SAPIENZA
UNIVERSITÀ DI ROMA

DOTTORATO DI RICERCA IN BIOLOGIA AMBIENTALE ED EVOLUZIONISTICA

XXXI CICLO

Evolution of larval development in marine gastropods

Ph.D. candidate: Valeria Russini

Tutor: Prof. Marco Oliverio

FACOLTÀ DI SCIENZE MATEMATICHE, FISICHE E NATURALI

DIPARTIMENTO DI BIOLOGIA E BIOTECNOLOGIE “CHARLES DARWIN”

CURRICULUM IN BIOLOGIA ANIMALE

ANNO ACCADEMICO 2018/2019

Table of content

Introduction

In marine habitats, benthic invertebrates usually have adult phases with relatively small dispersal ability, ranging from sessile Polychaeta or Bivalvia, to the slow Echinodermata, until the more vagile Crustacea. In the marine realm, connectivity among populations of benthic invertebrates is provided primarily by dispersion of larvae, or propagules, in the first life stages, given the small dispersal ability of the adult organisms. Almost all marine phyla comprise organisms that are provided with a planktonic stage in the first phases of life (e.g.: the parenchymula in Porifera, the free-swimming planula in Cnidaria, the cydippid in Ctenophora, Muller's and Gotte's larvae in Platyhelminthes, pilidium and planuliform larvae in Nemertea, cyphonauta in Bryozoa, trocophora in Annelida and Polychaeta, pseudotrocophora and veliger in Mollusca, nauplius in Crustacea and various larvae in Echinodermata: Argano et al., 2007; Young et al., 2002).

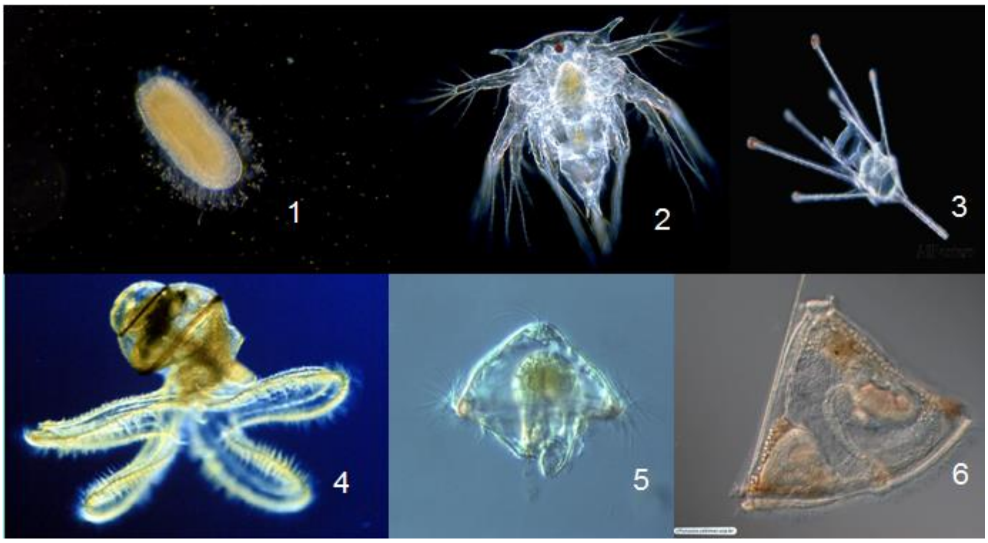


Figure 1. Examples of planktonic larvae: 1. Planula of Cnidaria (Craggs & Robson, 2012); 2. Nauplius of Crustacea (Wim Van Egmond, Science Photo Library); 3. Echinopluteus of Echinodermata (Wim Van Egmond, Science Photo Library); 4. Veliger of Gastropoda (©Peter Parks); 5. Trocophore of Annelida (T.C. Lacalli, University of Saskatchewan); 6. Cyphonauta of Bryozoa (Alvaro E. Migotto, http://www.usp.br/cbm/oceano/)

Dispersal is the movement of individual organisms, in the form of adult, larva, egg or gamete, from their birthplace to other locations for breeding. This process has impact at the community, species, and lineage level (Ellingson & Krug, 2015). At ecological time scales, dispersal can affect population size and persistence (Hansson, 1991), as well as metacommunity structure and diversity (Bie et al., 2012; Jones et al., 2015). Over evolutionary time, dispersal can influence genetic diversity (Méndez et al., 2014), range size (Kubisch et al., 2014), and diversification rate of a lineage (Krug et al., 2015). The process can last differently and it is under the influence of the characteristic of larvae, used as proxy of dispersal since direct measurement of dispersal can be difficult in marine invertebrates, and ends through a competence stage followed by the settlement in a suitable environment (Pineda et al., 2007). The larval development is a key feature in the ecology and biology of marine species, that influences population connectivity, areal occupation, species persistence and genetic structure (Cowen & Sponaugle, 2009; L. A. Levin, 2006).

In marine gastropods the larval development is a very important character due to a reduced mobility of the adult, in comparison with the potential dispersal range of the larvae.

In this taxon the various larval developmental strategies can be divided in two major types, Planktotrophic (P) and Non-Planktotrophic (NP) as first categorized and described by Thorson (1950) (Grahame & Branch, 1985; Levin & Bridges TS, 1995; Strathmann, 1978, 1985; Wray & Raff, 1991). The Planktotrophic larvae lead a free life in the water column and can swim and actively feed, mostly on phytoplankton. This stage persists in time for weeks or months, but can in some cases last until one year (Strathmann & Strathmann, 2007) before settling. Planktotrophy requires low quantity of parental resources for the larvae, that are originated by small eggs and with little or no yolk. This allows the production of very high numbers of offspring that have a stronger dispersal ability due to the long life. The larvae are strictly dependent on the environmental trophic availability and are exposed to predators.

The Non-Planktotrophic larval development includes lecithotrophic larvae and intracapsular development. In the intracapsular development the embryos and then the larvae develop entirely inside egg capsules, usually fixed to the bottom. Lecithotrophic larvae have pelagic life in the water column but with no active feeding on phytoplankton. The larvae feed only on yolk reserves and have usually lost some or all the specific feeding structures, like the velum, large ciliated lobs able to carry the food particles towards the mouth. Non-planktotrophic development requires high energy to produce the large amount of yolk of the eggs. This results in a lower number of individuals produced with a relatively short duration of the pelagic phase, directly correlated with the amount of yolk reserve. The shorter life of the larval stage results also in a reduced dispersal potential compared to planktotrophy. Despite this, an advantage for this kind of larval development is the independence of larvae from the food availability of the external environment.

The so-called Thorson's Rule (Mileikovsky, 1971) stated that at low latitudes the planktotrophic development is favoured by the constant amount of phyto- and zooplankton, whereas at higher latitudes, the non-planktotrophic development is favoured because of the high instability of the environment and the scarcity of resources, often available only in short periods during the year.

Caenogastropoda, Cox 1960, is an accepted subclass of Gastropoda (WoRMS - Appeltans et al., 2012) that comprises the large orders of Neogastropoda (mud whelks, rock shells, oyster drills, dove shells, tritons, miters, cone shells), and Littorinimorpha (periwinkles, cowries, creepers, slipper limpets, tuns, helmet shells, strombs, moon snails). The taxon comprises about 60% of living gastropod species and includes a large number of ecologically and commercially important marine families, worldwide distributed. Caenogastropods have undergone an extraordinary adaptive radiation, resulting in considerable morphological, ecological, physiological, and behavioural diversity. There is a wide array of often convergent shell morphologies, with the typically coiled shell being tall-spired to globose or flattened, with some uncoiled or limpet-like and others with the shells reduced or, rarely, lost (Ponder et al., 2008).

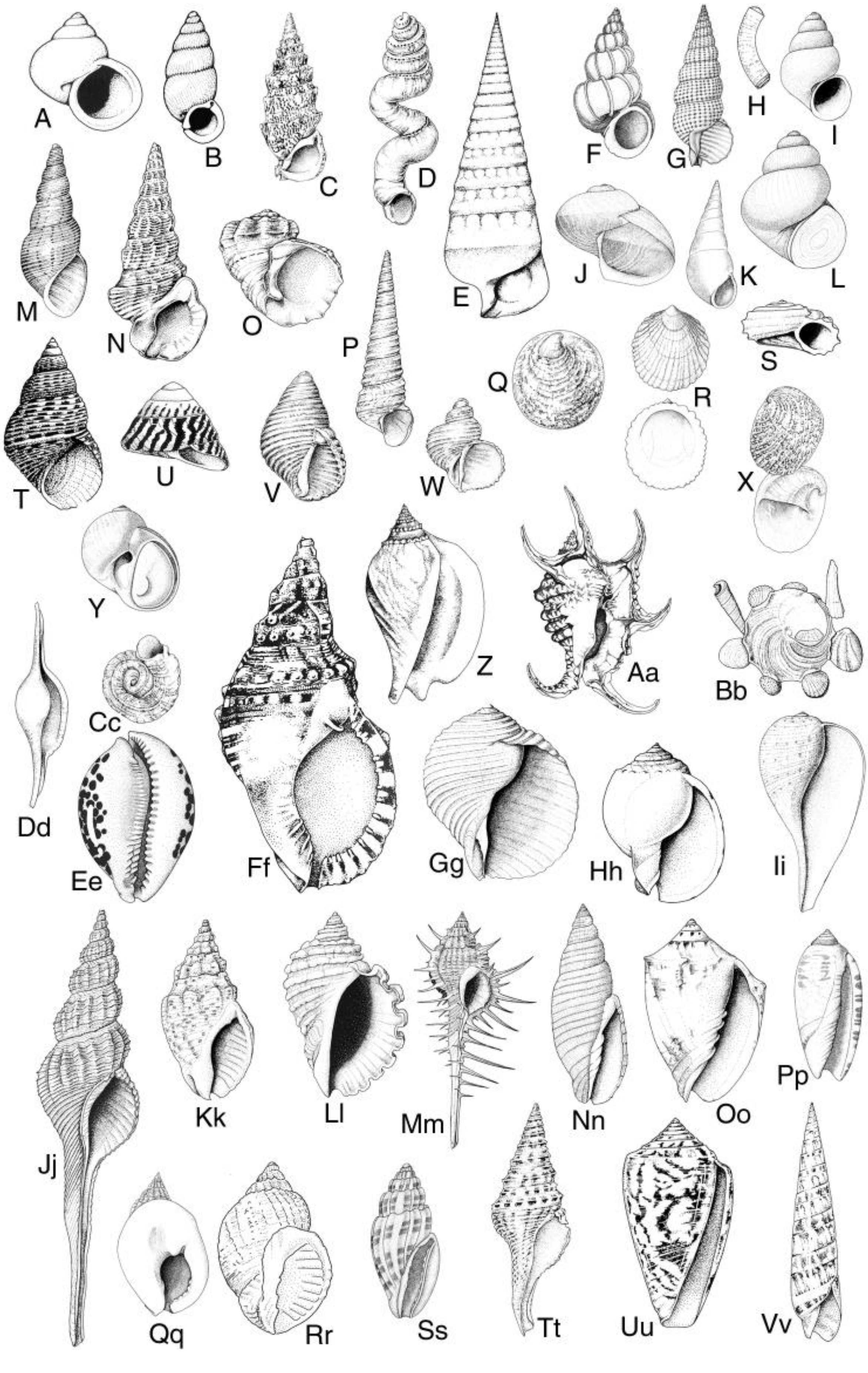

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
Aa
Bb
Cc
Dd
Ee
Ff
Gg
Hh
Ii
Jj
Kk
Ll
Mm
Nn
Oo
Pp
Qq
Rr
Ss
Tt
Uu
Vv

Figure 2. Shells of some Recent caenogastropods showing the range of morphology. (A) *Leptopoma* (Cyclophoridae); (B) *Pupinella* (Pupinidae); (C) *Pseudovertagus* (Cerithiidae); (D) *Tenagodus* (Siliquariidae); (E) *Campanile* (Campanilidae); (F) *Epitonium* (Epitoniidae); (G) *Ataxocerithium* (Newtoniellidae); (H) *Caecum* (Caecidae); (I) *Austropyrgus* (Hydrobiidae *sensu lato*); (J) *Janthina* (Janthinidae); (K) *Monogamus* (Eulimidae); (L) *Gabbia* (Bithyniidae); (M) *Melanoides* (Thiaridae); (N) *Pyrazus* (Batillariidae); (O) *Modulus* (Modulidae); (P) *Colpospira* (Turritellidae); (Q) *Capulus* (Capulidae); (R) *Sabia* (Hipponicidae); (S) *Circulus* (Vitrinellidae); (T) *Littoraria* (Littorinidae); (U) *Bembicium* (Littorinidae); (V) *Planaxis* (Planaxidae); (W) *Sirius* (Capulidae); (X) *Crepidula* (Calyptraeidae); (Y) *Notocochlis* (Naticidae); (Z) *Strombus* (Strombidae); (Aa) *Lambis* (Strombidae); (Bb) *Xenophora* (Xenophoridae); (Cc) *Serpulorbis* (Vermetidae); (Dd) *Volva* (Ovulidae); (Ee) *Cypraea* (Cypraeidae); (Ff) *Charonia* (Ranellidae); (Gg) *Tonna* (Tonnidae); (Hh) *Semicassis* (Cassidae); (Ii) *Ficus* (Ficidae); (Jj) *Fusinus* (Fasciolariidae); (Kk) *Cominella* (Buccinidae); (Ll) *Dicathais* (Muricidae); (Mm) *Murex* (Muricidae); (Nn) *Cancilla* (Mitridae); (Oo) *Cymbiola* (Volutidae); (Pp) *Oliva* (Olividae); (Qq) *Nassarius* (Nassariidae); (Rr) *Cancellaria* (Cancellariidae); (Ss) *Eucithara* (Turridae *sensu lato*); (Tt) *Lophiotoma* (Turridae); (Uu) *Conus* (Conidae); (Vv) *Terebra* (Terebridae). Not to scale. From Ponder et al., 2008

Primitive gastropods were (and still are) characterised by a lecithotrophic pelagic development (Nutzel, 2014; Nutzel et al., 2006). Larval planktotrophy evolved one or two times in gastropods, in Caenogastropoda and Heterobranchia, respectively. There is robust evidence that larval planktotrophy has supported and possibly also driven the radiation of caenogastropods. A planktotrophic development is the plesiomorphic state of almost all Caenogastropoda lineages. The loss of larval planktotrophy has occurred repeatedly during the evolution of the taxon. Indeed, the larval development is to be considered as a very plastic feature in caenogastropods and sibling species differing only or mostly in larval developmental type (e.g.: planktrotrophic v. non-planktotrophic) are a common phenomenon in most of the major family of the subclass, like Nassariidae, Raphitomidae, Muricidae, Columbellidae, Conidae, Calyptraeidae, Rissoidae etc. (Collin, 2001; Galindo et al., 2016; Giannuzzi-Savelli et al., 2018a; Modica et al., 2017; Oliverio, 1996a; Pusateri et al., 2012, 2013). Once lost, the reacquisition of planktotrophy is considered a very difficult and thus rare phenomenon, and largely excluded in Caenogastropoda (Strathmann, 1978). Loss or reduction of the complex larval structures for feeding and swimming in the plankton and the likely degeneration of the genetic machinery that supports their development is commonly regarded as very likely irreversible. Once lost, the difficulty of re-evolving a set of structures that function effectively for feeding and swimming makes it unlikely that feeding larvae will be reacquired from an ancestor with non-planktotrophic development (Collin et al., 2007). However, very few such cases of reversal (reacquisition of a lost plesiomorphic character) have been found in marine invertebrates: in Polychaeta (Rouse, 2000) and in three families of Caenogastropoda, Littorinidae (Reid, 1989), Calyptraeidae (Collin et al., 2007) and in the Muricidae (Pappalardo et al., 2014). The mechanism underlying the reacquisition of larval planktotrophy is still largely unknown.

The mode of larval development of a shelled gastropod (and bivalve) is reflected in the morphology of the protoconch (prodissoconch in bivalves) (Jablonski & Lutz, 1983; Rex & Warén, 1982; Shuto, 1974; Thorson, 1950) frequently preserved at the tip of the adult shell (teleoconch). This allows for the inference of many developmental features based on the comparative study of the protoconchs. By the morphology of the protoconch, developmental types can be mainly classified into two main types (Bouchet, 1990; Jablonski & Lutz, 1983; Shuto, 1974). The planktotrophic development, due to a longer life of larvae, produces a multispiral relatively thin protoconch of 2-5 whorls, often decorated with elaborated sculpture and a general tapered aspect (Figure 3. A and C). The non-planktotrophic development, where the larvae have a shorter life, produces a paucispiral large and stouter protoconch of 1-2 whorls with a simpler sculpture or no sculpture at all and an irregular and stocky general aspect (Figure 3. B and D).

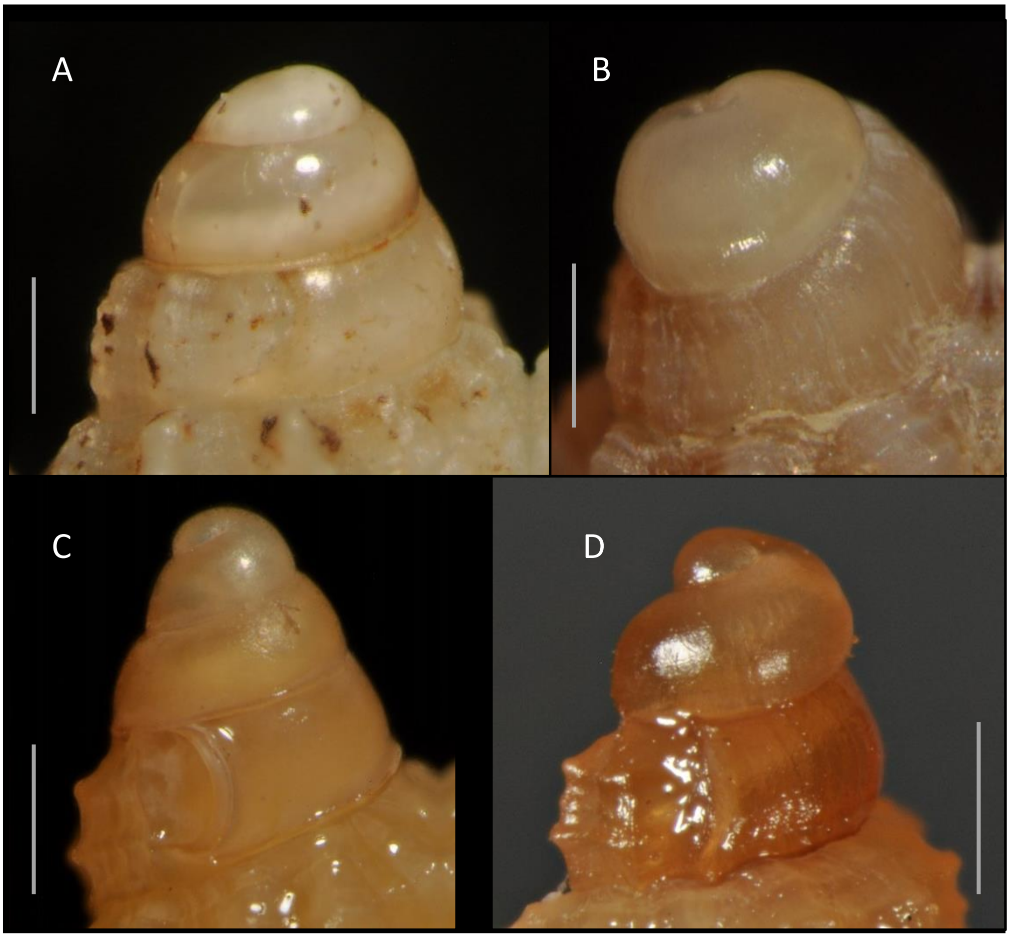


Figure 3. Protoconch of sibling species: multispiral protoconch (A) of *Phrontis alba* and paucispiral protoconch (B) of *Phrontis* sp. (Nassariidae). Multispiral protoconch (C) of *Murex tenuirostrum* and paucispiral protoconch (D) of *Murex africanus* (Muricidae). Russini V.

Shelled gastropods provide unique sources of data for the study of the evolution of larval development, allowing for the study of the same characters in extant and fossil lineages (Nutzel, 2014; Shuto, 1974). With the systematics of shelled gastropods based mostly on morphological character as shell, anatomy and radula (when genetic information is lacking), the protoconch was an important taxonomic character also for species identification (Oliverio, 1996b; Pusateri et al., 2013).

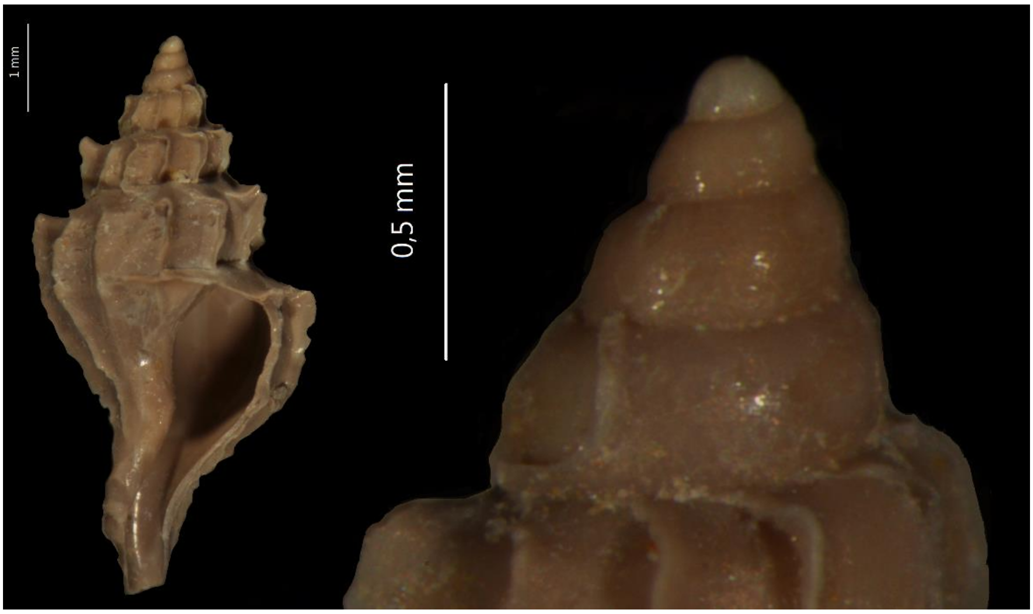


Figure 4. Teleoconch and multispiral protoconch of fossil specimens of *Flexopteron foliacea* (Melleville, 1843) (Muricidae), Ypresian, MNHN fossil collection. Russini V.

Poecilogony is defined as the intraspecific variation in developmental mode, with different larvae (e.g., free-swimming planktotrophic and brooded lecithotrophic) produced by the same individual, population or species. Since the first observation by Giard (1905) it has always been subject of considerable studies and discussions. It has been hypothesized (Hoagland & Robertson, 1988) that differences in egg size could be involved in this rare phenomenon (e.g., small eggs destined to planktotrophic development and large eggs that develop into non-planktotrophic larvae), or embryo size (e.g., some embryos consume nurse eggs but others do not). Poecilogony has been described in only a few groups of marine invertebrates (Knott & McHugh, 2012), whereas it has been long assumed that developmental strategies are strongly constrained within a species, and that poecilogony is not sufficiently documented in marine invertebrates (Hoagland & Robertson, 1988). In a landmark review for gastropods, Bouchet (1989) excluded the existence of intraspecific variation in the mode of larval development (poecilogony), in the Caenogastropoda. Following this assumption, the protoconch became often the only species identification character for sibling species, e.g. species identical in general morphology, that differ only in larval development (and thus larval shell morphology). Poecilogony has been so far documented with certainty only in a few groups of marine invertebrates as sacoglossan sea slugs (Krug, 2009), spionid polychaetes (Blake & Arnofsky, 1999), and just one case in caenogastropods, in the genus *Calyptraea* (Calyptraeidae: Mcdonald et al., 2014). Most marine invertebrates groups show evidence of evolutionary transitions in the larval phenotype, almost in all cases in terms of loss of planktotrophy, which occurred repeatedly in many lineages of marine caenogastropods (Oliverio, 1996b). The mechanisms underlying both the evolutionary transitions and the intraspecific variation are still largely unknown.

Given this background, some evolutionary issues have arisen about the change of larval development in Caenogastropoda. Are there any evolutionary patterns in these changes across phylogenetic lineages? Since modes of larval development of marine invertebrates are not homogeneously distributed in oceans (Strathmann & Strathmann, 1982; Thorson, 1950), can the changes in larval development be related to certain environmental condition or particular geographic area? The main purpose of this thesis is to investigate the evolution of larval development in some groups of gastropods, in order to clarify some aspect still less known in marine gastropods, as the bearing of larval development on population connectivity, the actual presence of poecilogony in Neogastropoda and the evolutionary pattern of development across lineages.

In this PhD research I have studied some of the major aspects of the evolution of larval development using four groups of Caenogastropoda, and the thesis is divided in four chapters, following a logic thread from particular to general.

The project started with the question on the bearing of different larval developmental strategies on connectivity among populations. To test the larval development influences connectivity, I have analysed populations of a pair of sibling species of marine gastropods, *Columbella rustica* and *Columbella adansoni,* allopatric species, that share a very similar adult shell and differ almost only in their larval development.

By analysing the sequence variation of the cytochrome c oxidase subunit I (COI), I found that *Columbella adansoni*, the Atlantic species with planktotrophic development and multispiral protoconch, showed no phylogeographic structure, lower levels of genetic diversity, interpopulational variance lower than the intrapopulational one and no spatial structure in the distribution of the genetic diversity; *Columbella rustica*, the species with lecithotrophic development, thus with evidently lower dispersal abilities, showed a clear phylogeographic structure, higher levels of genetic diversity, high interpopulational and low intrapopulational variance, and a clear signature of global spatial structure in the distribution of the genetic diversity. These species belong to a complex of at least three cryptic species, where the sister to the studied pair is the West-African *Columbella xiphitella,* identified during this study, with a planktotrophic development.

Then, I have investigated the presence of pairs of sibling species in another group of marine neogastropods, the genus *Raphitoma* Bellardi, 1847 (Raphitomidae: Conoidea). This genus is largely present in the Mediterranean Sea and the North-East Atlantic and several pairs of sibling species have been reported by morphological studies.

The chapter is divided in two studies. In the first work a new phylogenetic framework was reconstructed for the family Raphitomidae Bellardi, 1875*,* with over fifty extant species recently estimated in Mediterranean area (Giannuzzi-Savelli et al., 2018b), aimed to delimit the actual scope of the genus *Raphitoma* Bellardi, 1847. In this genus several pairs of species with contrasting larval developments have been identified. The systematic revision was based on three mitochondrial molecular markers: cytochrome c oxidase subunit I (COI), ribosomal 12S and 16S. The work allowed to clarify the systematic position of the species formerly ascribed to the genus *Raphitoma* and to delimit its scope and the set of species to study in the next step.

The aims of the second study were to reconstruct the relationships among *Raphitoma* species, to confirm or deny the pairs of sibling species, and to date the events of loss of planktotrophy across the lineages. The analyses were based on molecular data (the mitochondrial COI, 16S, 12S and the nuclear Internal transcribed spacer, ITS2). The results confirmed one pair of sibling species previously recognised morphologically. Two other such pairs (*Raphitoma philberti-R. locardi* and *R. laviae-R. bartolinorum*) were instead identified as two poecilogonous species, with syntopic specimens with different development, genetically indistinguishable. This study represents the first documented case of poecilogony in the Neogastropoda, the second in the whole Caenogastropoda (after the work of Mcdonald et al., 2014 that described poecilogony in *Calyptraea lichen* Broderip, 1834).

Once provided further evidence of the plasticity of larval development in the Ceanogastropoda, the evolutionary patterns across lineages were the next topic of my studies. To investigate how larval development changed – for instance - throughout a family, I needed a robustly resolved phylogeny and the knowledge of the larval development of a vast majority of the species in the family. For these reasons I started with a family recently revised for its phylogenetic framework, with larval development known for a high number of species, to attempt assessing the ancestral state of the character and dating the relevant changes across the tree.

The third chapter concern thus, the study of the evolution of larval development within the phylogenetic lineages of one important family of Caenogastropoda, the Nassariidae. Thanks to a recent large phylogenetic analysis of this family performed by Galindo et al. (2016), it has been possible to analyse the variation of larval development across the family's tree. The phylogeny has been dated by setting as calibration points twelve reliable fossil records. Then larval development was inferred for a large number of species. Two methods have been adopted to investigate the evolution of larval development in the family. In the first method, the change of larval development was constrained in coincidence with the nodes that lead to species with different larval developmental types. In the second method I have used a statistical tool in R (phytools) that estimated the events of loss of planktotrophy along the branches. The results of both analyses were largely congruent and suggested that the frequencies of loss of planktotrophy events varied statistically between biogeographic regions. Higher relative frequency occurred in the Atlantic and Mediterranean areas, the Caribbean region and South America (compared to the Indo-Pacific). Conversely, no significant variation was detected between different geological epochs. Geological history from the Paleogene of these biogeographic regions suggest that their long time of instability may have promoted geographic confinement of species (GCH) with increase of loss of planktotrophy.

The fourth and final chapter of this thesis presents a similar study on the family Muricidae. First, a complete phylogenetic reconstruction was made gathering all the data available on public databases along with new original data to make a complete phylogenetic framework for the evolution of larval development. The phylogeny has been dated with twelve calibration points obtained from as many reliable fossils record. Once a complete phylogenetic framework was available, the larval development was inferred for a large number of species. The evolution of larval development was estimated using the statistical tool in R (phytools), dating the events of loss of planktotrophy along the branches. The results clarified the systematic of this large and important family and the position and scope of each subfamily. The study proved that reversals in the evolution of larval development (from non-planktotrophic to planktotrophic), commonly excluded in marine invertebrates, occurred in two major subfamilies, Muricinae s.s. and Muricopsinae s.s.. The secondary re-acquisition of a planktotrophic larval development is anyway a very rare phenomenon, and in marine invertebrates only a few cases remain documented: in Polychaetes (Rouse, 2000) and in three family of Caenogastropoda: Littorinidae (Reid, 1989), Calyptraeidae (Rachel Collin et al., 2007) and Muricidae (Pappalardo et al., 2014; and this work).

References

Appeltans, W., Bouchet, P., Boxshall, G., & Fauchald, K. (2012). World register of marine species. Retrieved from http://www.marinespecies.org/index.php

Argano, R., Boero, F., Bologna, M., & Dallai, R. (2007). *Zoologia: Diversità animale*. (Monduzzi, Ed.).

Bie, T., Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D., et al. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters*, *15*(7), 740–747. https://doi.org/10.1111/j.1461-0248.2012.01794.x

Blake, J. A., & Arnofsky, P. L. (1999). Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. *Hydrobiologia*, *402*, 57–106. https://doi.org/10.1023/A:1003784324125

Bouchet, P. (1989). A review of poecilogony in gastropods. *Journal of Molluscan Studies*, *55*, 67–78.

Bouchet, Philippe. (1990). Turrid genera and mode of development : the use and abuse of protoconch morphology. *Malacologia*, *32*(1), 69–77.

Collin, R. (2001). The effects of mode of development on phylogeography and population structure of North Atlantic Crepidula(Gastropoda:Calyptraeidae). *Molecular Ecology*, *10*, 2249–2262.

Collin, Rachel, Chaparro, O. R., Winkler, F., & Véliz, D. (2007). Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. *Biological Bulletin*, *212*(2), 83–92.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, *1*(1), 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Craggs, J., & Robson, J. (2012). Observations of the life cycle of the scyphozoan jellyfish Aurelia aurita at the Horniman Museum Aquarium. *Quekett Journal of Microscopy*, *41*, 615–621.

Ellingson, R. A., & Krug, P. J. (2015). Reduced genetic diversity and increased reproductive isolation follow population-level loss of larval dispersal in a marine gastropod. *Evolution; International Journal of Organic Evolution*, *70*(I), 18–37. https://doi.org/10.1111/evo.12830

Galindo, L. A., Puillandre, N., Utge, J., Lozouet, P., & Bouchet, P. (2016). The phylogeny and systematics of the Nassariidae revisited (Gastropoda, Buccinoidea). *Molecular Phylogenetics and Evolution*, *99*(June), 337–353. https://doi.org/10.1016/j.ympev.2016.03.019

Giannuzzi-Savelli, R., Pusateri, F., & Bartolini, S. (2018a). A revision of the Mediterranean Raphitomidae (Gastropoda , Conoidea), 7 : on the sibling species Raphitoma densa (Monterosato , 1884) and R . griseomaculata n . sp . *Biodiversity Journal*, *9*(4), 429–440. https://doi.org/10.31396/Biodiv.Jour.2018.9.4.429.440

Giannuzzi-Savelli, R., Pusateri, F., & Bartolini, S. (2018b). A revision of the Mediterranean Raphitomidae (Gastropoda: Conoidea) 5: loss of planktotrophy and pairs of species, with the description of four new species. *Bollettino Malacologico*, *54*(suppl.11), 1–77.

Giard, A. (1905). La Poecilogonie. *Comp. Rend. 6 Eme Congr. International de Zoologie, Berne, 1904*, 617–646.

Grahame, J., & Branch, G. M. (1985). Reproductive patterns of marine invertebrates. *Oceanography and Marine Biology Annual Review*, *23*, 373–398. Retrieved from http://www.scielo.org.co/scielo.php?script=sci_nlinks&ref=000084&pid=S0122-97611993000100003000011&lng=en

Hansson, L. (1991). Dispersal and connectivity in metapopulations. *Biological Journal of the Linnean Society*, *42*(1–2), 89–103. https://doi.org/10.1111/j.1095-8312.1991.tb00553.x

Hoagland, K. E., & Robertson, R. (1988). An Assessment of Poecilogony in Marine Invertebrates: Phenomenon or Fantasy? *The Biological Bulletin*, *174*(2), 109–125. https://doi.org/10.2307/1541778

Jablonski, D., & Lutz, R. A. (1983). Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, *58*(1), 21–89. https://doi.org/10.1111/j.1469-185X.1983.tb00380.x

Jones, N. T., Germain, R. M., Grainger, T. N., Hall, A. M., Baldwin, L., & Gilbert, B. (2015). Dispersal mode mediates the effect of patch size and patch connectivity on metacommunity diversity. *Journal of Ecology*, *103*(4), 935–944. https://doi.org/10.1111/1365-2745.12405

Knott, K. E., & McHugh, D. (2012). Introduction to Symposium: Poecilogony--A Window on Larval Evolutionary Transitions in Marine Invertebrates. *Integrative and Comparative Biology*, *52*(1), 120–127. https://doi.org/10.1093/icb/ics037

Krug, P. J. (2009). Not my “type”: larval dispersal dimorphisms and bet-hedging in opisthobranch life histories. *The Biological Bulletin*, *216*(3), 355–72. https://doi.org/10.1086/BBLv216n3p355

Krug, P. J., Vendetti, J. E., Ellingson, R. A., Trowbridge, C. D., Hirano, Y. M., Trathen, D. Y., et al. (2015). Species Selection Favors Dispersive Life Histories in Sea Slugs, but Higher Per-Offspring Investment Drives Shifts to Short-Lived Larvae. *Systematic Biology*, *64*(6), 983–999. https://doi.org/10.1093/sysbio/syv046

Kubisch, A., Holt, R. D., Poethke, H.-J., & Fronhofer, E. A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, *123*(1), 5–22. https://doi.org/10.1111/j.1600-0706.2013.00706.x

Levin, L., & Bridges TS. (1995). Pattern and diversity in reproduction and development. *In: McEdward L (Ed) Marine Invertebrate Larvae. CRC Press, Oxford*, 1–47. Retrieved from https://ci.nii.ac.jp/naid/10018064784/

Levin, L. A. (2006). Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, *46*(3), 282–297. https://doi.org/10.1093/icb/icj024

Mcdonald, K. A., Collin, R., & Lesoway, M. P. (2014). Poecilogony in the caenogastropod Calyptraea lichen (Mollusca : Gastropoda). *Invertebrate Biology*, *133*(3), 213–220. https://doi.org/10.1111/ivb.12057

Méndez, M., Vögeli, M., Tella, J. L., & Godoy, J. A. (2014). Joint effects of population size and isolation on genetic erosion in fragmented populations: finding fragmentation thresholds for management. *Evolutionary Applications*, *7*(4), 506–518. https://doi.org/10.1111/eva.12154

Mileikovsky, S. A. (1971). Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Marine Biology*, *10*(3), 193–213.

https://doi.org/10.1007/BF00352809

Modica, M. V., Russini, V., Fassio, G., & Oliverio, M. (2017). Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Marine Environmental Research*, *127*, 92–101. https://doi.org/10.1016/j.marenvres.2017.04.001

Nutzel, A. (2014). Larval ecology and morphology in fossil gastropods. *Palaeontology*, 1–25. https://doi.org/10.1111/pala.12104

Nutzel, A., Lehnert, O., & Fryda, J. (2006). Origin of planktotrophy-evidence from early molluscs. *Evolution Development*, *8*(4), 325–330. https://doi.org/10.1111/j.1525-142X.2006.00105.x

Oliverio, M. (1996a). Contrasting developmental strategies and speciation in NE Atlantic prosobranchs: a preliminary analysis. *Origin and Evolutionary Radiation of the Mollusca*, *22*, 261–266.

Oliverio, M. (1996b). Life-histories, speciation, and biodiversity in Mediterranean prosobranch gastropods. *Vie et Milieu*, *46*(2), 163–169.

Pappalardo, P., Rodriguez-Serrano, E., & Fernàndez, M. (2014). Correlated Evolution between Mode of Larval Development and Habitat in Muricid Gastropods. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0094104

Pineda, J., Hare, J., & Sponaugle, S. (2007). Larval Transport and Dispersal in the Coastal Ocean and Consequences for Population Connectivity. *Oceanography*, *20*(3), 22–39. https://doi.org/10.5670/oceanog.2007.27

Ponder, W. F., Colgan, D. J., Healy, J. M., Alexander, N., Simone, L. R. L., & Strong, E. E. (2008). Caenogastropoda. In *Phylogeny and Evolution of the Mollusca* (pp. 331–383). https://doi.org/10.1525/california/9780520250925.003.0013

Pusateri, F., Giannuzzi-Savelli, R., & Oliverio, M. (2012). A revision of the Mediterranean Raphitomidae 1: on the sibling species Raphitoma contigua Monterosato, 1884 and Raphitoma spadiana n. sp. (Gastropoda, Conoidea). *Iberus*, *30*(1), 41–52.

Pusateri, F., Giannuzzi-Savelli, R., & Oliverio, M. (2013). A revision of the Mediterranean Raphitomidae 2: On the sibling species Raphitoma lineolata (B.D.D., 1883) and Raphitoma smriglioi n. sp. (Gastropoda, Conoidea). *Iberus*, *31*(1), 11–20.

Reid, D. G. (1989). The comparative morphology, phylogeny and evolution of the gastropod family littorinidae. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *324*(1220), 1–110. https://doi.org/10.1098/rstb.1989.0040

Rex, M., & Warén, A. (1982). Planktotrophic development in deep-sea prosobranch snails from the western North Atlantic. *Deep Sea Research Part A. Oceanographic Research Papers*, *29*(2), 171–184.

Rouse, G. W. (2000). Polychaetes have evolved feeding larvae numerous times. *Bulletin of Marine Science*, *67*(1), 391–409.

Shuto, T. (1974). Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia*, *7*, 239–256.

Strathmann, M. F., & Strathmann, R. R. (2007). An Extraordinarily Long Larval Duration of 4.5 Years from Hatching to Metamorphosis for Teleplanic Veligers of Fusitriton oregonensis. *Biological Bulletin*, *213*, 152–159.

Strathmann, R. R. (1978). The evolution and loss of feeding larval stages of marine invertebrates. *Evolution*, *32*(4), 894–906. https://doi.org/10.1111/j.1558-5646.1978.tb04642.x

Strathmann, R. R. (1985). Feeding anStrathmann, R. R. (1985). Feeding and Nonfeeding Larval Development and Life-History Evolution in Marine Invertebrates. Annual Review of Ecology and Systematics, 16(1), 339–361. https://doi.org/10.1146/annurev.es.16.110185.002011d Nonfeeding Lar. *Annual Review of Ecology and Systematics*, *16*(1), 339–361. https://doi.org/10.1146/annurev.es.16.110185.002011

Strathmann, R. R., & Strathmann, M. F. (1982). The Relationship Between Adult Size and Brooding in Marine Invertebrates. *The American Naturalist*, *119*(1), 91–101. https://doi.org/10.1086/283892

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, *25*(1), 1–45. https://doi.org/10.1111/j.1469-185X.1950.tb00585.x

Wray, G. A., & Raff, R. A. (1991). The evolution of developmental strategy in marine invertebrates. *Trends in Ecology & Evolution*, *6*(2), 45–50. https://doi.org/10.1016/0169-5347(91)90121-D

Young, C., Sewell, M., & Rice, M. (2002). *Atlas of marine invertebrate larvae*. San Diego: Academic press.

