rrnS*, 45° C for *cob*, 50°C for *H3*, and 52° C for 28S) for 80 s, and extension at 72° C for 90 s; followed by a final extension step at 72° C for 5 min. The amplified fragments were purified and sequenced using PCR primers with Sanger sequencing at Secugen, Spain.

2.3. Editing and alignment of sequences

Sequences were edited with Sequencher 5.0.1 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI, USA). The program TranslatorX (Abascal et al., 2010) was used to produce the alignments of each protein coding gene guided by the corresponding deduced open reading frames. The rRNA genes were aligned separately using MAFFT v. 7 (Katoh and Standley, 2013) with default parameters. No gaps were found in the protein-coding genes. Ambiguously aligned sites in the rRNA genes were removed with Gblocks, v.0.19b (Castresana, 2000) in the Phylogeny.fr server (Dereeper

et al., 2008) with the following settings: minimum sequence for flanking positions: 85%; maximum contiguous non-conserved positions: 8; minimum block length: 10; gaps in final blocks: no. Trees were reconstructed for each of the individual genes to check for potential PCR contaminations, numts (nuclear pseudogenes), or taxon misidentifications. Phylogenetic analyses were based on two multi-locus datasets; one constructed by concatenating four genes (*rrnS*, *rrnL*, *cox1*, and 28S *rRNA*; see Figs 1, 2A and 3A) and a second with two extra genes (*cob* and histone *H3*). The 6-gene dataset included a reduced taxon set with representative species of the two less-studied groups, Stomatellinae, and Mediterranean and NE Atlantic Cantharidinae (see Figs. 2B and 3B).

2.4. Phylogenetic analyses

Phylogenetic relationships of Cantharidinae and Stomatellinae were reconstructed using maximum likelihood (ML; Felsenstein, 1981) and Bayesian inference (BI; Huelsenbeck and Ronquist, 2001). ML analyses were conducted with RAXML v7.3.1 (Stamatakis, 2006) using the rapid hill-climbing algorithm and 10,000 bootstrap pseudoreplicates (BP). Bootstrap values >70%, between 70-50%, and <50% were considered to indicate high, moderate/ low, and non-significant statistical support, respectively (Hillis and Bull, 1993). BI analyses were conducted using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) and run with two independent analyses, each with four simultaneous Monte Carlo Markov chains (MCMC) run for 10 million generations. Trees were sampled every 1000 generations after a 25% burn-in when the analysis had reached stationarity (as judged by plots of ML scores and low SD of split frequencies). Statistical support of internal nodes was assessed using Bayesian posterior probabilities (BPP) with values >0.95, between 0.95-0.90, and <0.90 considered to indicate high, moderate/ low, and non-significant statistical support, respectively.

The best partition schemes and best-fit models of substitution for the different analyzed data sets were identified using Partition Finder (Lanfear et al., 2012) according to the Bayesian Information Criterion (BIC; Schwarz, 1978); see Supplementary Material 2).

2.5. Estimation of divergence times

We used BEAST v.1.7 (Drummond and Rambaut, 2007) to perform a Bayesian estimation of divergence times among major Cantharidinae lineages based on the four-gene data set. This software was used to infer branch lengths and nodal ages on a fixed topology (the reconstructed ML tree topology in Fig. 1). Only one individual per species was included in the analysis, except in the case of *Gibbula racketti*, *Jujubinus exasperatus* and *Jujubinus striatus* that had two because these taxa include highly divergent lineages that may represent cryptic species. For the clock model, we selected the lognormal relaxed-clock model, which allows rates to vary among branches without any *a priori* assumption of autocorrelation between adjacent branches. For the tree prior, we employed a Yule process of speciation. We employed the partitions and models selected by Partition Finder (see above). The final Markov chain was run twice, each for 50 million generations, sampling every 1,000 generations with the first 5 million discarded as the burn-in, after confirming convergence of chains with Tracer v.1.5. (Rambaut and Drummond, 2007). The effective sample size of all the parameters was > 200.

Estimated divergence times were obtained by specifying one calibration point based on fossil data using a lognormal distribution of prior probability. The origin of the New Zealand genus *Cantharidus* was constrained to have a minimum age corresponding to the Upper Eocene (35 MYA; 95% interval: 35.3–47.9 MYA) following Donald et al. (2012).

3. Results

Our taxon sampling strategy was designed to maximize representation of Cantharidinae species from the Mediterranean Sea and NE Atlantic Ocean, which have been lacking in previous studies (Table 1). A total of 25 specimens of *Gibbula*, 11 of *Jujubinus*, eight of *Phorcus*, one of *Clelandella*, and one *Callumbonella* representing 29 different species (including the type species of these five genera) from these regions were sampled and analyzed in combination with 14 new specimens of Indo-Pacific Cantharidinae and 14 new specimens of Stomatellinae (Table 1). Altogether 346 new sequences were determined for four-six partial genes (*coxI* with ~600 bp; *rrnL* with ~550 bp; *rrnS* with ~630 bp; *cob* with ~340 bp; 28S *rRNA* with ~1400 bp; *histone H3* with ~310 bp). Each of these genes was analyzed separately producing phylogenetic trees that essentially recover similar and congruent clades to those obtained in the phylogenetic analyses based on multi-locus matrices, but with poorer support (Supplementary Material 3).

3.1. Phylogenetic relationships of Cantharidinae

The newly determined sequences were combined with orthologous sequences available from GenBank for subfamilies Cantharidinae (36 specimens) and Stomatellinae (8 specimens), and phylogenetic analyses were based on four-gene (all Cantharidinae) and six-gene data sets (focused on Mediterranean and NE Atlantic Cantharidinae and on Stomatellinae) using probabilistic methods of inference (Figs. 1-3). The nucleotide alignment of the four-gene data set (*rrnS*, *rrnL*, *coxI*, and 28S) was 2,792 positions long. The ML (-lnL = 36651.18) and BI phylogenetic analyses arrived at the same topology using two species of the subfamily Umboniinae as outgroup (Fig. 1). Both

reconstructed trees recovered with high statistical support the monophyly of the subfamilies Stomatellinae (100% BP, 1 BPP) and Cantharidinae (94% BP, 1 BPP), as well as of a Mediterranean and NE Atlantic Cantharidinae group (91% BP, 1 BPP) (Fig. 1). Statistical support at the genus level was generally high, but other internal nodes in the four-gene trees received only moderate to poor or non-significant support (Fig. 1).

Within analyzed Cantharidinae, the genus *Oxystele* is recovered as sister to the remaining members of the subfamily, although with low support (Fig. 1). This genus is restricted to the South African region, and was represented in our analyses by four species (Table 1). Within the remaining Cantharidinae, four major clades and several independent lineages are recovered. Of these groups, only the one including the Mediterranean and NE Atlantic Ocean genera is highly supported (Fig. 1). One of the Indo-Pacific clades has high support (76% BP/ 1 BPP) and includes “*Jujubinus*” *geographicus* from Vanuatu as sister to “*Cantharidus*” from Japan and “*Jujubinus*” *gilberti* and Cantharidinae genus 2 from the Philippines (Fig. 1). Phylogenetic relationships within this clade are well resolved with nodes having high statistical support (Fig. 1). A second highly supported Indo-Pacific group (86% BP/ 1 BPP) includes the genera *Kanekotrochus* from Japan and *Agagus* from South East Africa, as well as “*Jujubinus*” *suarezensis* from Mozambique and Madagascar. A third group is moderately well-supported in ML (69% BP) and highly supported in BI (1 BPP), and includes New Zealand species of the genera *Micrelenchus*, *Cantharidus* and *Roseaplagis* (clade with high statistical support, 99% BP/ 1 BPP), the genus *Prothalotia* from the west coast of Australia, and Cantharidinae genus 1 from the Solomon Islands (Fig. 1). The relative phylogenetic position of *Priotrochus obscurus* from Mozambique, genera *Pictodiloma* and *Tosatrochus* both from Japan, “*Cantharidus*” and *Thalotia* both from Western Australia, “*Trochus*” *kotschy* from Abu Dhabi, and “*Cantharidus*”

sendersi from Vanuatu could not be confidently resolved. Of the three major Indo-Pacific clades, the one including Cantharidinae genus 1 from Solomon Islands, Australian *Prothalotia*, and *Micrelenchus* + *Cantharidus* + *Roseaplagis* from New Zealand, was recovered as sister group to the Mediterranean and NE Atlantic clade, with low and high statistical support in ML (54% BP) and BI (0.99 BPP), respectively (Fig. 1).

3.2. Phylogenetic relationships of Mediterranean and NE Atlantic Cantharidinae

Cantharidinae species living in the Mediterranean Sea and NE Atlantic Ocean form a highly supported (91% BP/ 1 BPP) monophyletic group in the phylogenetic analyses based on the four-gene data set (Fig. 1). Within this group, seven main lineages are recovered. The monophyly of both *Phorcus* and *Jujubinus* is maximally supported, as was a clade with the deep-sea genera *Clelandella* and *Callumbonella* sister to *Jujubinus* (99% BP/ 1BPP; Fig. 2A). Phylogenetic relationships within *Phorcus* are well resolved with *P. turbinatus* and *P. sauciatu*s recovered as sister group to two distinct clades, one including *P. mariae*, *P. punctulatus*, and *P. lineatus*, and the other including *P. atratus*, *P. mutabilis*, *P. articulatus* and *P. richardi* (Fig. 2A). Within *Jujubinus*, phylogenetic relationships are relatively well resolved. Three individuals identified as *J. striatus* but showing high sequence divergences form a clade that is the sister to the remaining *Jujubinus* and *Gibbula tingitana*. Within this clade, two lineages are distinguished, one including *Gibbula tingitana* from Alborán sea (south Spain), *J. gravinae* and *J. ruscurianus*, and the other having Macaronesian endemics as sister to *J. exasperatus*, which itself consist of two divergent lineages (Fig. 2A). However, the most striking result of the phylogenetic analysis is the cryptic diversity and non-monophyly of *Gibbula*, which shows at least three distinct and highly supported clades (Fig. 2A). One

clade includes *G. umbilicaris*, *G. cineraria*, *G. pennanti*, *G. racketti*, *G. albida*, and *G. adriatica*. Phylogenetic relationships within this clade are unresolved (Fig. 2A). A second, closely related clade includes *G. umbilicalis* as sister to *G. rarilineata* plus *G. varia*, and *G. divaricata*. A third clade includes *G. ardens* as sister group of *G. magus* and *G. fanulum*. Two additional *Gibbula* species, *G. philberti* and *G. turbinoides* show relatively long branches and were recovered together as sister group of *Gibbula* clade 3 without support (Fig. 2A). Finally, as mentioned above, *G. tingitana* is nested deeply within *Jujubinus* in all phylogenetic analyses (Fig. 2A).