Chapter I

- Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development.

- An assessment of the genus *Columbella* Lamarck, 1799 (Gastropoda: Columbellidae) from eastern Atlantic.

Marine Environmental Research 127 (2017) 92–101

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development

Maria Vittoria Modica [a, b], Valeria Russini [a], Giulia Fassio [a], Marco Oliverio [a, *]

[a] Department of Biology and Biotechnologies "Charles Darwin", 'La Sapienza' University, Viale dell'Università 32, I-00185 Roma, Italy
[b] Stazione Zoologica Anton Dohrn, Villa Comunale, I-80121 Napoli, Italy

A R T I C L E I N F O

Article history:
Received 30 June 2016
Received in revised form
10 March 2017
Accepted 1 April 2017
Available online 4 April 2017

Keywords:
Genetic connectivity
Larval development
Planktotrophy
Lecithotrophy
Gastropods

A B S T R A C T

In marine environments, connectivity among populations of benthic invertebrates is provided primarily by dispersion of larvae, with the duration of pelagic larval phase (PLD) supposed to represent one of the major factor affecting connectivity. In marine gastropods, PLD is linked to specific larval development types, which may be entirely intracapsular (thus lacking a pelagic dispersal), or include a short pelagic lecithotrophic or a long planktotrophic phase.

In the present study, we investigated two sibling species of the cosmopolitan neogastropod genus *Columbella* (commonly known as dove shells): *Columbella adansoni* Menke, 1853, from the Macaronesian Atlantic archipelagos, with planktotrophic development, and *Columbella rustica* Linnaeus, 1758, from the Mediterranean Sea, with intracapsular development.

We expected to find differences between these two sister species, in terms of phylogeographic structure, levels of genetic diversification and spatial distribution of genetic diversity, if PLD was actually a relevant factor affecting connectivity.

By analysing the sequence variation at the cytochrome *c* oxidase subunit I (COI) in 167 specimens of the two species, collected over a comparable geographic range, we found that *Columbella adansoni,* the species with planktotrophic development, and thus longer PLD, showed no phylogeographic structure, lower levels of genetic diversity, interpopulational variance lower than the intrapopulational one and no spatial structure in the distribution of the genetic diversity; *Columbella rustica*, the species with intracapsular development, thus with evidently lower dispersal abilities, showed a clear phylogeographic structure, higher levels of genetic diversity, high interpopulational and low intrapopulational variance, and a clear signature of global spatial structure in the distribution of the genetic diversity.

Thus, in this study, two sibling species differing almost only in their larval ecology (and PLD), when compared for their genetic variation showed patterns supporting the hypothesis that PLD is a major factor affecting genetic connectivity.

Therefore, it seems reasonable to expect that the ecological attributes of the marine communities - also in terms of the variation in larval ecology of the species involved – are taken into the due consideration in conservation actions, like the design of marine protected areas networks.

1. Introduction

Population connectivity is a key feature of organisms, influencing their genetic variability, persistence, genetic structure and range expansion, and as such has increasingly been investigated in the last years in different taxa (Cowen et al., 2000; Hellberg, 2009; Hastings and Botsford, 2006; Lowe and Allendorf, 2010; Weber et al., 2015). Clarifying the extent at which populations are connected allows the understanding of evolutionary and ecological processes shaping the distribution of individuals through their range, disentangling the effects of historical patterns and local adaptations (Laine, 2005; Sanford and Kelly, 2011). Additionally, connectivity studies are crucial to implement effective conservation and management strategies both in terrestrial and in marine environments (Webster et al., 2002; Palumbi, 2003; Shanks et al.,

* Corresponding author.
E-mail addresses: mariavittoria.modica@uniroma1.it (M.V. Modica), valeria.russini@uniroma1.it (V. Russini), giulia.fassio@uniroma1.it (G. Fassio), marco.oliverio@uniroma1.it (M. Oliverio).

http://dx.doi.org/10.1016/j.marenvres.2017.04.001

2003; Crooks and Sanjayan, 2006; Jones et al., 2007; Allendorf et al., 2010; Rabinowitz and Zeller, 2010; Funk et al., 2012).

In marine benthic invertebrates, dispersal is generally addressed by the earliest life history stages, while the adult stage is only slightly mobile, or even sessile (Knowlton and Jackson, 1993; Cowen and Sponaugle, 2009; Ellingson and Krug, 2015). Major factors affecting connectivity include both extrinsic (habitat characteristics and currents) and intrinsic factors, such as larval mortality, settlement competency features, and the duration of pelagic larval phase (PLD, the length of time that larva spends in water column before settling). The latter parameter is the most frequently used proxy of dispersal, since direct measurement of dispersal can be difficult in marine invertebrates. Early studies highlighted the presence of a correlation (with some exceptions) between PLD and dispersal distance (Shanks et al., 2003; Shanks, 2009; Siegel et al., 2003), although PLD is often assessed in laboratory settings that may not accurately represents actual conditions that larvae experiment in their natural environment (e.g.: Tyler and Young, 1999; Selkoe and Toonen, 2011; Villanueva et al., 2016).

The prediction that species with planktonic larvae displaying a longer PLD and larger dispersal kernels should also possess a lower level of genetic structure when compared with species lacking a dispersal phase (e.g. aplanktonic larvae, brooding) is supported by a number of studies (Berger, 1973; Duffy, 1993; Hunt, 1993; Hellberg, 1996; Hoskin, 1997; Arndt and Smith, 1998; Collin, 2001; Dawson et al., 2002; Teske et al., 2007; Sherman et al., 2008; Lee and Boulding, 2009; Steele et al., 2009; Hoffman et al., 2011; Guzmán et al., 2011; Tarnowska et al., 2012; Barbosa et al., 2013; Hoareau et al., 2013; Riginos et al., 2014). Anyway, the suitability of PLD as a good predictor of genetic connectivity has been questioned in a number of other cases, especially for species with a long PLD (Shanks, 2009), highlighting that other factors may have a major impact on connectivity, including habitat differences (Ayre et al., 2009) and past biogeographical events (Edmands, 2001; Marko, 2004).

The influence of PLD and dispersal abilities on genetic structure can be easily tested in most gastropods, as developmental type can be inferred from the structure of the protoconch, the shell produced by the embryo and the larva before metamorphosis or hatchling, and commonly retained at the top of the adult shell (Jablonski, 1980; Lima and Lutz, 1990).

In marine gastropods development can, as first described by Thorson (1949), either be entirely intracapsular, or include a pelagic phase during which larvae actively feed on plankton (planktotrophy), barely do so, or rely only on yolk reserves (lecithotrophy). Entirely intracapsular development is realized within the egg capsule, which is generally attached to the sea bottom; the eggs are provided with a large yolk supply and/or individuals may feed on nurse eggs until metamorphosis occurs, hatchling as benthic post-larvae. Yolk supply is also exploited by lecithotrophic planktonic larvae, which hatch as free living and spend a reduced time in the water column. Similarly, planktotrophic larvae hatch as free living, but they are able to actively collect phytoplankton using their velum; the life span of these larvae typically extends over weeks or months, and some cases can exceed several years (Strathmann and Strathmann, 2007).

Among Caenogastropoda, a large number of pairs of sibling species are known, differing only in their larval development (planktotrophic v. lecithotrophic), particularly studied in the North-eastern Atlantic (Oliverio, 1996) but well known on a global scale (Oliverio, 1997a). This offers the possibility to study the bearing of larval development on species otherwise very similar in their biology and ecology. In the present study, we investigated the genetic implications of different larval developments in two sibling species of the cosmopolitan neogastropod genus *Columbella* Lamarck, 1799, currently including 30 recognised species worldwide (Bouchet and Gofas, 2010). This genus has been recently reviewed in the East Atlantic region (Russini et al., 2017) and three species have been clearly identified by molecular data: *Columbella rustica* Linnaeus, 1758, *Columbella adansoni* Menke, 1853, and *Columbella xiphitella* Duclos, 1840. These three species share nearly identical adult shell morphology and anatomical features, occupy the same macrohabitat (all are shallow water, rock dwelling, algae associated, herbivorous), and their ranges do not overlap (Oliverio, 1995; Rolán, 2005; Russini et al., 2017). *C. rustica* is endemic to the Mediterranean Sea and its Atlantic approaches, where it is extremely common in shallow-water rocky habitats; *C. adansoni* inhabits the Macaronesian archipelagos; and *C. xiphitella* lives along East African coast from Ghana to Angola (including Sao Tomé and Principe Islands). According to molecular phylogenetic data, *C. rustica* and *C. adansoni* are sister species, whereas *C. xiphitella* is more distantly related (Russini et al., 2017). Planktotrophic larvae (39–73) hatch from the egg capsules of *C. adansoni* from Canary Islands and Cape Verde Islands (Knudsen, 1950, 1995), whereas the capsules of Mediterranean *C. rustica* have been described to contain 40-60 eggs, most of which are nurse eggs to nourish the 1–12 developing embryos (1–2: Franc, 1943; 6–12: Bacci, 1943). The only morphological features allowing separation of *C. rustica* and *C. adansoni* are, in fact, in their protoconchs. In *C. adansoni* the protoconch is multispiral with an evident ‘sinusigera mark’ i.e. a thin sigmoid sinus marking the protoconch-teleoconch boundary, clearly indicating a planktotrophic development (same protoconch of *C. xiphitella* for which a similar planktotrophic development can be inferred). The paucispiral protoconch of *C. rustica* possesses a very peculiar appearance, being irregularly cylindrical with a more or less pronounced apical keel and a flat top; its reduced whorl number, bluntness and the absence of a ‘sinusigera mark’ at the protoconch-teleoconch transition, attest a lecithotrophic development (Oliverio, 1995).

If PLD is a relevant factor affecting connectivity, we expect to find differences between these two sister species, in terms of phylogeographic structure, levels of genetic diversification and spatial distribution of genetic diversity. In particular, the species with planktotrophic development, and thus longer PLD, is expected to show weaker or no phylogeographic structure, lower levels of genetic diversity and no spatial structure in the distribution of the genetic diversity, when compared with the species with lecithotrophic development. The few samples available for the third species, *C. xiphitella*, did not allow their use for the same analyses as in the pair *C.adansoni*/*C. rustica*; however, they could serve as an optimal outgroup for phylogeographic analyses.

2. Material and methods

2.1. Samples collection and laboratory procedures

We obtained sequences from 99 specimens of *Columbella rustica* from the Mediterranean Sea, and 68 of *C. adansoni* from the Atlantic Ocean, in particular Azores, Madeira, Canary and Cape Verde Islands (Fig. 1). Details of collection localities are reported in Table 1. Sequences of *C. xiphitella* from Gabon were used as outgroup to root trees according to the phylogenetic pattern in Russini et al. (2017).

All specimens were collected in shallow-water rocky bottom, fixed and preserved in 95°–100° ethanol, and vouchers were stored in the Malacological Collection of Department of Biology and Biotechnologies “Charles Darwin” (acronym BAU) at Sapienza University of Rome (Italy). DNA was extracted from a fragment of foot tissue, using a modified phenol-chloroform protocol (Oliverio and Mariottini, 2001). A 658 bp fragment of the mitochondrial COI gene was PCR amplified, using the universal primers LCO1490 and

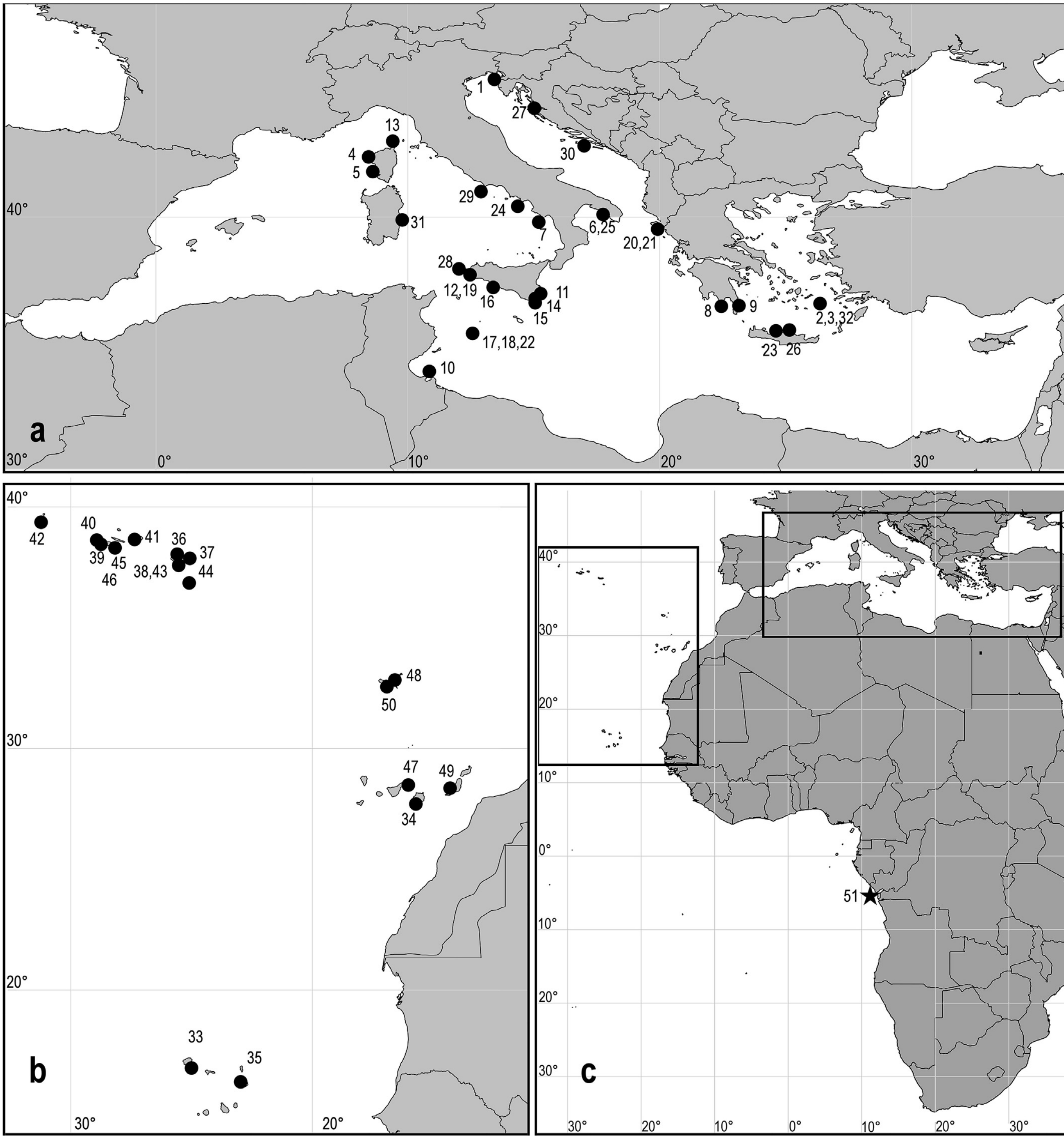


Fig. 1. Location of the sampling sites. **a** Mediterranean Sea (*Columbella rustica*). **b** Macaronesian archipelagos (*C. adansoni*). **c** East Atlantic area, with the sampling site for *C. xiphitella*.

HCO2198 (Folmer et al., 1994). Amplifications were performed in a total reaction volume of 25 µl, including 50–500 ng of DNA, 2.5 µl of 10x Reaction Buffer, 0.2 mM of dNTP, 1–2.5 µl of MgCl2, 1 µl of BSA, 100 ng of each primer and 2 U of BIOLINE Taq Polymerase. PCR conditions were as follows: initial denaturation step of 5′ at 94 °C, 35 amplification cycles (denaturation 94°C/30″, annealing 48–52°C/40″, elongation 72°C/50″), followed by a final elongation step of 7’ at 72 °C. PCR products were purified by ExoSAP-IT protocol (USB Corporation, Ohio, USA) and sequenced by Macrogen Inc. (Netherlands). Forward and reverse sequences were assembled, checked for contaminations and stop codons, aligned and edited with Geneious 4.8.5 (Biomatters Ltd.) and MEGA 6 (Tamura et al., 2013), and deposited in the GenBank (accession numbers: KX639827-KX639993).

Table 1
Collecting sites of the assayed samples, with BAU ID numbers for the lots. N indicate the number of the collecting site as reported in Fig. 1.

N	ID	Site	Coordinates
Columbella rustica			
1	BAU 1103	Mijiet Is., Croatia	42°45′54″N 017°23′50″E
2	BAU 1493	Analipsi, Astypalea Is., Greece	36°34′25″N 026°23′02″E
3	BAU 1582	Kotsoumiti Is., Greece	36°32′49″N 026°26′36″E
4	BAU 1608	Galeria, Corsica, France	42°25′04″N 008°39′23″E
5	BAU 1629	Tour d'Ancone, Corsica, France	42°02′36″N 008°43′15″E
6	BAU 1670	S. Isidoro, Italy	40°12′16″N 017°55′13″E
7	BAU 1755	Palinuro, Italy	40°01′31″N 015°16′03″E
8	BAU 1758	Simos beach, Elafonissi, Greece	36°28′33″N 022°57′38″E
9	BAU 1779	Cape Tenafo, Greece	36°24′33″N 022°29′29″E
10	BAU 1794	Sidi Jmour, Gabés, Tunisia	33°49′54″N 010°44′51″E
11	BAU 807	Siracusa, Sicily, Italy	36°58′15″N 015°14′55″E
12	BAU 808	Marsala, Sicily, Italy	37°48′10″N 012°25′29″E
13	BAU 811	Giraglia, Corsica, France	43°00′37″N 009°25′27″E
14	BAU 812	Vendicari, Sicily, Italy	36°49′25″N 015°06′31″E
15	BAU 813	Isola delle Correnti, Sicily, Italy	36°38′46″N 015°04′37″E
16	BAU 814	Siculiana, Sicily, Italy	37°20′14″N 013°23′08″E
17	BAU 816	Isola dei conigli, Lampedusa Is., Italy	35°30′35″N 012°33′27″E
18	BAU 817	Cala Greca, Lampedusa Is., Italy	35°30′16″N 012°35′04″E
19	BAU 818	Marsala, Sicily, Italy	37°47′37″N 012°25′52″E
20	BAU 819	Agios Georgios, Kerkyra, Greece	39°43′07″N 019°39′44″E
21	BAU 820	Afiona, Kerkyra, Greece	39°43′19″N 019°39′21″E
22	BAU 821	Cala francese, Lampedusa Is., Italy	35°29′37″N 012°37′21″E
23	BAU 822	Agia Pelagia, Crete, Greece	35°24′31″N 025°01′22″E
24	BAU 823	Napoli harbour, Italy	40°50′01″N 014°15′20″E
25	BAU 824	S. Isidoro, Italy	40°12′16″N 017°55′13″E
26	BAU 825	Lygaria Eraklion, Crete, Greece	35°25′13″N 024°45′16″E
27	BAU 826	Umag, Croatia	45°27′02″N 013°30′45″E
28	BAU 827	Marettimo, Sicily, Italy	37°59′07″N 012°04′13″E
29	BAU 829	Zannone Is., Italy	40°57′52″N 013°03′26″E
30	BAU 830	Starigrad Paklenikia, Croatia	44°16′45″N 015°27′11″E
31	BAU 831	Arbatax, Sardinia, Italy	39°55′19″N 009°42′49″E
32	BAU 832	Vai, Astypalea Is., Greece	36°36′13″N 026°23′28″E
Columbella adansoni			
33	BAU 1123	Mindelo, São Vicente Is., Cape Verde Islands	16°54′08″N 025°59′51″W
34	BAU 1124	Arguineguin, Gran Canaria Is., Canary Islands, Spain	27°45′18″N 015°41′04″W
35	BAU 1694	Sal Rei, Boavista Is., Cape Verde Islands	16°10′32″N 022°55′15″W
36	BAU 701	Riberinha, São Miguel Is., Azores Islands, Portugal	37°50′08″N 025°29′01″W
37	BAU 706	Nordeste, São Miguel Is., Azores Islands, Portugal	37°49′20″N 025°08′10″W
38	BAU 708	Caloura, São Miguel Is., Azores Islands, Portugal	37°42′24″N 025°30′31″W
39	BAU 710	Ponta dos Capelinhos, Fajal Is., Azores Islands, Portugal	38°35′30″N 028°49′43″W
40	BAU 713	Ponta de Eira, Fajal Is., Azores Islands, Portugal	38°38′03″N 028°40′23″W
41	BAU 716	Lajes, Pico Is., Azores Islands, Portugal	38°23′26″N 028°15′09″W
42	BAU 718	Santa Cruz, Flores Is., Azores Islands, Portugal	39°27′31″N 031°07′33″W
43	BAU 726	Caloura, São Miguel Is., Azores Islands, Portugal	37°42′24″N 025°30′31″W
44	BAU 728	Ilheu de Vila Franca do Campo, São Miguel Is., Azores Islands, Portugal	37°42′23″N 025°26′32″W
45	BAU 731	Biscoitos, Terceira Is., Azores Islands, Portugal	38°48′12″N 027°15′29″W
46	BAU 741	Portoes de São Pedro, Terceira Is., Azores Islands, Portugal	38°39′17″N 027°13′50″W
47	BAU 802	Puertito de Guimar, Tenerife Is., Canary Islands, Spain	28°17′11″N 016°22′48″W
48	BAU 804	Funchal, Madeira Is., Portugal	32°38′22″N 016°55′24″W
49	BAU 805	Ajuy, Fuerteventura Is., Canary Islands, Spain	28°26′90″N 014°09′17″W
50	BAU 806	São Lourenço, Madeira Is., Portugal	32°44′22″N 016°40′40″W
Columbella xiphitella			
51	BAU 1120	Cape Santa Clara, Libreville, Gabon	0°30′18″N 009°19′07″E

2.2. Phylogeographic analyses

Phylogenetic trees for phylogeographic analyses were obtained using Maximum Likelihood (ML) and Bayesian Inference (BI), under a HKY + I+Γ, as the best-fit substitution model indicated by jModelTest2 (Darriba et al., 2012), and rooted using as outgroup a COI sequence of a *C. xiphitella* specimen from Gabon, according to the phylogenetic pattern in Russini et al. (2017). ML analysis was performed using PHYML3.0 (Guindon et al., 2010) (http://www.atgc-montpellier.fr/phyml/), with 1000 bootstrap replicates. BI was performed using MrBayes 3.2.3 (Ronquist and Huelsenbeck, 2003), running two Markov chain Monte Carlo (MCMC) analyses in parallel for 10^7 generations, with a 25% burn-in and sampling every 1000 steps. Using TRACER 1.6 (Rambaut et al., 2014) chains convergence was assumed when the effective sample size values (ESS) were >200 and the potential scale reduction factor values (PSRF) were 1.

The genetic divergence between the resulting clades was calculated using a Kimura two-parameters (K2p) substitution model with the software MEGA 6 (Tamura et al., 2013).

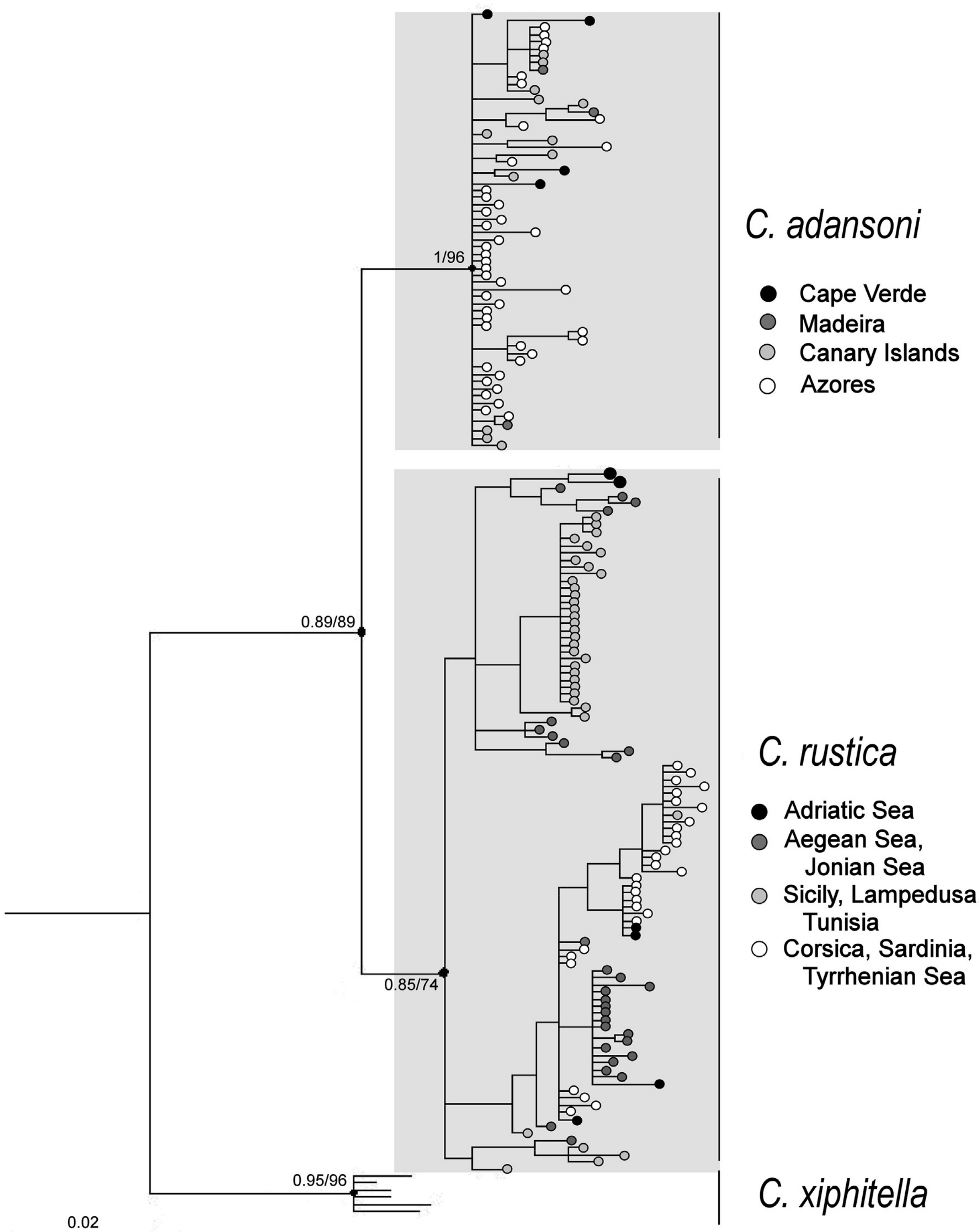


Fig. 2. Bayesian Inference tree based on the COI sequences (HKY + I+Γ model of evolution). Numbers at nodes indicate the Bayesian posterior probability supports (10^7 generations and 25% burnin) and the Maximum Likelihood bootstrap supports (1000 replicates). The colour in each tip-circle (white, ligth grey, dark grey, black) represents the collecting area of the specimen (same as in Figs 3–4). For details on the tip labels see Supplementary Fig. 1 (*C. adansoni*) and Supplementary Fig. 2 (*C. rustica*).

Relationships between haplotypes were investigated for each species using a Median Joining (MJ) approach (Bandelt et al., 1999) as implemented in PopART (http://popart.otago.ac.nz). Phylogenetic network analyses may perform better than tree-based phylogenetic methods when genetic divergence is low, as they allow multi-furcations and reticulate evolution patterns, more congruently with the intraspecific level of analysis (Posada and Crandall, 2001). In particular, MJ combines minimum spanning trees within a single network, and adds to the network median vectors (representing missing intermediates) using a parsimony criterion.

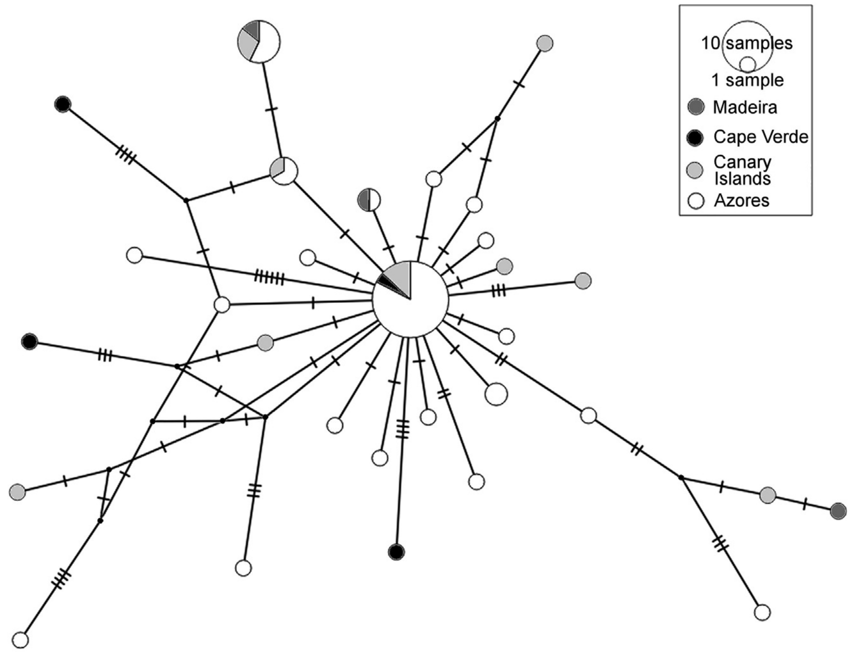


Fig. 3. Median-joining network of *C. adansoni* haplotypes. Each haplotype is represented by a circle. The colour in each circle (white, ligth grey, dark grey, black) represents the collecting archipelago of the haplotype (same as in Fig. 2). The size of each circle is proportional to the frequency of the haplotype.Single nucleotide base changes are indicated by solid bars on lines connecting each haplotype. Small filled circles represent inferred haplotypes that were not found.

2.3. Spatial distribution of genetic diversity

To investigate the spatial distribution of genetic diversity within each species, we carried out both a spatial principal component analysis (sPCA) as implemented in the R package ADEGENET (Jombart, 2008) and an isolation by distance (IBD) analysis using the IBDWebService (Jensen et al., 2005; ver. 3.23 http://ibdws.sdsu.edu/~ibdws/).

Isolation by distance (IBD), as proposed by Wright (1940), is defined as a decrease in the genetic similarity among populations as the geographic distance between them increases (Jensen et al., 2005). To verify the presence of an IBD pattern, a non-parametric Mantel test has been commonly used to test for non-random associations between the two matrices of genetic distances and geographical distances. We used MEGA 6 (Tamura et al., 2013) to build a genetic distance matrix with a K2p nucleotide substitution model, while the geographical distance matrix was created by calculating the shortest marine distance between every two points, using Google Earth 7.1.2.2041. Both matrices were used as input for the IBDWebService. Despite its widespread use, Mantel test has been recently questioned as a realistic approach to identify IBD patterns (e.g.: Meirmans, 2012), as it can be heavily biased by spatial autocorrelation. To avoid misinterpretation of the correlation patterns between genetic diversity and spatial distribution, we integrated IBD with a spatial principal component analysis (sPCA). This spatially explicit approach takes into account at the same time both the genetic variance among studied entities and their spatial autocorrelation (Jombart et al., 2008). The detection of spatial features in the input data is carried out incorporating Moran's *I* statistics (Moran, 1948, 1950) in geo-referenced genetic data. Moran's *I* ranges from -1 to $+1$, where values close to $+1.0$ indicate clustering, while values close to -1.0 indicate dispersion. To define neighbours for calculation of Moran's *I*, a Gabriel graph for individual sample locations was generated. Global and local tests based on Monte Carlo permutations ($N = 99{,}999$) were used to interpret global and local components of sPCA. The presence of a significant global structure can be related to patterns of spatial genetic structure (such as isolation by distance), whereas a local structure would refer to strong differences between local neighbourhoods (repulsion) (Jombart et al., 2008).

2.4. Molecular diversity

The number of haplotypes, nucleotide diversity (π) and haplotype diversity (HD) were calculated for each species using DnaSP 5.10 (Librado and Rozas, 2009) and Arlequin (Excoffier and Lischer, 2010). An AMOVA test (Excoffier et al., 1992) was performed to investigate intra- and interpopulation molecular diversity, on specimens grouped manually according to their geographical origin.

3. Results

3.1. Phylogeographic structure and spatial distribution of genetic diversity

Phylogenetic analyses of the entire dataset produced an identical branching pattern with both ML analysis and BI; here we used as reference the topology obtained by BI as it showed higher statistical supports at nodes (Fig. 2). The two sibling species, *C. adansoni* and *C. rustica* resulted both well supported and reciprocally monophyletic, but they showed different internal branching patterns. In fact, the *C. adansoni* clade displayed a geographically weaker structure compared to the *C. rustica* one, with subclades containing specimens from all sampled areas (Fig. 2, and Suppl. Fig. 1). Instead, the *C. rustica* clade showed a strong geographical structure, with three of the major internal clades, containing specimens from Sicily, Lampedusa Island and Tunisia (with $P = 0.934$), from the Tyrrhenian area (including Corsica, Sardinia, Zannone Island, Palinuro and Naples, with $P = 0.859$), and

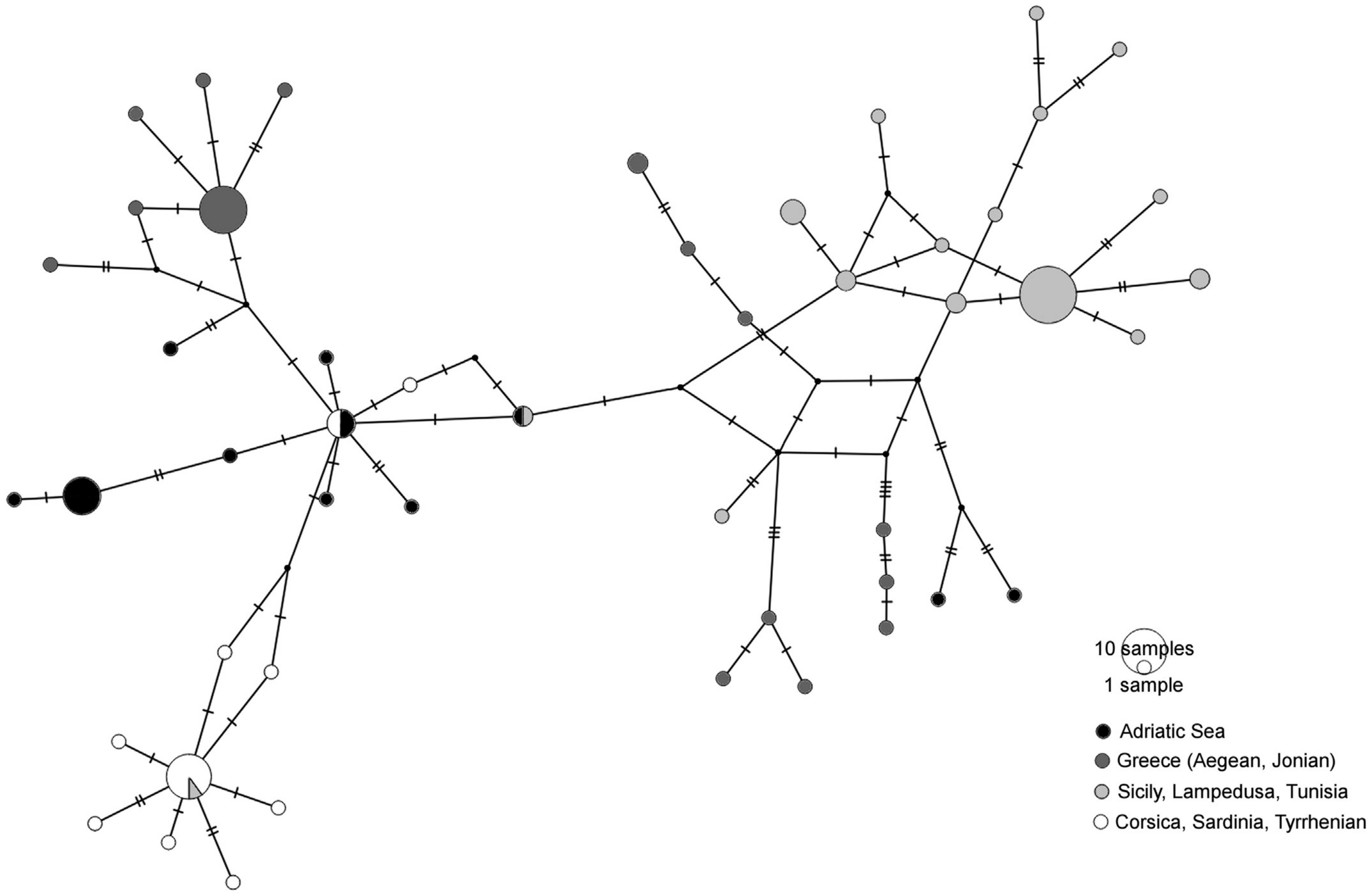


Fig. 4. Median-joining network of *C. rustica* haplotypes. Each haplotype is represented by a circle. The colour in each circle (white, ligth grey, dark grey, black) represents the collecting area of the haplotype (same as in Fig. 2). The size of each circle is proportional to the frequency of the haplotype. Single nucleotide base changes are indicated by solid bars on lines connecting each haplotype. Small filled circles represent inferred haplotypes that were not found.

from Jonian and Aegean Seas (with P = 0.898), respectively (Fig. 2, and Suppl. Fig. 2).

In the MJ network analysis *C. adansoni* (Fig. 3) 50 polymorphic sites defined 30 haplotypes, one of which was widely spread through the surveyed area. Conversely, in *C. rustica* (Fig. 4) 59 polymorphic sites yielded 49 haplotypes: a group of haplotypes from Sicilian sites was connected with haplotypes from Tunisia, and linked by median vectors to haplotypes of Tyrrhenian and Aegean populations.

Between-species genetic divergence ranged from 3.8% (*C. adansoni* - *C. rustica*) to 7% (*C. rustica* - *C. xiphitella*) (Table 2).

For *C. adansoni*, the Mantel's test (Figs Suppl. 3A–D) did not support any correlation between geographical and genetic distance ($0.272 \leq P \geq 0.483$). For *C. rustica*, Mantel's test (Figs Suppl. 4A–D) detected a statistically significant correlation between geographical and genetic distance ($P < 0.01$); the highest value was obtained with logarithmic transformation of both matrices ($r = 0.41$, $R^2 = 0.17$). In *C. rustica*, sPCA detected the presence of a significant global structure (Fig. 5a: $P < 0.001$) and the absence of a local spatial structure. In *C. adansoni*, sPCA did not recognise any genetic spatial structure either global or local (Fig. 5b).

3.2. Molecular diversity

Values of haplotypic diversity, nucleotide diversity, and intraspecific K2p genetic distance are reported for both species in Tables 2 and 3. Nucleotide diversity was higher in *C. rustica* than in *C. adansoni*. According to the AMOVA analysis, in *C. rustica* 74.80% of the variance was inter-populational, with a fixation index Fst = 0.74798 (P = 0.00000). In *C. adansoni* inter-population variance explained only 7.99% of total variance (intra-population variance 92.01% of the total), with a fixation index Fst = 0.07993 (P = 0.03226) (Table 3). Mean intraspecific genetic distance (K2p) was higher in *C. rustica* than in *C. adansoni* (Table 2).

4. Discussion

Although some cases of poecilogony were described for sacoglossan opisthobranch gastropod (e.g. West et al., 1984; Miles and Clark, 2002; Krug, 2007), such developmental plasticity was never observed in other gastropod taxa, for which only comparisons between, at best, sibling species can be drawn. This study addresses patterns of geographic structure and evolutionary history of lineages across a comparable spatial scale in *C. rustica* and *C. adansoni*, two species with contrasting developmental modes, using several approaches.

The analysis of the phylogeographic structure in this pair of sibling species revealed divergent patterns for the two species, congruently with their divergence in life history. Both the phylogenetic trees and the Median-joining networks showed geographically structured relationships in the Mediterranean lecithotrophic *C. rustica*, with many haplotypes clustering geographically. Conversely, the planktotrophic developing *C. adansoni* showed fewer haplotypes (with a star-like pattern of one widely

Table 2
Intra- and interspecific mean genetic divergence (K2p) among the assayed species (standard deviation in parentheses).

	Intraspecific			Interspecific	
	Min	Max	Mean		
C. adansoni	0.000	0.015	0.005 (0.00)		
C. rustica	0.002	0.030	0.020 (0.01)	0.038 (0.00)	
C. xiphitella	0.005	0.016	0.011 (0.00)	0.066 (0.01)	0.070 (0.01)
				C. adansoni	*C. rustica*

distributed haplotype), sometimes shared by sites thousands kilometres apart, suggesting that the pelagic stage of this species is long enough to partly counteract the effects of genetic differentiation, due to selection and/or genetic drift. A number of comparative population genetics studies of marine benthic invertebrates support the hypothesis that species with shorter or no PLD have a lower potential for gene flow and a reduced connectivity, and show higher degrees of spatial population structure relative to planktotrophic species (e.g. Wilke and Davis, 2000; Collin, 2001; Guzmán et al., 2011), with a limited number of exceptions (e.g. Hoskin, 1997; Arndt and Smith, 1998; Kyle and Boulding, 2000; Lee and Boulding, 2009).

C. adansoni, with planktotrophic development, and thus good larval dispersal capacities, showed no statistically significant correlation between genetic and geographic distance, and sPCA did not find any spatial structure either global or local. *C. rustica,* with lecithotrophic development, and thus potentially scarcer dispersal capacities, showed a clear pattern of isolation by distance (IBD) with genetic divergence increasing with geographic distance, which is congruent with the global spatial structure highlighted by sPCA.

Additionally, while *C. rustica* displayed high interpopulational *v.* low intrapopulational variance, in *C. adansoni* the interpopulational variance was remarkably lower than the intrapopulational one.

Weber et al. (2015) explicitly investigated the link between spatial distribution and genetic structure in brooder and broadcaster species of brittle stars belonging to the *Ophioderma longicauda* species complex, highlighting the strong influence of life history traits on connectivity. Also in our case, PLD seems to affect population connectivity, with bearing on phylogeography and genetic diversity of the studied species, although the effects of other factors cannot be ruled out.

According to genetic and paleontological data (Oliverio, 1995), the split of the two sibling species was dated to c. 2 million years ago, i.e. at the onset of the Pleistocene glacial cycles. The loss of planktotrophic development in *C. rustica* that likely accompanied the speciation event (if not actually drove it: Oliverio, 1996) occurred in the mainly Mediterranean species congruently with the more oligotrophic condition of this basin compared to the Atlantic (Pujo-Pay et al., 2011; Tanhua et al., 2013).

However, many more issues still need to be investigated into details, and compared among species, particularly the duration of the competence stage, the effects of different reproductive seasonality, and the relevance of environmental constraints. Additionally, the spatial dimension should be better and more realistically addressed by modelling direction and intensity of the marine currents (e.g. Treml et al., 2008; D'Agostini et al., 2015). The real path for a larva to link two populations might be different from the shortest marine distance (White et al., 2010). Strong currents and oceanographic features like eddies and fronts influence larval dispersal and can well connect two distant sites (Mitarai et al., 2009) as well as rarely allow the exchange of migrants between two populations from two different sites of a oceanographic front (Gilg and Hilbish, 2003). The possibility to include complex circulation dynamics in the analyses of the spatial distribution of genetic diversity might improve our ability to interpret population structure data and strengthen our result's supports. A detailed knowledge of circulation patterns can also be of great help in evaluating the importance of other larval characteristics, such as vertical migratory behaviour, which can affect dispersal by exposing the larvae to differential deep-water currents (White et al., 2010). In more realistic models, extrinsic factors such as the circulation pattern and the environmental conditions interact with intrinsic characteristic of species, including the seasonality and duration of PLD of their larvae and their ecological requirements, in shaping the distribution and connectivity of marine organisms.

Finally, while larval strategies have an important role in the evolutionary history of species (e.g.: Jablonski and Lutz, 1983; Oliverio, 1996), at smaller temporal scales management and conservation can greatly benefit from the understanding of mechanisms underlying population connectivity and patterns of genetic

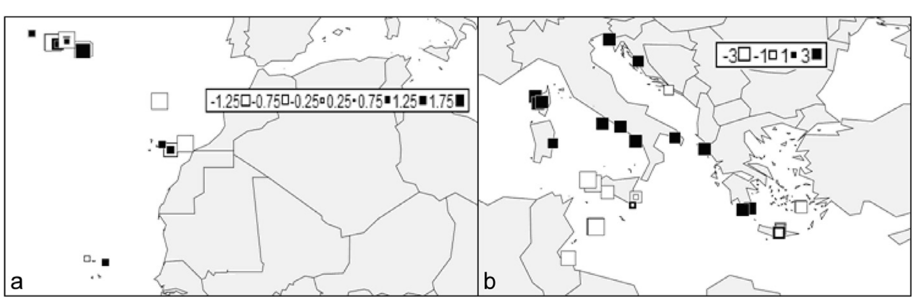


Fig. 5. Spatial distribution of the scores of the first principal component obtained from sPCA for *C. adansoni* (**a**) and *C. rustica* (**b**). Each square corresponds to the score of a haplotypev (positive if black, negative if white) and it is positioned by its spatial coordinates.

Table 3
Molecular diversity in the assayed species.

Species	Haplotypes	Haplotypic diversity Hd	Nucleotidic Diversity π	AMOVA	Fst
C. rustica	49	0.945	0.01146	74.80% interpopulation 25.20% intrapopulation	0.74798
C. adansoni	30	0.852	0.00493	7.99% interpopulation 92.01% intrapopulation	0.07993

structure of the species (Crooks and Sanjayan, 2006; Planes et al., 2009; Craig et al., 2007). Low v. high connectivity species may react differentially to environmental and climate changes; as a mere example, water temperature seems to be crucial to trigger the duration and success of larval stage (Rombough, 1997). It may be argued that the better the spatial genetic structure of a species and the underlying mechanisms are known, the better population response to the change of future years can be predicted. Different larval ecology may affect the success likelihood of invasive alien species, not necessarily favouring planktotrophic developers (e.g.: Chemello and Oliverio, 1997). In the Mediterranean Sea, the distribution of closely related species with different larval development (planktotrophic v. non-planktotrophic) is partitioned in the two major sub-basins (East v. West Mediterranean), resulting in communities (e.g. *Posidonia* meadows) comprising species with different attributes in different areas (Oliverio, 1996, 1997b). Therefore, while designing networks of marine protected areas, the knowledge of the ecological attributes of the communities as a whole will become crucial, also in terms of the variation in larval ecology of the species involved. Invertebrates are numerically and functionally important members of marine benthic communities, and show a vast array of developmental styles, often unknown, and the effect of which are still largely unexplored.

Acknowledgements

Emilio Rolán (Vigo), José Templado (Madrid), Marco Taviani (Bologna), Fabio Crocetta (Napoli), are thanked for help with collecting samples. Three reviewers significantly contributed with their criticisms on a previous version of this paper, to make clearer several points and skip some errors. Work partly supported by the Doctorate School in Evolutionary and Environmental Biology of “Sapienza” University (to GF), by Regione Lazio through “Torno Subito” programme (0031101/15, to VR) and by “Sapienza” University grant (C26A154R28, to MO).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.04.001.

References

Allendorf, F.W., Hohenlohe, P.A., Luikart, G., 2010. Genomics and the future of conservation genetics. Nat. Rev. Genet. 11, 697–709.
Arndt, A., Smith, M.J., 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol. Ecol. 7, 1053–1064.
Ayre, D.J., Minchinton, T.E., Perrin, C., 2009. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Mol. Ecol. 18, 1887–1903.
Bacci, G., 1943. Le capsule ovigere di *Columbella rustica* (L.) e di *Fascicolaria lignaria* (L.). (Prosobranchia Stenoglossa). Boll. Zool. 14 (4–6), 75–81.
Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.
Barbosa, S.S., Klanten, S.O., Puritz, J.B., Toonen, R.J., Byrne, M., 2013. Very fine-scale population genetic structure of sympatric asterinid sea stars with benthic and pelagic larvae: influence of mating system and dispersal potential. Biol. J. Linn. Soc. 108, 821–833.
Berger, E.M., 1973. Gene-enzyme variation in three sympatric species of *Littorina*. Biol. Bull. 145, 83–90.
Bouchet, P., Gofas, S., 2010. *Columbella* Lamarck, 1799. In: MolluscaBase (2016). Accessed through: World Register of Marine Species at. http://www.marinespecies.org/aphiaphp?p%3dtaxdetails%26id%3d137803 (Accessed 1 March 2017).
Chemello, R., Oliverio, M., 1997. Lessepsian migrations: a theoretical “island-jumping” model. Biol. Mar. Medit. 3 (1), 444–446.
Collin, R., 2001. The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Mol. Ecol. 10, 2249–2262.
Cowen, R.K., Lwiza, K.M.M., Sponaugle, S., Paris, C.B., Olson, D.B., 2000. Connectivity of marine populations: open or closed? Science 287 (5454), 857–859.
Cowen, R.K., Sponaugle, S., 2009. Larval dispersal and marine population connectivity. Annu. Rev. Mar. Sci. 1, 443–466. http://dx.doi.org/10.1146/annurev.marine.010908.163757.
Craig, M.T., Eble, J.A., Bowen, B.W., Robertson, D.R., 2007. High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). Mar. Ecol. Progr. Ser. 334, 245–254. http://dx.doi.org/10.3354/meps334245.
Crooks, K.R., Sanjayan, M., 2006. Connectivity conservation: maintaining connections for nature. In: Crooks, K.R., Sanjayan, M. (Eds.), Connectivity Conservation. Cambridge University Press, Cambridge, pp. 1–19.
D'Agostini, A., Gherardi, D.F.M., Pezzi, L.P., 2015. Connectivity of Marine Protected Areas and its relation with total kinetic energy. PLoS One 10 (10), e0139601. http://dx.doi.org/10.1371/journal.pone.0139601.
Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Meth. 9 (8), 772.
Dawson, M.N., Louie, K.D., Barlow, M., Jacobs, D.K., Swift, C.C., 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California transition zone. Mol. Ecol. 11, 1065–1075.
Duffy, J.E., 1993. Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. Mar. Biol. 116, 459–470.
Edmands, S., 2001. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Mol. Ecol. 10, 1743–1750.
Ellingson, R.A., Krug, P.J., 2015. Reduced genetic diversity and increased reproductive isolation follow population-level loss of larval dispersal in a marine gastropod. Evolution 70, 18–37. http://dx.doi.org/10.1111/evo.12830.
Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 35: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567. http://dx.doi.org/10.1111/j.1755-0998.2010.02847.x.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
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.
Franc, A., 1943. Études sur le développement de quelques Prosobranches méditerranéens (Dissertation, Alger).
Funk, W.C., McKay, J.K., Hohenlohe, P.A., Allendorf, F.W., 2012. Harnessing genomics for delineating conservation units. Tr. Ecol. Evol. 27, 489–496.
Gilg, M.R., Hilbish, T.J., 2003. The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. Ecology 84, 2989–2998. http://dx.doi.org/10.1890/02-0498.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. http://dx.doi.org/10.1093/sysbio/syq010.
Guzmán, B.E., Nuñez, J.J., Vejar, A., Barriga, E.H., Gallardo, C.S., 2011. Genetic diversity and population structure of two South American marine gastropods, *Crepipatella dilatata* and *C. fecunda* (Gastropoda: Calyptraeidae): distinct patterns based on developmental mode. It. J. Zool. 78, 444–454. http://dx.doi.org/10.1080/11250003.2011.576403.
Hastings, A., Botsford, L.W., 2006. Persistence of spatial populations depends on returning home. Proc. Natl. Acad. Sci. U. S. A. 103, 6067–6072.
Hellberg, M.E., 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50, 1167–1175.
Hellberg, M.E., 2009. Gene flow and isolation among populations of marine animals. Annu. Rev. Ecol. Evol. Syst. 40, 291–310.
Hoareau, T.B., Boissin, E., Paulay, G., Bruggemann, J.H., 2013. The Southwestern Indian Ocean as a potential marine evolutionary hotspot: perspectives from comparative phylogeography of reef brittle-stars. J. Biogeogr. 40, 2167–2179.
Hoffman, J.I., Clarke, A., Linse, K., Peck, L.S., 2011. Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. Mar. Biol. 158, 287–296.
Hoskin, M.G., 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. Mar. Biol. 127, 647–656.
Hunt, A., 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patiriella calcar* and *P. exigua*. Mar. Ecol. Progr. Ser. 92, 179–186.
Jablonski, D., 1980. Molluscan larval shell morphology ecology and paleontological implications. In: Rhoads, D.C., Lutz, R.A. (Eds.), Skeletal Growth of Aquatic Organisms. Plenum Publishing Corporation, New York, pp. 323–377.
Jablonski, D., Lutz, R.A., 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. Biol. Rev. 58, 21–89. http://dx.doi.org/10.1111/j.1469-185X.1983.tb00380.x.
Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genet. 6, 13–18.
Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. http://dx.doi.org/10.1093/bioinformatics/btn129.
Jombart, T., Devillard, S., Dufour, A.B., Pontier, D., 2008. Revealing cryptic spatial patterns in genetic variability by a new multivariate method. Heredity 101, 92–103. http://dx.doi.org/10.1038/hdy.2008.34.

Jones, G.P., Srinivasan, M., Almany, G.R., 2007. Population connectivity and conservation of marine biodiversity. Oceanography 20 (3), 100–111.
Knowlton, N., Jackson, J.B., 1993. Inbreeding and outbreeding in marine invertebrates. In: Thornhill, N.W. (Ed.), The Natural History of Inbreeding and Outbreeding. University of Chicago Press, Chicago, Illinois, pp. 200–249.
Knudsen, J., 1950. Egg capsules and development of some marine prosobranchs from tropical West Africa. Atlantide Rep. 1, 85–130.
Knudsen, J., 1995. Observations on reproductive strategy and zoogeography of some marine prosobranch gastropods (Mollusca) from the Azores (Supplement 1995). Açoreana 135–158.
Krug, P.J., 2007. Poecilogony and larval ecology in the gastropod genus *Alderia*. Amer. Malacol. Bull. 23, 99–211.
Kyle, C.J., Boulding, E.G., 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar. Biol. 137, 835–845. http://dx.doi.org/10.1007/s002270000412.
Laine, A.L., 2005. Spatial scale of local adaptation in a plant-pathogen metapopulation. J. Evol. Biol. 18, 930–938. http://dx.doi.org/10.1111/j.1420-9101.2005.00933.x.
Lee, H.J., Boulding, E.G., 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Mol. Ecol. 18, 2165–2184. http://dx.doi.org/10.1111/j.1365-294X.2009.04169.x.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452. http://dx.doi.org/10.1093/bioinformatics/btp187.
Lima, G.M., Lutz, R.A., 1990. The relationship of larval shell morphology to mode of development in marine prosobranch gastropods. J. Mar. Biol. Assoc. U. K. 70, 611–637. http://dx.doi.org/10.1017/S0025315400036626.
Lowe, W.H., Allendorf, F.W., 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19, 3038–3051.
Marko, P.B., 2004. 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol. Ecol. 13, 597–611.
Meirmans, P.G., 2012. The trouble with isolation by distance. Mol. Ecol. 21, 2839–2846. http://dx.doi.org/10.1111/j.1365-294X.2012.05578.x.
Miles, C., Clark, K.B., 2002. Comparison of biochemical composition and developmental mode in two populations of *Costasiella* [Opisthobranchia: Ascoglossa (= Sacoglossa)]. J. Mollusc. Stud. 68, 101–109.
Mitarai, S., Siegel, D.A., Watson, J.R., Dong, C., McWilliams, J.C., 2009. Quantifying connectivity in the coastal ocean with application to the Southern California Bight. J. Geophys. Res. 114, C10026. http://dx.doi.org/10.1029/2008JC005166.
Moran, P.A.P., 1948. The interpretation of statistical maps. J. R. Stat. Soc. Ser. B Stat. Methodol. 10 (2), 243–251.
Moran, P.A.P., 1950. Notes on continuous stochastic phenomena. Biometrika 37, 17–23. http://dx.doi.org/10.2307/2332142.
Oliverio, M., 1995. Larval development and allozyme variation in the East Atlantic *Columbella* (Gastropoda: Prosobranchia: Columbellidae). Sci. Mar. 59, 77–86.
Oliverio, M., 1996. Life-histories, speciation, and biodiversity in Mediterranean prosobranch gastropods. Vie Milieu 46 (2), 163–169.
Oliverio, M., 1997a. Global biodiversity and life-history evolution in prosobranchs gastropods. Iberus 16, 73–79.
Oliverio, M., 1997b. Biogeographical patterns in developmental strategies of gastropods from Mediterranean *Posidonia* beds. Boll. Malacol. 32, 79–88.
Oliverio, M., Mariottini, P., 2001. A molecular framework for the phylogeny of *Coralliophila* and related muricoids. J. Mollus. Stud. 67, 215–224. http://dx.doi.org/10.1093/mollus/67.2.215.
Palumbi, S.R., 2003. Population genetics, demographic connectivity,and the design of marine reserves. Ecol. Appl. 13 (1), S146–S158.
Planes, S., Jones, G.P., Thorrold, S.R., 2009. Larval dispersal connects fish populations in a network of marine protected areas. Proc. Natl. Acad. Sci. U.S.A. 106 (14), 5693–5697, 10.1073_pnas.0808007106.
Posada, D., Crandall, K.A., 2001. Intraspecific gene genealogies: trees grafting into networks. Tr. Ecol. Evol. 16, 37–45.
Pujo-Pay, M., Conan, P., Oriol, L., Cornet-Barthaux, V., Falco, C., Ghiglione, J.-F., Goyet, C., Moutin, T., Prieur, L., 2011. Integrated survey of elemental stoichiometry (C, N,P) from the western to eastern Mediterranean Sea. Biogeosciences 8, 883–899. http://dx.doi.org/10.5194/bg-8-883-2011.
Rabinowitz, A., Zeller, K.A., 2010. A range-wide model of landscape connectivity and conservation for the jaguar, *Panthera onca*. Biol. Cons. 143, 939–945.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. Available from: http://beast.bio.ed.ac.uk/Tracer.
Riginos, C., Buckley, Y.M., Blomberg, S.P., Treml, E.A., 2014. Disper- sal capacity predicts both population genetic structure and species richness in reef fishes. Am. Nat. 184, 52–64.
Rolán, E., 2005. Columbellidae (Gastropoda, Neogastropoda) of the gulf of Guinea with the description of eight new species. Iberus 23 (2), 119–156.
Rombough, P.J., 1997. The effects of temperature on embryonic and larval development. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for Freshwater and Marine Fish, vol. 61. Cambridge University Press, pp. 177–223.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180.
Russini, V., Fassio, G., Modica, M.V., deMaintenon, M., Oliverio, M., 2017. An assessment of the genus *Columbella* (Gastropoda: Columbellidae) from eastern Atlantic. Zoosystema 39 (2) in press.
Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Annu. Rev. Mar. Sci. 3, 509–535.
Selkoe, K.A., Toonen, R.J., 2011. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. Mar. Ecol. Progr. Ser. 436, 291–305. http://dx.doi.org/10.3354/meps09238.
Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. Biol. Bull. 216, 373–385.
Shanks, A.L., Grantham, B.A., Carr, M.H., 2003. Propagule dispersal distance and the size and spacing of marine reserves. Ecol. Appl. 13, 159–169.
Sherman, C.D.H., Hunt, A., Ayre, D.J., 2008. Is life history a barrier to dispersal? contrasting patterns of genetic differentiation along an oceanographically complex coast. Biol. J. Linn. Soc. 95, 106–116.
Siegel, D.A., Kinlan, B.P., Gaylord, B., Gaines, S.D., 2003. Lagrangian descriptions of marine larval dispersion. Mar. Ecol. Progr. Ser. 260, 83–96.
Steele, C.A., Baumsteiger, J., Storfer, A., 2009. Influence of life-history variation on the genetic structure of two sympatric salamander taxa. Mol. Ecol. 18, 1629–1639.
Strathmann, M.F., Strathmann, R.R., 2007. An extraordinarily long larval duration of 4.5 years from hatching to metamorphosis for teleplanic veligers of *Fusitriton oregonensis*. Biol. Bull. 213 (2), 152–159.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
Tanhua, T., Hainbucher, D., Schroeder, K., Cardin, V., Álvarez, M., Civitarese, G., 2013. The Mediterranean Sea system: a review and an introduction to the special issue. Ocean. Sci. 9, 789–803. http://dx.doi.org/10.5194/os-9-789-2013.
Tarnowska, K., Krakau, M., Jacobsen, S., Wołowicz, M., Féral, J.-P., Chenuil, A., 2012. Comparative phylogeography of two sister (congeneric) species of cardiid bivalve: strong influence of habitat, life history and post-glacial history. Estuar. Coast. Shelf Sci. 107, 150–158.
Teske, P.R., Zardi, G.I., McQuaid, C.D., Papadopoulos, I., Barker, N.P., Edkins, M.T., Griffiths, C.L., 2007. Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. Mar. Biol. 152, 697–711.
Thorson, G., 1949. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25, 1–45. http://dx.doi.org/10.1111/j.1469-185X.1950.tb00585.x.
Treml, E.A., Halpin, P.N., Urban, D.L., Pratson, L.F., 2008. Modelling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landsc. Ecol. 23, 19–36. http://dx.doi.org/10.1007/s10980-007-9138-y.
Tyler, P.A., Young, C.M., 1999. Reproduction and dispersal at vents and cold seeps. J. Mar. Biol. Ass. U.K. 79, 193–208.
Villanueva, R., Vidal, E.A.G., Fernández-Álvarez, F.Á., Nabhitabhata, J., 2016. Early mode of life and hatchling size in cephalopod molluscs: influence on the species distributional ranges. PLoS One 11 (11), e0165334. http://dx.doi.org/10.1371/journal.pone.0165334.
Weber, A.A.-T., Mérigot, B., Valiere, S., Chenuil, A., 2015. Influence of the larval phase on connectivity: strong differences in the genetic structure of brooders and broadcasters in the *Ophioderma longicauda* species complex. Mol. Ecol. 24, 6080–6094. http://dx.doi.org/10.1111/mec.13456.
Webster, M.S., Marra, P.P., Haig, S.M., Bensch, S., Holmes, R.T., 2002. Links between worlds: unravelling migratory connectivity. Tr. Ecol. Evol. 17, 76–83.
West, H., Harrigan, J., Pierce, S., 1984. Hybridization of two populations of a marine opisthobranch with different developmental patterns. Veliger 26, 199–206.
White, C., Selkoe, K.A., Watson, J., Siegel, D.A., Zacherl, D.C., Toonen, R.J., 2010. Ocean currents help explain population genetic structure. Proc. R. Soc. B 277 (1688), 1685–1694. http://dx.doi.org/10.1098/rspb.2009.2214.
Wilke, T., Davis, G.M., 2000. Intraspecific mitochondrial sequence diversity in *Hydrobia ulvae* and *Hydrobia ventrosa* (Hydrobiidae: Rissooidea: Gastropoda): do their different life histories affect biogeographic patterns and gene flow? Biol. J. Linn. Soc. 70, 89–105 Wright S: Breeding structure of populations in relation to speciation. Am. Nat. 1940, 74: 232-248. 10.1086/280891.
Wright, S., 1940. Breeding structure of populations in relation to speciation. Am. Nat. 74, 232–248. http://dx.doi.org/10.1086/280891.

An assessment of the genus *Columbella* Lamarck, 1799 (Gastropoda: Columbellidae) from eastern Atlantic

Valeria RUSSINI
Giulia FASSIO
Maria Vittoria MODICA
Department of Biology and Biotechnologies "Charles Darwin",
Sapienza University of Rome, Viale dell'Università 32, I-00185 Roma (Italy)

Marta J. deMAINTENON
University of Hawaii at Hilo, 200 W. Kawili Street, Hilo, HI 96720 (United States)

Marco OLIVERIO
Department of Biology and Biotechnologies "Charles Darwin",
Sapienza University of Rome, Viale dell'Università 32, I-00185 Roma (Italy)
marco.oliverio@uniroma1.it
(corresponding author)

Published on 30 June 2017

urn:lsid:zoobank.org:pub:57395522-BE12-40AC-B504-C448A31F8A8D

Russini V., Fassio G., Modica M. V., deMaintenon M. J. & Oliverio M. 2017. — An assessment of the genus *Columbella* Lamarck, 1799 (Gastropoda: Columbellidae) from eastern Atlantic. *Zoosystema* 39 (2): 197-212. https://doi.org/10.5252/z2017n2a2

ABSTRACT

Three species of the neogastropod genus *Columbella* Lamarck, 1799 are recognised from the northeastern Atlantic and the Mediterranean. One is the common Mediterranean *C. rustica* (Linnaeus, 1758), with paucispiral protoconch, extending its range in the Atlantic South to Senegal and North to Portugal. *Columbella adansoni* Menke, 1853, with multispiral protoconch is restricted to the Macaronesian archipelagoes. A third species, also with multispiral protoconch, from West Africa is recognised through molecular methods, and the name *C. xiphitella* Duclos, 1840 is employed by correcting the original erroneous locality ("Californie") to Gabon. Except for protoconch features, no major morphological characters are available to separate the three species; however diagnostic species-level differences in specific positions in the cytochrome c oxidase I (COI) sequences are present between all three species.

KEY WORDS
Columbellidae,
East Atlantic,
Mediterranean,
lectotypification,
DNA-Barcoding.

RÉSUMÉ

Étude du genre Columbella *Lamarck, 1799 (Gastropoda: Columbellidae) dans l'Est de l'océan Atlantique.*
Trois espèces du genre de néogastropode *Columbella* Lamarck, 1799 sont reconnues dans le nord est de l'Atlantique et en Méditerranée. L'une est courante en Méditerranée, *C. rustica* (Linnaeus, 1758), au protoconche paucispiralé : son aire de répartition s'étend en Atlantique du Sénégal au nord du Portugal. *Columbella adansoni* Menke, 1853, au protoconche multispiralé, se limite aux archipels Macaronésiens. Une troisième espèce, caractérisée également par un protoconche multispiralé, est originaire d'Afrique de l'Ouest : elle est reconnue par des méthodes moléculaires ; le nom de *C. xiphitella* Duclos, 1840 lui est attribué après correction de la localité originale erronée (« Californie ») en Gabon. Mis à part l'aspect du protoconche, aucun caractère morphologique majeur ne permet de séparer les trois espèces ; cependant des positions précises dans les séquences du cytochrome c oxidase I (COI) présentent des différences supportant des diagnoses spécifiques.

MOTS CLÉS
Columbellidae,
Atlantique de l'est,
Méditerranée,
lectotypification,
DNA-Barcoding.

INTRODUCTION

Columbella Lamarck, 1799 s.s. (type species *Voluta mercatoria* Linnaeus, 1758) is a genus of columbellid neogastropods (dove shells) including 17 recognised species, mostly from tropical America and the East Atlantic/Mediterranean (WoRMS: Bouchet & Gofas 2015). Based on Moolenbeek & Hoenselaar (1991), Oliverio (1995), Rolán (2005), and Rolán & Ryall (1999), two species are currently recorded in the eastern Atlantic and the Mediterranean Sea: *Columbella rustica* (Linnaeus, 1758), ranging over the entire Mediterranean Sea, and extending into the neighbouring Atlantic southward to Senegal, and northward to Portugal (it is absent in Galicia); and *Columbella adansoni* Menke, 1853, described from Cape Verde islands, and assumed to occur across Macaronesia, from the Azores to the Canary Islands, and along the West African coasts from Ghana to Angola (Oliverio 1995; Rolán & Ryall 1999; Rolán 2005). *Columbella rustica* has a paucispiral protoconch, indicating non-planktotrophic development (lecithotrophic, possibly entirely or mostly intracapsular), whereas *Columbella adansoni* has a multispiral protoconch, indicating planktotrophic larval development. This is the only consistent morphological diagnostic feature for the two species, which are otherwise quite variable in shell sculpture, colour and pattern. Preliminary to a study of the bearing of different larval developmental strategies on the genetic structure of populations (Modica *et al.* 2017), we decided to assay samples of *Columbella* from the eastern Atlantic and the Mediterranean to test the currently accepted species boundaries by molecular data. Therefore, we examined specimens collected from localities spanning as much as possible the known range for the genus in the eastern Atlantic. As a result, a third species of *Columbella* was discovered.

MATERIAL AND METHODS

Sampling locality data (Fig. 1), Identification (ID) catalogue numbers of the vouchers, and GenBank accession numbers are reported in Table 1. A total of 29 specimens from the East Atlantic and the Mediterranean were assayed. Specimens were sampled by SCUBA or snorkelling, and fixed in 95 to 100% ethanol. Vouchers are stored in the malacological collection at Department of Biology and Biotechnologies "Charles Darwin" ("La Sapienza" University of Rome) under BAU ID numbers and at Muséum national d'Histoire naturelle (Paris) under MNHN ID numbers. Genomic DNA was extracted using a proteinase K-phenol-chloroform protocol (Oliverio & Mariottini 2001). The DNA-barcode fragment of the mitochondrial cytochrome c oxidase I (COI) and part of the 16S rRNA were amplified by PCR using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) and 16SA (Palumbi *et al.* 2002) and CGLeuUUR (Hayashi 2003), respectively. For some crucial specimens from West Africa, fixed in alcohol but thereafter preserved dried, which were unsuccessfully assayed with the pair HCO2198-LCO1490, we employed HCO2198 with the primer mlCOIint-F (5'-GGWACWGGWTGAACWGTWTAYCCYCC-3') designed to amplify a shorter fragment (*c.* 300 bp) and employed in metabarcoding works (Leray *et al.* 2013). PCR amplifications were performed with the following conditions: initial denaturation of 5' at 94°C, 35 amplification cycles (30"/94°C, 40"/48-52°C, 50"/72°C), followed by a final phase of 7' at 72°C. PCR products were purified by ExoSAP-IT protocol (USB Corporation, Ohio, USA) and Sanger sequenced by Macrogen Inc. (The Netherlands). Forward and reverse sequences were assembled, checked for contamination and edited with Geneious 4.8.5 (Drummond *et al.* 2009).

Species delimitation in Columbellidae Swainson, 1840

A total of 106 COI sequences from columbellid specimens ascribed to the genera *Alia* H. Adams & A. Adams, 1853, *Amphissa* H. Adams & A. Adams, 1853, *Euplica* Dall, 1889, *Graphicomassa* Iredale, 1929, *Indomitrella* Oostingh, 1940, *Mitrella* Risso, 1826, *Pyrene* Röding, 1798, *Sulcomitrella* Kuroda, Habe & Oyama, 1971 and *Zafra* A. Adams, 1860 (plus some labelled as "columbellid indet.") were either provided by Nicolas Puillandre (ID MNHN-IM) or were retrieved from the GenBank (see Table 4). Sequences from *Cancellopollia* sp. (Gastropoda, Buccinoidea, Buccinidae) (EU015666.1; voucher MNHN-IM-2009-17854), and *Pisania striata* Duclos, 1840 (MNHN-IM-2009-30664, Gastropoda, Buccinoidea, Buccinidae) were retrieved from Genbank to be used as outgroups. COI sequences were manually aligned and checked for stop codons; 16S sequences were aligned using MAFFT 7 (Katoh *et al.* 2002), using the Q-INS-i algorithm (Katoh & Toh 2008), which accounts for secondary structures. Highly variable regions, resulting in gap-rich fragments with ambiguous alignment, were discarded using Gblocks 0.91b (Castresana 2000). All alignments are available from the authors on request.

To define species, we used Automatic Barcode Gap Discovery (ABGD, available at http://wwwabi.snv.jussieu.fr/public/abgd/), a distance-based method designed to detect the so-called "barcode gap" in the distribution of pairwise distances estimated in a COI alignment (Puillandre *et al.* 2012a, b), and the criteria of divergence and reciprocal monophyly (Knowlton 2000; Wheeler & Meier 2000; Reid *et al.* 2006; Malaquias & Reid 2009). The COI sequence alignments were processed in ABGD (excluding the outgroups) using the Kimura-2-parameter (K2p) model and the following settings: a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 25 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 0.1.

We ran ABGD on the whole columbellid dataset of 136 COI sequences, to define partition scheme(s) based on distance distribution. Then, species hypotheses as derived from ABGD were tested against taxonomic recognition for the assayed specimens and for phylogenetic congruence. Phylogenetic analyses of the COI, 16S and combined sequence alignments were conducted using Maximum likelihood (ML:

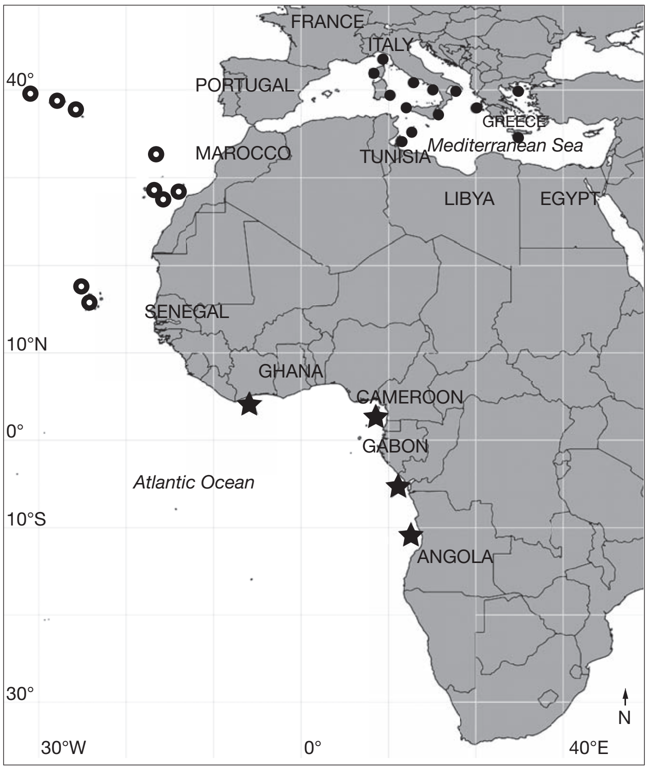


Fig. 1. — Map of the collecting sites (for details see Table 1). Symbols: ●, *Columbella rustica* (Linnaeus, 1758); ○, *Columbella adansoni* Menke, 1853; ★, *Columbella xiphitella* Duclos, 1840.

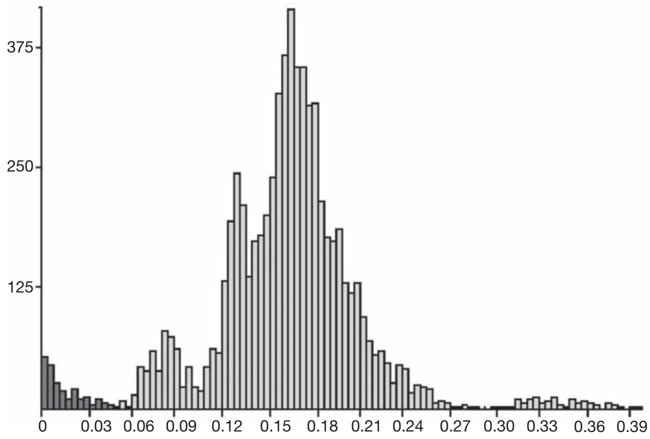


Fig. 2. — Histogram of the distribution of the pairwise estimated genetic distances (K2p) in intraspecific (left, dark grey) and interspecific (right, light grey) comparisons among Columbellidae Swainson, 1840.

with 1000 bootstrap replicates) by PhyML3.0 (http://www.atgc-montpellier.fr/phyml/) and Bayesian inference (BI: four-chain Markov chain Monte Carlo (MCMC) analysis, run twice in parallel for 10^7 generations; trees sampled every 1000 generations, burn-in 2500) by MrBayes 3.2.3 on the XSEDE resources on CIPRES Science Gateway V.3.3 portal (https://www.phylo.org/), both with the HKY + I + G (Hasegawa *et al.* 1985) nucleotide substitution model, as selected by jModelTest2. Same analyses (ABGD, ML and BI) were performed on a reduced dataset including sequences from the eastern Atlantic specimens (including full length and shorter COI sequences), sequences from *Columbella mercatoria* (Linnaeus, 1758) (type species of the genus *Columbella*) and *Columbella major* Sowerby, 1832, while those from *Euplica turturina* (Lamarck, 1822) (JQ950207.1 and JQ950143.1, voucher MNHN-IM-2007-33524) were used as outgroup.

Abbreviations

ABGD	Automatic Barcode Gap Discovery;
ICZN	International Commission on Zoological Nomenclature;
sh	shell(s).

Institutions

MNHN	Muséum national d'Histoire naturelle, Paris;
SMF	Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt.

RESULTS

For the eastern Atlantic/Mediterranean *Columbella* specimens, a total of 14 specimens from the Mediterranean, nine specimens from the Macaronesia, six specimens from Gabon, three from Ghana and one each from Angola and São Tomé, yielded full length 16S (723bp). Full length COI (658bp) were obtained from 14 specimens from the Mediterranean, nine specimens from Macaronesia, six specimens from Gabon; shorter COI sequences (288bp) were obtained from two specimens from Ghana and one specimen from Angola.

Species delimitation in worldwide Columbellidae

The 30 recursive steps in the ABGD analysis of the COI alignment converged toward a 46-species partition scheme, with the corresponding 46 species hypotheses largely congruent with the *a priori* morphological identification of the worldwide columbellid specimens included (Table 4). Accordingly, the intraspecific genetic divergence estimated on the COI dataset ranged from 0 to 5%, the interspecific ones from 5 to 30% (Fig. 2: K2p matrices available from the authors). ML and BI phylogenetic analyses of the same dataset recovered all 25 species with multiple specimens as monophyletic with very high bootstrap (> 95%) and BI (> 0.99) support.

Species delimitation in eastern Atlantic *Columbella*

The 658bp COI sequences of the eastern Atlantic/Mediterranean *Columbella* were split into three groups: 1) the Mediterranean specimens (corresponding to *Columbella rustica*); 2) the Macaronesian specimens (corresponding to *C. adansoni*); and 3) the specimens from Gabon. The pattern was exactly the same when the shorter sequences of specimens from Ghana and Angola were included.

Intraspecific distance ranged 0-1.5% in *C. adansoni*, 0.2-3% in *C. rustica*, and 0.5-1.6% in the West African species (see Table 2 for K2p indices). The estimated genetic distance was 4% between *C. rustica* and *C. adansoni*, and 7% between the new West African species and the other two (Table 2).

TABLE 1. — List of the examined material with ID numbers for voucher lots (**BAU**, Department of Biology and Biodiversity, Sapienza University of Rome; **MNHN**, Museum national d'Histoire naturelle, Paris), data on collecting sites (in parentheses the number used in Figure 1), and GenBank accession numbers for the sequences.