The nucleotide alignment of the six-gene (adding *cob* and *histone H3*) Mediterranean and NE Atlantic Cantharidinae data set was 3,507 positions long. The ML (-lnL = 18645.94) and BI phylogenetic analyses arrived at the same topology using *Agagus agagus* and “*Cantharidus*” *sendersi* as outgroups (Fig. 2B). The reconstructed trees confirmed the monophyly of *Jujubinus*, *Phorcus*, and of the three independent lineages of *Gibbula*, as well as the sister group relationship of *Clelandella* and *Callumbonella* to *Jujubinus* (Fig. 2B), providing higher statistical support for the corresponding tree nodes. Moreover, the addition of extra positions to the phylogenetic analyses produced higher resolution at deeper nodes connecting main lineages (Fig. 2B). A sister group relationship between *Gibbula* clades 1 and 2 was highly supported (98% BP, 1 BPP), and this clade was sister to *Phorcus* with moderate support (62% BP, 0.96 BPP). This clade had *G. philberti* as sister to the exclusion of *G. turbinoides* with low support (52% BP/ 0.93 BPP; Fig. 2B). The relative phylogenetic positions of *Gibbula* clade 3 and the clade with *Jujubinus* plus *Clelandella* and *Callumbonella* could not be resolved (Fig. 2B).

3.3. Phylogenetic relationships of Stomatellinae

The Indo-Pacific subfamily Stomatellinae has been poorly studied to date. Here, despite the fact that it was not the main focus of this study, we have significantly increased the number of species analyzed for this subfamily. In addition to the four-gene data set (see reconstructed trees in Fig. 3A) a six-gene (adding *cob* and *H3*) data set was also constructed for Stomatellinae. The nucleotide alignment of the six-gene Stomatellinae data set was 3,625 bp. The ML (-lnL = 13338.99) and BI phylogenetic analyses arrived at the same topology using *Agabus agabus* and “*Cantharidus*” *sendersi* as outgroups (Fig. 3B). Most internal nodes within Stomatellinae lacked resolution in both the four- and six-gene data sets (Fig. 3). However, phylogenetic analyses allowed delimitation of several independent lineages that indicate unrecognized diversity within the subfamily. One clade included species of the genera *Stomatia* and *Microtis* (Fig. 3). A second clade included four known and three unidentified species of the genus *Stomatella*. Individuals assigned to the species *S. impertusa* did not form a monophyletic group (Fig. 3). A third clade recovered the species *Pseudostomatella decolorata* as sister to *Stomatolina rubra* with maximal support (Fig. 3). The relative phylogenetic position of *Stomatolina angulata* and three unidentified species (Stomatellinae unknown genus 1 sp 1 and sp 2, and Stomatellinae unknown genus 2 sp 1) from the Philippines could not be confidently resolved. Lastly, *Calliotrochus marmoreus* was recovered as sister group of the Stomatellinae.

3.4. Estimation of divergence times

In order to date the major cladogenetic events within the Cantharidinae, a chronogram was estimated using a Bayesian uncorrelated relaxed molecular clock. One fossil calibration was used to constrain the origin of the New Zealand endemic clade to the Upper Eocene about 35 MYA (Donald et al., 2012). The resulting chronogram dated the

split between the two sister subfamilies Stomatellinae and Cantharidinae about 77.5 MYA, although with a large confidence interval (106-56, 95%HPD; Fig. 4). The origin of the Mediterranean and NE Atlantic clade was estimated to have occurred 47 MYA (58-39, 95%HPD), whereas its diversification into major lineages (*Phorcus*, *Jujubinus*, and the different *Gibbula* clades) was dated between 38-28 MYA (Fig. 4) coinciding with the early diversification of the New Zealand endemic Cantharidinae. Within the Mediterranean NE Atlantic clade, most of the main diversification events within each genus started about 14 MYA (as in the Indo-Pacific Ocean) and a few more recently, about 5 MYA (Fig. 4).

4. Discussion

4.1. Phylogenetic relationships of NE Atlantic and Mediterranean ridinae

In this study, we provide the first attempt to establish a robust phylogeny of Mediterranean and NE Atlantic Cantharidinae. All these taxa were recovered in a single, well-supported clade containing several well-supported subclades.

Unfortunately, phylogenetic relationships among groups within the Mediterranean and NE Atlantic Cantharidinae clade lack resolution, possibly pointing to a rapid radiation event soon after the origin of these lineages. Reciprocal monophyly of both *Phorcus* and *Jujubinus* is strongly supported, but *Gibbula* as currently defined is not monophyletic. Interestingly, a sister group relationship between the deep-sea genera *Clelandella* and *Callumbonella* is recovered, and their sister relationship with *Jujubinus* is highly supported. The species *Clelandella miliaris* was originally included in the genus *Calliostoma*, and later ascribed to the genus *Jujubinus*. More recently, Cretella et al. (1990) proposed the genus *Clelandella* was valid, and phylogenetically closer to *Jujubinus* than to *Calliostoma* based on external head-foot and radular characters and

allozyme data. The genus *Callumbonella* remained unassigned or variously assigned to either Umboniinae or Margaritinae (Rolán et al., 2009). Further work on deep sea Trochidae is on-going and will help determine the exact phylogenetic position of these enigmatic taxa (STW in prep).

The alpha-taxonomy of the genus *Jujubinus* and the exact distribution of many of its species remain unresolved despite considerable revisionary efforts (Mariottini et al., 2013; Smriglio et al., 2014; Oliverio pers. comm.). The reconstructed phylogenetic relationships within *Jujubinus* are relatively well resolved, and show a strong geographical component. The species from Madeira (*J. vexationis*) and Azores Islands (*J. pseudogravinae*) form a clade that is sister to the widespread *J. exasperatus*. This aptly named species is divided into two biogeographically distinct lineages: one found in the Mediterranean and one in the NE Atlantic. The divergence between these two lineages as well as that found among individuals assigned to *J. striatus* are similar to the one obtained between the Macaronesian endemic species, suggesting cryptic diversity within *J. exasperatus* and *J. striatus*. Hence, our results prompt for a revision of *Jujubinus* species with more thorough population sampling (currently being undertaken by M. Oliverio, pers. comm). One clear taxonomic outcome of the present phylogeny derives from the relative position of *Gibbula tingitana* from Alborán Sea, (south Spain), which is nested deeply within *Jujubinus* in all phylogenetic analyses next to species from the same region, *J. rucurianus* and *J. gravinae*. Thus, we propose officially reassigning this species to *Jujubinus*.

We have included in our study all nine species currently recognized within genus *Phorcus* (Donald et al., 2012) and recovered two main lineages within the genus, which correspond to the subgenera *Phorcus* (*P. richardi*, *P. articulatus*, *P. mutabilis*, *P. atratus*, *P. lineatus*, *P. punctulatus*, and *P. mariae*) and *Osilinus* (*P. sauciatus* and *P.*

turbinatus) (Donald et al., 2012). Within the subgenus *Phorcus*, the Atlantic species *P. punctulatus*, *P. mariae*, and *P. lineatus* were the sister to the Canary Island endemic and the Mediterranean species. Within the subgenus *Osilinus*, *P. sauciatus* is an Atlantic species and *P. turbinatus* is endemic to the Mediterranean Sea. Almost the same topology (varying only in the relative position of *P. mariae* as sister to *P. lineatus*) was recovered by Donald et al. (2012), who provided the first complete molecular phylogeny of *Phorcus* and clarified the evolution of this conspicuous group of intertidal grazers.

The reconstructed phylogeny divided species ascribed to the genus *Gibbula* into three major clades, with the exception of *G. turbinoides* and *G. philberti*, whose placement is uncertain. The relative position of the genus *Phorcus* (strongly supported in the six-gene phylogenetic analysis) makes *Gibbula* non-monophyletic. The clades obtained here largely coincide with those of a previous phylogeny of the genus based solely on *cox1* (Barco et al., 2013). All three *Gibbula* clades contain both Mediterranean and NE Atlantic species. In one clade, two eastern Mediterranean species, *G. albida* and *G. adriatica* are the sister group of a mix of Atlantic (*G. cineraria* and *G. pennanti*) and Mediterranean representatives (*G. umbilicaris*, *G. racketsi*). According to Barco et al. (2013) the species *G. spratti*, *G. nivosa*, *G. adansonii* and *G. nebulosa*, all from the Mediterranean Sea, could also belong to this clade. The high level of divergence found between two individuals assigned to *G. racketsi* may indicate the presence of cryptic species and deserves further study. A second clade embraces the Atlantic *G. umbilicalis* and the Mediterranean species *G. rarilineata*, *G. varia*, and *G. divaricata*. Hence, the internal division of the clade follows the geographical distribution of taxa. This clade was also obtained by Barco et al. (2013), although with *G. rarilineata* as sister to *G. umbilicalis* plus *G. varia*, and *G. divaricata*. The Mediterranean species *G. rarilineata*

and *G. divaricata* are known to form mixed populations in the same habitat, and morphologically intermediate forms can be found (López-Márquez, 2016).

Phylogenetic relationships among *Jujubinus*, *Phorcus* and the *Gibbula* clades are unresolved in phylogenetic trees reconstructed using the four-gene data set but some deeper nodes obtain statistical support in the six-gene phylogeny. In particular, the six-gene tree recovers high support for the sister group relationship of *Phorcus* and *Gibbula* clades 1 and 2 to the exclusion of *Gibbula* clade 3, *G. philberti*, and *G. turbinoides*, resulting in non-monophyly of the genus *Gibbula* as currently defined. Moreover, the mean sequence divergence among *Gibbula* clades is of the same magnitude as that estimated between *Phorcus* and *Jujubinus*, indicating that the three *Gibbula* clades plus the two independent species deserve treatment as separate genera. The clade 3 containing the type species, *G. magus*, plus *G. ardens* and *G. fanulus* should retain the genus name (*G. guttadauri*, not studied here, also belongs to this clade according to the results of Barco et al., 2013), whereas the remaining *Gibbula* clades could receive distinct generic names, as has been previously noted by Williams et al. (2010) and by Barco et al. (2013) employing *cox1* sequences. In fact, some junior synonyms are available, such as e.g., *Steromphalus* P. Fischer, 1875 (Type species *Trochus cinerarius* Linnaeus, 1758) for *Gibbula* clade 1, and *Gibbulastra* Monterosato, 1884 (Type species *Trochus divaricatus* Linnaeus, 1758) for *Gibbula* clade 2. However, we leave nomenclatural decisions for future studies with this specific aim and based on a more complete taxon sampling of European, Macaronesian and South African species.

Furthermore, some South African and Indo-Pacific species currently assigned to *Gibbula* and *Jujubinus* almost certainly belong to other genera. As a precedent, Hickman and McLean (1990) and Herbert (1991) assigned some western Indian Ocean species previously referred to *Gibbula* to the genera *Agagus* and *Calliotrochus*, and

Beck (1995) based on morphological and radular characters erected the new genus *Rubritrochus* (which belongs to the subfamily Trochinae) for two species previously included in *Gibbula* from the Indian Ocean and Red Sea (*G. pulcherrima* and *G. declivis*). Similarly, Donald et al. (2012) showed that *Osilinus kotschy*, from the Arabian and Red Seas, was not closely related to the other species of this NE Atlantic genus and tentatively referred it to the Indo-Pacific genus *Priotrochus* (see below). In particular, West African species currently attributed to *Gibbula* and *Jujubinus* should be studied molecularly to accurately determine the extent of both genera.

4.2. Phylogenetic relationships of Cantharidinae

The South African genus *Oxysteles* was recovered as sister to the remaining members of the subfamily Cantharidinae here analyzed, but only with poor support. Previous phylogenetic analyses based on concatenated genes were also rather inconclusive for this part of the tree, and recovered either *Kanekotrochus* (Williams et al., 2010) or *Kanekotrochus* + *C. jessoensis* (Donald and Spencer, 2016) as sister to the remaining Cantharidinae with *Oxysteles* in a more derived position. The different lineages within Cantharidinae are connected by short branches, suggesting a rapid radiation process soon after the origin of the subfamily. Genomic sequence data sets including relevant missing taxa may be needed to enhance resolution at these and other (e.g. sister group of Mediterranean and NE Atlantic taxa; see below) nodes in the phylogeny. A robust phylogeny is needed to shed light on the rather complex taxonomy of Indo-Pacific Cantharidinae. Most tropical species from this subfamily have been assigned to a specific genus based on shell or morphological characters, but the group requires extensive revision (Herbert, 1996; Vilvens, 2012), and many species will shift from one genus to another when robust phylogenies become available. For instance, our results

further support the suggestion that the genus *Cantharidus* (the type species, *C. opalus*, is included) should be restricted to New Zealand species, whereas species from other areas traditionally included in this genus should be assigned to other genera (Williams et al., 2010). In this regard, our results mostly matched those obtained by Donald and Spencer (2016), who recognized three New Zealand endemic genera *Cantharidus* s.s., *Micrelenchus* and *Roseaplagis*. The nomenclatural changes proposed by Donald and Spencer (2016) are accepted and followed here, and in fact, in our case the monophyly of New Zealand endemic taxa is highly supported.

A remarkable case is that of “*Trochus*” *kotschy* from the Arabian and Red Seas, whose taxonomic placement has been controversial historically, as it was variously assigned to genera *Cantharidus*, *Thalotia*, *Priotrochus* and *Gibbula* (Herbert, 1994). The species was once considered synonymous with *Priotrochus obscurus* (the type species of the genus) but Herbert (1988) confirmed its validity as an endemic species from the Persian Gulf. Later, the same author (Herbert, 1994) included “*Trochus*” *kotschy* in the European genus *Osilinus*. Nevertheless Donald et al. (2012) showed that this species is not sister to the European clade of *Osilinus* species, and suggested its tentative placement in *Priotrochus*. Our present results confirm that “*Trochus*” *kotschy* is neither related to the NE Atlantic and Mediterranean clade nor to the clade that includes the type species of *Priotrochus*. Therefore, the generic assignment of this species remains unresolved.

Due to lack of statistical support, it is not possible to determine with confidence, the exact sister to the Mediterranean and NE Atlantic Ocean genera. However, our results agree with previous phylogenetic analyses (Williams et al., 2010) in considering that a putative clade including New Zealand *Cantharidus* and related endemic genera (*Micrelenchus* and *Roseaplagis*; Donald and Spencer, 2016), *Prothalotia* from the west

coast of Australia, and an undescribed species from the Solomon Islands (species description in prep., Warén and Williams) could be the sister group to a clade containing Mediterranean and NE Atlantic genera. Donald and Spencer (2016) also add *Oxysteles* to the list of potential genera with affinities to Mediterranean and NE Atlantic Cantharidinae. If further supported, this phylogenetic relationship between antipode taxa would be favor an ancient Tethyan origin for the Mediterranean and NE Atlantic Cantharidinae (see below).

4.3. Phylogenetic relationships of Stomatellinae

The subfamily Stomatellinae has been long recognized as a natural group (Gray, 1840), but its phylogenetic placement was controversial until recently, when a robust sister group relationship with Cantharidinae was proposed based on morphology (Herbert, 1998), and further supported with molecules (Williams et al., 2010). The taxonomy of Stomatellinae has been neglected for many years and urgently demands monographic revisionary studies since nominal species were often inadequately described and type material has rarely been consulted by subsequent authors (Herbert, 2015). Therefore, it is not surprising that the phylogenetic relationships within Stomatellinae are largely unknown. Here, we provide an exploratory attempt to further resolve phylogenetic relationship within this subfamily, extending previous work (Williams et al., 2010) by adding new taxa, although several are pending formal identification or description due to the problems mentioned above (Supplementary Material 1). The reconstructed phylogeny is congruent with that of Williams et al. (2010), and shows that some of the newly added taxa likely represent new genera, and that some species like *Stomatella impertusa* may have served as a catch-all taxon. Surprisingly, our results could prove that *Calliotrochus* is not a member of the Cantharidinae (cf. WoRMS; Bouchet and

Gofas, 2010), but rather a basal and somewhat atypical (i.e., turbiniform rather than auriform) member of the Stomatellinae as suspected by Herbert (1998). Altogether, the phylogeny suggests that the diversity of the subfamily is highly underestimated at present.

4.4. Estimation of divergence times

The present-day distribution of the subfamily Cantharidinae extends from the Indo-West Pacific to the NE Atlantic-Mediterranean region and the coasts of Western Africa down to South Africa. This distribution strongly suggests a Tethyan origin for the group. The Tethys Ocean separated Laurasia and Gondwana supercontinents following the break-up of Pangea in the Triassic about 250 MYA. Continental drift produced collision of these two tectonic plates about 19 MYA and the final closure of the Eastern Tethys Seaway in the Middle Miocene around 14 MYA (Harzhauser et al., 2007; Okay et al., 2010). The closure definitively ended the previous exchange of water and tropical marine biota between the Indian Ocean and the proto-Mediterranean Basin (Hamon et al., 2013). It is striking that the sister group of the Mediterranean and NE Atlantic Cantharidinae lineage may comprise antipode temperate genera from Australia and New Zealand, which would support an original widespread distribution in the Tethys Ocean, and likely extinction events in tropical intermediate regions. However, this hypothesis requires the postulated sister group relationship receiving higher statistical support in future studies.

According to our results, the origin of the Mediterranean and NE Atlantic lineage occurred in the boundary between the Early and Middle Eocene around 47 MYA (about 51 MYA in Donald et al. (2012), which greatly predates the closure of the Eastern Tethys seaway. Many Cantharidinae species are associated with seagrass beds and

interestingly, the marine angiosperm genus *Posidonia* shows a similar geographic distribution with antipode (Australian and Mediterranean) clades. The divergence between these *Posidonia* clades is ancient, and was estimated based on ITS (internal transcribed spacer) sequence data to have occurred about 68 Myr (Aires et al., 2011), coinciding with the Cretaceous–Tertiary mass extinction (K–T boundary) event (Schulte et al., 2010). Other authors (Phillips and Meñez, 1988) estimated that the ancient *Posidonia* split could have occurred in the Late Eocene based on geological and tectonic history. This dating would be more in agreement with this study. While the Early Eocene was characterized by a warmer climate and sea level rise (which peaked in the so-called Eocene Climatic Optimum; 52 to 50 MYA), at the onset of Middle Eocene about 48 MYA, the *Azolla* phase started the transition from greenhouse conditions towards modern icehouse conditions (Speelman et al., 2009). The *Azolla* phase is named for massive blooms of this floating aquatic fern that sank in the Arctic Ocean at this time, producing a drop in atmospheric carbon dioxide. The consequent steady cooling of the climate (a 17-Myr-long trend towards cooler conditions that persisted until the latter part of the Oligocene about 26 to 27 MYA, Zachos et al., 2001) over this time period is likely to have favored temperate taxa.

The origin of the major Mediterranean and NE Atlantic lineages (*Jujubinus*, *Phorcus*, and the different clades of *Gibbula*) was dated in this study to have occurred towards the end of the Eocene. The traffic of nearshore gastropods across the Eastern Tethys Seaway continued throughout the Oligocene until the Upper Miocene (23 MYA), when it dropped to zero despite the passage remaining open for some time longer (Harzhauser et al., 2009). In fact, a re-establishment of migration pathways occurred about 16 MYA prior to the final closure (Harzhauser et al., 2009). Our estimates for the main diversification events within the different Cantharidinae lineages

were dated to have occurred after the final closure of the Eastern Tethys Seaway due to the consequent isolation of the Mediterranean and Atlantic Ocean basin from the Indo-Pacific region. The Messinian Salinity Crisis (5.9-5.3 MYA) presumably led to important background extinction rates in the Mediterranean Sea, which would have affected subtidal taxa such as almost all *Jujubinus* species and many *Gibbula* species, although having less effect on *Phorcus* species and some *Gibbula* species that are well adapted to the intertidal zone and thus are likely capable of surviving stressful conditions. The possibility of surviving the Messinian Salinity Crisis within the Mediterranean Sea has also been suggested for another intertidal group, the vermetid genus *Dendropoma* (Calvo et al., 2015). With the reopening of the Strait of Gibraltar (5.3 Ma), the refilling of the Mediterranean Sea by the Atlantic Ocean (the Zanclean flood; Garcia-Castellanos et al., 2009) allowed splitting among closely related species and population differentiation within species as suggested by our chronogram.

To conclude, this study builds on previous molecular phylogenetic studies of the hyperdiverse family Trochidae. It focuses on the subfamily Cantharidinae and in particular, Mediterranean and NE Atlantic species. The results of the study further corroborate the rampant levels of morphological homoplasy in top shell snails that have hindered their systematics, and are exemplified here by the non-monophyly of the genus *Gibbula*, which can be divided into at least three major clades. Moreover, it emphasizes the existence of unrecognized diversity both within the subfamily Cantharidinae and its sister subfamily Stomatellinae at the genus and species level, and the need for monographic works to thoroughly revise the taxonomy of both subfamilies. Future molecular phylogenies of the subfamily should add taxa from West Africa and use both mitochondrial and nuclear genomic data sets, which have proven to render highly resolved phylogenetic relationships within Vetigastropoda (Uribe et al., 2016).