ID	Site	Coordinates	Accession numbers	
			COI	16S
Columbella rustica (Linnaeus, 1758)				
BAU 1608	(1) Galeria, Corsica, France: 1-5 m depth	42°25'16"N, 8°37'26"E	KX639980	
BAU 1670	(2) S. Isidoro, Italy: 1-5 m depth	40°12'15"N, 17°55'12"E	KX639897	
BAU 1755	(3) Palinuro, Italy: 1-7 m depth	40°01'53"N, 15°16'07"E	KX639898	
BAU 1779	(4) Cape Tenafo, Greece: 1 m depth	36°23'07"N, 22°28'58"E	KX639914	KX664064 KX664065 KX664066
BAU 1794	(5) Sidi Jmour, Djerba, Tunisia: 0-1 m depth	33°49'53"N, 10°44'50"E	KX639919	
BAU 807	(6) Ognina Cuba, Sicily, Italy: 0-1 m depth	36°58'20"N, 15°14'55"E	KX639923 KX639925	
BAU 811	(7) Giraglia, Corsica, France: 0-1 m depth	43°00'37"N, 009°25'27"E	KX639976	KX664073 KX664074 KX664075
BAU 816	(8) Isola dei conigli, Lampedusa, Italy: 0-2 m depth	35°30'35"N, 12°33'27"E	KX639933	
BAU 818	(9) Marsala, Sicily, Italy: 0-1 m depth	37°47'32"N, 12°25'50"E	KX639940	KX664076 KX664077 KX664078
BAU 819	(10) Agios Georgos, Corfù, Greece: 1-3 m depth	39°43'07"N, 19°39'44"E	KX639946	
BAU 822	(11) Agia Pelagia, Crete, Greece: 0-3 m depth	35°24'25.6"N, 25°01'05.5"E	KX639959	
BAU 829	(12) Zannone Island, Italy: 0-10 m depth	40°58'10"N, 13°02'44"E	KX639983	
BAU 831	(13) Arbatax, Sardinia, Italy: 0-12 m depth	39°55'19.0"N, 9°42'54.9"E	KX639987	
Columbella adansoni Menke, 1853				
BAU 1123	(14) Mindelo, São Vicente, Cape Verde: intertidal	16°54'08"N, 24°59'51"W	KX639833	KX664059
BAU 1124	(15) Arguineguin, Gran Canaria, Canary Islands: 0-1 m depth	27°45'18"N, 15°41'04"W	KX639835	
BAU 1694	(16) Sal Rei, Boavista, Cape Verde: intertidal	16°11'5.18"N, 22°55'26.70"W	KX639841	KX664061 KX664062 KX664063
BAU 708	(17) Caloura, São Miguel, Azores: 0-3 m depth	37°42'26.8"N, 25°30'16.4"W	KX639851	
BAU 716	(18) Lajes, Pico, Azores: 0-2 m depth	38°23'05.7"N, 28°15'04.2"W	KX639859	KX664067 KX664068 KX664069
BAU 718	(19) Santa Cruz, Flores, Azores: 0-2 m depth	39°27'07.3"N, 31°07'26.6"W	KX639867	
BAU 802	(20) Puertito de Guimar, Tenerife, Canary Islands: 1-2 m depth	28°17'11"N, 16°22'48"W	KX639885	
BAU 804	(21) Funchal, Madeira: 1-2 m depth	32°38'22"N, 16°55'24"W	KX639888	
BAU 805	(22) Ajuy, Fuerteventura, Canary Islands: 0-1 m depth	28°24'14"N, 14°09'20"W	KX639890	KX664070 KX664071 KX664072
Columbella xiphitella Duclos, 1840				
BAU 1120 MNHN-IM-2000-32497/32498	(23) Cape Santa Clara, Libreville, Gabon: intertidal to 1 m depth	0°30'18"N, 9°19'07"E	KX639827 KX639828 KX639829 KX639830 KX639831 KX639832	KX664053 KX664054 KX664055 KX664056 KX664057 KX664058
BAU 1118	(24) Praia da Corimba, Luanda, Angola: dredged in c. 20 m depth	8°51'S, 13°10'E	KY464898	KX664049
BAU 1119	(25) Miemia, Ghana: 1-10 m depth	4°47'39"N, 2°10'15"W	KY464900 KY464899	KX664050 KX664051 KX664052
BAU 1693	(26) Lagoa Azul, São Tomé: 1-10 m depth	0°24'22"N, 6°36'29"E		KX664060
Columbella major Sowerby, 1832				
184659143	Venado Is., Panama.		KY464894	KY464896
Columbella mercatoria (Linnaeus, 1758)				
184659120	Guadeloupe		KY464895	KY464897
Euplica turturina (Lamarck, 1822)				
MNHN-IM-2007-33524	Vanuatu, SW Tutuba Is., SANTO 2006 Stn. NR04	15°34'59.52"S, 167°15'23.7"E	JQ950207.1	JQ950143.1

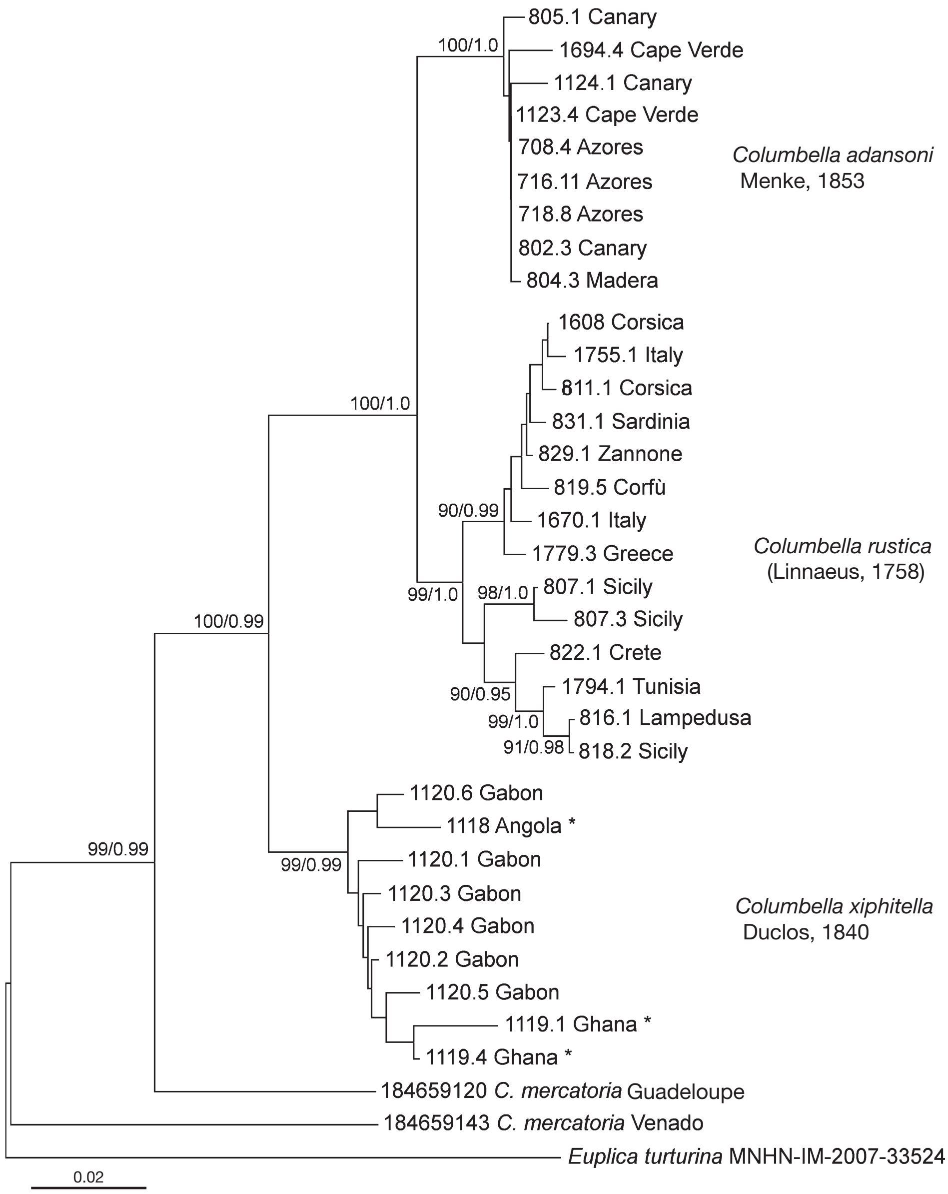


Fig. 3. — ML tree based on the COI dataset (HKY + I + G model of evolution). Numbers at nodes indicate the support by BI Bps (10^7 generations and 25% burnin) and ML bs (1000 replicates). Asterisks indicate shorter sequences (288 bp).

Table 2. — K2p genetic distance between East Atlantic and Mediterranean species of *Columbella* (standard deviation in parentheses).

	intraspecific			interspecific	
	min	max	mean		
C. adansoni Menke, 1853	0.000	0.015	0.005 (0.00)		
C. rustica (Linnaeus, 1758)	0.002	0.030	0.020 (0.01)	0.04 (0.00)	
C. xiphitella Duclos, 1840	0.005	0.016	0.011 (0.00)	0.07 (0.01)	0.07 (0.01)
				C. adansoni	*C. rustica*

Table 3. — Autapomorphic (diagnostic) position in the COI sequences of the three species.

species	Diagnostic positions
C. adansoni Menke, 1853	61 [G], 91 [G], 160 [C], 181 [T], 352 [C], 549 [A], 586 [T].
C. rustica (Linnaeus, 1758)	238 [C], 310 [T], 447 [G].
C. xiphitella Duclos, 1840	34 [T], 55 [T], 78 [G], 100 [T], 115 [T], 117 [A], 130 [A], 133 [C/G], 178 [C], 309 [C], 346 [C], 385 [T], 430 [C], 463 [T], 472 [G], 565 [T], 598 [T], 619 [T].

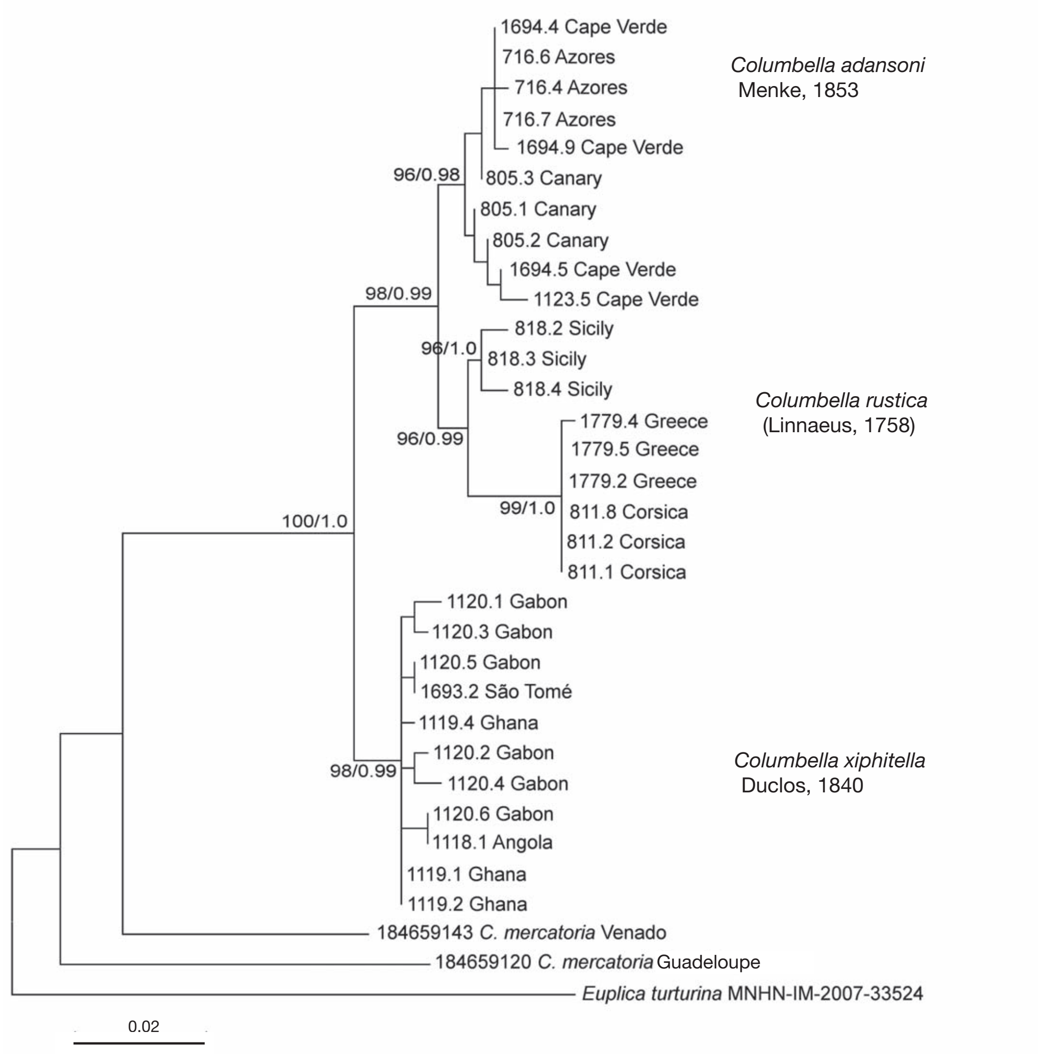


FIG. 4. — ML tree based on the 16S dataset (HKY+I+G model of evolution). Numbers at nodes indicate the support by BI Bps (10^7 generations and 25% burn in) and ML bs (1000 replicates).

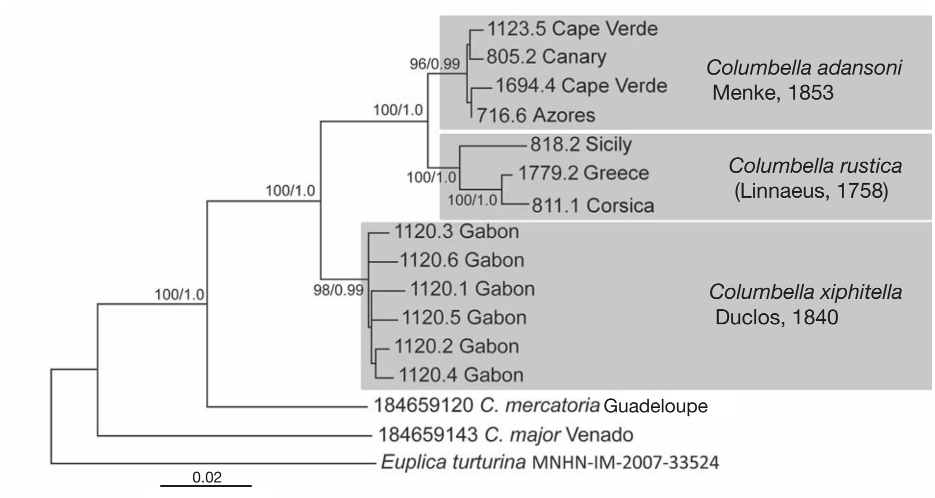


FIG. 5. — ML tree based on the combined COI-16S dataset (HKY+I+G model of evolution). Numbers at nodes indicate the support by BI Bps (10^7 generations and 25% burn in) and ML bs (1000 replicates).

All phylogenetic analyses (ML and BI) of the single gene (16S, COI: including shorter sequences) and of the combined datasets of the eastern Atlantic/Mediterranean *Columbella* retrieved the same topology, with the sequences corresponding to the species hypotheses of ABGD grouped as reciprocally monophyletic clades with high bootstrap values and posterior probabilities (ML bootstrap ≥ 96%, BI support ≥ 0.99: Figs 3-5). In all trees, *C. adansoni* was restricted to the Macaronesian specimens, *C. rustica* to the Mediterranean specimens, and the African specimens comprised a third lineage. The pair including *C. adansoni*/*C. rustica* (ML bootstrap ≥ 98%, BI support ≥ 0.99) was the sister to the African species, which according to the phylogenetic patterns from COI and 16S, included samples from Ghana, São Tomé, Gabon and Angola. Autapomorphic (diagnostic) nucleotides were scored for each species by comparing their COI sequences and are reported in Table 3.

SYSTEMATICS

Family Columbellidae Swainson, 1840
Genus *Columbella* Lamarck, 1799

Type species. — *Voluta mercatoria* Linnaeus, 1758, by monotypy.

Remark

The list of available names for eastern Atlantic *Columbella* is rather long. According to Tryon (1883) and updated with more recent works (e.g., Moolenbeek & Hoenselaar 1991; Bouchet & Gofas 2010; Monsecour & Gofas 2010a, b), we have scored 26 nominal taxa (some under the incorrect subsequent spelling *Colombella*) referable to the *Columbella rustica* complex. All nominal taxa with an explicit Macaronesian type locality can be ascribed to *C. adansoni*: *Columbella adansoni* Menke, 1853, *C. rufa* Menke, 1853, *C. rustica* var. *azorica* Drouët, 1858, *C. striata* var. *minor* Dautzenberg, 1900. All nominal taxa with an explicit type locality from Senegal to Mediterranean (where a single species is known) and/or with a paucispiral protoconch are easily ascribed to *Columbella rustica*. This is the case of *Voluta rustica* Linnaeus, 1758, *C. rustica* var. *elongata* Philippi, 1836, *C. spongiarum* Duclos, 1840, *C. striata* Duclos, 1840, *C. fustigata* Kiener, 1841, *C. striata* Duclos *in* Chenu, 1846, *C. simpronia* Duclos *in* Chenu, 1846, *C. rustica* var. *cuneatiformis* Pallary, 1900, *C. rustica* var. *lutea* Pallary, 1900, *C. rustica* var. *minor* Pallary, 1900, *C. rustica* var. *obesula* Pallary, 1900. The other synonymies currently implemented in WoRMS for this complex are almost all accepted (where necessary by correcting or imposing Mediterranean as type locality, see below) since they maintain stability of current usage, with two exceptions: *C. xiphitella* Duclos, 1840 and *C. nucleus* Kiener, 1841. Among ten potential syntypes of the latter at MHNG, eight have eroded apices, while two have protoconchs partly eroded but clearly multispiral; if we imposed a Macaronesia type locality, this would make *C. nucleus* a senior synonym of *C. adansoni* (which has been the accepted valid name for the Macaronesian species for the last 25 years: Moolenbeek & Hoenselaar, 1991). The same holds for *C. xiphitella* Duclos, 1840: two of the 16 syntypes at MNHN have clearly multispiral protoconchs, the locality indicated ("Californie") is clearly erroneous, and imposing a Macaronesian type locality would make *C. xiphitella* also a senior synonym of *C. adansoni*. Therefore, to preserve nomenclatural stability in this group, we have decided to impose as first reviewers, "Gabon" as type locality to both *C. xiphitella* Duclos, 1840 and *C. nucleus* Kiener, 1841.

Columbella rustica (Linnaeus, 1758)
(Figs 6A, B; 7A-D; 8C)

Voluta rustica Linnaeus, 1758: 731.

Columbella reticulata Lamarck, 1822: 295.

Columbella gualteriana Risso, 1826: 206, n°533.

Columbella rustica var. *elongata* Philippi, 1836: 228.

Colombella tumida Duclos, 1840: pl. 1, figs 13, 14.

Colombella spongiarum Duclos, 1840: pl. 3, figs 13-16.

Columbella striata Duclos, 1840: pl. 6, figs 5-8 (not Menke 1829).

Columbella ambigua Kiener, 1840: 11, pl. 2, fig. 3 [note: plate issued in 1840].

Columbella fustigata Kiener, 1841: 20-21, pl. 5, fig. 3.

Columbella modesta Kiener, 1841: 22, pl. 11, fig. 2.

Colombella aureola Duclos *in* Chenu, 1846: pl. 6, figs 17, 18.

Colombella simpronia Duclos *in* Chenu, 1846: pl. 15, figs 19, 20.

Colombella vestalia Duclos *in* Chenu, 1846: pl. 15, figs 15, 16.

Colombella zulmis Duclos *in* Chenu, 1848: pl. 24, figs 21, 22.

Columbella rustica var. *cuneatiformis* Pallary, 1900: 278, pl. 6, fig. 17.

Columbella rustica var. *lutea* Pallary, 1900: 278.

Columbella rustica var. *minor* Pallary, 1900: 277.

Columbella rustica var. *obesula* Pallary, 1900: 278, pl. 6, fig. 18.

Type material. — *Voluta rustica*: 6 sh in the Linnaean Society (LSL.348 [Dance label image ref: P-Z 0010728] http://linnean-online.org/17388/). — Type locality: Mediterranean.
Columbella reticulata: 5 probable syntypes MHNG-MOLL-92487. — Type locality: Mediterranean (imposed herein, ICZN 1999: rec. 76A.1.4).
Columbella gualteriana: lectotype (Arnaud 1978) MNHN-IM-2000-6899. — Type locality: Mediterranean (imposed herein, ICZN 1999: rec. 76A.1.4).
Columbella rustica var. *elongata*: lectotype ZMB 13.994, 2 paralectotypes ZMB 112.717. — Type locality: Palermo (Sicily).
Colombella tumida: 2 syntypes MNHN-IM-2000-6373. — Type locality: "China", erroneous, corrected to Mediterranean (ICZN 1999: rec. 76.A.2).
Colombella spongiarum: 2 syntypes, MNHN-IM-2000-6385. — Type locality: Senegal.
Columbella striata: syntypes, 15 sh without locality label

MNHN-IM-2000-6381, and 5 sh from Senegal MNHN-IM-2000-6382. — Type locality: Senegal.
Columbella ambigua: 6 syntypes MNHN-IM-2000-6935. — Type locality: "Asia", erroneous, corrected to Mediterranean (ICZN 1999: rec. 76.A.2).
Columbella fustigata: 7 syntypes MNHN-IM-2000-6904. — Type locality: "Iles Saintes" (Îles des Saintes, Antilles), erroneous, corrected to Mediterranean (ICZN 1999: rec. 76.A.2).
Columbella modesta: MHNG-MOLL-95504 (5 probably not types from Delessert coll. and not "Mus coll" as in description). — Type locality: Mediterranean (imposed herein, ICZN 1999: rec. 76A.1.4).
Colombella aureola: 1 shell MNHN-IM-2000-6346). — Type locality: "Californie", erroneous, corrected to Mediterranean (ICZN 1999: rec. 76.A.2).
Colombella simpronia: 4 syntypes MNHN-IM-2000-6389. — Type locality: Mediterranean.
Colombella vestalia: Not found, not present in MNHN. — Type locality: Mediterranean (imposed herein, ICZN 1999: rec. 76A.1.4).
Colombella zulmis: MNHN-IM-2000-9609. — Type locality: "China", erroneous, corrected to Mediterranean (ICZN 1999: rec. 76.A.2).
Columbella rustica var. *cuneatiformis*: not found at MNHN. — Type locality: Oran, Algeria.
Columbella rustica var. *lutea*: not found at MNHN. — Type locality: Oran, Algeria.
Columbella rustica var. *minor*: not found at MNHN. — Type locality: Oran, Algeria.
Columbella rustica var. *obesula*: not found at MNHN. — Type locality: Oran, Algeria.

Distribution. — According to the present data, *Columbella rustica* ranges throughout the entire Mediterranean Sea, and extends in the Atlantic South to Senegal, and North to Portugal.

Diagnosis. — Shell of medium size for the family 12-20 mm long, biconic/strombiform.
Protoconch of 1.5-1.6 smooth, convex whorls; protoconch-teleoconch boundary marked by a slightly opisthocline scar.
Teleoconch of 7-9 almost straight-sided whorls, penultimate whorl slightly convex, body whorl rounded and inflated, about ⅔ to ¾ shell length.
Sculpture of nodulose axial ridges on the first whorls, fading after 2-3 whorls, and very weak, irregular spiral striae. Aperture narrow, elongate and sinuous.
Outer lip angulate posteriorly in some, thickened, especially medially, with 13-16 denticles, and rust coloured markings between denticles. Columellar wall with two weak ridges medially; parietal wall with 5-7 denticles anteriorly, sometimes with rust coloured markings between. Siphonal canal open.
Colour very variable, with white-whitish background and yellow, orange, brown, grey or black irregular spots, sometimes arranged into axial flames or sinuous bands.
Periostracum thin, brown.
Animal with whitish to yellowish background and tawny-orange spots, very dense on propodium, head and mantle; tip of cephalic tentacles white; siphon grey. Radula rachiglossate, with central tooth reduced to a slightly arched plate with no cusps. One pair of massive lateral teeth with a small, basal, outer cusp and a tall, sinuous inner primary cusp with three secondary cusps along the posterior edge: a narrow, pointed distal cusp, a flat central cusp slightly enlarged at the base, and a quadrangular and apically curved basal cusp.

Remarks

We correct herein (ICZN 1999: rec. 76.A.2) to "Mediterranean" the evidently erroneous localities indicated for *Colombella tumida*, *Columbella ambigua*, *Columbella fustigata*, *Colombella aurola*, *Colombella zulmis*; and impose (ICZN 1999: rec. 76A.1.4) "Mediterranean" for *Colombella vestalia*, *Columbella modesta*, *Columbella reticulata*, *Columbella gualteriana*. The five possible syntypes of *Columbella reticulata* Lamarck (MHNG-MOLL-92487, ex Delessert collection) bear "Bresil" as locality, quite probably a posthumous erroneous labelling.

Very variable in coloration, but also in size, with some populations of very small adult size (12 mm) and others attaining much larger length (20 mm).

Franc (1943) described the egg capsules and embryos of *C. rustica*: the capsules contained 39-57 eggs, 250-280 µm in diameter, of which most were nurse eggs to nourish the 1-2 developing embryos (shell length at hatching 660-850 µm). See also Bandel (1975) for a description of the protoconch in specimens from Banyuls. Pelorce & Boyer (2005: fig. 11) described samples from Central Senegal as 10-14 mm long, with paucispiral protoconch of 1.5-2 whorls, the animal milky white or cream with amber-brown speckles, which matches remarkably the appearance of Mediterranean samples.

As already noticed by Moolenbeek & Hoenselaar (1991), *Columbella striata* Duclos (originally described from Senegal) is a junior homonym of *Columbella striata* Menke, 1829 and therefore is not usable as the valid name for any species. In Senegal two distinct protoconch types have been sometimes cited and interpreted as multispiral and paucispiral, respectively (Thorsson 2003). However, based on Oliverio (1995), Rolán & Ryall (1999), Hernández & Boyer (2005) and Pelorce & Boyer (2005), all intact protoconchs of *Columbella* from Morocco to Mauritania, including Senegal, are paucispiral. Unfortunately, material from Senegal or Mauritania properly fixed for DNA extraction was not available for this study and the actual identity of the *Columbella* from this area could not be unequivocally assessed herein.

Three autapomorphic positions were scored in the COI sequences: 238 [C], 310 [T], 447 [G].

Columbella adansoni Menke, 1853
(Figs 6C, D; 7G-H; 8A)

Columbella Adansoni [sic] Menke, 1853: 74, 75.

Columbella rufa Menke, 1853: 75.

Columbella rustica var. *azorica* Drouët, 1858: 169.

Columbella striata var. *minor* Dautzenberg, 1900: 183.

Type material. — *Columbella adansoni*: lectotype (Moolenbeek & Hoenselaar 1991) SMF. — Type locality: Cape Verde Islands.
Columbella rufa: lectotype (Moolenbeek & Hoenselaar, 1991) SMF. — Type locality: Cape Verde Islands.
Columbella rustica var. *azorica*: unknown (Moolenbeek & Hoenselaar, 1991). — Type locality: Azores.
Columbella striata var. *minor*. — Type locality: Ilhéu Branco (Cape Verde Islands).

Distribution. — According to the data presented herein, *Columbella adansoni* ranges throughout Macaronesia, and is not present in continental African waters.

Diagnosis. — Shell of medium size for the family, 16-25 mm long, biconic/strombiform.

FIG. 6. — *Columbella* spp. types: **A**, **B**, *Columbella rustica* (Linnaeus, 1758), syntype of *Voluta rustica* (LSL.348), Mediterranean (permission of The Linnean Society of London); **C**, **D**, *Columbela adansoni* Menke, 1853, lectotype SMF, Cape Verde (after Moolenbeek & Hoenselaar 1991, figs 1, 2); **E**-**G**, *Columbella xiphitella* Duclos, 1840, lectotype (**F**, **G**) and paralectotypes (**E**) from lot MNHN-IM-2000-9599, Gabon; **H**-**L**, *Columbella xiphitella*, lectotype (**H**, **J**, **K**) and paralectotypes (**I**, **L**) of *C. nucleus* Kiener, 1841 from lot MHNG-MOLL-95502. Symbols: *, the selected lectotypes; o, the paralectotype with close-up of the protoconch (**L**). Scale bars: 10 mm; G, H, L, not to scale.

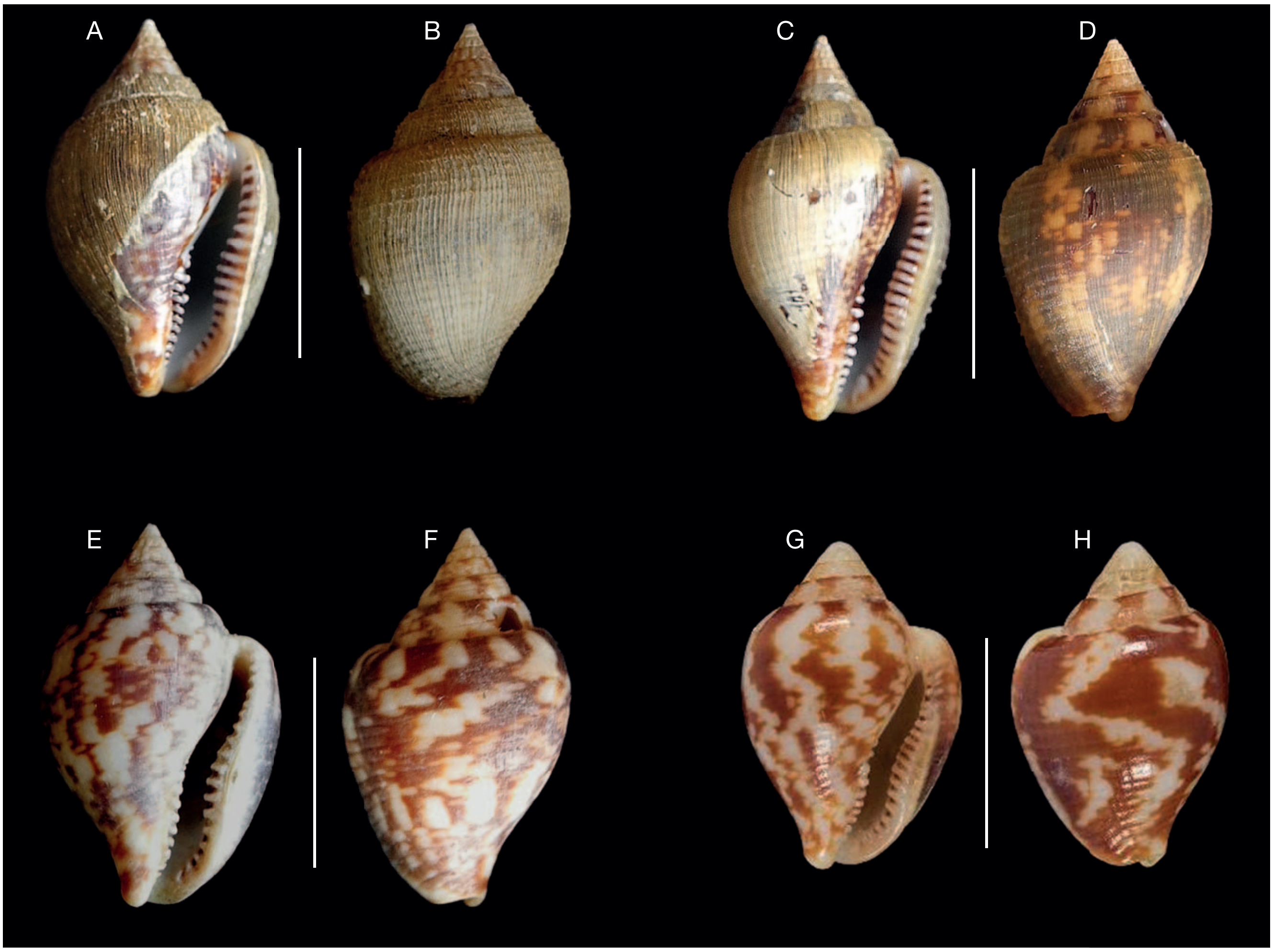


Fig. 7. — *Columbella xiphitella* Duclos, 1840 Gabon: **A**, **B**, MNHN IM-2000-32498; **C**, **D**, MNHN IM-2000-32498; **E**, **F**, BAU 1120.7; **G**, **H**, paralectotype from lot MNHN-IM-2000-9599. Scale bars: 10 mm.

Protoconch of 2.5-2.6 convex whorls, entirely covered by densely spaced microgranules; embryonic shell (protoconch I) of 0.8-0.9 whorls, and larval shell (protoconch II) of 1.6-1.7 whorls; protoconch-teleoconch boundary marked by a sinusigera scar.
Teleoconch of 7-9 almost straight-sided whorls, penultimate whorl slightly convex, body whorl rounded and inflated, about ⅔ to ¾ shell length.
Sculpture of nodulose axial ridges on the first whorls, fading after 2-3 whorls, and very weak, irregular spiral striae. Aperture narrow, elongate and sinuous.
Outer lip angulate posteriorly in some, thickened, especially medially, with 13-16 denticles, and rust coloured markings between denticles. Columellar wall with two weak ridges medially; parietal wall with 5-7 denticles anteriorly, and rust coloured markings between. Siphonal canal open.
Colour very variable, with white-whitish background and yellow, orange, brown, grey or black irregular spots, sometimes arranged into axial flames or sinuous bands. Periostracum thin, brown.
Animal yellowish with tawny-orange spots, very dense on propodium, head and mantle; tip of cephalic tentacles white, siphon grey. Radula rachiglossate, with central tooth reduced to a slightly arched plate with no cusps. One pair of massive lateral teeth with a small, basal, outer cusp and a tall, sinuous inner primary cusp with three secondary cusps along the posterior edge: a narrow, pointed distal cusp, a flat central cusp slightly enlarged at the base, and a quadrangular and apically curved basal cusp.

Remarks

Knudsen (1995) summarized his own (Knudsen 1950) and Gunnar Thorson's (unpublished) notes on the egg capsules of *C. adansoni* from Cape Verde Islands and Canary Islands, respectively. The egg capsules contained 39-73 eggs, *c.* 200 µm in diameter, developing into pelagic larvae attaining at metamorphosis 450 µm shell width (1000 µm length).

Seven autapomorphic positions were scored in the COI sequences: 61 [G], 91 [G], 160 [C], 181 [T], 352 [C], 549 [A], 586 [T].

Columbella xiphitella Duclos, 1840
(Figs 6E-L; 7A-H; 8B; 9A, B)

Colombella xiphitella Duclos, 1840: pl. 9, figs 13, 14.

Columbella nucleus Kiener, 1841: 14-15, pl. 3, fig. 4.

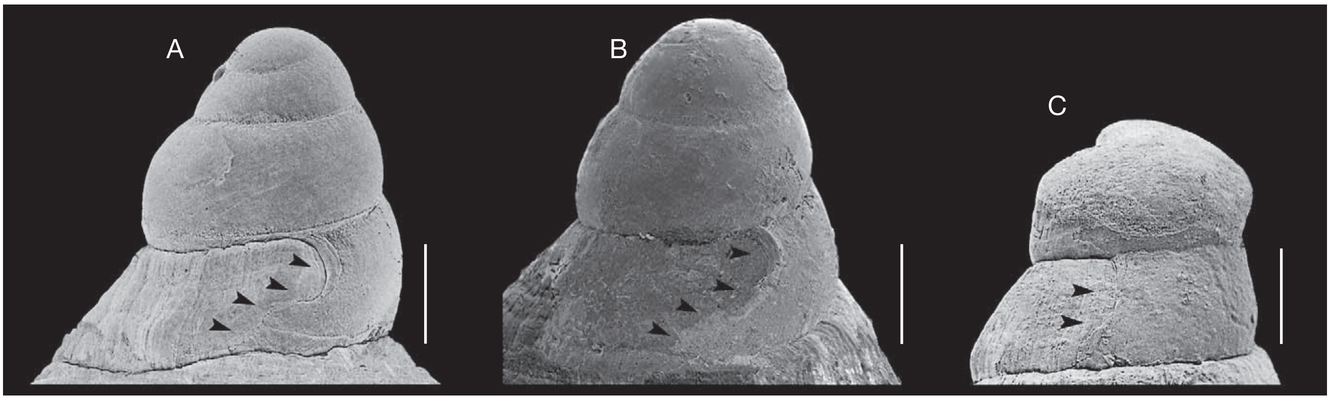


Fig. 8. — Protoconchs of *Columbella* spp.: **A**, *Columbella adansoni* Menke, 1853, Tenerife Is., Canary Islands; **B**, *Columbella xiphitella* Duclos, 1840, Miemia, Ghana; **C**, *Columbella rustica* (Linnaeus, 1758), San Domino Is., Italy. Arrows indicate the protoconch-teleoconch boundary. Scale bars: 100 µm.

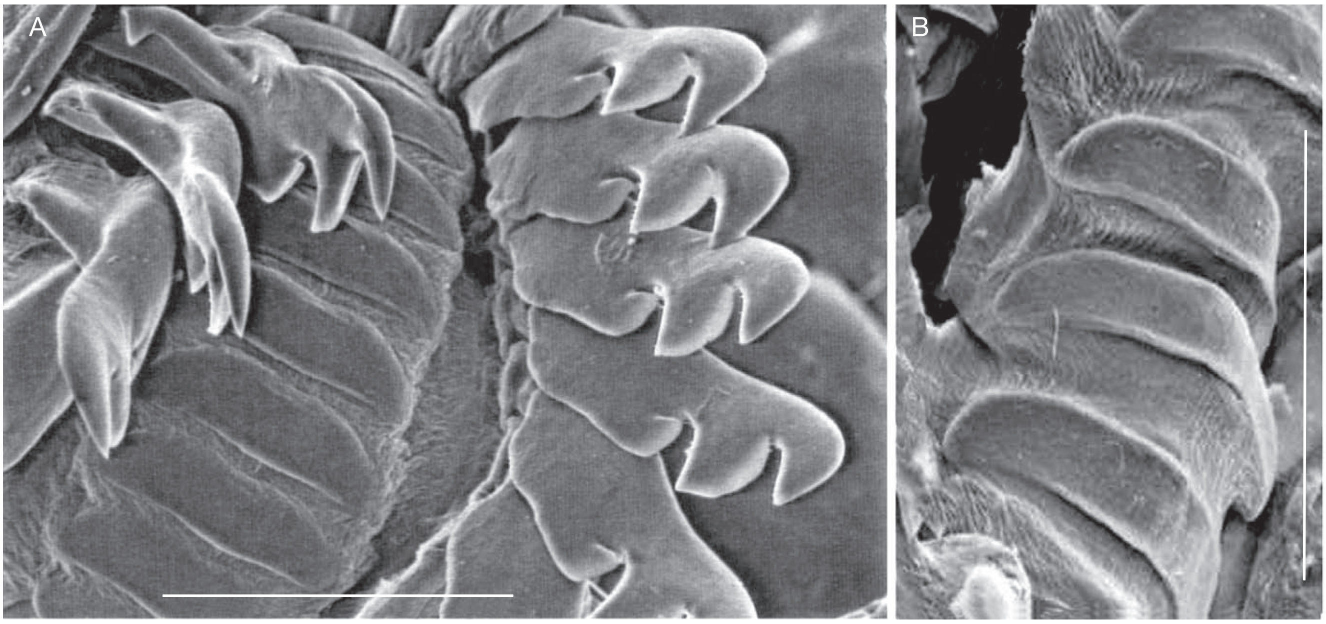


Fig. 9. — Radula of *Columbella xiphitella* Duclos, 1840: **A**, Miemia, Ghana; **B**, Lagoa Azul, São Tomé, detail of the rachidian. Scale bars: 100 µm.

Type material. — *Colombella xiphitella*: lectotype (here designated: Fig. 6F, G) and 11 paralectotypes MNHN-IM-2000-9599, 4 paralectotypes MNHN-IM-2000-9598. — Type locality: "Californie", erroneous, corrected to Gabon (ICZN 1999: rec. 76.A.2).
Columbella nucleus: MHNG-MOLL-95502 lectotype (here selected: Fig. 6H, J, K) and 9 paralectoypes (from Delessert collection). — Type locality: Gabon (imposed herein, ICZN 1999: rec. 76A.1.4).

Distribution. — According to the material examined genetically herein, *Columbella xiphitella* ranges along West African coasts from Ghana to Angola, including *São Tomé and Principe*.

Diagnosis. — Shell of medium size for the family, 10-18 mm long, biconic/strombiform.
Protoconch of 2.5-2.6 convex whorls, entirely covered by densely spaced microgranules; embryonic shell (protoconch I) of 0.8 whorls, and larval shell (protoconch II) of 1.7 whorls; protoconch-teleoconch boundary marked by a sinusigera scar.
Teleoconch of 7-9 almost straight-sided whorls, penultimate whorl slightly convex, body whorl rounded and inflated, about ¾ shell length. Sculpture of nodulose axial ridges on the first whorls, fading after 2-3 whorls, and very weak, irregular spiral striae. Aperture narrow, elongate and sinuous.
Outer lip angulate posteriorly in some, thickened, especially medially, with 14-19 strong denticles, and rust coloured markings between denticles. Columellar wall with two weak ridges medially; parietal wall with 5-8 strong denticles anteriorly, usually with rust coloured markings between. Siphonal canal open.
Colour very variable, with white-whitish background and yellow, orange, brown, grey or black irregular spots, sometimes arranged into axial flames or sinuous bands. Periostracum thin, brown.
Animal observed only in alcohol preserved specimens: whitish background and dark brown to dark tawny spots, dense on propodium and head, very dense on mantle; tip of cephalic tentacles white, siphon dark grey. Radula rachiglossate, with central tooth reduced to a slightly arched plate with no cusps. One pair of massive lateral teeth with a small, basal, outer cusp and a tall, sinuous inner primary

Table 4. — COI sequences of worldwide columbellids, with the ABGD group assignation (alternate grey/white lines, according to ABGD groups numbers), voucher ID (or GenBank accession number), a-priori morphological identification, a-posteriori MOTU assignation.