Additionally, the study highlights interesting biogeographical patterns as it supports a single origin of the Mediterranean and NE Atlantic clade from Indo-Pacific lineages, and shows important divergences within each genus between Mediterranean and NE Atlantic species. The dating of the phylogeny using a fossil-calibrated molecular clock suggests that the *Azolla* phase, the eastern closure of the Tethys Ocean, and the Messinian Salinity Crisis of the Mediterranean Sea had major effects on the diversification of the group.

5. Acknowledgements

We are grateful to Serge Gofas for donating species from south Spain, and Emilio Rolán, who provided specimens from northwest Spain. The MNHN Indo-Pacific material originates from a series of expeditions and workshops, conducted in the context of the "Our Planet Reviewed" programme with Pro-Natura International (SANTO 2006 to Vanuatu, ATIMO VATAE 2010 to Madagascar "Deep South" with Université de Tuléar, and its spin-off INHACA 2011 at Estação de Biologia Marítima, Universidade Eduardo Mondlane), of the "Tropical Deep-Sea Benthos" programme with Institut de Recherche pour le Développement (SALOMON 2 to the Solomon Is), or stand-alone projects (PANGLAO 2004 to the Philippines, with University San Carlos; WESTERN AUSTRALIA 2011, with the Western Australian Museum). We thank the Total Foundation Total, Prince Albert II of Monaco Foundation, and Stavros Niarchos Foundation for their support to these expeditions. Jose Rosado and Hugh Morrison facilitated the field logistics in Mozambique and Australia, respectively. Alexander Fedosov, Yuri Kantor, Nicolas Puillandre and Ellen Strong processed the specimens in the field for molecular sequencing, and we are grateful to Philippe Bouchet for giving us access to this collection. All expeditions operated under the regulations then in force

in the countries in question and satisfy the conditions set by the Nagoya Protocol for access to genetic resources. We particularly thank Raquel Álvarez for her technical assistance in the molecular laboratory at early stages of this work. We are indebted to Jesús Marco and Luis Cabellos who provided access to the supercomputer Altamira at the Institute of Physics of Cantabria (IFCA-CSIC), member of the Spanish Supercomputing Network, for performing phylogenetic analyses. Dai Herbert and an anonymous referee provided very useful comments that greatly improved a previous version of the manuscript. This work was supported by the Spanish Ministry of Science and Innovation (CGL2010-18216 and CGL2013-45211-C2-2-P to RZ; BES-2011-051469 to JEU), and by funding from the NHM Department of Life Sciences to STW.

6. References

- Abascal, F., Zardoya, R., Telford, M.J., 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res.* 38, W7-13.
- Aires, T., Marbà, N., Cunha, R.L., Kendrick, G.A., Walker, D.I., Serrão, E.A., Duarte, C.M., Arnaud-Haond, S., 2011. Evolutionary history of the seagrass genus *Posidonia*. *Mar. Ecol. Prog. Ser.* 421, 117-130.
- Barco, A., Evans, J., Schembri, P.J., Taviani, M., Oliverio, M., 2013. Testing the applicability of DNA barcoding for Mediterranean species of top-shells (Gastropoda, Trochidae, *Gibbula* s.l.). *Mar. Biol. Res.* 9, 785-793.
- Beck, L., 1995. *Rubritrochus*, a new genus name for *Gibbula pulcherrima* A. Adams 1855 and *Gibbula declivis* (Forsk. 1775) (Gastropoda: Prosobranchia: Trochidae). *Arch. Molluskenkd.* 124, 65-85.
- Bouchet, P.; Gofas, S. (2010). Cantharidinae Gray, 1857. In: MolluscaBase (2016). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=382171> on 2016-06-02
- Bouchet, P., Gofas, S., 2015. Cantharidinae Gray, 1857. In: MolluscaBase (2015). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=382171> on 2016-04-30
- Bouchet, P., Rocroi, J.P., Frýda, J., Hausdorf, B., Ponder, W.F., Valdés, Á., Warén, A. 2005. Classification and nomenclator of gastropod families. *Malacologia* 47, 1-397.
- Calvo, M., Alda, F., Oliverio, M., Templado, J., Machordom, A., 2015. Surviving the Messinian Salinity Crisis? Divergence patterns in the genus *Dendropoma*

(Gastropoda: Vermetidae) in the Mediterranean Sea. *Mol. Phylogenet. Evol.* 91, 17-26.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540-552.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Molecular phylogenetics of the Arthropoda: relationships based on histone H3 and U2 snRNA DNA sequences. *Aust. J. Zool.* 46, 419-437.

Cretella, M., Scillitani, G., Picariello, O., 1990. The systematic position of '*Trochus miliaris* Brocchi, 1814 (Gastropoda: Trochidae); morphological and biological evidences. *Lavori della Societa' Italiana di Malacologia* 23, 51-81.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M., Gascuel, O., 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 36, W465-W469.

Donald, K.M., Kennedy, M., Spencer, H.G., 2005. The phylogeny and taxonomy of austral monodontine topshells (Mollusca: Gastropoda: Trochidae), inferred from DNA sequences. *Mol. Phylogenet. Evol.* 37, 474-483.

Donald, K.M., Preston, J., Williams, S.T., Reid, D.G., Winter, D., Alvarez, R., Buge, B., Hawkins, S.J., Templado, J., Spencer, H.G., 2012. Phylogenetic relationships elucidate colonization patterns in the intertidal grazers *Osilinus* Philippi, 1847 and *Phorcus* Risso, 1826 (Gastropoda: Trochidae) in the northeastern Atlantic Ocean and Mediterranean Sea. *Mol. Phylogenet. Evol.* 62, 35-45.

Donald, K.M., Spencer, H.G., 2016. Phylogeographic patterns in New Zealand and temperate Australian cantharidines (Mollusca: Gastropoda: Trochidae:

Cantharidinae): Trans-Tasman divergences are ancient. *Mol. Phylogenet. Evol.* 100, 333-344.

Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* 17, 368-376.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294-299.

Garcia-Castellanos, D., Estrada, F., Jiménez-Munt, I., Gorini, C., Fernàndez, M., Vergés, J., De Vicente, R., 2009. Catastrophic flood of the Mediterranean after the Messinian salinity crisis. *Nature* 462, 778-781.

Gofas, S., 2005. Geographical Differentiation In *Clelandella* (Gastropoda: Trochidae) In The Northeastern Atlantic. *J. Mollus. Stud.* 71, 133-144.

Gofas, S., 2009. Stomatellinae Gray, 1840. In: MolluscaBase (2016). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=382176> on 2016-05-16

Gofas, S., 2015. Trochidae. In: MolluscaBase (2015). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=443> on 2016-04-30

Gofas, S., Bouchet, P., 2015a. *Gibbula* Risso, 1826. In: MolluscaBase (2016). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=138590> on 2016-05-16

Gofas, S., Bouchet, P., 2015b. *Jujubinus Monterosato*, 1884. In: MolluscaBase (2016).

Accessed through: World Register of Marine Species at

<http://www.marinespecies.org/aphia.php?p=taxdetails&id=138591> on 2016-05-16

Gray, J.E., 1840. Shells of molluscous animals. Synopsis of the contents of the British Museum. Woodfall and Son, London, pp. 106-156.

Hamon, N., Sepulchre, P., Lefebvre, V., Ramstein, G., 2013. The role of eastern Tethys seaway closure in the Middle Miocene Climatic Transition (ca. 14 Ma). *Clim. Past* 9, 2687-2702.

Harzhauser, M., Kroh, A., Mandic, O., Piller, W.E., Göhlich, U., Reuter, M., Berning, B., 2007. Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene Tethyan Seaway. *Zool. Anz.* 246, 241-256.

Harzhauser, M., Reuter, M., Piller, W.E., Berning, B., Kroh, A., Mandic, O., 2009. Oligocene and Early Miocene gastropods from Kutch (NW India) document an early biogeographic switch from Western Tethys to Indo-Pacific. *Paläontol. Z.* 83, 333-372.

Hawkins, S.J., Watson, D.C., Hill, A.S., Harding, S.P., Kyriakides, M.A., Hutchinson, S., Norton, T.A., 1989. A comparison of feeding mechanisms in microphageous, herbivorous, intertidal prosobranchs in relation to resource partitioning. *J. Mollus. Stud.* 55, 151-165.

Herbert, D.G., 1988. Studies of *Priotrochus obscurus* and the systematic position of *Priotrochus* (Mollusca: Gastropoda: Trochidae). *J. Zool. Lond.* 214, 261-268.

Herbert, D.G., 1991. A revision of the genus *Agagus* Jousseaume, 1894 (Mollusca: Gastropoda: Trochidae). *J. Nat. Hist.* 25, 883-900.

Herbert, D.G., 1994. *Trochus kotschy*, the first Indian Ocean record of the genus *Osilinus* (Mollusca: Gastropoda: Trochidae). *J. Zool. Lond.* 233, 345-357.

- Herbert, D.G., 1996. A critical review of the trochoidean types in the Muséum d'Histoire Naturelle, Bordeaux (Mollusca: Gastropoda). Bull. Mus. Natl. Hist. Nat. Series 4, 18, Section A, 3-4, 409-445.
- Herbert, D.G., 1998. Revision of the genus *Calliotrochus* Fischer, 1879 (Gastropoda : Trochoidea). Invertebr. Syst. 12, 545-565.
- Herbert, D.G., 2015. An annotated catalogue and bibliography of the taxonomy, synonymy and distribution of the Recent Vetigastropoda of South Africa (Mollusca). Zootaxa 4049, 1-98.
- Hickman, C.S., 2005. Seagrass fauna of the temperate southern coast of Australia I: the cantharidine trochid gastropods. In: Wells, F.E., Walker, D.I., Kendrick, G.A. (Eds.), The Marine Flora and Fauna of Esperance, Western Australia. Western Australia Museum, Perth.
- Hickman, C.S., McLean, J.H., 1990. Systematic revision and suprageneric classification of trochacean gastropods. Science series of Natural History Museum of Los Angeles County 35, 1-169.
- Hillis, D.M., and Bull, J. J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182-192.
- Huelsenbeck, J., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754-755.
- Kano, Y., 2008. Vetigastropod phylogeny and a new concept of Seguenzioidea: independent evolution of copulatory organs in the deep sea habitats. Zool. Scr. 37, 1-21.
- Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol. 30, 772-780.

- Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Mol. Biol. Evol.* 29, 1695-1701.
- López-Márquez, V., García-Jiménez, R., Templado, J., Machordom, A., 2016. Development and characterization of 26 novel microsatellite loci for the Trochidae *Gibbula divaricata* (Linnaeus, 1758), using Illumina MiSeq next generation sequencing technology. *Peer J.* 4:e1789
- Mariottini, P., Di Giulio, A., Appolloni, M., Smriglio, C., 2013. Phenotypic diversity, taxonomic remarks and updated distribution of the Mediterranean *Jujubinus baudoni* (Monterosato, 1891) (Gastropoda, Trochidae). *Biodivers. J.* 4, 343-354.
- Merritt, T.J., Shi, L., Chase, M.C., Rex, M.A., Etter, R.J., Quattro, J.M., 1998. Universal cytochrome b primers facilitate intraspecific studies in molluscan taxa. *Mol. Mar. Biol. Biotechnol.* 7, 7-11.
- Okay, A.I., Zattin, M., Cavazza, W., 2010. Apatite fission-track data for the Miocene Arabia-Eurasia collision. *Geology* 38, 35-38.
- Oliverio, M., Mariottini, P., 2001. A molecular framework for the phylogeny of *Coralliophila* and related muricoids. *J. Mollus. Stud.* 67, 215-224.
- Palumbi, S., Martin A, McMillan, W., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR. Available from <http://palumbi.stanford.edu/SimpleFoolsMaster.pdf>, 1-45.
- Phillips, R.C., Meñez, E.G., 1988. Seagrasses. Smithsonian Institution Press, Washington, DC.
- Rambaut, A., Drummond, A.J., 2007. Tracer v1.4, Available from <http://beast.bio.ed.ac.uk/Tracer>.

- Rolán, E., Gonzalez-Porto, M., de Matos-Pita, S., 2009. The genus *Callumbonella* (Gastropoda, Trochacea), with description of a new species from Namibia. J. Conchol. 39, 634-657.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.
- Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual. 2nd. Edition. Cold Spring Harbor Laboratory, New York.
- Schulte, P., Alegret, L., Arenillas, I., Arz, J.A., Barton, P.J., Bown, P.R., Bralower, T.J., Christeson, G.L., Claeys, P., Cockell, C.S., Collins, G.S., Deutsch, A., Goldin, T.J., Goto, K., Grajales-Nishimura, J.M., Grieve, R.A.F., Gulick, S.P.S., Johnson, K.R., Kiessling, W., Koeberl, C., Kring, D.A., MacLeod, K.G., Matsui, T., Melosh, J., Montanari, A., Morgan, J.V., Neal, C.R., Nichols, D.J., Norris, R.D., Pierazzo, E., Ravizza, G., Rebolledo-Vieyra, M., Reimold, W.U., Robin, E., Salge, T., Speijer, R.P., Sweet, A.R., Urrutia-Fucugauchi, J., Vajda, V., Whalen, M.T., Willumsen, P.S., 2010. The Chicxulub Asteroid Impact and Mass Extinction at the Cretaceous-Paleogene Boundary. Science 327, 1214-1218.
- Schwarz, G., 1978. Estimating the Dimension of a Model. Ann. Statist. 6, 461-464.
- Smriglio, C., Di Giulio, A., Mariottini, P., 2014. Description of two new *Jujubinus* species (Gastropoda: Trochidae) from the Sicily Channel, with notes on the *Jujubinus curinii* species complex. Zootaxa 381, 583-590.
- Speelman, E.N., Van Kempen, M.M.L., Barke, J., Brinkhuis, H., Reichart, G.J., Smolders, A.J.P., Roelofs, J.G.M., Sangiorgi, F., De Leeuw, J.W., Lotter, A.F., Sinninghe Damsté, J.S., 2009. The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown. Geobiology 7, 155-170.

- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688-2690.
- Templado, J., 2011. Familia Trochidae. In: Gofas, S., Moreno, D., Salas, C. (eds.). *Moluscos marinos de Andalucía*, vol 1. Servicio de Publicaciones e Intercambio Científico, Universidad de Málaga. pp 104-117.
- Uribe, J.E., Kano, Y., Templado, J., Zardoya, R., 2016. Mitogenomics of Vetigastropoda: insights into the evolution of pallial symmetry. *Zool. Scr.* 45, 145-159.
- Vilvens, C., 2012. New species and new records of Seguenzioidea and Trochoidea (Gastropoda) from French Polynesia. *Novapex* 13, 1-23.
- Vilvens, C., Swinnen, F., Deniz Guerra, F. 2011. A new species of *Clelandella* (Gastropoda: Trochoidea: trochidae: Cantharidinae) from Western Sahara. *Novapex* 12(1-2): 49-55.
- Williams, S.T., 2012. Advances in molecular systematics of the vetigastropod superfamily Trochoidea. *Zool. Scr.* 41, 571-595.
- Williams, S.T., Donald, K.M., Spencer, H.G., Nakano, T., 2010. Molecular systematics of the marine gastropod families Trochidae and Calliostomatidae (Mollusca: Superfamily Trochoidea). *Mol. Phylogenet. Evol.* 54, 783-809.
- Williams, S.T., Karube, S., Ozawa, T., 2008. Molecular systematics of Vetigastropoda: Trochidae, turbinidae and trochoidea redefined. *Zool. Scr.* 37, 483-506.
- Williams, S.T., Ozawa, T., 2006. Molecular phylogeny suggests polyphyly of both the turban shells (family Turbinidae) and the superfamily Trochoidea (Mollusca: Vetigastropoda). *Mol. Phylogenet. Evol.* 39, 33-51.
- Zachos, J., Pagani, M., Sloan, L., Thomas E., Billups, K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292, 686-693.

Legends to Figures

Figure 1. Phylogenetic relationships of Stomatellinae and Cantharidinae. The reconstructed ML tree based on the four-gene data set is shown. Numbers at nodes are statistical support values for ML (bootstrap proportions)/ BI (posterior probabilities). Only phylogenetic relationships among Indo-Pacific Cantharidinae are shown in full. The Mediterranean and NE Atlantic Cantharidinae and Stomatellinae clades are collapsed and shown separately in full in Figs. 2A and 3A, respectively. Type species are shown in bold.

Figure 2. Phylogenetic relationships of Mediterranean and NE Atlantic Cantharidinae. The reconstructed ML trees based on the four-gene (A) and six-gene (B) data sets are shown. Numbers at nodes are statistical support values for ML (bootstrap proportions)/ BI (posterior probabilities). Type species are shown in bold.

Figure 3. Phylogenetic relationships within Stomatellinae. The reconstructed ML trees based on the four-gene (A) and six-gene (B) data sets are shown. Numbers at nodes are statistical support values for ML (bootstrap proportions)/ BI (posterior probabilities). Type species are shown in bold. Photos of unidentified taxa are shown. More detailed photos can be found in Supp. Data 1.

Figure 4. Chronogram with age estimates of major divergence events among Mediterranean and North-eastern Atlantic Cantharidinae based on the four-gene data set. A Bayesian uncorrelated relaxed lognormal clock was used in BEAST. Horizontal bars represent 95% credibility intervals for time estimates, and the calibration constraint is indicated with an asterisk on the corresponding node. Dates (and credibility intervals) are in millions of years.

Paleogene and Neogene periods are shown. The *Azolla* phase, the eastern closure of Tethys Ocean, and the Messinian Salinity Crisis of the Mediterranean Sea are indicated with red, orange and green dashes lines, respectively. Type species are shown in bold.

Figure 1

Figure 2

Figure 3

Figure 4

Table 1

Table 1. Species analyzed in this study. Specimens, expedition, locality, voucher, GenBank accession numbers and availability of photos are shown.