ABGD group	Voucher ID	a priori morphological identification	a posteriori MOTU assignation
1	IM-2007-33580	*Aesopus cumingii* (Duclos, 1846)	*A. cumingii*
1	IM-2007-33581	*Aesopus cumingii*	*A. cumingii*
2	KF643896.1	*Amphissa columbiana* (Dall, 1916)	*A.* aff. *columbiana*
2	KF644010.1	*Amphissa columbiana*	*A.* aff. *columbiana*
2	KF644101.1	*Amphissa columbiana*	*A.* aff. *columbiana*
2	KF643694.1	*Amphissa versicolor* (Dall, 1871)	*A.* aff. *columbiana*
3	KF644285.1	*Amphissa reticulata* (Dall, 1916)	*A. reticulata*
4	KF643489.1	*Alia carinata* (Hinds, 1844)	*A. carinata*
4	KF643493.1	*Alia carinata*	*A. carinata*
4	KF643566.1	*Alia carinata*	*A. carinata*
4	KF643846.1	*Alia carinata*	*A. carinata*
4	KF643937.1	*Alia carinata*	*A. carinata*
4	KF644175.1	*Alia carinata*	*A. carinata*
4	KF644247.1	*Alia carinata*	*A. carinata*
4	KF644276.1	*Alia carinata*	*A. carinata*
4	KF643354.1	*Alia carinata*	*A. carinata*
5	IM-2009-11313	*Anachis* sp.	*Anachis* sp.
6	BAU 710_7	*Columbella adansoni* Menke, 1853	*C. adansoni*
6	BAU 726_7	*Columbella adansoni*	*C. adansoni*
6	BAU 741_11	*Columbella adansoni*	*C. adansoni*
7	BAU 1120_1	*Columbella adansoni*	*C. xiphitella*
7	BAU 1120_2	*Columbella adansoni*	*C. xiphitella*
7	BAU1120_3	*Columbella adansoni*	*C. xiphitella*
8	BAU 817_1	*Columbella rustica* (Linnaeus, 1758)	*C. rustica*
8	BAU 818_5	*Columbella rustica*	*C. rustica*
8	BAU 821_2	*Columbella rustica*	*C. rustica*
9	IM-2009-18927	columbellid indet.	columbellid indet.
9	IM-2007-35775	columbellid indet.	columbellid indet.
10	IM-2007-35599	columbellid indet.	columbellid indet.
11	IM-2007-33570	columbellid indet.	columbellid indet.
12	IM-2007-33521	*Euplica borealis* (Pilsbry, 1904)	*E . borealis*
13	IM-2007-33515	*Euplica scripta* (Lamarck, 1822)	*E. scripta*
14	JN052985.1	*Euplica scripta*	*E. scripta*
15	JN052986.1	*Euplica scripta*	*E. scripta*
15	JN052987.1	*Euplica scripta*	*E. scripta*
15	HQ834054.1	*Euplica scripta*	*E. scripta*
16	IM-2007-33519	*Euplica turturina* (Lamarck, 1822)	*E. turturina*
16	IM-2007-33522	*Euplica turturina*	*E. turturina*
16	IM-2007-33524	*Euplica turturina*	*E. turturina*
16	IM-2007-33539	*Euplica turturina*	*E. turturina*
16	JQ950207.1	*Euplica turturina*	*E. turturina*
17	IM-2007-33537	*Euplica varians* (Sowerby, 1832)	*E. varians*
17	IM-2007-33583	*Euplica varians*	*E. varians*
18	IM-2007-33493	*Graphicomassa albina* (Kiener, 1841)	*G. adiostina* (Duclos, 1840)
18	IM-2007-33494	*Graphicomassa albina*	*G. adiostina*
19	IM-2007-33514	*Graphicomassa ligula* (Duclos, 1835)	*G. ligula*
19	IM-2007-33517	*Graphicomassa ligula*	*G. ligula*
19	IM-2007-33523	*Graphicomassa ligula*	*G. ligula*
19	IM-2007-33534	*Graphicomassa ligula*	*G. ligula*
19	IM-2007-33542	*Graphicomassa ligula*	*G. ligula*
19	JQ950206.1	*Graphicomassa ligula* as *Mitrella ligula* (Duclos, 1840)	*G. ligula*
20	IM-2007-35779	*Indomitrella* cf. *conspersa* (Gaskoin, 1851)	*I.* cf. *conspersa*
21	IM-2007-33532	*Indomitrella puella* (Sowerby, 1844)	*I. puella*
22	IM-2007-33548	*Indomitrella schepmani* K. Monsecour & D. Monsecour, 2007	*I. schepmani*
22	IM-2007-35594	*Indomitrella schepmani*	*I. schepmani*
23	HM180683.1	*Mitrella bicincta* (Gould, 1860)	*M.* aff. *bicincta*
23	HM180684.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180685.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180687.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180688.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180690.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180691.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	HM180692.1	*Mitrella bicincta*	*M.* aff. *bicincta*
23	JN053028.1	*Mitrella burchardi* (Dunker, 1877)	*M.* aff. *bicincta*
23	HQ834098.1	*Mitrella burchardi*	*M.* aff. *bicincta*
24	JN052988.1	*Mitrella bicincta* (Gould, 1860)	*M. bicincta*
24	JN052989.1	*Mitrella bicincta*	*M. bicincta*
24	JN052990.1	*Mitrella bicincta*	*M. bicincta*
24	JN052991.1	*Mitrella bicincta*	*M. bicincta*

Table 4. — Continuation.

ABGD group	Voucher ID	a priori morphological identification	a posteriori MOTU assignation
24	HQ834055.1	*Mitrella bicincta* (Gould, 1860)	*M. bicincta*
24	HM180686.1	*Mitrella bicincta*	*M. bicincta*
24	HM180689.1	*Mitrella bicincta*	*M. bicincta*
25	IM-2007-30282	*Mitrella* cf. *philia* (Duclos, 1846)	*M.* cf. *philia*
26	IM-2007-35498	*Mitrella essingtonensis* (Reeve, 1859)	*M. essingtonensis*
27	IM-2007-33485	*Metanachis jaspidea* (Sowerby, 1844)	*M. jaspidea*
27	IM-2007-33529	*Metanachis jaspidea*	*M. jaspidea*
27	IM-2007-33585	*Metanachis jaspidea*	*M. jaspidea*
28	IM-2007-33490	*Mitrella moleculina* (Duclos, 1835)	*M. moleculina*
29	IM-2007-33504	*Mitrella nympha* (Kiener, 1841)	*M. nympha*
29	IM-2007-33565	*Mitrella nympha*	*M. nympha*
30	IM-2007-35750	columbellid indet.	*Mitrella* sp.
30	IM-2007-35749	*Mitrella* cf. *moleculina* (Duclos, 1840)	*Mitrella* sp.
30	IM-2007-35495	*Mitrella* sp.	*Mitrella* sp.
31	KF643804.1	*Mitrella* cf. *tuberosa* (Carpenter, 1865)	*Mitrella* sp.
32	IM-2007-33582	*Mitrella* sp.	*Mitrella* sp.
33	IM-2007-35626	*Mitrella* sp.	*Mitrella* sp.
34	IM-2013-20589	*Nassarina metabrunnea* (Dall & Simpson, 1901)	*N. metabrunnea*
35	IM-2007-36625	*Pyrene flava* (Bruguière, 1789)	*P. flava*
35	IM-2007-36760	*Pyrene flava*	*P. flava*
35	IM-2007-36685	*Pyrene flava*	*P. flava*
36	IM-2007-33560	*Pyrene punctata* (Bruguiere, 1789)	*P. punctata*
36	IM-2007-33578	*Pyrene punctata*	*P. punctata*
37	HQ834097.1	*Pseudamycla* sp.	*Pseudamycla* sp.
38	IM-2007-39377	columbellid indet.	*S.* cf. *kanamaruana* A
38	IM-2007-32142	*Sulcomitrella* cf. *kanamaruana* (Kuroda, 1953)	*S.* cf. *kanamaruana* A
38	IM-2007-33555	*Sulcomitrella* sp.	*S.* cf. *kanamaruana* A
39	IM-2009-11298	*Sulcomitrella* cf. *kanamaruana* (Kuroda, 1953)	*S.* cf. *kanamaruana* B
39	IM-2009-11301	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-32150	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-33479	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-33482	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-33574	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-33575	*Sulcomitrella* cf. *kanamaruana*	*S.* cf. *kanamaruana* B
39	IM-2007-33540	*Sulcomitrella circumstriata* (Schepman, 1911)	*S.* cf. *kanamaruana* B
39	IM-2007-36339	*Sulcomitrella circumstriata*	*S.* cf. *kanamaruana* B
40	IM-2007-35773	*Sulcomitrella* cf.. *monodonta* (Habe, 1958)	*S.* cf.. *monodonta* A
41	IM-2009-11304	*Sulcomitrella monodonta* (Habe, 1958)	*S.* cf.. *monodonta* B
42	IM-2007-33551	*Sulcomitrella circumstriata* (Schepman, 1911)	*S. circumstriata*
42	IM-2007-33552	*Sulcomitrella circumstriata*	*S. circumstriata*
43	IM-2007-30246	*Zafra* cf. *pumila* (Dunker, 1858)	*Z.* cf. *pumila*
44	IM-2007-33480	*Zafrona isomella* (Duclos, 1840)	*Z. isomella*
45	IM-2007-30355	*Zafra pumila* (Dunker, 1858)	*Z. pumila*
46	IM-2007-33535	*Metanachis laingensis* Sleurs, 1985	*Mitrella* sp.
46	IM-2007-33536	*Mitrella* cf. *alizonae* (Melvill & Standen, 1901)	*Mitrella* sp.
46	IM-2007-33488	*Mitrella chinoi* Monsecour & Dekkers, 2013	*Mitrella* sp.

cusp with three secondary cusps along the posterior edge: a narrow, pointed distal cusp, a flat central cusp slightly enlarged at the base, and a quadrangular and apically curved basal cusp.

Remarks

Dunker (1853: 24) used *Columbella striata* Duclos for his specimens from Luanda and Annobon, quite certainly referring to this species. However, *Columbella striata* Duclos (described from Senegal and here provisionally included in the synonymy of *C. rustica*) is preoccupied by *Columbella striata* Menke 1829, a nomen dubium without type(s) availables. The 10 syntypes of *Columbella nucleus* Kiener at MHNG are to be considered as syntypes as they originate from the Delessert collection, as reported for this species in the original description.

C. xiphitella differs from *Columbella rustica* by its multispiral protoconch (v. paucispiral in *C. rustica*). Morphological (including colour pattern) variation in the teleoconch of the three eastern Atlantic species (*C. rustica*, *C. adansoni* and *C. xiphitella*) largely overlaps with no evident diagnostic characters. All shells of *C. xiphitella* examined (including the type series) have strong dentition on columellar and outer lips, and very dark marks between the denticles, features only occasionally present in the other two species. However, the three species are unequivocally separated by molecular data from COI and 16S. Eighteen autapomorphic positions were scored in the COI sequences: 34 [T], 55 [T], 78 [G], 100 [T], 115 [T], 117 [A], 130 [A], 133 [C/G], 178 [C], 309 [C], 346 [C], 385 [T], 430 [C], 463 [T], 472 [G], 565 [T], 598 [T], 619 [T].

DISCUSSION

The combined use of molecular data with morphological, geographical and ecological attributes is revealing a growing number of cases of hidden biodiversity in gastropods, often with virtually no morphological distinction in shell characters, among genetically well-separated species (e.g., Modica *et al.* 2013). In the present case, the three species of *Columbella* detected in the eastern Atlantic and the Mediterranean are virtually indistinguishable by their teleoconch features, whereas they are neatly separated by genetic data.

Two species were previously accepted after Moolenbeek & Hoenselaar (1991), Oliverio (1995) and Rolán & Ryall (1999): *Columbella rustica* Linnaeus, 1758, ranging through the entire Mediterranean Sea, and extending into the neighbouring Atlantic South to Senegal and Mauritania, and North to Portugal (it is absent in Galicia); and *Columbella adansoni* Menke, 1853, described from Cape Verde islands, and assumed to occur across Macaronesia, from the Azores to the Canary Islands, and along the West African coast from Mauritania to Angola (Oliverio 1995).

Based on the present data, *Columbella adansoni* is restricted with certainty only to populations from Macaronesia. West African populations from Mauritania and Senegal North to Morocco (with paucispiral protoconch) are conservatively included in *Columbella rustica* pending genetic analysis; those from Ghana South to Angola belong to *Columbella xiphitella* (type locality corrected herein), while those from Mauritania to Ghana should also be assayed genetically, since *C. adansoni* and *C. xiphitella* (albeit clearly defined genetically) are indistinguishable morphologically.

As already highlighted by Moolenbeek & Hoenselaar (1991) and Oliverio (1995), *Columbella adansoni* has a multispiral protoconch indicating planktotrophic larval development, whereas *Columbella rustica* has a paucispiral protoconch, indicating non-planktotrophic development. *Columbella xiphitella*, which is phylogenetically the sister to the other two species, has a multispiral protoconch (similar to *Columbella adansoni*), thus suggesting that the plesiomorphic state in this group was a planktotrophic larva, as is typical of most (if not all) caenogastropod lineages. This is also paralleled by *Columbella moinensis* deMaintenon, 2000, from the Pliocene to Pleistocene(?) of Costa Rica and Colombia, with planktotrophic development (and multispiral protoconch); this is a clear sibling of *Columbella mercatoria* (Pliocene to Recent, Caribbean) with lecithotrophic development (and a paucispiral protoconch) (deMaintenon 2000). Within columbellids, sibling species differing mainly or only in their larval development (and thus in their protoconch morphology) are known also in the genera *Zafrona* Iredale, 1916, *Mitrella* and *Euplica*.

The study of large geographic samples in the species involved herein may yield crucial data to analyse the genetic structure and dynamics of populations from closely related species with contrasting larval ecology. These may in turn prove important to define larval ecology drivers in speciation events related to the loss of planktotrophy (Oliverio 1996b), which has produced pairs of sibling species in many lineages of caenogastropods (e.g., Oliverio 1996a, 1997; Duda & Palumbi 1999).

Acknowledgements
Emilio Rolán (Vigo) provided samples from West Africa. Laurent Charles (Muséum Sciences et Nature, Bordeaux), Andrea Deneau (The Linnean Society, London), Virginie Héros (MNHN, Paris), Alan J. Kohn (University of Washington, Seattle), Gary Rosenberg (ANSP, Philadelphia), Emmanuel Tardy (MHNG, Genève), are thanked for bibliographic help and precious information on and pictures of type material. Nicolas Puillandre (MNHN) gave us access to unpublished sequences obtained from MNHN expedition material. Serge Gofas (University of Malaga) and Kevin Monsecour (Aarschot) provided very useful criticisms.

REFERENCES

Adams A. 1860. — On some new genera and species of Mollusca from Japan. *Annals and Magazine of Natural History* (3) 5: 299-303 [April 1860], 405-413.

Arnaud P. M. 1978. — Révision des taxa malacologiques méditerranéens introduits par Antoine Risso. *Annales du Muséum d'Histoire naturelle de Nice* "1977"5: 101-150.

Bandel K. 1975. — Das Embryonalgehäuse mariner Prosobranchier der Region von Banyuls-Sur-Mer. 1. Teil. *Vie et Milieu* 25: 83-118.

Bouchet P. & Gofas S. 2010. — *Columbella*, *in* MolluscaBase (2015). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=137803 on 2016-12-29

Castresana J. 2000. — Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540-552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chenu J. C. 1846-50. — *Illustrations Conchyliologiques ou Description et figures de Toutes les Coquilles Connues Vivantes et Fossiles.* Paris, A. Franck. Tome Premier. Genre *Colombelle*, pls 1-27.

Dautzenberg P. 1900. — Croisière du Yacht Chazalie dans l'Atlantique. Mollusques. *Mémoires de la Société zoologique de France* 13: 145-265, pls 9-10.

deMaintenon M. J. 2000. — A new species of *Columbella* (Neogastropoda: Columbellidae) from the Caribbean Neogene. *The Nautilus* 114: 14-17. https://doi.org/10.5962/bhl.part.29124

Drouët H. 1858. — Mollusques marins des Îles Açores. *Mémoires de la Société académique de l'Aube* 22: 53 p.

Drummond A. J., Ashton B., Cheung M., Helen J., Kearse M., Moir R., Stones-Havas S., Thierer T.& Wilson A. 2009. — Geneious v 4.7. Biomatters, Ltd., Auckland, New Zealand.http://www.geneious.com/.

Dunker G. 1853. — *Index Molluscorum, quae in itinere ad Guineam Inferiorem collegit Georgius Tams med. dr. / Auctore Guilielmo Dunker ... accedunt novarum specierum diagnosis, Cirripedia nonnulla et X tabulae iconum*. T. Fisher,Casselis Cattorum: vi+74, pls 10.

Duclos P. L. 1840. — *Histoire naturelle générale et particulière de tous les genres de coquilles univalves marines à l'état vivant et fossiles, publiée par monographie; ou description et classification méthodique de toutes les espèces connues jusqu'à ce jour représentées en couleur avec la figure et l'anatomie d'un assez grand nombre de Mollusques nouvellement découverts, Genre Colombelle.* Didot, Paris. 13 pls. https://doi.org/10.5962/bhl.title.84659

DUDA T. F JR. & PALUMBI S. R. 1999. — Developmental shifts and species selection in gastropods. *Proceedings of the National Academy of Science of USA* 96: 10272-10277.

FOLMER O., BLACK M., HOEH W., LUTZ R. & VRIJENHOEK R. 1994. — DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

FRANC A. 1943. — *Études sur le développement de quelques Prosobranches méditerranéens*. Thesis, Alger, 159 p.

HASEGAWA M., KISHINO H. & YANO T. 1985. — Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 22: 160-174. https://doi.org/10.1007/BF02101694

HAYASHI S. 2003. — The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives. *Molluscan Research* 25: 85-98.

HERNÁNDEZ J. M. & BOYER F. 2005. — Notes on the columbellid fauna from the infralittoral and circalittoral levels of the Canary Islands. *Iberus* 23 (2): 69-93.

IREDALE T. 1916. — On some new and old molluscan names. P*roceedings of the Malacological Society of London*. 12: 27-37.http://biodiversitylibrary.org/page/15770460

KATOH K. & TOH H. 2008. — Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286-298. https://doi.org/10.1093/bib/bbn013

KATOH K., MISAWA K., KUMA K. & MIYATA T. 2002. — MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059-3066. https://doi.org/10.1093/nar/gkf436

KIENER L. C. 1834-1850. — *Spécies général et iconographie des coquilles vivantes comprenant la collection du Muséum d'Histoire naturelle de Paris, la collection Lamarck, celle du prince Masséna (appartenant maintenant à M. le M. le Baron Delessert) et les découvertes récentes des voyageurs.* 138 'livraisons' ['Colombelles': figures in livraisons 59 (1840) and 60-61 (1841), text in livraisons 62-65 (1841)]. Paris, P. Duménil (later Rousseau) and J. B. Ballière, 2132 p., 827 pls.

KNOWLTON N. 2000. — Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420: 73-90. https://doi.org/10.1023/A:1003933603879

KNUDSEN J. 1950. — Egg capsules and development of some marine prosobranchs from tropical West Africa. *Atlantide Report* 1: 85-130.

KNUDSEN J. 1995. — Observations on reproductive strategy and zoogeography of some marine prosobranch gastropods (Mollusca) from the Azores. *Açoreana, Supplement 1995*: 135-158.

LAMARCK J.-B. M. DE 1799. — Prodrome d'une nouvelle classification des coquilles, comprenant une rédaction appropriée des caractères génériques, et l'établissement d'un grand nombre de genres nouveaux. *Mémoires de la Société d'Histoire naturelle de Paris* 1: 63-91.

LAMARCK J.-B. M. DE. 1822. — *Histoire naturelle des animaux sans vertèbres. Tome septième*. Paris, published by the Author, 711 p.

LERAY M., YANG J. Y., MEYER C. P., MILLS S. C., AGUDELO N., RANWEZ V., BOEHM J. T. & MACHIDA R. J. 2013. — A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology* 10: 34. https://doi.org/10.1186/1742-9994-10-34

LINNAEUS C. 1758. — *Caroli Linnaei… systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum caracteribus, differentiis, synonymis, locis… Editio decima, reformata. Impensis Direct. Laurentii Salvii: Holmiae, Tome 1. Animalia.* [Systema Naturae, 10th Edition]. Stockholm, Sweden, v + 823 p. https://doi.org/10.5962/bhl.title.542

MALAQUIAS M. A. E. & REID D. G. 2009. — Tethyan vicariance, relictualism and speciation: evidence from a global molecular phylogeny of the opisthobranch genus *Bulla*. *Journal of Biogeography* 36 (9): 1760-1777. https://doi.org/10.1111/j.1365-2699.2009.02118.x

MENKE K. T. 1829. — *Verzeichniss der ansehnlichen Conchylien-Sammlung des Freiherrn von der Malsburg...: welche hierdurch zum verkaufe dargeboten wird*. H. Gelpke, Pyrmont.: vi + 123 p.

MENKE K. T. 1853. — Conchylien von St. Vincent mit kritischen Anmerkungen. *Zeitschrift für Malakozoologie* 10 (5-6): 67-80.

MODICA M. V., MARIOTTINI P., PRKIC J. & OLIVERIO M. 2013. — DNA-barcoding of sympatric species of ectoparasitic gastropods of the genus *Cerithiopsis* (Mollusca: Gastropoda: Cerithiopsidae) from Croatia. *Journal of the Marine Biological Association of the United Kingdom* 93 (4): 1059-1065. https://doi.org/10.1017/S0025315412000926

MODICA M. V., RUSSINI V., FASSIO G. & OLIVERIO M. 2017. — Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Marine Environmental Research* 127: 92-101. https://doi.org/10.1016/j.marenvres.2017.04.001

MONSECOUR K. & GOFAS S. 2010a. — *Columbella adansoni*, *in* MolluscaBase (2016). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=139195 on 2016-12-29

MONSECOUR K. & GOFAS S. 2010b. — *Columbella rustica* (Linnaeus, 1758), *in* MolluscaBase (2016). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=139196 on 2016-12-29

MOOLENBEEK R. G. & HOENSELAAR H. J. 1991. — On the identity of *Columbella rustica* from West Africa and the Macaronesian Islands. *Bulletin of the Zoological Museum, University of Amsterdam* 13 (6): 65-70.

OLIVERIO M. 1995. — Larval development and allozyme variation in the East Atlantic *Columbella*. *Scientia Marina* 59 (I): 77-86.

OLIVERIO M. 1996a. — Life-histories, speciation and biodiversity in Mediterranean prosobranchs gastropods. *Vie et Mileu* 46: 163-169.

OLIVERIO M. 1996b. — Contrasting developmental strategies and speciation in N. E. Atlantic prosobranchs: a preliminary analysis, *in* TAYLOR J. D. (ed.), *Origin and Evolutionary Radiation of the Mollusca*, Vol. 22. Oxford University Press, Oxford: 261-266.

OLIVERIO M. 1997. — Global biodiversity and life-history evolution in prosobranchs gastropods. *Iberus* 16: 73-79.

OLIVERIO M. & MARIOTTINI P. 2001. — A molecular framework for the phylogeny of Coralliophila and related muricoids. *Journal of Molluscan Studies* 67: 215-224. https://doi.org/10.1093/mollus/67.2.215

PALLARY P. 1900. — Coquilles marines du littoral du Départment d'Oran. *Journal de Conchyliologie* 48 (3): 211-422, pls 6-8.

PALUMBI S., MARTIN A., ROMANO S., MCMILLAN W. O., STICE L. & GRABOWSKI G. 2002. — *The simple fool's guide to PCR Version 2.0*. Honolulu, HI: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, 45 p.

PELORCE J. & BOYER F. 2005. — La famille Columbellidae (Gastropoda: Muricoidea) dans l'infralittoral de la Péninsule du Cap Vert (Sénégal). *Iberus* 23 (2): 95-118.

PHILIPPI R. A. 1836. — *Enumeratio molluscorum Siciliae cum viventium tum in tellure tertiaria fossiliumquae in itinere suo observavit*. S. Shropp, Berlin, xiv + 267 p., pls 1-12.

PUILLANDRE N., LAMBERT A., BROUILLET S. & ACHAZ G. 2012a. — ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* 21: 1864-1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

PUILLANDRE N., MODICA M. V., ZHANG Y., SIROVICH L., BOISSELIER M. C., CRUAUD C., HOLFORD M.& SAMADI S. 2012b. — Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology* 21: 2671-2691. https://doi.org/10.1111/j.1365-294X.2012.05559.x

REID D. G., LAL K., MACKENZIE-DODDS J., KALIGIS F., LITTLEWOOD D. T. J. & WILLIAMS S. T. 2006. — Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal* of *Biogeography* 33: 990-1006. https://doi.org/10.1111/j.1365-2699.2006.01469.x

RISSO A. 1826-1827. — *Histoire naturelle des principales productions de l'Europe Méridionale et particulièrement de celles des environs de Nice et des Alpes Maritimes*. Paris, Levrault: Vol. 4 IV + 439 + 12 pl. (molluscs) [november 1826]. https://doi.org/10.5962/bhl.title.58984

ROLÁN E. 2005. — Columbellidae (Gastropoda, Neogastropoda) of the gulf of Guinea with the description of eight new species. *Iberus* 23 (2): 119-156.

ROLÁN E. & RYALL P. 1999. — The genus *Columbella* Swainson, 1840 in the East Atlantic. *La Conchiglia* 31 (290): 57-58.

THORSSON W. 2003. — Living Columbellidae Part 9. *Internet Hawaiian Shell News*, November 2003 Feature-3: 1-17, at http://internethawaiishellnews.org/IHSN/2003/0311fea3.pdf

TRYON G. W. JR. 1883. — *Manual of Conchology, Structural and Systematic, with Illustrations of the Species. (1) 5: Marginellidae, Olividae, Columbellidae.* Philadelphia, published by the author: 1-276, pls 1-63. http://doi.org/10.5962/bhl.title.6534

WHEELER Q. & MEIER R. 2000. — *Species Concepts and Phylogenetic Theory: A Debate*. Columbia University Press, New York, 230 p.

Submitted on 21st June 2016;
accepted on 24 January 2017;
published on 30 June 2017.

Chapter II

- An assessment of *Raphitoma* and allied genera (Neogastropoda: Raphitomidae)

- Genetic evidence of poecilogony in Neogastropoda: implications for the systematics of the genus Raphitoma Bellardi, 1847

An assessment of *Raphitoma* and allied genera (Neogastropoda: Raphitomidae)

Giulia Fassio[1], Valeria Russini[1], Francesco Pusateri [2], Riccardo Giannuzzi-Savelli[3], Tore Høisæter[4], Nicolas Puillandre[5], Maria Vittoria Modica[6,7] and Marco Oliverio[1]

[1]Department of Biology and Biotechnologies "Charles Darwin" Zoology, Viale dell'Università 32, I-00185 Roma, Italy;
[2]Via Castellana, 64 - 90135 Palermo;
[3]Via Mater Dolorosa, 54 - 90146 Palermo;
[4]University of Bergen, Museum of Natural History, P.O. Box 7800 (Thormøhlens gate 53A), N-5020 Bergen, Norway;
[5]Institut de Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, 57 Rue Cuvier, CP 26, 75005 Paris, France;
[6]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy; and
[7]UMR5247, University of Montpellier, France

Running head: Systematics of *Raphitoma*

(Received 9 January 2019; editorial decision 9 April 2019)

Correspondence: M. Oliverio; email: marco.oliverio@uniroma1.it

ABSTRACT

The systematics of several Eastern Atlantic conoidean species, traditionally ascribed to the genus *Raphitoma* Bellardi, 1847, are revised on the basis of DNA sequence data from three gene regions (cytochrome *c* oxidase subunit I, 16S rRNA and 12S rRNA). We assign genus ranking to three major lineages (*Raphitoma*, *Cyrillia* Kobelt, 1905 and *Leufroyia* Monterosato, 1884), and suggest that two West African species belong in the subgenus *Daphnella* (*Paradaphne*) Laseron, 1954. A new classification, based on molecular systematics and critical study of morphology, is provided for of all Eastern Atlantic and Mediterranean species that are currently ascribed to *Raphitoma s. l.* The genus *Clathromangelia* Monterosato, 1884 is confirmed as belonging to Raphitomidae. Phylogenetic relationships and genetic distances suggest that *R. maculosa* Høisæter, 2016 and *R. obesa* Høisæter, 2016 may be deviating morphotypes of *R. bicolor* (Risso, 1826) and *Cyrillia aequalis* (Jeffreys, 1867), respectively.

INTRODUCTION

The Raphitomidae are probably the most diverse family of Conoidea, in terms of species richness, ecological range and anatomy (Kantor & Taylor, 2002; Bouchet *et al.*, 2011). The name Raphitomidae Bellardi, 1875 is based on the genus *Raphitoma* Bellardi, 1847. At the time of its introduction, this genus comprised 34 fossil and Recent species (Bellardi, 1847: 85) that had previously been classified in various genera, such as *Pleurotoma* and *Clathurella*. The genus *Raphitoma* has been particularly well studied in the northeastern Atlantic and Mediterranean, where a recent estimate (Giannuzzi-Savelli *et al.*, 2018) suggested that over 50 extant species occur. These snails, which are usually active at night, live mostly in marine soft-bottom environments at depths ranging from 0–100 m (*R. pseudohystrix* has been collected at 700 m). While they inhabit a wide variety of habitats ranging from coastal bioclastic coarse sands to muddy bioclastic coarse sands, they also occur in sandy pockets, between rocks and in seagrass meadows, with individuals hiding buried under sand or concealed under stones and in crevices during the day. The limits of the genus are still under debate and *Raphitoma s. l.*, as currently conceived, comprises species with the following shell characters: turreted to biconic-pupoidal shape; small to medium size (5–25 mm) in relation to the family Raphitomidae as whole; a frequently keeled last whorl; protoconch consisting of 3–4.5 whorls when multispiral, with the typical raphitomid diagonally cancellate sculpture (Giannuzzi-Savelli *et al.*, 2018; Manousis *et al.*, 2019; Fig. 1). While available data on the morphology of the soft parts are scarce, they nonetheless suggest that there is substantial variation in the anatomy of the foregut. Some species, such as *R. villaria* and *R. linearis*, have neither a radula nor a venom gland. Others, such as *R. purpurea* and *R. leufroyi*, do have a radula, a venom gland or both (Sheridan *et al.*, 1973: 177; Pusateri & Giannuzzi-Savelli, 2008: 124). The arrangement of the foregut has been described for *R. purpurea* (Miller, 1989: 173; Sheridan *et al.*, 1973: 177), but there is a different arrangement in *R. linearis* and *R. leufroyi*, where a rhynchodeal introvert or pseudoproboscis is present (Taylor *et al.*, 1993: 128; Sheridan *et al.*, 1973: 178). The systematic implications of this variability are still unknown, and the problem is further complicated by the lack of a comprehensive phylogenetic framework for the family Raphitomidae.

The type species of *Raphitoma* is *R. histrix* Bellardi, 1847 [ex *Pleurotoma hystrix* Cristofori & Jan, 1832, *nomen nudum*] by subsequent designation (Monterosato, 1872: 54). *Raphitoma histrix* as almost always conceived is a fossil species (Miocene–Pleistocene) and has a complex nomenclatural history that has been summarized by Giannuzzi-Savelli *et al.* (2018: 9; see also Dall, 1918: 316; van Aartsen *et al.*, 1984: 89-90; Rolán *et al.*, 1998: 105). *Raphitoma pseudohystrix* (Sykes, 1906) appears to be the extant closest relative of *R. histrix*; while the teleoconch of the former is almost identical to that of the latter, the protoconch in the extant species is paucispiral whereas in *R. histrix* it is multispiral.

According to current taxonomy, at least eight nominal genera are included in the synonymy of *Raphitoma s. l.* (see Systematic Descriptions below).

Høisæter (2016) argued that DNA-sequence-based phylogenetic studies would most likely show that *Raphitoma s. l.* consists of several genus-level taxa, for which available names could be employed. By carrying out a molecular phylogenetic study of the raphitomids, we seek to explore this issue. Our dataset consists of representatives of at least 13 recognized genera of Raphitomidae (18% of the *c.* 70 genera known for this family; MolluscaBase, 2018), as well as two species of *Clathromangelia*, a genus that has been considered to be a raphitomid (Oliverio, 1995) or a clathurellid (Bouchet *et al.*, 2011). The dataset also includes 14 species which, on the basis of morphology, have been ascribed to *Raphitoma s. l.*; these include the type species of *Cenodagreutes*, *Cyrillia*, *Leufroyia*, *Lineotoma* and *Philbertia*, the apparent closest relatives of the type species of *Cordieria* and *Cyrtoides*, and the closest extant relative of the (fossil) type species of *Raphitoma*.

Table 1. List of material used in the study along with voucher registration numbers, collection localities, GenBank accession numbers for sequences and relevant references.

			GenBank accession numbers			
Taxon	**Voucher ID**	**Locality**	**COI**	**16S rRNA**	**12S rRNA**	**References**
Raphitomidae						
Cyrillia aequalis (Jeffreys, 1867)	ZMBN-020209-O	Norway, 60°13′48″N 5°12′E	JF834219	JF834214		Høisæter (2016)
Cyrillia aequalis (Jeffreys, 1867)	ZMBN-E-345-66a	Norway, 60°18′N 5°10′48″E	JF834221			Høisæter (2016)
Cyrillia aequalis (Jeffreys, 1867)	ZMBN-E-345-66b	Norway, 60°18′N 5°10′48″E	JF834225			Høisæter (2016)
Cyrillia aequalis (Jeffreys, 1867)	MT09383	North Sea, 57°53′56.4″N 0°54′57.6″W	KR084567			Barco *et al.* (2016)
Cyrillia aequalis (Jeffreys, 1867)	MT09222	North Sea, 55°22′15.6″N 0°12′25.2″W	KR084390			Barco *et al.,* 2016
Cyrillia linearis (Montagu, 1803)	BAU-2234	Italy, Giannutri Is., loc. Le Cerniette, 42°15′10″N 011°05′32″E	MK410632	MK410605	MK410585	This study
Cyrillia linearis (Montagu, 1803)	BAU-2912.1	Italy, Giglio Is., Cala Cupa, 42°22′06′′N 10°55′12′′E, 10-20 m	MK410623	MK410599		This study
Cyrillia obesa (Høisæter, 2016)	ZMBN-E-37-68	Norway, 60°18′N 5°07′48″E	JF834220	MK410610		Høisæter (2016); this study
Clathromangelia granum (Philippi, 1844)	BAU-3082.1	Italy, Scilla, 38°15′23″N 15°42′45″E, 35-37 m	MK410624	MK410600		This study
Clathromangelia loiselieri Oberling, 1970	BAU-1545	Greece, Astypalea Is., VYLLAS, 36°35′02″N 026°25′24′′E, 1-7 m, under rocks	MK410627	MK410601		This study
Daphnella sp.	MNHN-IM-2007-17927	Salomon Is., Vella Gulf, SALOMON 2, 8°3′32.4′ S 156°54′32.4″E	EU015740	HQ401674	HQ401607	Puillandre *et al.* (2008)
Daphnella (Paradaphne) corimbensis Rolán, Otero-Schmitt & Fernandes, 1998	BAU-2989	Canary Islands, Tenerife, Radazul, 28°24′08″N 16°19′5″W, 20 m	MK410635	MK410608	MK410587	This study
Eucyclotoma cymatodes (Hervier, 1897)	MNHN-IM-2007-17903	Philippines, Pamilacan Is., PANGLAO 2004, 9°29′24′′N 123°56′0′′E	EU015678	HQ401676	HQ401610	Puillandre *et al.* (2008)
Hemilienardia acinonyx Fedosov, Stahlschmidt, Puillandre, Aznar-Cormano & Bouchet, 2017	MNHN-IM-2009-33593	Philippines, Panglao Is., Momo beach	KX233238	KX233249		Fedosov *et al.* (2017)
Hemilienardia calcicincta (Melvill & Standen, 1895)	MNHN-IM-2007-17861	Philippines, Panglao Is., Sungcolan Bay, PANGLAO 2004, 9°38′30′′N 123°49′12′′E	EU015683	HQ401684	HQ401618	Puillandre *et al.* (2008)
Leufroyia concinna (Scacchi, 1836)	ZMBN-H-3-69a	Norway, 60°33′N 4°52′12″E	JF834222			Høisæter (2016)
Leufroyia concinna (Scacchi, 1836)	ZMBN-E-23-67	Norway, 60°18′N 5°10′48″E	JF834223			Høisæter (2016)
Leufroyia concinna (Scacchi, 1836)	ZMBN-020209-F	Norway, 60°13′48″N 5°12′E	JF834224	JF834218		Høisæter (2016)
Leufroyia concinna (Scacchi, 1836)	BAU-2254.1	Croatia, Biograd, 43°55′51″N 15°26′42″E	MK410616	MK410593	MK410580	This study
Leufroyia concinna (Scacchi, 1836)	BAU-2237	France, La Ciotat, Figuerolles, 43°09′53″N 5°35′45″E, 15 m	MK410633	MK410606		This study
Leufroyia leufroyi (Michaud, 1828)	BAU-2240.1	Croatia, Sevid, 43°28′46″N 16°02′08″E, 2-4 m	MK410613			This study

Leufroyia leufroyi (Michaud, 1828)	BAU-1742	Sardinia, Villasimius, 39°07′43″N 9°32′17″E Mid-	MK410628		MK410584	This study
'Phymorhynchus' sp.	MCR-1256	Cayman Spreading Centre, Beebe vent chimneys	KJ566952	KM979537		Plouviez *et al.* (2015)
Pleurotomella sp.	MNHN-IM-2007-17848	New Caledonia, Lansdowne, EBISCO, 20°4′52.32′′S 160°20′2.34′′E	EU015657	HQ401701	HQ401640	Puillandre *et al.* (2008)
Pseudodaphnella aureotincta (Hervier, 1897)	MNHN-IM-2007-17878	Philippines, Pamilacan Is., PANGLAO 2004, 9°29′24′′N 123°56′6′′E	EU015700	HQ401688	HQ401624	Puillandre *et al.* (2008)
Raphitoma bicolor (Risso, 1826)	BAU-1897	France, St. Maxime, 43°18′49″N 6°40′22″E, intertidal	MK410630	MK410603		This study
Raphitoma cordieri (Payraudeau, 1826)	BAU-2262.1	Croatia, Sukosan, 44°02′04″N 15°18′57″E	MK410619	MK410595	MK410582	This study
Raphitoma cordieri (Payraudeau, 1826)	BAU-2262.2	Croatia, Sukosan, 44°02′04″N 15°18′57″E	MK410625			This study
Raphitoma densa (Monterosato, 1884)	BAU-2257.1	Croatia, Sukosan, 44°02′10″N 15°18′55″E	MK410617	MK410594	MK410581	This study
Raphitoma densa (Monterosato, 1884)	BAU-1895	Italy, Torre Colimena, 40°17′39″N 17°45′17″E, 3 m	MK410629	MK410602		This study
Raphitoma horrida (Monterosato, 1884)	BAU-2264.1	Croatia, Dugi Otok, 43°59′N 15°05′34″E	MK410620	MK410596	MK410583	This study
Raphitoma horrida (Monterosato, 1884)	BAU-1900	Corsica, Tour d'Ancone, 42°02′36″N 8°43′20″E, 10 m	MK410631	MK410604		This study
Raphitoma horrida (Monterosato, 1884)	BAU-1906.1	France, St. Maxime, 43°18′49″N 6°40′22″E, intertidal	MK410612	MK410590	MK410577	This study
Raphitoma laviae (Philippi, 1844)	BAU-2253.1	Croatia, Telascjca, 43°53′30″N 15°09′33″E	MK410615	MK410592	MK410579	This study
Raphitoma laviae (Philippi, 1844)	BAU-2246.1	Croatia, Zaton, 44°13′07″N 15°09′41″E	MK410614	MK410591	MK410578	This study
Raphitoma maculosa Høisæter, 2016	ZMBN-040809_X	Norway, 60°18′N 5°07′48″E	MK410638			Høisæter (2016); this study
Raphitoma philberti (Michaud, 1829)	BAU-2365.1	Croatia, Biograd, 43°55′51″N 15°26′42″E	MK410622	MK410598		This study
Raphitoma philberti (Michaud, 1829)	BAU-2258.1	Croatia, Vrsi, 44°16′56″N 15°12′35″E	MK410618			This study
Raphitoma philberti (Michaud, 1829)	BAU-1893.1	Greece, Limnos, Koukonisi Bay, 39°53′07″N 25°16′16″E	MK410611			This study
Raphitoma philiberti (Michaud, 1829)	BAU-3046	Greece: Astypalea Is., Vai, VYLLAS 2017, 36°35′13′′N 026°24′10′′E, 1-6 m, under rocks	MK410636		MK410588	This study
Raphitoma pseudohystrix (Sykes, 1906)	BAU-3205	Malta, SW, off Gnejna Bay, 35°49′54.3″N 14°17′15.2″E, 220 m, fine sand and mud	MK410637	MK410609	MK410589	This study
Raphitoma purpurea (Montagu, 1803)	BAU-2337.1	France, Ploubazlanec, 48°48′5″N 3°00′10″W, intertidal	MK410621	MK410597		This study
Raphitoma purpurea (Montagu, 1803)	BAU-2337.3	France, Ploubazlanec, 48°48′5″N 3°00′10″W, intertidal	MK410626			This study
Raphitoma purpurea (Montagu, 1803)	BAU-2338	France, Ploubazlanec, 48°48′5″N 3°00′10″W, intertidal	MK410634	MK410607	MK410586	This study
'Raphitoma' rubroapicata (E. A. Smith, 1885)	MNHN-IM-2007-17890	Philippines, Panglao Is., off Momo beach, PANGLAO 2004, 9°36′30′′N 123°45′18′′E	EU015713	HQ401703	HQ401642	Puillandre *et al.* (2008)
'Raphitoma' sp.	MNHN-IM-2007-17882	Philippines, Balicasag Is., PANGLAO 2004, 9°30′54′′N 123°41′12′′E	EU015704			Puillandre *et al.* (2008)
Rimosodaphnella sp.	MNHN-IM-2007-17836	New Caledonia, Koumac Sector, around Ouaco, BOA1, 20°48′42′′S 164°24′12′′E	EU015645	HQ401704		Puillandre *et al.* (2008)
Spergo sp.	MNHN-IM-2007-17841	New Caledonia, SE Fairway, EBISCO, 21°32′36′′S 162°28′36′′E	EU015650	HQ401682	HQ401616	Puillandre *et al.* (2008)
Taranis sp.	MNHN-IM-2007-42296	Philippines, AURORA 2007, 15°56′34.2′′N 121°50′11.4′′E	HQ401584	HQ401707	HQ401645	Puillandre *et al.* (2011)

Taranis sp.	MNHN-IM-2013-52046	Papua New Guinea, Bismarck Archipelago, W Kairiru I., 3°19′26.4″S 143°27′14.4″E	KR087296	KR088045	KR087382	Fedosov *et al.* (2015)
Teretiopsis cf. *hyalina* Sysoev & Bouchet, 2001	MNHN-IM-2007-17845	New Caledonia, SE Fairway, EBISCO, 21°28′8′′S 162°33′54′′E	EU015654	HQ401708	HQ401646	Puillandre *et al.* (2008)
Thatcheria mirabilis Angas, 1877	MNHN-IM-2007-17924	Salomon Is., SE Isabel, SALOMON 2, 8°16′54′′S 159°59′42′′E	EU015736	FJ868138	FJ868124	Puillandre *et al.* (2008)
Veprecula cf. *spanionema* (Melvill, 1917)	MNHN-IM-2007-17883	Philippines, Balicasag Is., PANGLAO 2004, 9°30′54′′N 123°41′12′′E	EU015705	HQ401717	HQ401654	Puillandre *et al.* (2008)
Clathurellidae						
Lienardia crassicostata (Pease, 1860)	NA	NA	JF823629	JF823611	JF823590	Cabang *et al.* (2011)
Lienardia nigrotincta (Montrouzier in Souverbie & Montrouzier, 1873)	MNHN-IM-2007-42607	Vanuatu, E Luganville, Segond Channel, SANTO 2006, 15°30′58′′S 167°11′52′′E	HQ401575	HQ401666	HQ401599	Puillandre *et al.* (2011)
Nannodiella ravella (Hedley, 1922)	MNHN-IM-2007-17904	Philippines, Panglao Is., off San Isidro, PANGLAO 2004, 9°33′54′′N 123°50′30′′E	EU015679	HQ401698	HQ401634	Puillandre *et al.* (2008)
Mangeliidae						
Anticlinura sp. Thiele, 1934	MNHN-IM-2007-42513	Salomon Is., Sta Isabel, SALOMON 2, 8°47′0′′S 159°37′54′′E	HQ401572	HQ401660	HQ401590	Puillandre *et al.* (2011)
Propebela cf. *scalaris* (Møller, 1842)	MNHN-IM-2007-42325	Norway, Hornsund, Svalbard	HQ401582	HQ401699	HQ401635	Puillandre *et al.* (2011)
Toxicochlespira pagoda Sysoev & Kantor, 1990	MNHN-IM-2007-17925	Salomon Is., Choiseul, SALOMON 2, 6°37′12.6′′S 156°12′44.4′E	EU015738	HQ401711	HQ401649	Puillandre *et al.* (2008)
Conidae						
Conus radiatus Gmelin, 1791	MNHN-IM-2007-30883	Philippines, Bohol Is., Ubajan, PANGLAO 2004, 9°41′30′′N 12350′60′′E	KJ550437	KJ550900	KJ551133	Puillandre *et al.* (2014)
Conus textile Linnaeus, 1758	MNHN-IM-2007-30900	Vanuatu, NW Aésé Is., SANTO 2006, 15°25′7′′S 167°14′10′′E	KJ550497	KJ550930	KJ551134	Puillandre *et al.* (2014)
Conus ventricosus Gmelin, 1791	NA	Djerba, Tunisia	KJ550006	KJ550745	KJ551370	Puillandre *et al.* (2014)

Institutional abbreviations are as follows: BAU, Department of Biology and Biotechnologies, 'Sapienza' University, Rome; MNHN, Muséum national d'Histoire naturelle, Paris; MT, German Centre for Marine Biodiversity Research, Senckenberg Institute, Wilhelmshaven; ZMBN, University Museum of Bergen Natural History Collections. NA indicates that specimen registration data were not available.