Subfamily	Species	Expedition	Locality	Voucher No	28S	COI	16S	12S	H3	COB	PHOTO
Cantharidinae											
	<i>Gibbula umbilicaris</i> (Linnaeus, 1758) 1	Mediterranean 2005	El Mohon, Murcia, SE Spain	MNCN:ADN:86692	KX277459	KX277553	KX277387	KX277313	–	–	
	<i>Gibbula umbilicaris</i> 2	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86693	KX277460	KX277554	KX277388	KX277314	KX277612	KX277506	
	<i>Gibbula cineraria</i> (Linnaeus, 1758)	Galicia 2005	A Guarda, Pontevedra, NW Spain	MNCN:ADN:86694	KX277461	KX277555	KX277389	KX277315	KX277613	–	
	<i>Gibbula pennanti</i> (Philippi, 1846) 1	—	Roscoff, W France	NHMUK 20080944	GQ232393	GQ232365	GQ232295	GQ232330	–	–	MorphoBank
	<i>Gibbula pennanti</i> 2	Galicia 2005	A Guarda, Pontevedra, NW Spain	MNCN:ADN:86695	KX277462	KX277556	KX277390	KX277316	KX277614	KX277507	
	<i>Gibbula racketti</i> (Payraudeau, 1826) 1	Mediterranean 2005	Islas Chafarinas, S Spain	MNCN:ADN:86696	KX277463	KX277557	KX277391	KX277317	KX277615	KX277508	
	<i>Gibbula racketti</i> 2	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86697	KX277464	KX277558	KX277392	KX277318	–	–	
	<i>Gibbula albida</i> (Gmelin, 1791)	Galicia 2005	O Grove, Pontevedra, NW Spain	MNCN:ADN:86698	KX277465	KX277559	KX277393	KX277319	KX277616	–	
	<i>Gibbula adriatica</i> (Philippi, 1844)	Galicia 2005	O Grove, Pontevedra, NW Spain	MNCN:ADN:86699	KX277466	KX277560	KX277394	KX277320	KX277617	–	
	<i>Gibbula varia</i> (Linnaeus, 1758) 1	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86700	KX277467	KX277561	KX277395	KX277321	–	–	
	<i>Gibbula varia</i> 2	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86701	KX277468	KX277562	KX277396	KX277322	–	–	
	<i>Gibbula varia</i> 3	Mediterranean 2005	Islas Chafarinas, S Spain	MNCN:ADN:86702	KX277469	KX277563	KX277397	KX277323	KX277618	KX277509	
	<i>Gibbula divaricata</i> (Linnaeus, 1758)	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86703	KX277470	KX277564	KX277398	KX277324	KX277619	–	
	<i>Gibbula rarilineata</i> (Michaud, 1829) 1	Croatia 2011	Dugi otok, Telašćica, Croatia	MNCN:ADN:86704	KX277471	KX277565	KX277399	KX277325	indv 2*	indv 2*	
	<i>Gibbula rarilineata</i> 2	—	Lumbarda, Korcula I., Croatia	NHMUK 20080375	GQ232394	GQ232366	GQ232296	–	KX277610	KX277504	MorphoBank
	<i>Gibbula umbilicalis</i> (da Costa, 1778) 1	Galicia 2005	O Grove, Pontevedra, NW Spain	MNCN:ADN:86705	indv 2*	KX277566	KX277400	KX277326	KX277620	KX277510	
	<i>Gibbula umbilicalis</i> 2	Galicia 2005	A Guarda, Pontevedra, NW Spain	MNCN:ADN:86706	KX277472	KX277567	KX277401	KX277327	–	–	
	<i>Gibbula umbilicalis</i> 3	—	Wembury, Plymouth, UK	NHMUK 20080946	GQ232395	GQ232367	GQ232297	–	–	–	–
	<i>Gibbula umbilicalis</i> 4	Galicia 2005	O Grove, Pontevedra, NW Spain	MNCN:ADN:86707	KX277473	KX277568	KX277402	KX277328	–	–	
	<i>Phorcus richardi</i> (Payraudeau, 1826) 1	—	Almuñecar, Granada, S Spain	See Donald et al. 2012	JN686264	JN686361	JN686181	–	–	–	–
	<i>Phorcus richardi</i> 2	Mediterranean 2005	Cala Rajá, Cabo de Gata, SE Spain	MNCN:ADN:86708	KX277474	KX277569	KX277403	KX277329	KX277621	KX277511	
	<i>Phorcus articulatus</i> (Lamarck, 1822) 1	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86709	KX277475	KX277570	KX277404	KX277330	–	–	
	<i>Phorcus articulatus</i> 2	Mediterranean 2005	Islas Chafarinas, S Spain	MNCN:ADN:86710	KX277476	KX277571	KX277405	KX277331	KX277622	KX277512	
	<i>Phorcus mutabilis</i> (Philippi, 1846)	—	Fano, Italy	See Donald et al. 2012	JN686268	JN686364	JN686179	–	–	–	–
	<i>Phorcus atratus</i> (Wood, 1828)	—	La Gofa, Lanzarote, Canary Islands	See Donald et al. 2012	JN686209	JN686295	JN686113	–	–	–	–
	<i>Phorcus lineatus</i> (da Costa, 1778) 1	Galicia 2005	A Guarda, Pontevedra, NW Spain	MNCN:ADN:86711	JN686232	KX277572	KX277406	KX277332	KX277623	KX277513	–
	<i>Phorcus lineatus</i> 2	—	Pembrokeshire, Wales, UK	See Donald et al. 2012	JN686241	JN686328	JN686125	–	–	–	–
	<i>Phorcus punctulatus</i> (Lamarck, 1822)	—	Dakar, Senegal	See Donald et al. 2012	JN686259	JN686350	JN686158	–	–	–	–
	<i>Phorcus mariae</i> Templado & Rolán, 2012	—	Mordeira Bay, Sal, Cape Verde Islands	See Donald et al. 2012	JN686249	JN686337	JN686156	–	–	–	–
	<i>Phorcus sauciatius</i> (Koch, 1845) 1	Galicia 2005	Ria de Aldán, Pontevedra, NW Spain	MNCN:ADN:86712	KX277477	KX277573	KX277407	KX277333	–	–	
	<i>Phorcus sauciatius</i> 2	Azores & Madeira 2008	Funchal, Madeira	MNCN:ADN:86713	JN686227	KX277574	KX277408	KX277334	–	–	
	<i>Phorcus sauciatius</i> 3	Galicia 2005	Cabo Estai, Canido, Pontevedra, NW Spain	MNCN:ADN:86714	KX277478	KX277575	KX277409	KX277335	KX277624	KX277514	
	<i>Phorcus turbinatus</i> (Born, 1778) 1	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86715	JN686167	JN686340	KX277410	KX277336	KX277625	KX277515	
	<i>Phorcus turbinatus</i> 2	—	Coral Bay, Cyprus	See Donald et al. 2012	GQ434030	GQ434018	GQ434024	GQ434013	–	–	–
	<i>Jujubinus exasperatus</i> (Pennant, 1777) 1	Balearic Islands 2007	Cala San Esteve, Menorca, E Spain	MNCN:ADN:86716	KX277479	KX277576	KX277411	KX277337	–	–	
	<i>Jujubinus exasperatus</i> 2	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86717	KX277480	KX277577	KX277412	KX277338	KX277626	KX277516	
	<i>Jujubinus exasperatus</i> 3	—	Lumbarda, Korcula I., Croatia	NHMUK 20080387	GQ232396	GQ232368	GQ232298	GQ232333	–	–	MorphoBank
	<i>Jujubinus exasperatus</i> 4	Galicia 2005	Cabo Estai, Canido, Pontevedra, NW Spain	MNCN:ADN:86718	KX277481	KX277578	KX277413	KX277339	–	–	
	<i>Jujubinus exasperatus</i> 5	—	Ile Callot, France	NHMUK 20080943	GQ232387	GQ232361	GQ232288	GQ232323	–	–	–
	<i>Jujubinus pseudogravinae</i> Nordsieck, 1973	Azores & Madeira 2008	San Miguel, Azores	MNCN:ADN:86719	KX277482	KX277579	KX277414	KX277340	KX277627	KX277517	
	<i>Jujubinus vexationis</i> Curini-Galletti, 1990	Azores & Madeira 2008	Funchal, Madeira	MNCN:ADN:86720	KX277483	KX277580	KX277415	KX277341	KX277628	KX277518	
	<i>Jujubinus ruscurianus</i> (Weinkauff, 1868) 1	Mediterranean 2005	Banalmádena, Málaga, S Spain	MNCN:ADN:86721	KX277484	KX277581	KX277416	KX277342	KX277629	KX277519	
	<i>Jujubinus ruscurianus</i> 2	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86722	–	KX277582	KX277417	KX277343	–	–	
	<i>Jujubinus gravinae</i> (Dautzenberg, 1881)	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86723	KX277485	KX277583	KX277418	KX277344	KX277630	KX277520	
	<i>Gibbula tingitana</i> Pallary, 1901	Mediterranean 2005	Calahonda, Málaga, S Spain	MNCN:ADN:86724	–	KX277584	KX277419	KX277345	KX277631	KX277521	
	<i>Jujubinus striatus</i> (Linnaeus, 1758) 1	Balearic Islands 2008	Menorca, E Spain	MNCN:ADN:86725	–	KX277585	KX277420	KX277346	–	–	
	<i>Jujubinus striatus</i> 2	Galicia 2005	Cabo Estai, Canido, Pontevedra, NW Spain	MNCN:ADN:86726	KX277486	KX277586	KX277421	KX277347	KX277632	KX277522	
	<i>Jujubinus striatus</i> 3	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86727	–	KX277587	KX277422	KX277348	–	–	
	<i>Callumbonella suturalis</i> (Philippi, 1836)	Fauna I, stn 26A	La Herradura Seamount, Alboran Sea, SE Spain	MNCN:ADN:86728	KX277487	–	KX277423	KX277349	–	–	
	<i>Clelandella militaris</i> (Brocchi, 1814)	Fauna I, stn 5A	off Vléz-Málaga, Alborán Sea, SE Spain	MNCN:ADN:86729	KX277488	KX277588	KX277424	KX277350	–	–	
	<i>Gibbula magus</i> (Linnaeus, 1758) 1	Galicia 2005	Cabo Estai, Canido, Pontevedra, Spain	MNCN:ADN:86730	KX277489	KX277589	KX277425	KX277351	KX277633	–	
	<i>Gibbula magus</i> 2	Mediterranean 2005	Cabo Pino, Málaga, Spain	MNCN:ADN:86731	KX277490	KX277590	KX277426	KX277352	–	–	
	<i>Gibbula magus</i> 3	—	Faro, Portugal	NHMUK 20030329	GQ232392	GQ232364	GQ232294	GQ232329	–	–	–
	<i>Gibbula famulum</i> (Gmelin, 1791)	—	Korcula I., Croatia	NHMUK 20080378	GQ232391	GQ232363	–	GQ232328	KX277611	KX277505	MorphoBank
	<i>Gibbula ardens</i> (Salis Marschlin, 1793) 1	Balearic Islands 2008	Menorca, Spain	MNCN:ADN:86732	KX277491	KX277591	KX277427	KX277353	–	–	
	<i>Gibbula ardens</i> 2	Balearic Islands 2007	Cala San Esteve, Menorca, E Spain	MNCN:ADN:86733	KX277492	KX277592	KX277428	KX277354	–	–	
	<i>Gibbula ardens</i> 3	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86734	KX277493	KX277593	KX277429	KX277355	KX277634	KX277523	
	<i>Gibbula philberti</i> (Récluz, 1843) 1	Mediterranean 2005	El Mohon, Murcia, SE Spain	MNCN:ADN:86735	KX277494	KX277594	KX277430	KX277356	KX277635	–	
	<i>Gibbula philberti</i> 2	Mediterranean 2005	Banalmádena, Málaga, S Spain	MNCN:ADN:86736	KX277495	KX277595	KX277431	KX277357	–	–	
	<i>Gibbula turbinoides</i> (Deshayes, 1835)	Mediterranean 2005	Cabo de Palos, Murcia, SE Spain	MNCN:ADN:86737	KX277496	KX277596	KX277432	KX277358	KX277636	KX277524	
	<i>Cantharidus opalus</i> (Marty, 1784)	—	Stewart I., New Zealand	NMNZ M.287117	GQ249739	GQ249696	GQ249830	GQ249799	–	–	MorphoBank