MATERIAL AND METHODS

The dataset is composed of 62 specimens representing 14 raphitomid genera from the Mediterranean Sea, North Sea and Indo–Pacific region. DNA sequence data were generated by us for28 of these specimens; sequence data for the remaining individuals were obtained from GenBank (Table 1). The specimens sampled included 17 species ascribed to the genus *Raphitoma s. l.*: *Raphitoma aequalis*, *R. bicolor*, *R. concinna*, *R. cordieri*, *R. corimbensis*, *R. densa*, *R. horrida*, *R. laviae*, *R. leufroyi*, *R. linearis*, *R. maculosa, R. obesa, R. philberti*, *R. pseudohystrix*, *R. purpurea*, *R. rubroapicata*, an unidentified *Raphitoma* sp. The dataset also included 13 other raphitomid or putative raphitomid genera: *Clathromangelia* Monterosato, 1884; *Hemilienardia* Boettger, 1895; *Eucyclotoma* Boettger, 1895; *Rimosodaphnella* Cossmann, 1916; *Veprecula* Melvill, 1917; *Pleurotomella* Verrill, 1872; *Phymorhynchus* Dall, 1908; *Pseudodaphnella* Boettger, 1895; *Spergo* Dall, 1895; *Taranis* Jeffreys, 1870; *Thatcheria* Angas, 1877; *Daphnella* Hinds, 1844; and *Teretiopsis* Kantor & Sysoev, 1989. Specimens from two other conoidean families were also included. These groups are the Clathurellidae (the putative sister group of the raphitomids) and the Mangeliidae (considered to be sister to the clade comprising the Raphitomidae and Clathurellidae) (Abdelkrim *et al.,* 2018). The outgroup comprised three species of Conidae.

DNA was isolated from a piece of foot tissue following a standard proteinase K/phenol–chloroform extraction protocol (Oliverio & Mariottini, 2001). Three mitochondrial gene fragments were amplified: the 658-bp barcode region of cytochrome *c* oxidase subunit I (COI), with universal primers LCO1490 and HC02198 (Folmer *et al.,* 1994); a *c.* 500-bp region of the 16S rRNA gene, with primers 16SA (Palumbi, 1996), and CGLeuR (Hayashi, 2003) or 16SH (Espiritu *et al.,* 2001); and a *c.* 600 bp region of the 12S rRNA, with primers 12SI and 12SIII (Oliverio & Mariottini, 2001). The following PCR conditions were used: initial denaturation (94 °C for 4 min); 35 cycles of denaturation (94 °C for 30 s); annealing (48–51 °C for COI, 52 °C for 16S rRNA, 58–60 °C for 12S rRNA for 40 s) and extension (94 °C for60''); final extension (72 °C for 10 min). Amplicons were purified using Exosap-IT (USB Corporation) and sequenced by Macrogen Inc. (The Netherlands).

COI sequences were aligned using Geneious v. 11 (Kearse *et al.*, 2012). Sequences for 16S rRNA and 12S rRNA were aligned with the online version of MAFFT v. 7 (Katoh *et al.,* 2017, Kuraku *et al.,* 2013), using the Q-INS-I algorithm. Ambiguous regions in the 16SrRNA and 12S rRNA alignments were discarded using Gblocks v. 0.91b (Castresana, 2000) with respectively 76% and 64% of the original positions being retained; we used default options.

In our phylogenetic analyses we used the three single-gene datasets as well as a combined dataset (COI+12S rRNA+16S rRNA). The Bayesian information criterion (BIC) implemented in jModelTest v. 2.1.7 (Posada, 2008) was used to identify the best substitution models and parameters for each gene partition; the substitution model selected for all datasets was GTR+I+G. Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian approaches; all analyses were run on the CIPRES Science Gateway (Miller Pfeiffer & Schwartz, 2010). ML analyses were done using RAxML v. 8 (Stamatakis, 2014). Branch support estimates were based on 1000 bootstrap replicates. Bayesian analyses were performed using MrBayes v. 3.2.3 (Huelsenbeck & Ronquist, 2001); analyses were run for 10^7 generations, with trees sampled every 1000 generations and 25% burn-in (for all other parameters we used default settings). Convergence of MCMC was assumed to have occurred when the effective sample size was >200 and the potential scale reduction factor was ~1, as calculated with Tracer v. 1.6. Branches with bootstrap values (BS) ≥70% and posterior probabilities (PP) ≥0.95 were considered to be strongly supported.

RESULTS

The final datasets consisted of 62 COI sequences, 47 16S rRNA sequences and 34 12S rRNA sequences. Single-gene and combined analyses yielded topologically similar trees. The trees obtained from the concatenated dataset tended to show higher branch support values, and this was especially so in the case of the Bayesian analysis (Fig. 2, Supplementary Material Figs S1–S7). The three families Raphitomidae, Clathurellidae and Mangeliidae together formed a strongly supported monophyletic group. Our Bayesian analyses recovered the Clathurellidae as sister to the raphitomid clade, but this relationship was not strongly supported (e.g. PP = 0.71 for combined dataset, Fig. 2). We found consistently strong support for the monophyly of the Raphitomidae.

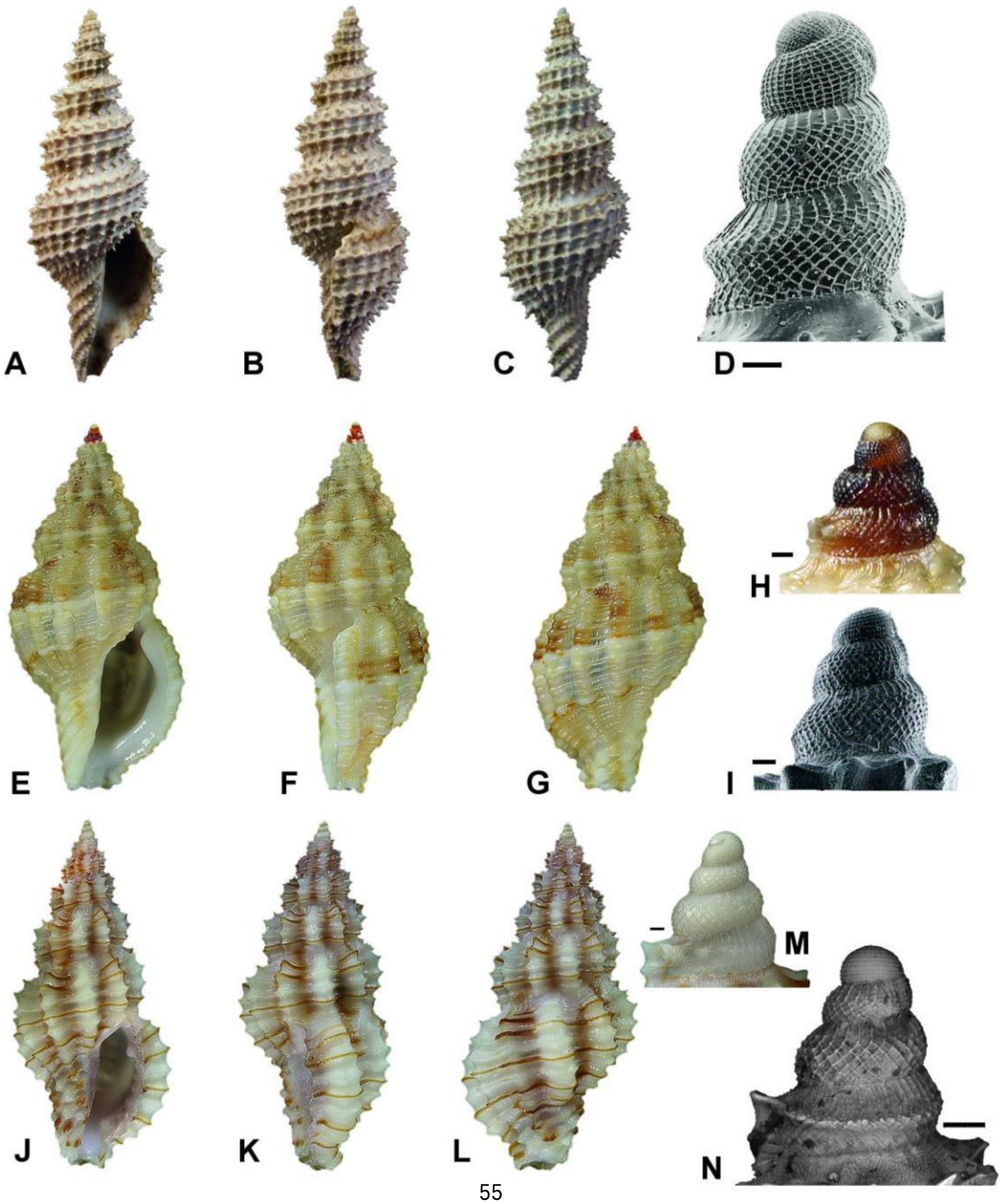


Figure 1. Type species of Raphitomidae illustrated by representative shell material. **A–D.** *Raphitoma hystrix* Bellardi, 1847, the type species of the genus *Raphitoma* Bellardi, 1847. Neotype (MRSN n. cat. 011.16.008) from Colli Astesi (Italy; Pliocene, Piacentian); shell height is 17.6 mm. **E–I.** *Leufroyia leufroyi* (Michaud, 1828), the type species of the genus *Leufroyia* Monterosato, 1884. Shell from a depth of 40 m, Ile Rousse (Corsica); shell height is 11 mm. **J–M.** *Cyrillia linearis* (Jeffreys, 1867), the type species of the genus *Cyrillia* Kobelt, 1905. Shell from a depth of 1 m, Lastovo (Croatia); shell height is 7 mm. All scale bars are 100 µm in length.

Within the Raphitomidae, specimens of the genus *Raphitoma s. l.* were distributed across five clades. *Raphitoma leufroyi* and *R. concinna* were strongly supported as sister species (BS = 99%, PP = 1); these two species together with *R. rubroapicata* and the genus *Hemilienardia* formed a clade that was strongly supported in the ML analysis (BS = 85%), but not in the Bayesian analysis (PP = 0.94). The Bayesian analysis showed strong support for the clade comprising *R. corimbensis*, *Rimosodaphnella* and *Veprecula* (PP = 0.95), and the clade comprising the '*Raphitoma*' sp. from the Philippines (MNHN-IM-2007-17882) and *Eucyclotoma cymatodes* (PP = 0.99). Relationships between these two clades and other raphitomids were unresolved. The two species of *Clathromangelia*, which were strongly supported as sister taxa (BS = 99%, PP = 1), formed a clade with *Pseudodaphnella*, *Eucyclotoma* and a '*Raphitoma*' sp. (MNHN-IM-2007-17882) in the Bayesian analysis (PP = 1). This clade was nested within the raphitomid clade.

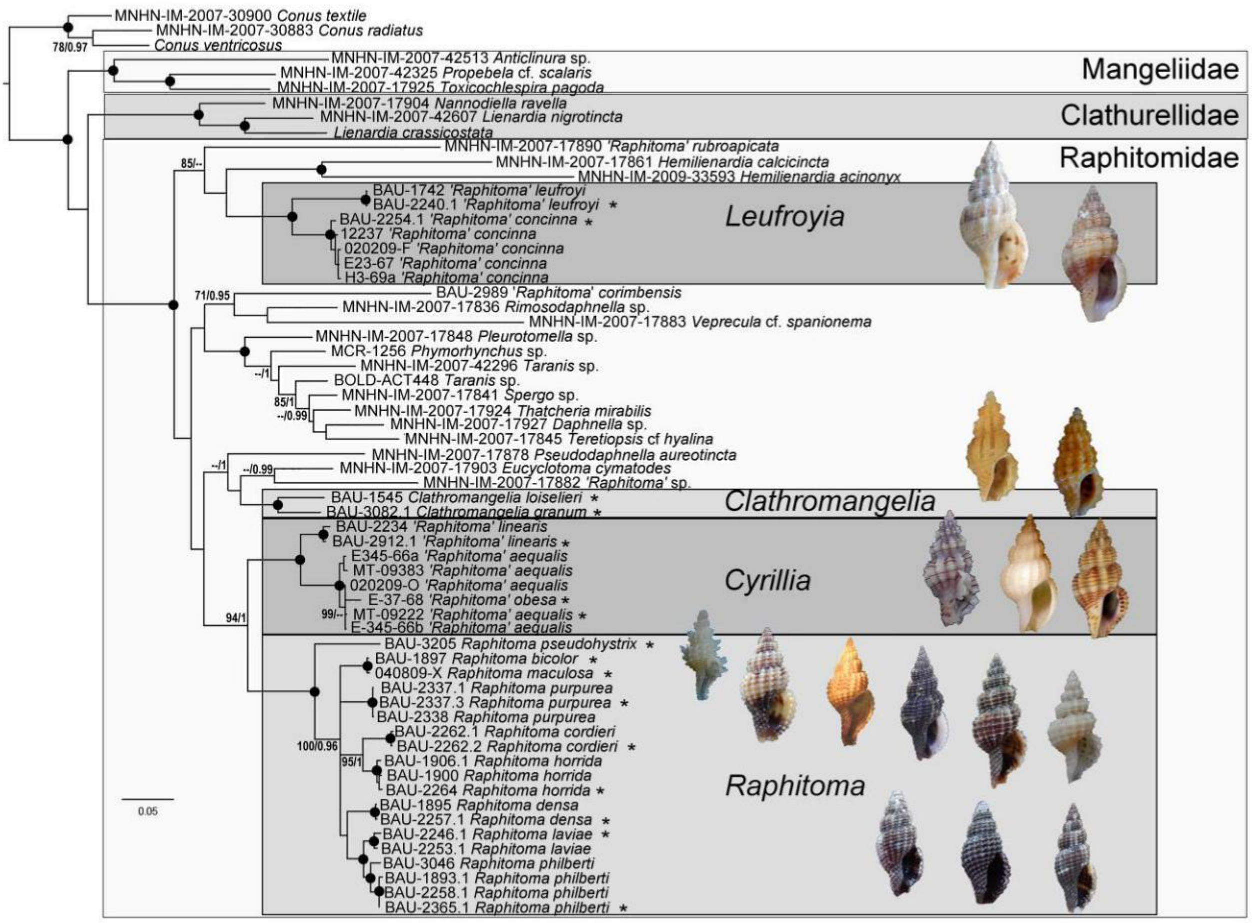


Figure 2. Phylogenetic relationships among conoideans, as illustrated by the Bayesian majority consensus tree of the combined dataset (COI+12S rRNA+16S rRNA). The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as posterior probabilities for the Bayesian analysis (only values ≥0.95 are shown) and as bootstrap percentages for the ML analysis (only values ≥70% are shown). Closed circles indicate branches with bootstrap support >95% and posterior probabilities >0.98. Shells of vouchers are indicated by asterisks and are not to scale. Scale bar indicates substitutions per site.

Most of the specimens ascribed to *Raphitoma s. l.* formed a strongly supported clade only in the Bayesian analyses of the 12S rRNA, 16S rRNA and combined datasets (PP = 1 in Fig. 1; see also Supplementary Material Figs S1, S3, S5); this large clade was not strongly supported in most of the remaining analyses (Supplementary Material Figs S2, S4, S6, S7). However we consistently found strong support for two lineages within this clade. The first sublineage comprised *Raphitoma linearis, R. aequalis* and *R. obesa* (BS = 100%, PP = 1). The second sublineage consisted of *R. pseudohystrix*, *R. bicolor, R. cordieri, R. densa, R. horrida, R. laviae, R. maculosa, R. philberti* and *R. purpurea* (BS = 100%, PP = 1); in this sublineage, *R. pseudohystrix* was sometimes strongly supported as sister to the clade containing the other members of the sublineage.

DISCUSSION

The Bayesian analyses showed a sister-group relationship between the Raphitomidae and Clathurellidae; this agrees with the most recent phylogenetic hypotheses on the Conoidea, which are based on the most extensive taxon sampling achieved to date (Puillandre *et al.,* 2011, Abdelkrim *et al.,* 2018), but was not strongly supported. The genus *Clathromangelia* was confirmed as belonging to the Raphitomidae, as has previously been suggested on the basis of anatomical and protoconch data (Oliverio, 1995). Our finding that *Clathromangelia* is a member of a clade containing *Pseudodaphnella* and *Eucyclotoma*, is not surprising given the similarity in shell morphology between these three taxa, and particularly between *Clathromangelia* and *Pseudodaphnella*. This study shows that most of the species ascribed to *Raphitoma s. l.* fall into three clades, and we propose that these distinct lineages should be ranked as genera.

We propose to use the name *Raphitoma* for the clade containing *R. pseudohystrix* (believed to be the closest extant relative of the type species of *Raphitoma*), *R. bicolor, R. cordieri*, *R. densa*, *R. horrida*, *R. laviae*, *R. maculosa*, *R. philberti and R. purpurea*. We note that a strongly supported sister-group relationship of *R. pseudohystrix* to the other species in the sublineage was recovered in some analyses. We also note that *R. pseudohystrix* never formed a clade with other morphologically similar spiny-shelled raphitomids, such as *R. cordieri* and *R. horrida.*

The clade comprising *R. linearis*, *R. aequalis* and *R. obesa* may be the sister group of *Raphitoma s. s.*, but this relationship was strongly supported in only three of the eight analyses we carried out. We propose, therefore, to treat the clade of *R. linearis*, *R. aequalis* and *R. obesa* as a distinct genus for which the name *Cyrillia* Kobelt, 1905 is available (see Systematic Descriptions, below). Our results show that the *R. leufroyi* + *R. concinna* lineage is not nested within the clade that contains most of the *Raphitoma* species or the clade of *R. linearis* + *R. aequalis* + *R. obesa*. We use the generic name *Leufroyia* Monterosato, 1884 for the *R. leufroyi* + *R. concinna* lineage.

Raphitoma corimbensis was not related to any of these lineages (*Raphitoma*, *Cyrillia* or *Leufroyia*) and, as suggested by its shell morphology (and by that of its certainly close relative, *R. bedoyai* Rolán, Otero-Schmitt & Fernandes, 1998), further studies on its systematic position should explore the relationship between this species and lineages currently placed in the genus *Daphnella* Hinds, 1844 (which may prove to be polyphyletic). We suggest a provisional classification of *R. corimbensis* and *R. bedoyai* in *Paradaphne* Laseron, 1954 (type species: *Daphnella botanica* Hedley, 1922 by original designation), which is currently ranked as a subgenus of *Daphnella* Hinds,

1844. The rationale for this classification is that the shell features of the type species of *Paradaphne* is strikingly similar to *R. bedoyai* and *R. corimbensis*.

Our findings suggest that *Raphitoma rubroapicata* (E.A. Smith, 1885), and the '*Raphitoma*' sp. (IM-2007-17882) do not belong in the genus *Raphitoma*, but further work involving broader taxon sampling is needed to clarify their relationships.

On the basis of the phylogenetic results presented here and shell morphological data, we propose the following new classification for the bulk of Mediterranean/East Atlantic species currently ascribed to *Raphitoma s. l.*, as previously conceived.

Table 2. List of Recent species of the genus *Raphitoma* with their geographic range (NEA, North East Atlantic; WA, West Africa; Mac, Macaronesia; Med, Mediterranean) and the type of protoconch (m, multispiral; p, paucispiral).

	Species	NEA	WA	Mac	Med	P
	R. alida Pusateri & Giannuzzi-Savelli, 2016				+	p
	R. alleryana (Sulliotti, 1889)				+	p
	R. alternans (Monterosato, 1884)				+	p
	R. arnoldi (Pallary, 1906)				+	p
	R. atropurpurea (Locard & Caziot, 1900)	+		+	+	m
	R. bartolinorum Pusateri & Giannuzzi-Savelli, 2018				+	p
	R. bernardoi Rolán, Otero-Schmitt & Fernandes, 1998		+			m
*	*R. bicolor* (Risso, 1826) = *R. maculosa* Høisæter, 2016	+		+	+	m
	R. bourguignati (Locard, 1891)	+			+	m
	R. bracteata (Pallary, 1904)				+	p
	R. brunneofasciata Pusateri, Giannuzzi-Savelli & Oliverio, 2013				+	m
	R. christfriedi Rolán, Otero-Schmitt & Fernandes, 1998		+		+	m
	R. contigua (Monterosato, 1884)	+			+	m
	R. corbis (Potiez & Michaud, 1838)				+	m
*	*R. cordieri* (Payraudeau, 1826)	+	+	+	+	m
*	*R. densa* (Monterosato, 1884)			+	+	m
	R. digiulioi Pusateri & Giannuzzi Savelli, 2017				+	m
	R. ebreorum Pusateri & Giannuzzi-Savelli, 2018				+	m
	R. echinata (Brocchi, 1814) sensu Auctores	+		+	+	m
	R. farolita F. Nordsieck, 1977				+	p
	R. formosa (Jeffreys, 1867)	+				m
	R. griseomaculata Pusateri & Giannuzzi-Savelli 2018				+	p
	R. hispida (Monterosato, 1890)	+			+	m
*	*R. horrida* (Monterosato, 1884)				+	p
	R. kharybdis Pusateri & Giannuzzi-Savelli, 2018				+	p
*	*R. laviae* (Philippi, 1844)				+	m
	R. lineolata (Bucquoy, Dautzenberg & Dollfus, 1883)	+			+	m
	R. locardi Pusateri, Giannuzzi-Savelli & Oliverio, 2013				+	m
*	*R. maculosa* Høisæter, 2016 [=? *R. bicolor*]	+				m
	R. mirabilis (Pallary, 1904)				+	p
	R. nivea (J. T. Marshall in Sykes, 1906)				+	p
	R. oblonga (Jeffreys, 1867)	+				m
	R. papillosa (Pallary, 1904)				+	p
*	*R. philberti* (Michaud, 1829)			+	+	p
	R. pruinosa (Pallary, 1906)				+	p
*	*R. pseudohystrix* (Sykes, 1906)			+	+	p
	R. pumila (Monterosato, 1890)				+	m
	R. pupoides (Monterosato, 1884)				+	m
*	*R. purpurea* (Montagu, 1803)	+	+	+	+	m
	R. radula (Monterosato, 1884)				+	m
	R. skylla Pusateri & Giannuzzi-Savelli, 2018				+	m
	R. smriglioi Pusateri & Giannuzzi-Savelli, 2013				+	p
	R. spadiana Pusateri & Giannuzzi-Savelli, 2012				+	p
	R. strucki (Maltzan, 1883)		+			?
	R. syrtensis F. Nordsieck, 1977				+	p

R. zelotypa Rolán, Otero-Schmitt & Fernandes, 1998	+	m

Species included in our molecular systematic analyses are indicated by an asterisk.

SYSTEMATIC DESCRIPTIONS

Family RAPHITOMIDAE Bellardi, 1875
Genus *Raphitoma* Bellardi, 1847
(Fig. 1A–D; Table 2)

Raphitoma Bellardi, 1847: 612. [type species *Raphitoma histrix* Bellardi, 1847 (ex *Pleurotoma hystrix* Cristofori & Jan, 1832, *nomen nudum*) SD, Monterosato, 1872: 54].

Homotoma Bellardi, 1875: 22 (type species *Murex reticulatus* Renier, 1804; SD, Powell, 1966). *Cordieria* Monterosato, 1884: 131 (type species *Murex reticulatus* Renier, 1804.; SD, Crosse, 1885; erroneously credited to Brocchi, 1814, ICZN, 1999, Art. 67.7; not Rouault, 1848). *Philbertia* Monterosato, 1884: 132 (type species *Pleurotoma bicolor* Risso, 1826; SD, Crosse, 1885). *Peratotoma* Harris & Burrows, 1891: 113 (replacement name for *Homotoma* Bellardi, 1875, not Guérin-Méneville, 1844). *Cyrtoides* F. Nordsieck, 1968: 176 [type species *Pleurotoma rudis* Scacchi, 1836 (not G.B. Sowerby I, 1834; renamed *Cordieria pupoides* Monterosato, 1884 and *Raphitoma neapolitana* F. Nordsieck, 1977) OD].

Diagnosis: Shell small to medium size for family, ranging in height from 5 mm (*R. laviae*) to 25 mm (*R. cordieri, R. bourguignati*); shape turreted to biconic-pupoidal; suture impressed. Protoconch: if multispiral, then 3–4.5 whorls, with protoconch I (embryonic shell) of 0.5–0.7 whorls, with reticulate sculpture of spirals and orthocline axial striae, and protoconch II (larval shell) of 2.3–3.5 whorls, with diagonally cancellate sculpture and often keeled last whorl; if paucispiral, then of 2 whorls, with large nucleus and reticulate sculpture. Teleoconch with slender spire of 5 (*R. brunneofasciata*) to 9 (*R. cordieri*) uniformly convex whorls; reticulate-cancellate sculpture, axials broader than spirals. Fine granular microsculpture occasionally present on whole teleoconch (*R. papillosa*) or on first whorl only (*R. philberti*). Outer lip thickened, with 7–13 inner denticles. Columella simple, slightly sinuous anteriorly. Siphonal canal very short (*R. contigua*) to moderately long (*R. cordieri*). Siphonal notch wide, plain or intorted.

Remarks: As type species of *Cordieria*, Crosse (1885) designated '*Murex reticulatus* Brocchi, 1814' (following the indication by Monterosato: 1884: 131 "*C. reticulata*, (Ren.) Brocc. / = *Murex reticulatus* ed *echinatus*, Brocc. - 1814, p. 423, t. 8, f. 3"). However, *Murex reticulatus* Brocchi (1814: 435, pl. 9, fig. 12) is not a raphitomid, but a species of *Genota* Gray, 1847 (Borsoniidae). It is clear that Monterosato (1884: 131) confused *Murex reticulatus* Brocchi with *M. reticulatus* Renier (which is also invalid: ICZN, 1999: Op. 316); the latter is probably the same as *Murex echinatus* Brocchi, 1814 (=*Raphitoma echinata*) and it was this species that Monterosato (1884) was indicating. Therefore, we retain Crosse's (1885) designation but as an incorrect citation (ICZN, 1999: Art. 67.7) and use Renier's name which, even if unavailable, can be designated as the type species for *Cordieria* and *Homotoma*; see ICZN, 1999: Art 67.1.2).

The phylogenetic results presented here do not support any further splitting of this genus. In this respect in it important to note that the species traditionally ascribed to the 'genera' *Philbertia* and *Cordieria* (=*Peratotoma*) are distributed across the tree. Similarly, the grouping of species in the phylogeny does not correspond to differences in larval development (as indicated by their multispiral or paucispiral protoconch), and this is consistent with the currently accepted view that larval development is not a reliable taxonomic character at the genus level (Bouchet, 1990). The genetic distance between *Raphitoma maculosa* and *R. bicolor* is small (<1%), and this level of variation could well fall within the variation of the latter species when a denser sampling of *R. bicolor* is carried out. In contrast, our phylogenetic data indicate that a DNA-barcode-based approach could potentially be used to discriminate between closely related species of *Raphitoma* (e.g. *R. philberti* and *R. densa* in the COI phylogeny; see Supplementary Material Figs S1, S2). DNA barcodes should be used in combination with shell morphology to define species limits in this difficult group of neogastropods.

Table 3. List of Recent species of the genus *Cyrillia* with their geographic range (NEA, North East Atlantic; WA, West Africa; Mac, Macaronesia; Med, Mediterranean) and the type of protoconch (m, multispiral; p, paucispiral).

Species	NEA	WA	Mac	Med	P
* *C. aequalis* (Jeffreys, 1867)	+		+	+	m
C. ephesina (Pusateri, Giannuzzi-Savelli & Stahlschmidt, 2017)				+	m
C. kabuli (Rolán, Otero-Schmitt & Fernandes, 1998)		+			m
* *C. linearis* (Montagu, 1803)	+		+	+	m
* *C. obesa* (Høisæter, 2016) [=? *C. aequalis*]	+				m
C. zamponorum (Horro, Gori & Rolán, 2019)		+			m

Species included in our molecular systematic analyses are indicated by an asterisk.

Genus *Cyrillia* Kobelt, 1905

(Fig. 1J–M; Table 3)

Cirillia Monterosato, 1884: 133 [type species *Murex linearis* Montagu, 1803, SD Crosse, 1885; not Rondani, 1856 (Diptera)].

Cyrillia Kobelt, 1905: 367 (unjustified emendation of *Cirillia* Monterosato, 1884).

Cenodagreutes E. H. Smith, 1967: 1 (type species *Cenodagreutes aethus* E. H. Smith, 1967 = *Defranciaaequalis* Jeffreys, 1867; OD). *Lineotoma* F. Nordsieck, 1977 (replacement name for *Cirillia* Monterosato, 1884, not Rondani, 1856).

Diagnosis: Shell small in size for family, from 5 mm (*C. linearis*) to 10 mm (*C. ephesina*); biconic, suture impressed. Protoconch 3.5–4 whorls, multispiral, with protoconch I (embryonic shell) of 0.5– 0.7 whorls, with reticulate sculpture of spirals and orthocline axial striae, and protoconch II (larval shell) of 3.3–3.5 whorls, with diagonally cancellate sculpture and weakly keeled last whorl. Teleoconch with slender spire of 5 (*C. linearis*) to 7 (*C. ephesina*) convex whorls, with reticulate-cancellate sculpture; axials broader than spirals. Microsculpture of granules or pustules; growth lines seldom obvious. Outer lip thickened, with 7–13 inner denticles, the 2 anterior-most stronger. Columella simple, slightly sinuous anteriorly. Siphonal canal short; siphonal notch plain.

Remarks: *Cirillia* Monterosato, 1884 is preoccupied by *Cirillia* Rondani, 1856, but the emended name *Cyrillia* Kobelt, 1905 is available, and has already been used (e.g. Ceulemans *et al*., 2018). This is a clear case of a demonstrably intentional emendation (ICZN, 1999: Art. 33.2), since the prescriptions of the Code are met: "there is an explicit statement of intention" ... and "both the original and the changed spelling are cited and the latter is adopted in place of the former" (ICZN, 1999: Art. 33.2.1). As an intentional, yet unjustified emendation, the name that should be used is *Cyrillia* Kobelt, 1905 (ICZN, 1999: Art. 33.2.3).

Cirillia aequalis and *C. linearis* lack radula and venom gland. Our phylogenetic results suggest that denser sampling may show *C. obesa* to be simply a colour variant of *C. aequalis*. *Cyrillia zamponorum* from São Tomé Island and another probably undescribed species from Madagascar (N. Puillandre & M. Oliverio, unpubl.) indicate that this lineage has a wide geographical distribution.

Table 4. List of Recent species of the genus *Leufroyia* with their geographic range (NEA, North East Atlantic; WA, West Africa; Mac, Macaronesia; Med, Mediterranean) and the type of protoconch (m, multispiral; p, paucispiral).

	Species	NEA	WA	Mac	Med	P
*	*L. concinna* (Scacchi, 1836)	+		+	+	m
	L. erronea Monterosato, 1884				+	m
*	*L. leufroyi* (Michaud, 1828)	+	+	+	+	m
	L. villaria (Pusateri & Giannuzzi-Savelli, 2008)		+		+	m

Species included in our molecular systematic analyses are indicated by an asterisk.

Genus *Leufroyia* Monterosato, 1884

(Fig. 1E–I; Table 4)

Leufroyia Monterosato, 1884: 134 (type species *Pleurotoma leufroyi* Michaud, 1828; SD Crosse, 1885).

Diagnosis: Shell medium to large size for family, from 15 mm *(L. concinna)* to 24 mm *(L. villaria*); shape suboval (*L. erronea*) to fusiform (*L. leufroyi*). Protoconch of 3–3.5 whorls with protoconch I (embryonic shell) of 0.5–0.7 whorls, with reticulate sculpture of spirals and orthocline axial striae, and protoconch II (larval shell) of 2.5–3 whorls, with diagonally cancellate sculpture, sometimes lightly keeled last whorl. Teleoconch with slender spire of 5 (*L. concinna*) to 7 (*L. villaria*) uniformly convex whorls; sculpture of thin, numerous low spiral cords and broader, wavy axial ribs. Microsculpture of dense, rather conspicuous growth lines, or rugae; no granules or pustules. Inner lip smooth with no denticles. Columella simple, slightly sinuous anteriorly. Siphonal canal short (*L. erronea*) to moderately long (*L. leufroyi*); siphonal notch wide, plain.

Remarks: The protoconch is wider (diameter = *c.* 220–250 µm) and lower than in the 'multispiral' propoconch of species of *Raphitoma* and *Cyrillia*.

ACKNOWLEDGEMENTS

We thank Jean Louis Delemarre, Michel Le Quement, Constantin Mifsud and Jakov Prkić for providing critical specimens for our molecular work. Extralimital material was collected as part of the MNHN *Tropical Deep-Sea Benthos* (Aurora, BOA1, EBISCO, Salomon 2) and *Our Planet Reviewed* (Papua Niugini, Santo 2006) programmes, or stand-alone expeditions (Panglao 2004); Bouchet (2009) and Bouchet *et al.* (2008, 2016) provide details on the context of the expeditions and the partnerships involved. All expeditions operated under permits provided by the host countries and satisfy the conditions set by the Convention on Biological Diversity for access to genetical resources. Stefano Bartolini, Vittorio Garilli, Andrea Nappo and Bruno Sabelli provided help with photography. Part of the molecular work was conducted by Louise Lindblom in the DNA Lab at the University of Bergen. SEM photos were taken at the Laboratory of Technological and Functional Analyses of Prehistoric Artifacts of Sapienza University of Rome, with the kind help of Cristina Lemorini (Department of Classics). The work was funded partly by the Sapienza University of Rome (grant AR11715C7E17226C/2017 to VR and RM11715C818F7955/2017 to MO). Virginie Héros and Philippe Bouchet (MNHN) commented on initial drafts of the manuscript; two anonymous reviewers provided constructive feedback; and David Reid suggested a number of editorial improvements.

REFERENCES

AARTSEN, J.J. VAN, MENKHORST, H.P.M.G. & GITTENBERGER, E. 1984. The marine Mollusca of the Bay of Algeciras, Spain, with general notes on *Mitrella*, Marginellidae and Turridae. *Basteria,* **Supplement 2**: 1–135.

ABDELKRIM, J., AZNAR-CORMANO, L., FEDOSOV, A. E., KANTOR, Y. I., LOZOUET, P., PHUONG, M. A., ZAHARIAS, P. & PUILLANDRE, N. 2018. Exon-capture-based phylogeny and diversification of the venomous gastropods (Neogastropoda, Conoidea). *Molecular Biology and Evolution*, **35**: 2355–2374.

BARCO, A., RAUPACH, M.J., LAAKMANN, S., NEUMANN, H. & KNEBELSBERGER, T. 2016. Identification of North Sea molluscs with DNA barcoding. *Molecular Ecology Resources,* **16**: 288–297.

BELLARDI, L. 1847 [1848]. Monografia delle Pleurotome fossili del Piemonte. *Memorie della Reale Accademia delle Scienze di Torino*, serie 2, **9**: 531–650, pls 1–4. [Journal issue published in 1848, but a separate distributed in 1847 as: *Monografia delle Pleurotome Fossili del Piemonte*. Torino].

BONFITTO, A., & MORASSI, M. 2013. New Indo–Pacific species of *Rimosodaphnella* Cossmann, 1916 (Gastropoda: Conoidea): a genus of probable Tethyan origin. *Molluscan Research*, **33**: 230–236.

BOUCHET, P. 1990. Turrid genera and mode of development: the use and abuse of protoconch morphology. *Malacologia,* **32**: 69–77.

BOUCHET, P., 2009. From specimens to data, and from seashells to molluscs: the Panglao Marine Biodiversity Project. *Vita Malacologica*, **8**: 1–8.

BOUCHET, P., HEROS, V., LOZOUET, P. & MAESTRATI, P. 2008. A quarter-century of deep-sea malacological exploration in the South and West Pacific: Where do we stand? How far to go? Tropical Deep-Sea Benthos, Volume 25. *Mémoires du Muséum National d'Histoire Naturelle*, **196**: 9–40.

BOUCHET, P., LE GUYADER, H. & PASCAL, O. 2011. The "making of" Santo 2006.*Patrimoines Naturels*, **70**: 529–548.

BOUCHET, P., KANTOR, Y.I., SYSOEV, A. & PUILLANDRE, N. 2011. A new operational classification of the Conoidea. *Journal of Molluscan Studies,* **77**: 273–308.

CABANG, A.B., IMPERIAL, J.S., GAJEWIAK, J., WATKINS, M., CORNELI, P.S., OLIVERA, B.M. & CONCEPCION, G.P. 2011. Characterization of a venom peptide from a crassispirid gastropod. *Toxicon,* **58**: 672–680.

CASTRESANA, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution,* **17**: 540–552.

CEULEMANS, L., VAN DINGENEN, F. & LANDAU, B.M. 2018. The lower Pliocene gastropods of Le Pigeon Blanc (Loire- Atlantique, northwest France). Part 5 – Neogastropoda (Conoidea) and Heterobranchia (fine). *Cainozoic Research.* **18**: 89–176.

CROSSE, H. 1885. Nomenclature generica e specifica di alcune Conchiglie Mediterranee, pel marchese di Monterosato. *Journal de Conchyliologie,* **33**: 139–142.

DALL, W.H. 1918. Notes on the nomenclature of the mollusks of the family Turritidae. *Proceedings of the United States National Museum*, **54**: 313–333.

ESPIRITU, D.J.D., WATKINS, M., DIA-MONJE, V., CARTIER, G.E., CRUZ, L.J., OLIVERA, B.M., 2001. Venomous cone snails: molecular phylogeny and the generation of toxin diversity. *Toxicon* **39**: 1899–1916.

FEDOSOV, A.E., PUILLANDRE, N., KANTOR, Y.I. & BOUCHET, P. 2015. Phylogeny and systematics of mitriform gastropods (Mollusca: Gastropoda: Neogastropoda). *Zoological Journal of the Linnean Society*, **175**: 336–359.

FEDOSOV, A.E., STAHLSCHMIDT, P., PUILLANDRE, N., AZNAR-CORMANO, L. & BOUCHET, P. (2017). Not all spotted cats are leopards: evidence for a *Hemilienardia ocellata* species complex (Gastropoda: Conoidea: Raphitomidae). *European Journal of Taxonomy,* **268**: 1–20.

FISCHER, P. 1880–1887. *Manual de conchyliologie et de paléontologie conchyliologique*. Vol. xx. Savy, Paris.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.

GIANNUZZI-SAVELLI, R., PUSATERI, F. & BARTOLINI, S. 2018. A revision of the Mediterranean Raphitomidae (Gastropoda: Conoidea) 5: loss of planktotrophy and pairs of species, with the description of four new species. *Bollettino Malacologico*, **54** (Suppl 11): 1– 77.

HARRIS, G.F. & BURROWS, H.W. 1891. *The Eocene and Oligocene beds of the Paris Basin*. University College, London.

HAYASHI, S., 2003. The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives. *Molluscan Research*, **25**: 85–98.

HØISÆTER, T. 2016. A taxonomic review of the Norwegian species of *Raphitoma* (Gastropoda: Conoidea: Raphitomidae). *Fauna Norvegica,* **36**: 9–32.

HORRO, J., GORI, S. & ROLÁN, E. 2019. *Raphitoma zamponorum* a new species from São Tomé Island (Gastropoda: Raphitomidae). *Iberus*, **37**: 1–6.

HUELSENBECK, J.P. & RONQUIST, F. 2001. MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.

KANTOR, YU.I. & TAYLOR, J.D. 2002. Foregut anatomy and relationships of raphitomine gastropods (Gastropoda: Conoidea: Raphitominae). In: *Systematics, phylogeny and biology of the Neogastropoda* (M. Oliverio & R. Chemello, eds). *Bollettino Malacologico*, **Supplement 5**: 161–174.

KATOH, K., ROZEWICKI, J. & YAMADA K.D. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, **30**: 3059.

KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK S., BUXTON S., COOPER A., MARKOWITZ S., DURAN C., THIERER T., ASHTON B., MEINTJES, P. & DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647–1649.

KOBELT, W. 1905 1887-1908. *Iconographie der schalentragenden europäischen Meeresconchylien*. Part 3: 1-24, pls 59–62 [1902]; 25–200, pls 63–78 [1903]; 201–272, pls 79– 84, 86–87 [1904]; 273–406 [1905]. T. Fischer, Cassel.

KURAKU, S., ZMASEK, C.M., NISHIMURA, O. & KATOH, K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research,* **41**: W22–W28.

MANOUSIS, T., KONTADAKIS, C., MBAZIOS, G., & POLYZOULIS, G. 2018. The family Raphitomidae (Mollusca: Gastropoda: Conoidea) in the Greek Seas with the description of two new species. *Journal of Biological Research-Thessaloniki*, **25**: 14.

MILLER, J.A. 1989. The toxoglossan proboscis: structure and function. *Journal of Molluscan Studies,* **55**: 167–182.

MILLER, M.A., PFEIFFER, W. & SCHWARTZ, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA*, pp 1–8.MONTEROSATO T.A. di 1872. *Notizie intorno alle conchiglie Mediterranee*. Michele Amenta, Palermo.

MONTEROSATO T.A. di 1884. *Nomenclatura generica e specifica di alcune conchiglie mediterranee*. Virzi, Palermo.

NORDSIECK, F. 1968. *Die europäischen Meeres-Gehäuseschnecken (Prosobranchia). Vom Eismeer bis Kapverden und Mittelmeer.* Gustav Fischer, Stuttgart.

NORDSIECK, F. 1977. *The Turridae of the European Seas.* Ed. La Piramide, Rome.

OLIVERIO, M. 1995. The systematics of the radula-less gastropod *Clathromangelia* (Caenogastropoda, Conoidea). *Zoologica Scripta*, **24**: 193–201.

OLIVERIO, M., & MARIOTTINI, P. 2001. A molecular framework for the phylogeny of *Coralliophila* and related muricoids. *Journal of Molluscan Studies*, **67**: 215–224.

PLOUVIEZ, S., JACOBSON, A., WU M. & VAN DOVER, C.L. 2015. Characterization of vent fauna at the Mid-Cayman Spreading Center. *Deep-Sea Research* I, **97**: 124–133.

POSADA, D. 2008. JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution,* **25**: 1253–1256.

POWELL, A.W.B. 1966. The molluscan families Speightiidae and Turridae. An evaluation of the valid taxa both recent and fossil, with lists of characteristic species. *Bulletin of the Auckland Institute and Museum* **5**: 1–184.

PUILLANDRE, N., BOUCHET, P., DUDA, T.F. JR., KAUFERSTEIN, S., KOHN, A.J., OLIVERA, B.M., WATKINS, M. & MEYER, C. 2014. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). *Molecular Phylogenetics and Evolution,* **78C**: 290–303.

PUILLANDRE, N., KANTOR, Y. I., SYSOEV, A., COULOUX, A., MEYER, C., RAWLINGS, T., TODD, J.A. & BOUCHET, P. 2011. The dragon tamed? A molecular phylogeny of the Conoidea (Gastropoda). *Journal of Molluscan Studies*, **77**: 259–272.

PUILLANDRE, N., SAMADI, S., BOISSELIER, M.C., SYSOEV, A.V., KANTOR, Y.I., CRUAUD, C., COULOUX, A. & BOUCHET, P. 2008. Starting to unravel the toxoglossan knot: molecular phylogeny of the 'turrids' (Neogastropoda: Conoidea) *Molecular Phylogenetics and Evolution,* **47**: 1122–1134.

ROLÁN, E., OTERO SCHMITT, J. & FERNANDES, F. 1998. The family Turridae *s.l.* (Mollusca, Gastropoda) in Angola (West Africa), 1. Subfamily Daphnellinae. *Iberus,* **16**: 95–118.

SHERIDAN, R., VAN MOL, J.-J. & BOUILLON, J. 1973. Étude morphologique du tube digestif de quelques Turridae (Moll.-Gastr.-Prosobr.-Toxogl.) de la région de Roscoff. *Cahiers de Biologie Marine,* **14**: 159–188.

SMITH, E.H. 1967. Two new species of British turrids. *Veliger* **10**: 1–4.

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics,* **30**: 1312–1313.

TAYLOR, J.D., KANTOR, Y.I. & SYSOEV, A.V. 1993. Foregut anatomy, feeding mechanisms, relationships and classification of Conoidea (Toxoglossa) (Gastropoda). *Bulletin of the Natural History Museum, London (Zoology)*, **59**: 125–170.

Supplementary Figures to:

An assessment of *Raphitoma* and allied genera (Neogastropoda: Raphitomidae)

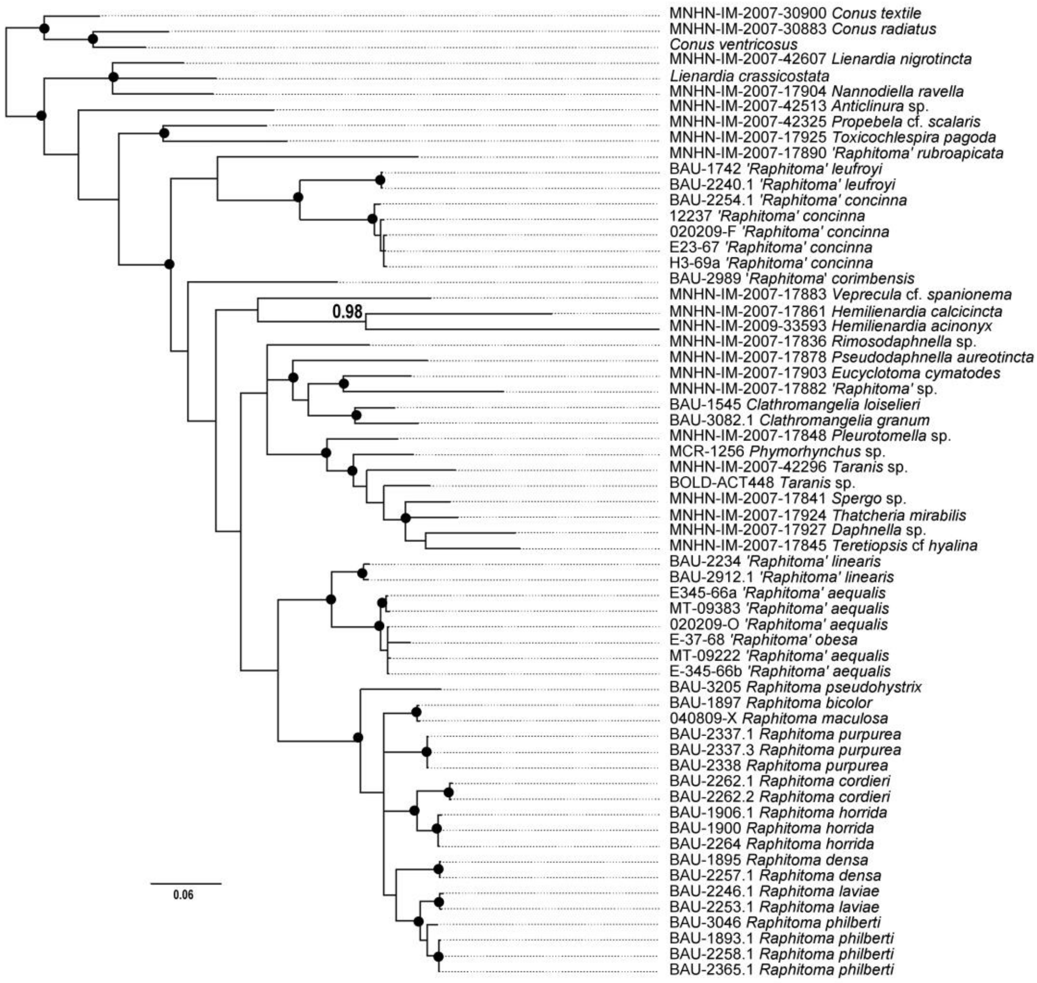
Giulia Fassio[1], Valeria Russini[1], Francesco Pusateri[2], Riccardo Giannuzzi-Savelli[3], Tore Høisæter[4], Nicolas Puillandre[5], Maria Vittoria Modica[6,7] and Marco Oliverio[1]

[1]Department of Biology and Biotechnologies "Charles Darwin" Zoology, Viale dell'Università 32, I-00185 Roma, Italy;
[2]Via Castellana, 64 - 90135 Palermo;
[3]Via Mater Dolorosa, 54 - 90146 Palermo;
[4]University of Bergen, Museum of Natural History, P.O. Box 7800 (Thormøhlens gate 53A), N-5020 Bergen, Norway;
[5]Institut de Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, 57 Rue Cuvier, CP 26, 75005 Paris, France;
[6]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy; and UMR5247, University of Montpellier, France

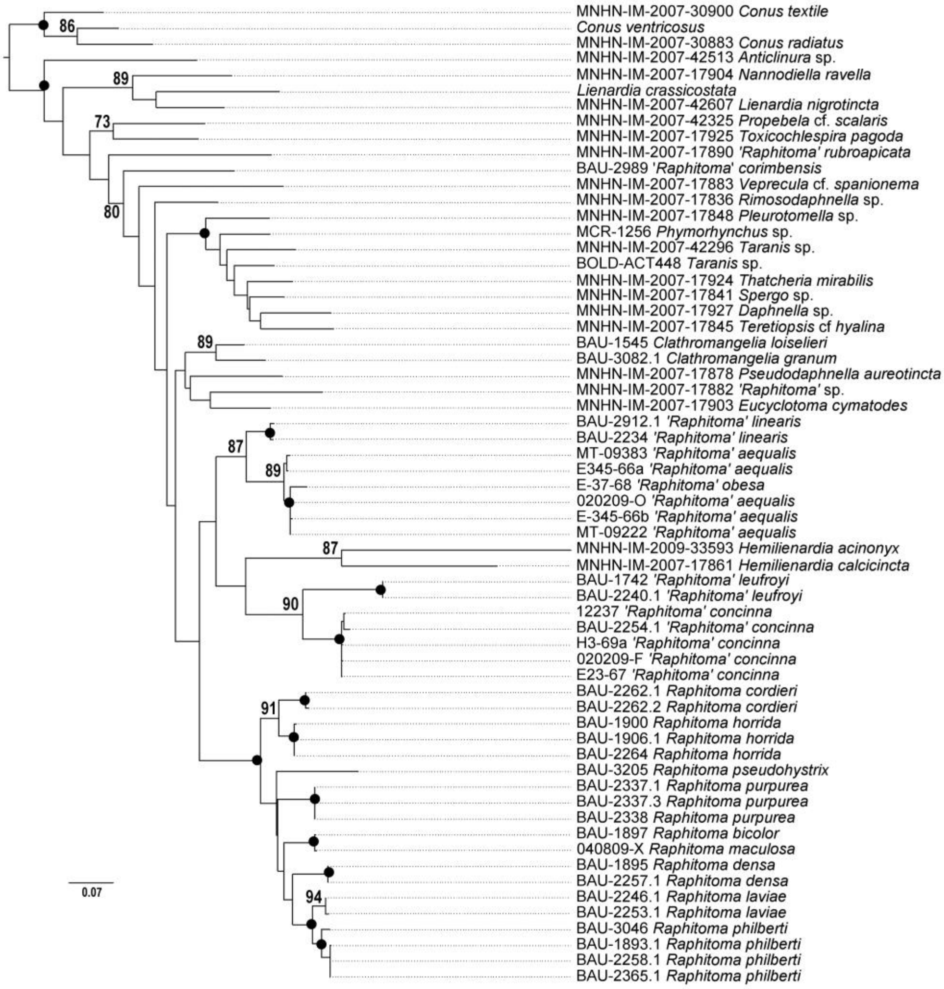
Running head: Systematics of *Raphitoma*

(Received 9 January 2019; editorial decision 9 April 2019)

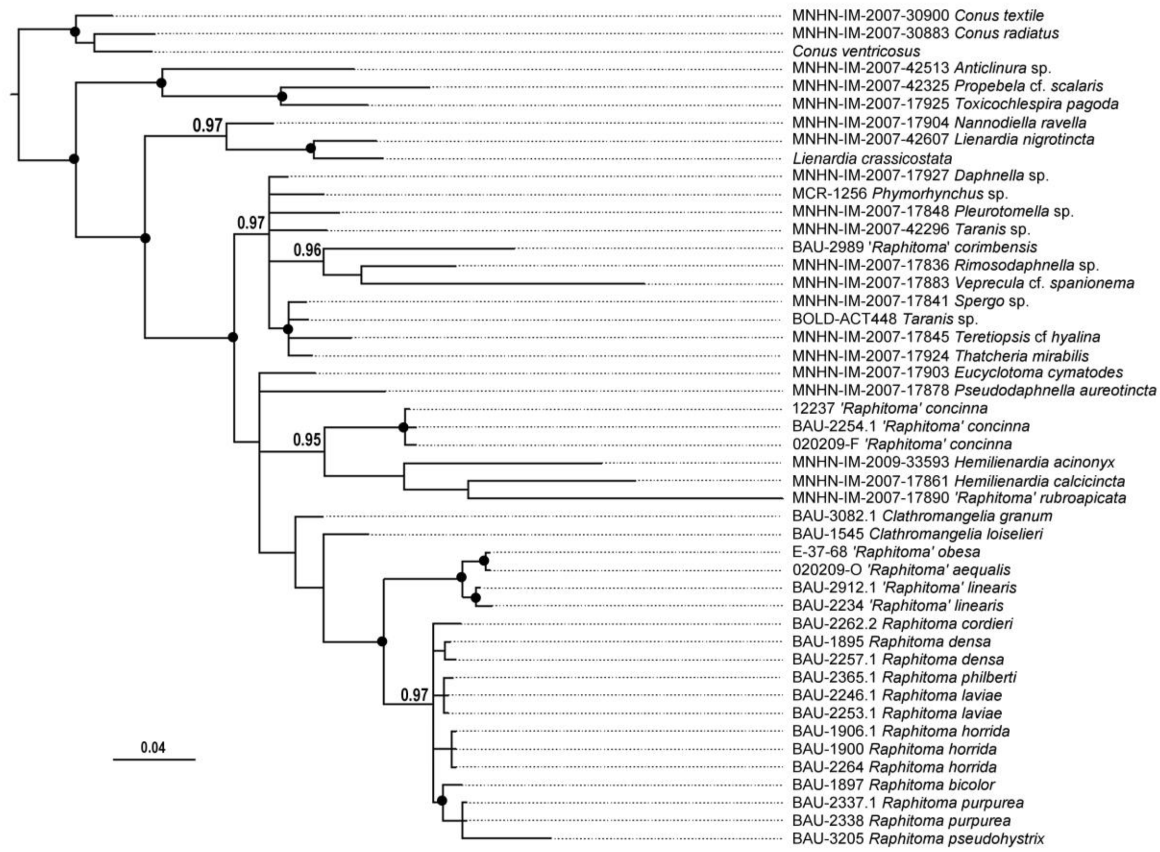
Correspondence: M. Oliverio; email: marco.oliverio@uniroma1.it



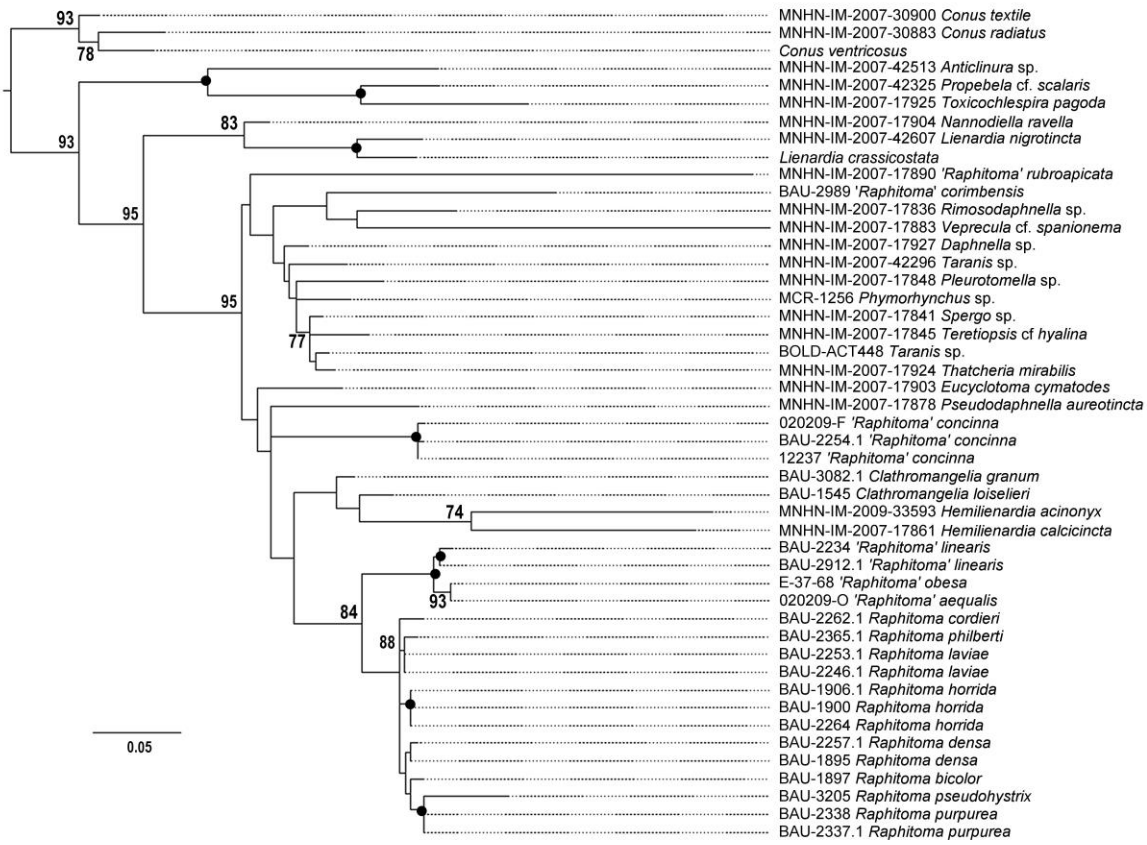
Suppl. Figure 1. Phylogenetic relationships among conoideans as illustrated by the Bayesian majority consensus tree of the COI alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as posterior probabilities for the Bayesian analysis based on 10^7 generations, 25% burnin (only values ≥0.95 are shown); closed circles indicate branches with posterior probabilities >0.98.



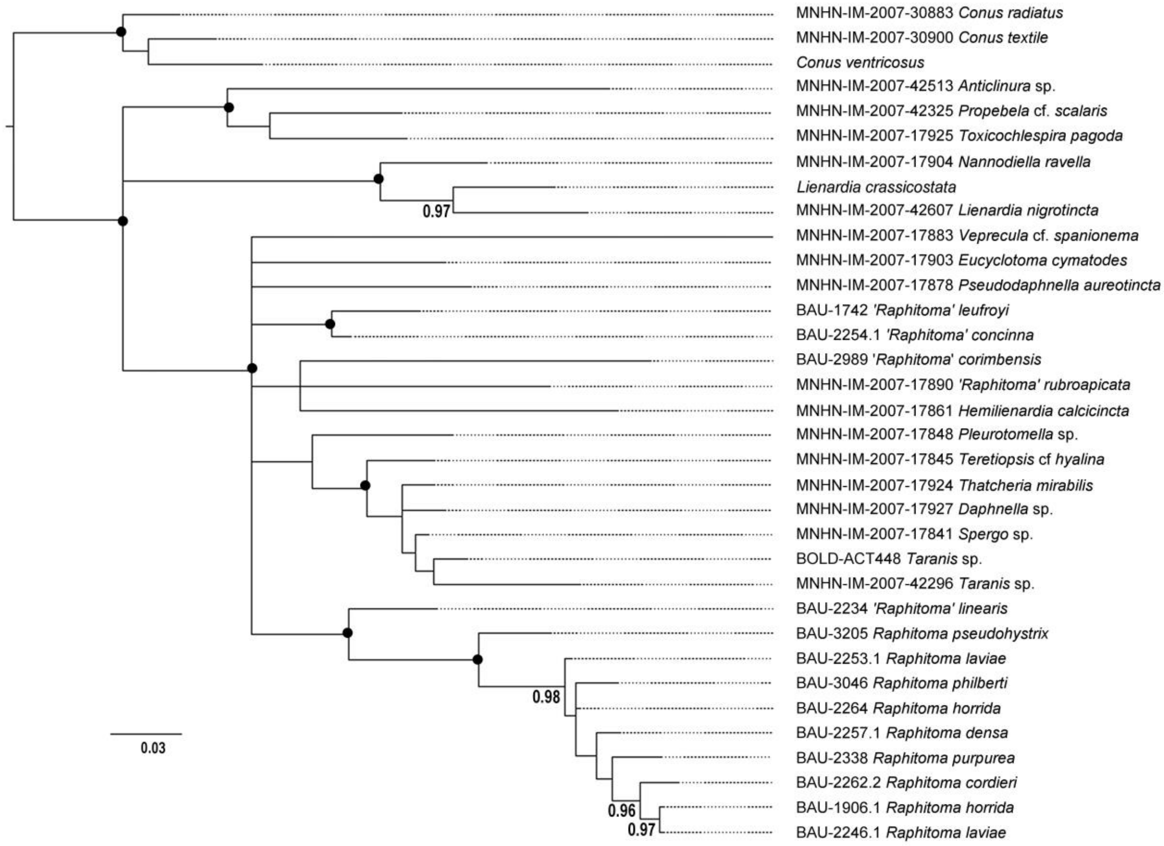
Suppl. Figure 2. Phylogenetic relationships among conoideans as illustrated by the ML majority consensus tree of the COI alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as bootstrap support after ML analysis of 1000 pseudoreplicates (only values ≥70% are shown); closed circles indicate branches with bootstrap support >95%.



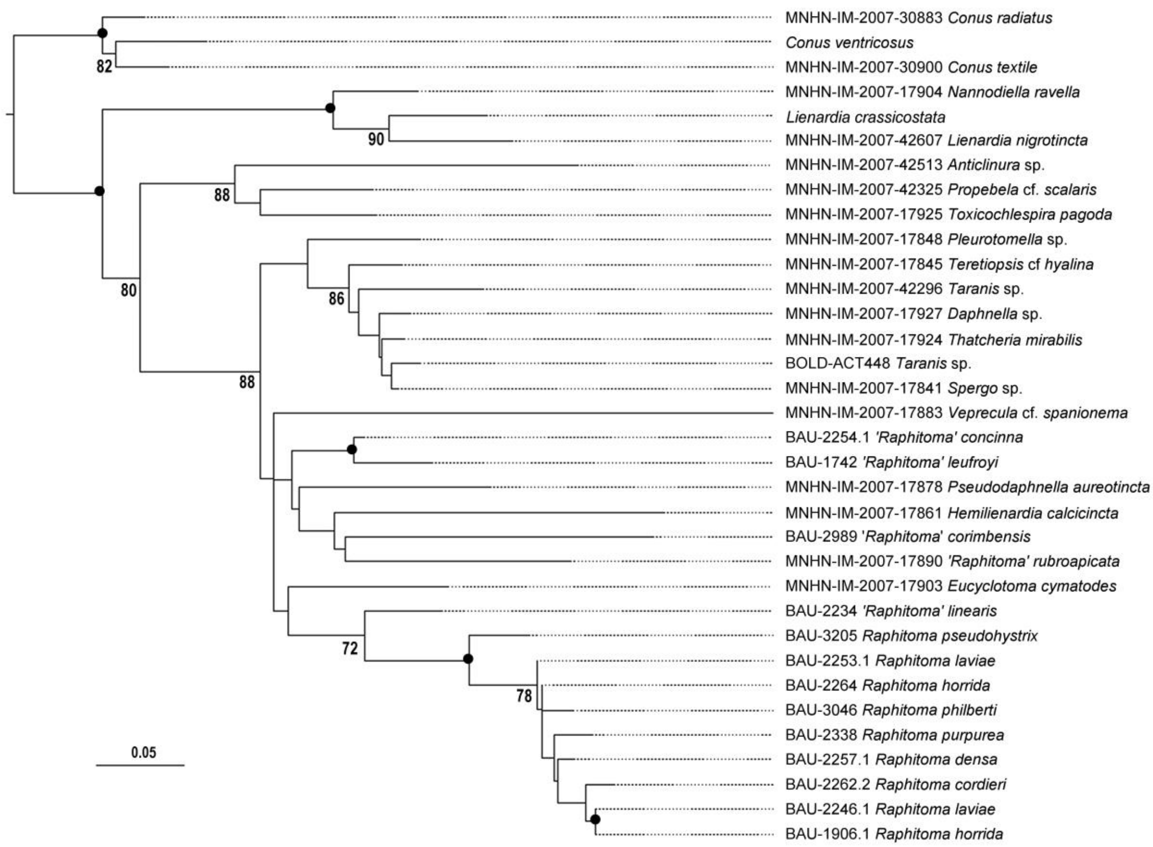
Suppl. Figure 3. Phylogenetic relationships among conoideans as illustrated by the Bayesian majority consensus tree of the 16S rRNA alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as posterior probabilities for the Bayesian analysis based on 10^7 generations, 25% burnin (only values ≥0.95 are shown); closed circles indicate branches with posterior probabilities >0.98.



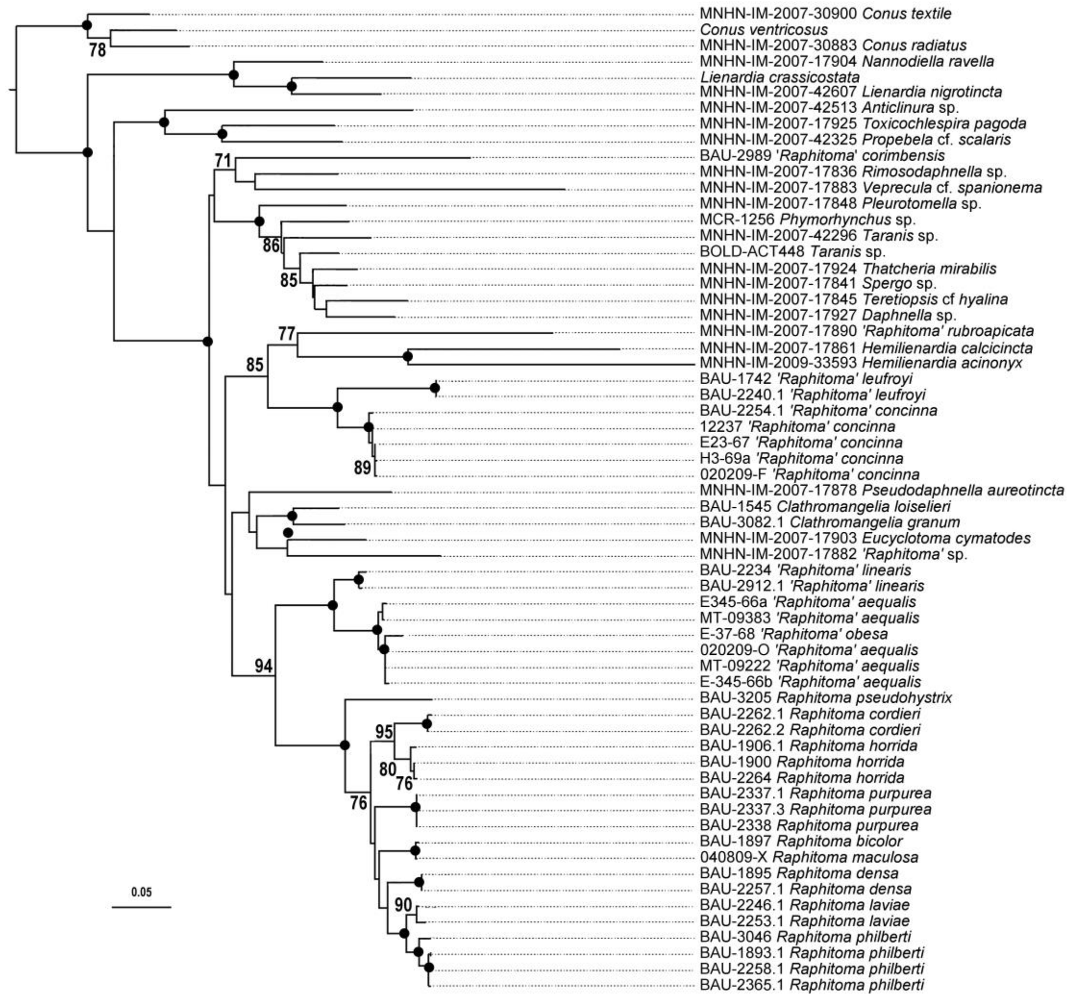
Suppl. Figure 4. Phylogenetic relationships among conoideans as illustrated by the ML majority consensus tree of the 16S rRNA alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as bootstrap support after ML analysis of 1000 pseudoreplicates (only values ≥70% are shown); closed circles indicate branches with bootstrap support >95%.



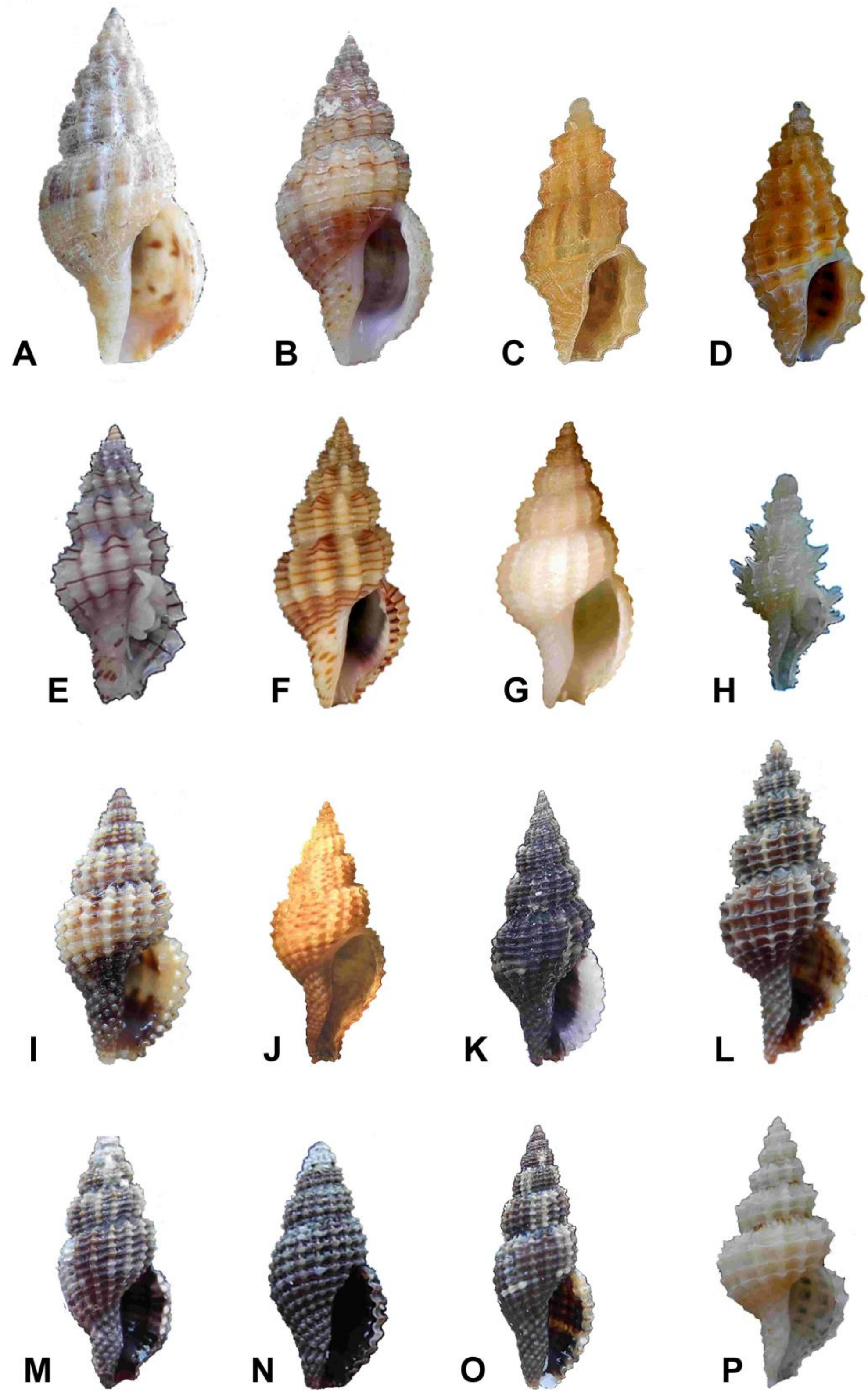
Suppl. Figure 5. Phylogenetic relationships among conoideans as illustrated by the Bayesian majority consensus tree of the 12S rRNA alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as posterior probabilities for the Bayesian analysis based on 10^7 generations, 25% burnin (only values $\geq$0.95 are shown); closed circles indicate branches with posterior probabilities >0.98.



Suppl. Figure 6. Phylogenetic relationships among conoideans as illustrated by the ML majority consensus tree of the 12S rRNA alignment. The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as bootstrap support after ML analysis of 1000 pseudoreplicates (only values ≥70% are shown); closed circles indicate branches with bootstrap support >95%.



Suppl. Figure 7. Phylogenetic relationships among conoideans as illustrated by the ML majority consensus tree of the combined dataset (COI+12S rRNA+16S rRNA). The tree is rooted on a composite outgroup comprising three species of *Conus*. Support values are given as bootstrap support after ML analysis of 1000 pseudoreplicates (only values ≥70% are shown); closed circles indicate branches with bootstrap support >95%.



Suppl. Figure 8. Representative vouchers as in Figure 1. A. *Leufroyia leufroyi* (Michaud, 1828), BAU-2240.1, Croatia. B. *L. concinna* (Scacchi, 1836), BAU-2254.1, Croatia. C. *Clathromangelia loiselieri* Oberling, 1970, BAU-1545, Greece. D. *Clathromangelia granum* (Philippi, 1844), BAU-3082.1, Italy. E. *Cyrillia linearis* (Montagu, 1803), BAU-2234, Italy. F, G. *C. aequalis* (Jeffreys, 1867); F, ZMBN-E-37-68, form '*obesa*', Norway. G, MT09222, typical form, North Sea. H. *Raphitoma pseudohystrix* (Sykes, 1906), BAU-3205, Malta. I, J. *R. bicolor* (Risso, 1826); I, BAU-1897, typical form, France; J, form '*maculosa*', ZMBN-040809-X, Norway. K. *R. purpurea* (Montagu, 1803), BAU-2337.3, France. L. *R. cordieri* (Payraudeau, 1826), BAU-2262.2, Croatia. M. *R. densa* (Monterosato, 1884), BAU-2257.1, Croatia. N. *R. laviae* (Philippi, 1844), BAU-2246.1, Croatia. O. *R. philberti* (Michaud, 1829), BAU-2365.1, Croatia. P. *R. horrida* (Monterosato, 1884), BAU-2264.1, Croatia.

Manuscript submitted to: Invertebrate Systematics

Genetic evidence of poecilogony in Neogastropoda: implications for the systematics of the genus *Raphitoma* Bellardi, 1847

Valeria Russini[A], Riccardo Giannuzzi-Savelli[B], Francesco Pusateri[C], Jakov Prkić[D], Giulia Fassio[A], Maria Vittoria Modica[E], Marco Oliverio[A,F]

[A] Department of Biology and Biotechnologies "Charles Darwin" Zoology, Viale dell'Università 32, I-00185 Roma, Italy.

[B] Via Mater Dolorosa 54, I-90146 Palermo.

[C] Via Castellana 64, I-90135 Palermo.

[D] Getaldićeva 11, HR-21000 Split, Croatia.

[E] Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy; and UMR5247, University of Montpellier, France.

[F] Corresponding author. Email: marco.oliverio@uniroma1.it

Abstract

Poecilogony is the intraspecific variation in developmental mode, with larvae of different types produced by the same individual, population or species. It is very rare among marine invertebrates, and in gastropods has long been described only in a few opisthobranchs. The physiological and regulatory mechanisms underlying larval evolutionary transitions, such as loss of planktotrophy that occurred repeatedly in many caenogastropod lineages, are still largely unknown. We have studied the inter- v. intraspecific variation in larval development in the NE Atlantic neogastropod genus *Raphitoma,* starting with an integrative taxonomy approach: 17 morpho-species were tested against a COI molecular-distance based method (ABGD), and the retained species hypotheses were eventually inspected for reciprocal monophyly on a multilocus dataset. We subsequently performed an ancestral state reconstruction on an ultrametric tree of the 10 final species hypotheses, time-calibrated by fossils, revealing that all the interspecific changes were planktotrophy>lecithotrophy, and all have occurred after 2.5 Million years ago (mya). This is suggestive of a major role played by Pleistocene Mediterranean oceanographic conditions - enhanced oligotrophy, unpredictable availability of water column resources - likely to favour loss of planktotrophy. Within this group of species, that has diversified after the Miocene, we identified one pair of sibling species differing in their larval development, evidence of a speciation event associated to the loss of planktotrophy. However, we also identified two poecilogonous species, each characterized by individuals with both larval developmental types. This is the first documentation of poecilogony in the Neogastropoda, and the second in the whole Caenogastropoda. Although sibling species with different developmental strategies may offer good models to study some evolutionary aspects, poecilogonous taxa are optimally suited for identifying regulatory and developmental mechanisms underlying evolutionary transitions.

Additional keywords: Gastropoda, planktotrophy, lecithotrophy, Integrative Taxonomy

Introduction

Marine gastropod molluscs, like many other benthic invertebrates, generally have a biphasic life cycles, with sedentary adults and pelagic larvae to which dispersal is mostly committed (Cowen and Sponaugle 2009; Ellingson and Krug 2015; Jablonski and Lutz 1983; Strathmann 1985). The most primitive gastropods possess a pelagic larva that does not feed actively on plankton (non-planktotrophic) but in the course of their evolutionary history the members of the class have evolved several developmental strategies that fall into two fundamental categories:

[P] planktotrophic development, with larvae feeding on plankton, spending a relatively long time in the planktonic stage;

[NP] non-planktotrophic development, mostly lecithotrophic but including also direct development and brooding: larvae, if present, have at their disposal a more or less large yolk supply (lecithotrophy) or nurse eggs, reach metamorphosis without feeding on plankton (with only limited uptake of dissolved organic material: see Jaeckle and Manahan 1989; Manahan 1990), and usually spend less time than P-larvae or no time at all in the plankton.