<i>Cantharidus capillaceus</i> (Philippi, 1849)	—	Enderby Island, New Zealand	—	GQ249742	GQ249700	GQ249834	GQ249804	—	—	—
<i>Cantharidus dilatatus</i> (G. B. Sowerby II, 1870)	—	Wellington, New Zealand	—	GQ249727	GQ249689	GQ249809	GQ249771	—	—	—
<i>Cantharidus antipoda</i> Hombrodt & Jackinot, 1854	—	Stewart Island, New Zealand	NMNZ M.287119	GQ249726	GQ249682	GQ249807	GQ249767	—	—	MorphoBank
<i>Micrelenchus tessellatus</i> (A. Adams, 1853)	—	Warrington, New Zealand	NMNZ M.288193	GQ434028	GQ434017	GQ434022	GQ434011	—	—	MorphoBank
<i>Micrelenchus huttoni</i> (E.A. Smith, 1876)	—	Purakaunui Bay, New Zealand	NMNZ M.288196	GQ434027	GQ434016	GQ434021	GQ434010	—	—	MorphoBank
<i>Micrelenchus sanguineus</i> (Gray, 1843)	—	Warrington, New Zealand	NMNZ M.287121	GQ249728	GQ249685	GQ249812	GQ249773	—	—	MorphoBank
<i>Micrelenchus purpureus</i> (Gmelin, 1791)	—	Bay of Islands, New Zealand	NMNZ M.287118	GQ249744	GQ249702	GQ249835	GQ249805	—	—	—
<i>Roseaplagis rufozonus</i> (A. Adams, 1853)	—	Tauranga, New Zealand	NMNZ M.284054	GQ249740	GQ249697	GQ249831	GQ249800	—	—	MorphoBank
<i>Prothaltia lehmanni</i> (Menke, 1843)	—	Dunborough, Australia	NHMK 20070156	EU530021	EU530123	GQ232302	GQ232338	—	—	MorphoBank
Cantharidinae unknown genus 1 sp. 1	Salomon 2, st. CP2203	Sta Isabel I., Solomon Islands	MNHN IM-2007-18160	GQ232384	GQ232358	GQ232286	FN435321	—	—	—
<i>"Cantharidus" sendersi</i> Poppe, Tagaro & Dekker, 2006	Santo 2006, st. EP39	Tutuba I., Vanuatu	MNHN IM-2007-18458	—	KX277545	KX277379	KX277305	—	—	—
<i>"Jujubinus" suarezensis</i> (P. Fischer, 1878) 1	Atimo Vatae, st. TM19	Sainte Luce, Madagascar	MNHN IM-2009-13642	KX277436	KX277528	KX277362	KX277290	—	—	—
<i>"Jujubinus" suarezensis</i> 2	Atimo Vatae, st. BS08	Rocher de l'Albatros, Madagascar	MNHN IM-2009-13612	KX277435	KX277527	KX277361	KX277289	—	—	—
<i>"Jujubinus" suarezensis</i> 3	Inhaca 2011, st. MR06	Ilha dos Portugueses, Maputo Bay, Mozambique	MNHN IM-2009-23230	KX277457	KX277551	KX277385	KX277311	—	—	—
<i>"Jujubinus" suarezensis</i> 4	Inhaca 2011, st. MR06	Ilha dos Portugueses, Maputo Bay, Mozambique	MNHN IM-2009-23229	KX277456	KX277550	KX277384	KX277310	—	—	—
<i>"Jujubinus" suarezensis</i> 5	Inhaca 2011, st. MM11	Xixuane, Maputo Bay, Mozambique	MNHN IM-2009-23217	KX277453	KX277547	KX277381	KX277307	KX277609	KX277503	—
<i>"Jujubinus" suarezensis</i> 6	Inhaca 2011, st. MM04	Ponta Pundiue, Maputo Bay, Mozambique	MNHN IM-2009-23257	KX277458	KX277552	KX277386	KX277312	—	—	—
<i>Kanekotrochus infuscatus</i> (Gould, 1861)	—	Katakuchihama, Kagoshima, Japan	NSMT Mo76821	AB505235	AB505238	AB505328	AB505371	—	—	MorphoBank
<i>Agagus agagus</i> Jousseume, 1894	—	Aliwal Shoal, KwaZulu-Natal, South Africa	NMSA W2743	KX277498	KX277598	KX277434	KX277360	KX277638	KX277526	—
<i>Priotrochus kotschy</i> (Philippi, 1849)	—	Abu Dhabi, United Arab Emirates	OKOT.ABD.1	JN686228	JN686315	JN686118	—	—	—	—
<i>Thalotia conica</i> (Gray, 1827)	Western Australia 2011, st. WA10	Pagoda Point, Princess Royal Harbour, Australia	MNHN IM-2009-23270	EU530022	FN435322	GQ232309	GQ232345	—	—	—
<i>"Jujubinus" gilberti</i> (Montouzier in Fischer, 1878) 1	Panglao 2004, st. B8	Napaling, Panglao I., Philippines	MNHN IM-2007-18363	KX277443	KX277535	KX277369	KX277297	—	—	—
<i>"Jujubinus" gilberti</i> 2	Panglao 2004, st. S2	Baclayon Takot, Bohol I., Philippines	MNHN IM-2007-18375	—	KX277376	KX277302	—	—	—	—
Cantharidinae unknown genus 2 sp. 1	Panglao 2004, st. B8	Napaling, Panglao I., Philippines	MNHN IM-2007-18362	KX277442	KX277534	KX277368	KX277296	—	—	Supp. Data 1
<i>"Cantharidus" callichroa</i> (Philippi, 1849)	—	Omaezaki, Shizuoka, Japan	NHMK 20050419	AM048703	AM049338	AM048892	GQ232314	—	—	MorphoBank
<i>"Cantharidus" jessoensis</i> (Scherenck, 1863)	—	Otuchi, Iwate, Japan	NSMT Mo76819	AB505405	AB505280	AB505326	AB505369	—	—	MorphoBank
<i>"Jujubinus" geographicus</i> Poppe, Tagaro & Dekker, 2006	Santo 2006, st. EP36	E Aoré I., Aimbuei Bay, Vanuatu	MNHN IM-2007-18457	KX277451	KX277544	KX277378	KX277304	—	—	—
<i>Tosatrochus attenuatus</i> (Jonas, 1844)	—	Marukihama, Bounotsu, Kagoshima, Japan	—	AB505236	AB505283	AB505329	AB505372	—	—	MorphoBank
<i>Pictodiloma suavis</i> (Philippi, 1850)	—	Susaki, Ogasawara I., Japan	NSMT Mo76820	AB505234	AB505281	AB505327	AB505370	—	—	MorphoBank
<i>"Cantharidus" lepidus</i> (Philippi, 1846)	—	Duke of Orleans Bay, Esperance, Australia	NHMK 20080950	GQ232402	GQ232305	GQ232305	GQ232341	—	—	MorphoBank
<i>Priotrochus obscurus</i> (W. Wood, 1828) 1	Inhaca 2011, st. MM07	Ponta Pundiue, Maputo Bay, Mozambique	MNHN IM-2009-23222	KX277454	KX277548	KX277382	KX277308	—	—	—
<i>Priotrochus obscurus</i> 2	Inhaca 2011, st. MM02	Ponta Pundiue, Maputo Bay, Mozambique	MNHN IM-2009-23224	KX277455	KX277549	KX277383	KX277309	—	—	—
<i>Oxysteles impervia</i> (Menke, 1843)	—	False Bay, South Africa	NMNZ M.287122	GQ249748	DQ061093	DQ061084	GQ249769	—	—	MorphoBank
<i>Oxysteles variegata</i> (Anton, 1838)	—	False Bay, South Africa	NMNZ M.287126	GQ249751	DQ061092	DQ061083	GQ249770	—	—	MorphoBank
<i>Oxysteles tabularis</i> (Krauss, 1848)	—	East London, South Africa	NMNZ M.287124	GQ249750	DQ061090	DQ061081	GQ249766	—	—	MorphoBank
<i>Oxysteles sinensis</i> (Gmelin, 1791)	—	East London, South Africa	NMNZ M.287123	GQ249749	DQ061089	DQ061080	GQ249765	—	—	MorphoBank
Stomatellinae										
<i>Stomatella planulata</i> (Lamarck, 1816)	—	Teniyu, Nago City, Okinawa, Japan	NHMK 20080949	EU530029	EU530130	GQ232307	GQ232343	—	—	MorphoBank
<i>Stomatella</i> sp. 1	Panglao 2004, st. M3	Danao, Panglao I., Philippines	MNHN IM-2007-18370	KX277447	KX277539	KX277373	KX277300	KX277604	—	Supp. Data 1
<i>Stomatella</i> sp. 2	Panglao 2004, st. B9	Napaling, Panglao I., Philippines	MNHN IM-2007-18365	KX277445	KX277537	KX277371	—	KX277603	KX277501	Supp. Data 1
<i>Stomatella</i> sp. 3	Panglao 2004, st. B13	Baclayon Takot, Bohol I., Philippines	MNHN IM-2007-18355	KX277438	KX277530	KX277364	KX277292	KX277600	—	Supp. Data 1
<i>Stomatella impertusa</i> (Burrow, 1815) 1	Jordan 2005	Aqaba, Jordan	MNCN-ADN-86738	KX277497	KX277597	KX277433	KX277359	KX277637	KX277525	—
<i>Stomatella impertusa</i> 2	—	Esperance, Australia	NHMK 20080948	GQ232400	GQ232369	GQ232303	GQ232339	—	—	MorphoBank
<i>Stomatella impertusa</i> 3	Santo 2006, st. DB8	Bruat Channel, Vanuatu	MNHN IM-2007-18154	GQ232401	GQ232370	GQ232304	GQ232340	—	—	MorphoBank
<i>Stomatolina rubra</i> (Lamarck, 1822) 1	Panglao 2004, st. B9	Napaling, Panglao I., Philippines	MNHN IM-2007-18366	KX277446	KX277538	KX277372	KX277299	—	—	Supp. Data 1
<i>Stomatolina rubra</i> 2	Panglao 2004, st. B39	Pontod Lagoon, Panglao I., Philippines	MNHN IM-2007-18156	GQ232404	GQ232373	GQ232308	GQ232344	—	—	MorphoBank
<i>Stomatolina rubra</i> 3	Panglao 2004, st. B39	Pontod Lagoon, Panglao I., Philippines	MNHN IM-2007-18359	KX277440	KX277532	KX277366	KX277294	—	—	Supp. Data 1
<i>Stomatolina rubra</i> 4	Panglao 2004, st. B39	Pontod Lagoon, Panglao I., Philippines	MNHN IM-2007-18360	KX277441	KX277533	KX277367	KX277295	—	—	Supp. Data 1
<i>Stomatolina angulata</i> (A. Adams, 1850)	—	Marukihama, Bounotsu, Kagoshima, Japan	—	AB505248	AB505295	AB505339	AB505382	—	—	MorphoBank
<i>Pseudostomatella decolorata</i> (Gould, 1848)	—	Benoki, Okinawa, Japan	NSMT Mo76824	AB505244	AB505291	AB505335	AB505379	—	—	MorphoBank
Stomatellinae unknown genus 1 sp. 1	Panglao 2004, st. R31	Pamilacan I., Philippines	MNHN IM-2007-18371	KX277448	KX277540	KX277374	KX277301	KX277605	—	—
Stomatellinae unknown genus 1 sp. 2	Panglao 2004, st. R43	Cortes Takot, Bohol I., Philippines	MNHN IM-2007-18372	KX277449	KX277541	KX277375	—	KX277606	—	Supp. Data 1
Stomatellinae unknown genus 2 sp. 1	Panglao 2004, st. B13	Baclayon Takot, Bohol I., Philippines	MNHN IM-2007-18356	KX277439	KX277531	KX277365	KX277293	KX277601	KX277499	Supp. Data 1
<i>Microtis</i> sp. 1	Santo 2006, st. DB40	Palikulo Bay, Vanuatu	MNHN IM-2007-18449	KX277450	KX277543	KX277377	KX277303	KX277607	—	Supp. Data 1
<i>Microtis tuberculata</i> H. & A. Adams, 1850	Panglao 2004, st. B9	Napaling, Panglao I., Philippines	MNHN IM-2007-18364	KX277444	KX277536	KX277370	KX277298	KX277602	KX277500	—
<i>Microtis?</i> sp. 2	Santo 2006, st. LD8	Santo, Malparavu I., Vanuatu	MNHN IM-2007-18463	KX277452	KX277546	KX277380	KX277306	KX277608	KX277502	Supp. Data 1
<i>Stomatia obscura</i> Sowerby G.B. III, 1874	—	Marukihama, Bounotsu, Kagoshima, Japan	—	AB505247	AB505294	AB505338	AB505381	—	—	MorphoBank
<i>Stomatia phymotis</i> Helbling, 1779	Santo 2006, st. VM59	SE Malparavu Island, Vanuatu	MNHN IM-2007-18155	GQ232403	GQ232372	GQ232306	GQ232342	—	—	MorphoBank
<i>Calliotrochus marmoreus</i> (Pease, 1861)	Panglao 2004, st. B11	Pamilacan I., Philippines	MNHN IM-2007-18352	KX277437	KX277529	KX277363	KX277291	KX277599	—	—
Umboniinae										
<i>Isanda coronata</i> A. Adams, 1854	—	Karratha Back Beach, Australia	NHMK 20050421	AM048705	AM049340	AM048894	GQ232332	—	—	MorphoBank
<i>Umbonium costatum</i> (Kiener, 1839)	—	Pusan, South Korea	NHMK 20050422	AM048706	AM049341	AM048895	GQ232348	—	—	MorphoBank

*indv2: the corresponding sequence comes from specimen 2

Supplementary Material 1

Photos of unknown genera and species

No photo

Cantharidinae unknown genus 1 sp. 1

Cantharidinae unknown genus 2 sp. 1

Stomatella sp. 1

Stomatella sp. 2

Stomatella sp. 3

Stomatellinae unknown genus 1 sp. 1

Stomatellinae unknown genus 1 sp. 2

Stomatellinae unknown genus 2 sp. 1

Microtis sp. 1

Microtis? sp. 2

Supplementary Material 2. Best fit partitions and evolutionary substitution models* as selected by Partition Finder

Four genes matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =76613.79)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.68	0.35	0.23	8.71	1.63	0.55	6.32	0.30	0.13	0.22	0.33
	2 <i>28S</i>	GTR+I+G	1.02	0.88	0.19	1.05	0.38	0.05	4.10	0.22	0.24	0.32	0.21
	3 <i>coxI</i> 1st+2nd	SYM+I+G	0.82	0.83	0.21	3.37	1.48	0.00	21.32	0.18	0.25	0.23	0.32
	4 <i>coxI</i> 3rd	GTR+G	1.81	0.00	2.24	82.09	4.93	11.51	50.04	0.34	0.17	0.09	0.37
Six gene Mediterranean and NE Atlantic matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =39345.53)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.70	0.50	0.00	11.23	1.73	1.38	9.15	0.30	0.13	0.21	0.34
	2 <i>28S</i>	TrN+I+G	2.79	0.93	0.22	1.31	0.46	0.16	4.31	0.21	0.24	0.32	0.21
	3 <i>cob</i> 1st + <i>coxI</i> 1st	SYM+I+G	1.42	0.62	0.00	0.92	1.18	0.10	25.29	0.21	0.23	0.29	0.25
	4 <i>cob</i> 2nd + <i>coxI</i> 2nd	TrN+I+G	0.56	0.92	1.59	25.38	0.93	1.25	2.66	0.17	0.24	0.15	0.42
	5 <i>cob</i> 3rd + <i>coxI</i> 3rd	TIM+G	1.78	0.00	2.31	73.20	3.04	12.89	56.23	0.31	0.15	0.13	0.39
	6 <i>H3</i> 1st+2nd	JC+I	2.60	0.93	1.30	1.29	0.86	0.00	1.95	0.29	0.27	0.24	0.19
	7 <i>H3</i> 3rd	TVM+G	0.42	0.28	8.41	7.02	0.95	0.00	5.32	0.08	0.39	0.28	0.23
Six gene Stomatellinae matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =29758.58)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.40	0.22	0.37	10.72	2.82	0.28	8.00	0.30	0.14	0.23	0.31
	2 <i>28S</i>	TIM+I	7.80	0.91	0.42	1.35	0.00	0.06	6.73	0.21	0.24	0.32	0.20
	3 <i>cob</i> 1st + <i>coxI</i> 1st	TrNef+G	2.98	0.67	0.00	1.64	0.76	0.00	40.47	0.22	0.23	0.29	0.25
	4 <i>cob</i> 2nd + <i>coxI</i> 2nd	HKY+I	1000.00	0.91	0.00	2.39	0.00	1.78	2.55	0.16	0.24	0.16	0.42
	5 <i>cob</i> 3rd + <i>coxI</i> 3rd	HKY+I+G	0.51	0.00	14.16	389.12	9.64	0.00	577.33	0.32	0.16	0.12	0.38
	6 <i>H3</i> 1st+2nd	TIMef+I	1000.00	0.95	3.89	0.00	0.00	0.00	12.14	0.29	0.26	0.24	0.19
	7 <i>H3</i> 3rd	K81uf+I	6.95	0.38	3.38	6.49	0.10	0.89	7.29	0.10	0.37	0.30	0.21
Time tree matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =37407.14)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.43	0.41	2.05	1.08	0.11	0.13	0.15	0.33	0.10	0.19	0.36
	2 <i>28S</i>	GTR+I+G	0.45	0.84	6.88	0.31	0.13	4.36	0.15	0.22	0.24	0.32	0.21
	3 <i>coxI</i> 1st+2nd	GTR+I+G	0.55	0.81	1.64	0.29	0.15	3.14	3.26	0.18	0.25	0.23	0.32
	4 <i>coxI</i> 3rd	GTR+I+G	1.92	9.96	6.89	1.88	4.29	0.19	0.15	0.34	0.17	9.87	0.38

* Model parameter values as estimated empirically with RaxML or BEAST

Supplementary Material 3 (individual gene trees)

rrnS ML Tree (cont.)

rrnS ML Tree

rrnL ML Tree

rrnL ML Tree (cont.)

rrnL ML Tree (cont.)

28S ML Tree

28S ML Tree (cont.)

28S ML Tree (cont.)

cox1 ML Tree (cont.)

cox1 ML Tree (cont.)

H3 ML Tree

cob ML Tree

Supplementary Material 2. Best fit partitions and evolutionary substitution models* as selected by Partition Finder

Four genes matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =76613.79)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.68	0.35	0.23	8.71	1.63	0.55	6.32	0.30	0.13	0.22	0.33
	2 <i>28S</i>	GTR+I+G	1.02	0.88	0.19	1.05	0.38	0.05	4.10	0.22	0.24	0.32	0.21
	3 <i>coxI</i> 1st+2nd	SYM+I+G	0.82	0.83	0.21	3.37	1.48	0.00	21.32	0.18	0.25	0.23	0.32
	4 <i>coxI</i> 3rd	GTR+G	1.81	0.00	2.24	82.09	4.93	11.51	50.04	0.34	0.17	0.09	0.37
Six gene Mediterranean and NE Atlantic matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =39345.53)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.70	0.50	0.00	11.23	1.73	1.38	9.15	0.30	0.13	0.21	0.34
	2 <i>28S</i>	TrN+I+G	2.79	0.93	0.22	1.31	0.46	0.16	4.31	0.21	0.24	0.32	0.21
	3 <i>cob</i> 1st + <i>coxI</i> 1st	SYM+I+G	1.42	0.62	0.00	0.92	1.18	0.10	25.29	0.21	0.23	0.29	0.25
	4 <i>cob</i> 2nd + <i>coxI</i> 2nd	TrN+I+G	0.56	0.92	1.59	25.38	0.93	1.25	2.66	0.17	0.24	0.15	0.42
	5 <i>cob</i> 3rd + <i>coxI</i> 3rd	TIM+G	1.78	0.00	2.31	73.20	3.04	12.89	56.23	0.31	0.15	0.13	0.39
	6 <i>H3</i> 1st+2nd	JC+I	2.60	0.93	1.30	1.29	0.86	0.00	1.95	0.29	0.27	0.24	0.19
	7 <i>H3</i> 3rd	TVM+G	0.42	0.28	8.41	7.02	0.95	0.00	5.32	0.08	0.39	0.28	0.23
Six gene Stomatellinae matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =29758.58)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.40	0.22	0.37	10.72	2.82	0.28	8.00	0.30	0.14	0.23	0.31
	2 <i>28S</i>	TIM+I	7.80	0.91	0.42	1.35	0.00	0.06	6.73	0.21	0.24	0.32	0.20
	3 <i>cob</i> 1st + <i>coxI</i> 1st	TrNef+G	2.98	0.67	0.00	1.64	0.76	0.00	40.47	0.22	0.23	0.29	0.25
	4 <i>cob</i> 2nd + <i>coxI</i> 2nd	HKY+I	1000.00	0.91	0.00	2.39	0.00	1.78	2.55	0.16	0.24	0.16	0.42
	5 <i>cob</i> 3rd + <i>coxI</i> 3rd	HKY+I+G	0.51	0.00	14.16	389.12	9.64	0.00	577.33	0.32	0.16	0.12	0.38
	6 <i>H3</i> 1st+2nd	TIMef+I	1000.00	0.95	3.89	0.00	0.00	0.00	12.14	0.29	0.26	0.24	0.19
	7 <i>H3</i> 3rd	K81uf+I	6.95	0.38	3.38	6.49	0.10	0.89	7.29	0.10	0.37	0.30	0.21
Time tree matrix.		Best Model	Alpha	Pinvar	A <-> C	A <-> G	A <-> T	C <-> G	C <-> T	pi(A)	pi(C)	pi(G)	pi(T)
Best Partition (BIC =37407.14)	1 <i>rrnS</i> + <i>rrnL</i>	GTR+I+G	0.43	0.41	2.05	1.08	0.11	0.13	0.15	0.33	0.10	0.19	0.36
	2 <i>28S</i>	GTR+I+G	0.45	0.84	6.88	0.31	0.13	4.36	0.15	0.22	0.24	0.32	0.21
	3 <i>coxI</i> 1st+2nd	GTR+I+G	0.55	0.81	1.64	0.29	0.15	3.14	3.26	0.18	0.25	0.23	0.32
	4 <i>coxI</i> 3rd	GTR+I+G	1.92	9.96	6.89	1.88	4.29	0.19	0.15	0.34	0.17	9.87	0.38

* Model parameter values as estimated empirically with RaxML or BEAST

Supplementary Material 3 (individual gene trees)

rrnS ML Tree (cont.)

rrnS ML Tree

rrnL ML Tree

rrnL ML Tree (cont.)

rrnL ML Tree (cont.)

28S ML Tree

28S ML Tree (cont.)

28S ML Tree (cont.)

cox1 ML Tree (cont.)

cox1 ML Tree (cont.)

H3 ML Tree

cob ML Tree