Poecilogony is defined as the intraspecific variation in developmental mode, with different larvae (e.g., free-swimming planktotrophic and brooded lecithotrophic) produced by the same individual, population or species (Giard 1905; Chia *et al.* 1996). Such variations have been documented in a few groups of marine invertebrates only (Knott and McHugh 2012), whereas it has long been assumed that within Caenogastropoda development strategies are strongly constrained within a species, and that poecilogony is not present (Bouchet 1989; Hoagland and Robertson 1988). Since poecilogony has been documented with certainty and with biological details only in a few groups of marine invertebrates – sacoglossan sea slugs (Krug 2009), and spionid polychaetes (Blake and Arnofsky 1999) – any further evidence is certainly of great relevance. In fact, most marine invertebrate groups show evidence of evolutionary transitions in larval phenotype, such as the loss of planktotrophy that occurred repeatedly in many lineages of marine caenogastropods (Oliverio 1996*b*). The mechanisms underlying both the evolutionary transitions and the intraspecific variation (poecilogony) are still largely unknown. Although sibling species with different developmental strategies may offer good models to such study, it is clear that poecilogonous taxa would be optimally suited for identifying regulatory and physiological mechanisms of evolutionary developmental transitions, since this very transition exists within a single species and is not confounded by variation occurring during or after speciation (Knott and McHugh 2012).

Knott and McHugh (2012) provided a schematic approach to the study of poecilogony, aimed at describing the mechanisms of poecilogony and their role in evolutionary transitions. The first step in this approach is obviously the identification of reliable cases of poecilogony, ruling out potentially cryptic species. In fact, following Hoagland and Robertson (1988) and Bouchet (1989), who discredited most cases of putative poecilogony, further studies confirmed that developmental variability subtended unrecognized cryptic species (Collin 2002; Kruse *et al.* 2003; Russini *et al*. 2017), in what has been assumed as the most conservative interpretation (Knowlton 2000, Bickford *et al*. 2007). However, in a few cases genetic data supported poecilogony (Ellingson and Krug 2006; Mcdonald *et al*. 2014; Vendetti *et al*. 2012*a*).

Shelled gastropods can serve as unique models for evolutionary developmental studies, since many aspects of larval development are incorporated in the morphology of the larval shells (protoconch), which are very frequently preserved at the tip of the adult shell (teleoconch). This characteristic allows for the inference of a number developmental features based on the comparative study of the protoconch, and the extension of such studies to both extant and fossil lineages (Nutzel 2014; Shuto 1974).

Recently, the taxonomic revision of the North-Eastern Atlantic and Mediterranean neogastropods of the family Raphitomidae (Giannuzzi-Savelli *et al*. 2018*b*) has yielded the description of many pairs of sibling 'species' differing exclusively in their larval development, as inferred by the morphology of the larval shell, and assuming that poecilogony is not present in this group following Bouchet (1989, 1990). A similar pattern of sibling species differing mainly in their larval development was recently observed also in Indo-Pacific species of the raphitomid genus *Pseudodaphnella* Boettger, 1895, with at least three such pairs identified by molecular data (Fedosov and Puillandre 2012). The possibility of testing this assumption also in the genus *Raphitoma* Bellardi, 1847 with molecular data prompted us to scrutinize this issue.

Materials and methods

Dataset

Our dataset consisted of 96 specimens from the Mediterranean and the NE Atlantic spanning as much as possible of the morphological variation within the genus *Raphitoma* as recently redefined (Fassio *et al.* 2019). Taxonomic authorities and dates, localities and accession numbers for all specimens are reported in Table 1. Identification upon collection, based on gross examination of overall shell morphology, suggested the presence of at least a dozen distinct morpho-species. Almost all specimens were collected in shallow water, 0-10 m depth, fixed and preserved in 95°-100° EtOH (in some cases after microwave oven treatment: Galindo *et al*. 2014) and are stored in the malacological collection at the Department of Biology and Biotechnologies "Charles Darwin" (acronym BAU), Sapienza University of Rome (Italy). Details of the collecting localities, accession numbers, morphological identification and final species attribution are reported in Table 1. In addition to the specimens sequenced herein, we have included in our dataset all available sequences of the genus *Raphitoma* after the work of Fassio *et al.* (2019).

DNA was extracted from a small piece of foot tissue using a modified Proteinase k-Phenol-Cloroform protocol (Oliverio and Mariottini 2001). We amplified one nuclear marker (the internal transcribed spacer 2 of the ribosomal cluster, ITS2, ~500 bp with primers ITS-3d and ITS-4r: Oliverio and Mariottini 2001), and three mitochondrial markers: the barcode fragment of the cytochrome *c* oxidase subunit I (COI), 658 bp, with primers LCO1490 and HCO 2198 (Folmer *et al*. 1994); a ~ 540 bp fragment of the 12S rDNA with primers 12S I and 12S III (Oliverio and Mariottini 2001); a ~ 484 bp fragment of the 16S rDNA with primers 16SA (Palumbi *et al.* 1991) and CGLeu^{UUR}R (Hayashi 2014). PCR products were amplified using the follow general conditions: initial denaturation (94°C/4'); 35 cycles of denaturation (94°C/30''), annealing (48 - 60°C /40''), extension (94°C/60''); final extension (72°C/10'). PCR product were purified using Exosap-IT (USB Corporation) and sequenced by Macrogen Inc. (Spain).

Sequences were aligned using Geneious 11 (Kearse *et al.* 2012) or the online version of MAFFT 7 (Katoh *et al.* 2017; Kuraku *et al.* 2013) with the Q-INS-I algorithm. Intraspecific genetic distance for each putative species were estimated with MEGA 7 (Kumar *et al.* 2016), with the Kimura-2-parameters (K2p) model.

The final molecular dataset was composed by 96 COI (658 bp), 12 12S (534 bp), 16 16S (486 bp), 54 ITS2 (577 bp), of which 137 original sequences (Table 1).

Species delimitation

We used an integrative approach to species delimitation, where species are considered as hypotheses to be subsequently tested by independent evidences (Puillandre *et al.* 2009, 2012, 2014). In a first step, we assigned each specimen to a nominal morpho-species based on the most recent taxonomy of the group (Giannuzzi-Savelli *et al.* 2017, 2018*a*, 2018*b*; Pusateri *et al.* 2012, 2013, 2016, 2018), relying on characters of the teleoconch to identify putative species or species-pairs. Through the observation of the protoconch we inferred the larval development for each specimen, i.e. planktotrophic with multispiral protoconch *v*. non-planktotrophic with paucispiral protoconch; accordingly, we identified a putative member within each pair under the assumption that the dichotomy multispiral/paucispiral protoconch can be used to identify sisters species (Bouchet 1989; Oliverio 1997, 1996*a*, 1996*b*). This step allowed to identify a series of morphologically based Preliminary Species Hypotheses (PSH).

After the morphological identification, we have tested the PSH against a molecular approach, with the Automatic Barcode Gap Discovery (ABGD, available at http://wwwabi.snv.jussieu.fr/public/abgd/), a distance-based method designed to detect the "barcode gap" in the distribution of pairwise distances within a COI alignment (Puillandre *et al.* 2012), which proved useful in delimiting closely related raphitomid species in a recent work (Fassio *et al.* 2019). The analysis was run using the Kimura-2-parameter (K2P) model, a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 20 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 1.5.

Finally, we tested the species hypotheses retained after the ABGD analysis for their reciprocal monophyly (yielding Final Species Hypotheses, FSH) by performing a phylogenetic analysis by Maximum Likelihood (ML) and Bayesian inference (BI) on single-gene alignments and on a concatenated dataset (COI+16S+12S+ITS2). The best fitting substitution models and parameters for each partition were chosen with Partition Finder2 (Lanfear *et al.* 2016) using the Bayesian Information Criterion (BIC) for model selection. ML analyses were done using IQ-TREE (Nguyen *et al.* 2014), on 10,000 bootstrap replicates for node support with ultrafast bootstrap (UFBoot) (Hoang *et al.* 2017). BI analyses were performed using MrBayes 3.2.3 (Ronquist *et al.* 2012) with four-chain Markov chain Monte Carlo (MCMC), run twice in parallel for 10^7 generations, trees sampled every 1000 generations, and a burn-in of 25%. All analyses were run on the CIPRES Science Gateway (Miller *et al.* 2010). We used 2 samples of *Cyrillia linearis* and 6 samples of *Cyrillia aequalis* as outgroup for the genus *Raphitoma* based on the most recent phylogenetic framework for the raphitomids (Fassio *et al.* 2019). Nodes with Bootstraps support (BS) of 70-90% and Posterior Probabilities (PP) of 0.90-0.95 have been considered as moderately supported; BS > 90% and PP > 0.95 have been consider as highly supported.

Ancestral character reconstruction

To investigate the evolution of larval development through the lineages of the ingroup, we performed an ancestral state reconstruction (using the package phytools in R: Revell 2012) on a calibrated ultrametric tree, generated with the software BEAST (v. 1.8.0) (Suchard *et al.* 2018) using the concatenated dataset. For the estimate of node ages, we relied on the fossil record of the extinct *Raphitoma histrix* Bellardi, 1847 (the type species of the genus *Raphitoma*), very likely representing the ancestor of the extant *R. pseudohystrix,* from which it differed only in the multispiral protoconch*. Raphitoma histrix* is known since the Zanclean stage (3.6–5.33 mya) (Giannuzzi-Savelli *et al.* 2018*a*). Another calibration point was set with the first appearance of *R. cordieri*, not known before the Piacenzian stage (2.58–3.6 mya) (Pinna and Spezia 1978). The two calibration points were set under exponential prior (Ho and Phillips 2009), with the major distributions within the boundaries of the respective stage age of identification. The heterogeneity of the mutation rate across lineages was set under uncorrelated, lognormal distributed relaxed clocks for the five partitions, and the Yule process (Gernhard 2008) was chosen.

Based on the state of the character in *R. histrix* (planktotrophic development), and under the assumption that planktotrophy is generally the ancestral state of caenogastropod lineages (Haszprunar 1995; Oliverio 1996*b*) we set planktotrophy as prior for the plesiomorphic state of the genus as represented in our dataset.

Results

Species delimitation

After a refined morphological analysis based on teleoconch and protoconch features, the specimens used in this work were assigned to 17 Preliminary Species Hypotheses (PSHs):

Raphitoma pseudohystrix, R. cf *bicolor, R. maculosa, R. purpurea, R.* sp. C (cf *echinata*)*, R. cordieri, R. horrida, R. densa, R. philberti, R. locardi, R. spadiana, R. laviae, R. contigua, R. atropurpurea, R. bartolinorum* and two species, namely *R.* sp*.* A and *R.* sp*.* B, for which no nominal taxon matching their morphology was found and that are thus potentially undescribed (Table 1). In particular, the species within each of four putative pairs of siblings (*R. cordieri/R. horrida, R. philberti*/*R. locardi, R. spadiana/R. contigua* and *R. laviae/R. bartolinorum*) were identified by the different protoconch (multispiral in *R. cordieri, R. locardi, R. contigua* and *R. laviae* v. paucispiral in *R. horrida, R. philberti, R. spadiana* and *R. bartolinorum*, respectively), whereas the nominal taxa within each pair shared almost the same variation in teleoconch characters according to traditional taxonomy (Giannuzzi-Savelli *et al.* 2018*b*).

The recursive ABGD analysis on the COI alignment identified 10 putative species (Fig. 2). Seven PSH were confirmed by the distance-based analysis (*R. pseudohystrix, R. purpurea, R. cordieri, R. horrida, R. densa, R.* sp. A and *R.* sp. B). However, some PSH were not confirmed by the genetic data: BAU-3047 from Croatia, a juvenile specimen morphologically identified as *R.* sp. C (cf *echinata*), was clearly indicated as conspecific with Atlantic specimens of *R. purpurea*; *R.* cf *bicolor* and *R. maculosa* were considered as conspecific; the specimens identified as *R. philberti, R. laviae, R. contigua, R. spadiana, R. locardi, R. atropurpurea, R. bartolinorum* were rearranged into two genetic species hypotheses.

In the phylogenetic analyses, the single-gene trees showed similar topologies but lower node support values. The trees (BI and ML) based on the concatenated dataset have similar topologies and differed mostly in the branch length and support values (Fig. 2). The clade representing the genus *Raphitoma* was highly supported (100/1). All the species hypotheses retrieved by ABGD formed monophyletic clades with high supports (in all trees, by single-gene and concatenated datasets). These 10 groups corresponded to the Final Species Hypotheses (FSH1–10) eventually retained.

Accordingly, the estimated intraspecific genetic divergence at the barcode fragment (COI) ranged from 0.2% to 0.9%, whereas the interspecific genetic divergence within the genus *Raphitoma* ranged from 4.3% to 18.9%

The phylogenetic analyses showed three pairs of sister clades: *R. cordieri* and *R. horrida*; FSH-5 (*R.* cf *bicolor* and *R. maculosa*) and *R. purpurea*; *R. densa* and *R.* sp. B; FSH-9 and FSH-10. The latter two FSHs were the most interesting for their composition: FSH-9 included all the specimens morphologically identified as *R. laviae, R. contigua* and *R. atropurpurea* (all with multispiral protoconch and planktotrophic larval development), plus those identified as *R. spadiana* and *R. bartolinorum* and some of the specimen identified as *R. philberti* (all with paucispiral protoconch and lecithotrophic larval development); FSH-10 included all the specimen identified as *R. locardi* (with multispiral protoconch and planktotrophic development) and the remaining specimens morphologically ascribed to *R. philberti* (with paucispiral protoconch and lecithotrophic development).

Ancestral state reconstruction

The tree in Fig. 3 portrays the estimated dating of the nodes based on the calibration from the known fossil data. The split between the *R. histrix-pseudohystrix* lineage and the other assayed species of *Raphitoma* was estimated at 4.92 mya (95% HPD: 3.16–6.57), i.e. around the Miocene-Pliocene boundary. The divergence of the two poecilogonous species (FSH-9 and FSH-10) was estimated at 2.56 mya (95% HPD: 1.59–3.77), i.e. at the Pliocene-Pleistocene boundary.

For the ancestral character reconstruction, we estimated the distribution of changes from stochastic mapping on 100 tree, and found an average of 7.63 P>NP and 4.48 NP>P changes, the latter due exclusively to the presence of poecilogony in FSH-9 and FSH-10 (all interspecific changes were P>NP). In Figure 4, the colour of the branches shows the probability of the appearance of non-planktotrophic development, and the hypothetical timing of the change P>NP. All changes were estimated to have occurred after 2.5 mya. The ancestral state for the poecilogonous FSH-9 was estimated to be non-planktotrophic development, whereas for the FSH-10 it was uncertain, with slightly higher probability for planktotrophic development. The transition P>NP in the *R. histrix-pseudohystrix* lineage was estimated at 1.5–2 mya.

Discussion

Our integrative taxonomy approach performed well in identifying species boundaries within the genus *Raphitoma*. Particularly, several species recognised on the basis of morphological features of the teleoconch as traditionally used in this group have been confirmed by the genetic data, including two undescribed species preliminarily identified through subtle morphological features: *Raphitoma* sp. A and *R*. sp. B, which are under formal description elsewhere (Prkić *et al.* 2019). This is reassuring for the systematics of both extant and fossil taxa of this group. However, the complex of specimens morphologically ascribed mostly to *R. philberti* and *R. laviae* (but also to *R. contigua, R.* cf *spadiana*, *R. atropurpurea* and *R. bartolinorum*) based on adult and larval shell features, have been reassigned to only two distinct Final Species Hypotheses, FSH-9 and FSH-10. These two species hypotheses do not completely correspond to any of the traditional morphospecies for which the various binomens (and especially *R. philberti* and *R. laviae*) may be employed based on the morphotypes in each clade. Both FSHs included specimens with mixed protoconch types (and thus having undergone two different developments): in some instances, specimens of the same FSH collected sympatrically or even syntopically displayed identical teleoconchs but different protoconchs, strongly indicating the existence of two clearly poecilogonous species. The alternative hypothesis that the COI is unable to recognise sibling species does not hold, since the integrative taxonomy approach we have used has proven very efficient in various groups of Conoidea, including Raphitomidae (e.g.: Fedosov and Puillandre 2012, Fassio *et al.* 2019) and in this work it has detected species very closely related such as *R. horrida* and *R. cordieri*, or *R. densa* and *R.* sp. B. This is the first genetically supported evidence of poecilogony in the Neogastropoda.

The split between the *R. histrix-pseudohystrix* lineage and the other species of *Raphitoma*, estimated at 4.92 mya, is congruent with the oldest documented appearance in the fossil record of *R. histrix* in the Lower Pliocene. All interspecific changes in the larval development within *Raphitoma* were P>NP, congruently with the assumption that loss of planktotrophy has frequently accompanied speciation in caenogastropods (Oliverio 1996*a*, 1996*b*). All changes were estimated to have occurred after 2.5 mya, i.e. after the onset of the glacial cycles, and the transition P>NP in the *R. histrix-pseudohystrix* (estimated at 1.5-2 mya) perfectly fits the data from fossils on the two protoconch types in this lineage. This is congruent with the suggestion that oceanographic conditions during the Pleistocene favoured loss of planktotrophy (Oliverio 1996*b*), particularly the enhanced oligotrophy in the Mediterranean Sea in the period following the onset of the glaciations and their southward extension (Tunnell and Douglas 1983; Thunnell *et al.* 1984), with unpredictable availability of resources in the water column. In particular, during the cold phases, sea level lowering produced extreme reductions of the Sicily Channel width, which, along with inversion of water flows at the Gibraltar and the Siculo-Tunisian sills, may have contributed to periodic confinement of large areas of the eastern Mediterranean (Bethoux 1979, 1984). Such conditions (fluctuations in the energy/food input, restricted areas, higher predatory pressure in the water column) are those expected to counter select the planktotrophic larvae (Strathmann 1978*a*, 1978*b*).

Based on the present results, the taxonomy of the involved species is provisionally modified as follows.

SYSTEMATICS

Family **Raphitomidae Bellardi, 1875**

Genus ***Raphitoma* Bellardi, 1847**

Raphitoma Bellardi 1847: 612 – Type species: *Raphitoma histrix* Bellardi, 1847 [ex *Pleurotoma hystrix* Cristofori and Jan, 1832, *nomen nudum*] by subsequent designation (Monterosato 1872: 54).

Homotoma Bellardi, 1875: 22 – Type species: *Murex reticulatus* Renier, 1804 by subsequent designation (Powell, 1966).

Cordieria Monterosato, 1884: 131 – Type species: *Murex reticulatus* Renier, 1804 by subsequent designation (Crosse, 1885).

Philbertia Monterosato, 1884: 132 – Type species: *Pleurotoma bicolor* Risso, 1826 by subsequent designation (Crosse, 1885).

Peratotoma Harris and Burrows, 1891: 113 – Replacement name for *Homotoma* Bellardi, 1875, not Guérin-Ménéville, 1844).

Cyrtoides F. Nordsieck, 1968: 176 – Type species: *Pleurotoma rudis* Scacchi, 1836 (not G.B. Sowerby I, 1834 by original designation.

Diagnosis: Shell of small to medium size for the family, from 5 mm to 25 mm, from turreted to biconic-pupoid, suture impressed.

Protoconch of 3-4.5 whorls when multispiral, with protoconch I (embryonic shell) of 0.5-0.7 whorls, with a reticulate sculpture of spirals and orthocline axial striae, and protoconch II (larval shell) of 2.3-3.5 whorls, with a diagonally cancellate sculpture and a frequently keeled last whorl; paucispiral protoconch of 2 whorls, with large nucleus and reticulate sculpture.

Teleoconch with slender spire of 5 to 9 uniformly convex whorls, with reticulate-cancellate sculpture, axials broader than spirals. Microsculpture of fine granules occasionally present, on the whole teleoconch (*R. papillosa*) or on the first whorl only.

Outer lip thickened, with 7-13 inner denticles.

Columella simple, slightly sinuous anteriorly.

Siphonal canal from very short to moderately long. Siphonal notch wide, plain or intorted.

Remarks: See Fassio et al. (2019) for a recent redefinition of the scope of the genus in a molecular phylogenetic framework.

FSH-1 *Raphitoma pseudohystrix* (Sykes, 1906)

Clathurella pseudohystrix Sykes 1906: 187

Distribution

Middle Pleistocene of Italy. Recent: Northeastern Atlantic (Madeira), Western and Central Mediterranean and Adriatic. In rather deep waters (120-700 m), on the continental slope, but also in bathyal depths, found also in the white coral assemblages of the Central Tyrrhenian Sea (Smriglio *et al.* 1987).

Diagnosis

Shell of small-medium size for the genus (height: 5-15 mm), fragile, fusiform, slender. Protoconch paucispiral of 1.9 convex whorls. Teleoconch of 5-7 convex and stepped whorls, weak suture and strong sculpture. No microgranules on the surface. Axial sculpture of 12-29 orthocline or slightly opisthocline ribs. Spiral sculpture of up to 9 primary cords and secondary cordlets above the aperture. Cancellation sharp rectangular, with spinulose processes. Subsutural ramp wide, smooth, sligthly concave. Siphonal canal long and sinuose. Outer lip with 12-20 weak plications in correspondence of spiral cords and cordlets. Siphonal fasciole with 7-9 spinulose cords. Coloration uniformly whitish or yellowish often with brownish blotches of variable size. Soft parts body entirely white. Foot sharply bilobed anteriorly.

Remarks

As noted by Giannuzzi-Savelli *et al.* (2018), old authors frequently confused the nominal taxa *R. histrix* and *R. pseudohystrix*. At that time, only Jeffreys (1870: 82) had already distinguished the extant form by its paucispiral larval shell (“twisted and spirally striated, like that of *Trophon*”), at variance with the multispiral protoconch of the fossil. In this lineage, fossils from the Lower Pliocene to the Lower Pleistocene showed exclusively a multispiral protoconch (and thus, had a planktotrophic larval development) (see Giannuzzi-Savelli *et al.* 2018 for a review); in the Middle Pleistocene the two protoconch types coexisted, but starting with the Upper Pleistocene the multispiral protoconch disappeared.

FSH-2 *R*. sp. A

Distribution

So far know only from Croatia (Adriatic Sea) and Sicily (Tyrrhenian Sea).

Diagnosis

Shell of medium size for the genus (height: 10-19 mm), robust and broad. Protoconch multispiral of 2.1-2.5 convex whorls. Teleoconch of 6-7 convex and stepped whorls, suture incised and strong sculpture. Microgranules on the surface. Axial sculpture of 13-18 orthocline or slightly opisthocline ribs. Spiral sculpture of 5-7 primary cords stronger than the axials above the aperture, and occasional 1-4 secondary cordlet (1 on subsutural ramp). Cancellation rectangular to squared, with elongate and elevated tubercles. Subsutural ramp wide, inclined, flat. Siphonal canal short. Outer lip with 9-10 strong inner plicate denticles. Siphonal fasciole with 7-9 spinulose cords. Coloration brown, brown-reddish or grey-blackish background, with cream-yellowish or light brownish blotches of variable size. Soft parts body translucent yellow or yellowish-white, siphon black, with sparse minute white speckles. Foot sharply bilobed anteriorly.

Remarks

Raphitoma sp. A belongs to the complex of *R. echinata* (Brocchi, 1814), from which it differs in its broader shell and shorter protoconch (2.1-2.5 vs 2.7-3.3 whorls). This species is under formal description elsewhere (Prkić *et al.* 2019).

FSH-3 *Raphitoma maculosa* Høisæter, 2016

Raphitoma maculosa Høisæter 2016: 13

Distribution

Raphitoma maculosa is known from the Norwegian waters. *Raphitoma bicolor* ranges throughout the entire Mediterranean Sea, and in the Atlantic, from Wales to Canary Islands (but see below in the remarks).

Diagnosis

Shell of medium size for the genus (height: 7-11 mm), solid, fusiform-acute. Protoconch multispiral of 3 to 3.5 convex whorls. Teleoconch of 4.5-5.5 convex and stepped whorls, suture not incised and strong sculpture. Microgranules on the surface. Axial sculpture of 18-27 orthocline or slightly opisthocline ribs. Spiral sculpture of 5 primary cords stronger than the axials. Cancellation subquadrate or rectangular, with tubercles. Subsutural ramp narrow. Siphonal canal long. Outer lip thin (all immature specimens), without inner denticles. Siphonal fasciole with 7-8 cords. Coloration yellowish-white background, reddish-brown on the spirals. Soft parts body translucent grey-white, siphon greyish, with sparse minute white speckles.

Remarks

The diagnosis is based on *R. maculosa*. The specimen BAU-1897 from St. Maxime (France, Mediterranean), that genetically has been assessed as conspecific with a topotype of *R. maculosa*, differs morphologically in some aspects: beside the thickened outer lip with 9 inner denticles (all types of *R. maculosa* are immature), the siphonal canal is shorter, and the coloration is different. The outline recalls *Raphitoma bicolor* (Risso, 1826), but the latter is devoid of any microsculpture, and the coloration is different. The actual identity of this species, which is present in the Atlantic and the Mediterranean, must be assessed by a larger sampling.

FSH-4 *R. purpurea* (Montagu, 1803)

Murex purpureus Montagu 1803:260, pl. 9, fig. 3

Distribution

Norteastern Atlantic, from northern Norway to Great Britain, south to the Azores and Canary Islands, Mauritania, and westernmost Mediterranean.

Diagnosis

Shell of large size for the genus (height: 11-24 mm), robust, fusiform, acute. Protoconch multispiral of 2.8-3 convex whorls. Teleoconch of 6-9 convex and not stepped whorls, suture not incised and strong sculpture. Microgranules on the surface. Axial sculpture of 15-26 opisthocline ribs. Spiral sculpture of strong primary cords. Cancellation squared to rectangular, with tubercles. Subsutural ramp narrow, with few thin cordlets. Siphonal canal short. Outer lip thick, crenulated, white, with 10-21 robust inner lyrate denticles. Siphonal fasciole with 8-10 nodulose cords. Coloration light to very dark brown with whitish blotches or spots. Soft parts body translucent whitish, siphon greyish, with sparse minute white speckles.

Remarks

This is a well-known and rather unmistakable species, remarkably without synonyms. The specimen BAU-3047.1 from Croatia, is a juvenile, and shell features would diagnose it as *Raphitoma echinata*. Additional comparisons with adult specimens are necessary to assess the relationships of Mediterranean specimens with the prevalently Atlantic *R. purpurea*.

FSH-5 *R. cordieri* (Payraudeau, 1826)

Pleurotoma cordieri Payraudeau 1826: 144, pl. 7 fig. 11

Clathurella dollfusi Locard 1886: 115

Distribution

Northeastern Atlantic and Mediterranean.

Diagnosis

Shell of large size for the genus (height: 16-24 mm), fragile, fusiform, acute. Protoconch multispiral of 2.3 convex whorls. Teleoconch of 7 convex and not stepped whorls, suture not incised and strong sculpture. Microgranules on the surface. Axial sculpture of 16 strong orthocline ribs. Spiral sculpture of 5 primary cords above the aperture. Cancellation subquadrate to rectangular, with spinose tubercles. Subsutural ramp narrow, sometimes with a secondary, spinulose cordlet. Siphonal canal long. Outer lip thick, 9 inner lyrate denticles. Siphonal fasciole with 7 nodulose cords. Coloration ligth to dark brown, occasionally with darker blotches. Soft parts body translucent yellow or yellowish-white, siphon grey or black, with sparse coarse white speckles. Foot sharply bilobed anteriorly.

Remarks

Very similar to *R. horrida* but with larger shell, and protoconch multispiral (*v*. paucispiral in *R. horrida*).

FSH-6 *Raphitoma horrida* (Monterosato, 1884)

Cordieria horrida Monterosato 1884: 131-132

Distribution

Mediterranean.

Diagnosis

Shell of medium size for the genus (height: 12-16 mm), solid, fusiform, acute. Protoconch paucispiral of 1.15-1.5 convex whorls. Teleoconch of 6-7 convex and not stepped whorls, suture not incised and strong sculpture. Microgranules on the surface. Axial sculpture of 13 orthocline ribs. Spiral sculpture of 4 strong primary cords above the aperture, and one subsutural cordlet. Cancellation subquadrate to rectangular, with spinose tubercles. Subsutural ramp wide, inclined. Siphonal canal short. Outer lip thik, 8-9 inner lyrate denticles. Siphonal fasciole with 7-8 nodulose cords. Coloration light to very dark brown with whitish blotches or spots. Soft parts body and siphon translucent whitish, with sparse minute white speckles, occasionally with a blackish area on the head.

Remarks

Similar to *R. cordieri*, but with only four spiral cordlets above the aperture, smaller shell and paucispiral protoconch (*v*. multispiral in *R. cordieri*).

FSH-7 *Raphitoma densa* (Monterosato, 1884)

Philbertia densa Monterosato 1884: 133

Clathurella decorata Locard 1891: 67-68

Raphitoma (Philbertia) bourguignati tarentina F. Nordsieck, 1977: 55, pl. 17 fig. 136

Raphitoma (Philbertia) flavida F. Nordsieck, 1977: 54, pl. 17 fig. 132

Distribution

Northeastern Atlantic (Canary Islands) and Mediterranean.

Diagnosis

Shell of medium size for the genus (height: 8-16 mm), solid, fusiform, acute. Protoconch multispiral of 3 convex whorls. Teleoconch of 7-9 convex and not stepped whorls, suture incised and strong sculpture. Microgranules on the surface. Axial sculpture of 16-29 orthocline ribs. Spiral sculpture of strong 6-9 primary cords above the aperture. Cancellation rectangular, with tubercles. Subsutural ramp narrow. Siphonal canal short. Outer lip thick, 10-14 strong inner lyrate denticles. Siphonal fasciole with 6-10 nodulose cords. Coloration orange-brown with ash-grey blotches. Soft parts body translucent white or yellowish-white, siphon grey-brownish, with sparse minute white speckles.

Remarks

Similar to *R.* sp. B*,* but with fewer, stronger and broader axial ribs, and less slender shell especially in juveniles. Additionally, protoconch whorls number is slightly lower in *R. densa* than in *R.* sp. B, 2.5-3.0 (mean 2.79) *vs* 2.6-3.25 (mean 2.98), and there are 5-6 (mean 5.85) primary cords in *R. densa* above the aperture, *vs* 6-8 (mean 6.96) in *R.* sp. B*.*

FSH-8 *R.* sp. B

Distribution

So far known only from Croatian (Adriatic).

Diagnosis

Shell of medium size for the genus (height: 8-12 mm), solid, fusiform-acute. Protoconch multispiral of 2.6-3.25 convex whorls. Teleoconch of 6-7 slightly convex and not stepped whorls, suture incised and strong sculpture. Microgranules on the surface. Axial sculpture of 18-27 orthocline or slightly opisthocline ribs. Spiral sculpture of 6-8 primary cords stronger than the axials, and 2 secondary cordlet on subsutural ramp. Cancellation subquadrate or rectangular, with tubercles. Subsutural ramp narrow, quite inclined. Siphonal canal of medium length. Outer lip thick, with 9-14 strong inner plicate denticles. Siphonal fasciole with 9-12 strong nodulose cords. Coloration brown background, darker interspaces, usually with white to ash-grey blotches and spots of variable size. Soft parts body translucent white or yellowish-white, siphon grey-brownish, with sparse minute white speckles.

Remarks

This species can be confused only with *Raphitoma densa* (Monterosato, 1884) with which is often found living in sympatry in Croatia, and by which it differs in the more numerous, weaker and narrower axial ribs, and the more slender shell especially in juveniles. Additionally, protoconch whorls number is slightly higher in *R.* sp. B than in *R. densa*, 2.6-3.25 (mean 2.98) *vs* 2.5-3.0 (mean 2.79), and there are 6-8 (mean 6.96) primary cords above the aperture in *R.* sp. B, *vs.* 5-6 (mean 5.85) in *R. densa.*

FSH-9 *Raphitoma philberti* (Michaud, 1829)

Pleurotoma philberti Michaud 1829: 261-262, figs 2, 3

? Raphitoma locardi Pusateri, Giannuzzi-Savelli and Oliverio, 2013: 18 [replacement name for *Clathurella cylindrica* Locard and Caziot, 1899, non Pease, 1860]

Distribution

Northeastern Atlantic (Canary Islands), and the entire Mediterranean Sea.

Diagnosis

Shell of small size for the genus (height: 5-9 mm), solid, subfusiform-acute. Protoconch multispiral of 3 convex whorls, or paucispiral of 1.3-1.8 convex whorls. Teleoconch of 5-7 slightly convex and not stepped whorls, suture incised and strong sculpture. Microgranules present. Axial sculpture of 15-20 orthocline ribs. Spiral sculpture of 6-8 primary cords above the aperture. Cancellation rectangular, with tubercles. Subsutural ramp narrow, with two cordlets. Siphonal canal short. Outer lip thick, with 9-11 strong inner denticles. Siphonal fasciole with 8-9 nodulose cords. Coloration light to dark tawny-reddish background, with whitish blotches vanishing towards the suture. Soft parts body translucent white or yellowish-white, siphon dark grey, with sparse minute white speckles.

Remarks

FSH-10 included all the specimen identified as *R. locardi* (with multispiral protoconch and planktotrophic development) and most of specimens morphologically ascribed to *R. philberti* (with paucispiral protoconch and lecithotrophic development). We use *Pleurotoma philberti* Michaud, 1829 for this species, since the largely most represented morphotype in our material correspond to this taxon as defined by the neotype recently designated (Giannuzzi-Savelli *et al*. 2018), and this is also the oldest available name. The final decision on the synonymy of *R. locardi* will be taken after topotypical samples will be assayed.

FSH-10 *Raphitoma laviae* (Philippi, 1844)

Pleurotoma laviae Philippi 1844: 170, pl. XXVI fig. 17

? Raphitoma bartolinorum Pusateri and Giannuzzi-Savelli, in Giannuzzi-Savelli, Pusateri and Bartolini 2018: 35-36, figs 38-39B

? Raphitoma spadiana Pusateri and Giannuzzi-Savelli, in Pusateri, Giannuzzi-Savelli and Oliverio 2012: 41-52

? Raphitoma contigua Monterosato 1884: 133

? Clathurella atropurpurea Locard and Caziot 1900: 193-274

Distribution

Known from the entire Mediterranean Sea.

Diagnosis

Shell of small size for the genus (height: 5-9 mm), solid, subfusiform-acute. Protoconch multispiral of 2.75 convex whorls, or paucispiral of 1.5-1.7 convex whorls. Teleoconch of 5-6 slightly convex and not stepped whorls, suture incised and strong sculpture. Microgranules present. Axial sculpture of 16-23 orthocline or slightly prosocline ribs. Spiral sculpture of 6-7 primary cords above the aperture. Cancellation squared, with tubercles. Subsutural ramp narrow. Siphonal canal short. Outer lip thick, with 8-10 strong inner denticles. Siphonal fasciole with 5-6 nodulose cords. Coloration light yellow to dark brown background, suprasutural cordlet white, usually with whitish blotches. Soft parts body translucent white or yellowish-white, siphon grey-brownish, with sparse minute white speckles.

Remarks

FSH-10 included all the specimens morphologically identified as *R. laviae, R. contigua* and *R. atropurpurea* (all with multispiral protoconch and planktotrophic larval development) and *R. spadiana, R. bartolinorum* and some *R. philberti* (with paucispiral protoconch and lecithotrophic larval development).

We use *Pleurotoma laviae* Philippi, 1844 for this species, since the largely most represented morphotype in our material corresponded to this taxon as defined by the recently designated neotype (Giannuzzi-Savelli *et al.* 2018). We have highlighted some potential synonyms based on the assayed materials, but obviously, from the nomenclatural point of view, a wider coverage and the analysis of topotypical samples will be necessary to stabilize the use of the multiple binomens potentially referable to this complex. For instance, *R. bartolinorum* should be devoid of microgranules (based on type material) whereas the specimen BAU 2245.2 has microgranules. Further check on this feature are needed to ascertain its actual taxonomic value, and to assess the status of *R. bartolinorum*.

Conclusions

Poecilogony in gastropods is well known among sacoglossans (Ellingson and Krug 2015; Krug 1998; Vendetti *et al.* 2012a), but it has long been considered as unproven in caenogastropods, for which the presence of different larval developmental types (as indicated by the morphology of larval shells) have been regarded as a clue of species distinction (Hoagland and Robertson 1988; Bouchet 1989). In fact, several cases of sister species differing only or mostly in their larval development and protoconch morphology have been reported (Collin 2001, 2002, 2004; Galindo *et al.* 2016; Modica *et al.* 2017). In the family Raphitomidae, Fedosov and Puillandre (2012) have scored at least three pairs of sister species of the genus *Pseudodaphnella* differing in their protoconch morphology (*P. punctifera / P. boholensis* Fedosov and Puillandre, 2012; *P. martensi* (G. Nevill and H. Nevill, 1875) / *P. nynpha* Fedosov and Puillandre, 2012; *P. crypta* Fedosov and Puillandre, 2012 / *P. philippinensis* Fedosov and Puillandre, 2012). A similar pair of species has been discovered also in the genus *Raphitoma*, where we have found that *R. cordieri* and *R. horrida*, very similar morphologically, are evidently sister species, and with P *v*. NP development, respectively. These cases confirm the existence of a mechanism of speciation related to the loss of planktotrophy (Oliverio 1996*a*). However, at least one case of poecilogony in Caenogastropoda has been reliably presented in recent literature: *Calyptraea lichen* Broderip, 1834 (Mcdonald *et al.* 2014). We have reported here the case of two species in the genus *Raphitoma*, each including specimens with two different protoconchs, unequivocally addressing to the presence of both planktotrophic and non-planktotrophic larval development, among specimens otherwise hardly separable morphologically and clearly conspecific based on nuclear and mitochondrial markers. For supraspecific systematics this is the final word on the status of conoidean genera or subgenera based only on difference in larval development (as marked in the protoconch morphology) proposed by Powell (1966), followed for some time by some European malacologists, but rejected as phylogenetically inconsistent (Bouchet 1990: Fassio *et al*. 2019): the occurrence of poecilogony strongly supports such rejection.

At lower taxonomic level, a high number of gastropod species descriptions have been mostly or exclusively based on larval shell morphology, under the assumption that poecilogony was not present in caenogastropods. The new evidence of poecilogony in some caenogastropods raises issues about the reliability of sibling species identification based only on different protoconch shape, questioning the protoconch as a unique source of diagnostic characters at the species level. The problems linked to the use of morphological characters is amplified in palaeontology; although a screening at a broad taxonomic scale will be necessary to asses every single case by genetic data in extant groups, we suggest maintaining the use of different names in the fossils since this helps preserving the information on larval development. The case of the *Raphitoma histrix* – *R. pseudohystrix* lineage is paradigmatic: the use of different names for specimens with different protoconch in paleontological literature allowed to define the temporal span of the two entities, with the possibility of testing the reliability of the molecular time calibration adopted in this work.

Crucial aspects for understanding larval development evolutionary patterns lie in the adaptive implications of poecilogony, and the definition of the regulatory and physiological mechanisms involved in the switch of larval development. Poecilogony remains a rare phenomenon, and is documented in a few groups of marine invertebrates only: polychaetes (Blake and Arnofsky 1999; David *et al.* 2014; Duchêne 2000; Morgan *et al.* 1999); sacoglossan heterobranch gastropods (Ellingson and Krug 2006; Vendetti *et al.* 2012*b*), and Caenogastropoda with *Calyptraea lichen* (Mcdonald *et al.* 2014) and the cases reported herein for *Raphitoma*. Knott and McHugh (2012) summarised the three major features common to poecilogonous groups:

1- poecilogonous species seem to be restricted to taxa with at least some degree of brooding (Krug 2007), or with eggs developing inside egg capsules, egg masses, or other brood structures;

2- often different larvae do not develop from eggs of different sizes: an external source of yolk is usually provided by the mother to offsprings that develop via lecithotrophic or adelphophagic (where nurse eggs are consumed) strategies. Maternal provisioning is thus expected to play a significant role in determining different developmental modes (Moran and McAlister 2009; Prowse *et al.* 2008; Smith and Gibson 1999; Vance 1973);

3- poecilogonous species do not have a catastrophic metamorphosis between the larval and adult stages, unlike other marine invertebrates.

At least features 1 (egg capsules) and 2 (yolk provision) are observed in most caenogastropods (including Raphitomidae), and feature 3 (non-catastrophic metamorphosis) should be assessed on each case. Poecilogony has always been a controversial issue, but despite its rarity, poecilogonous species can provide a unique model to understand the regulatory and physiological mechanisms underlying the evolution of larval development.

Acknowledgement

We thank Jean Louis Delemarre, Michel Le Quement, Constantin Mifsud, Tore Høisæter, André Hoarau, Alen Petani, Rino Stanic, Bruno Sabelli, Bruno Fumanti, António Frias Martins, Javier Martin, José Enrique García Raso, Renato Chemello for providing critical specimens for our molecular work. SEM photos were partially provided by Bruno Sabelli and Andrea Di Giulio, and partially made at the Laboratory of Technological and Functional Analyses of Prehistoric Artifacts of Sapienza University of Rome, with the kind help of Cristina Lemorini (Department of Classics). Special thanks to Sara Palomba, Sapienza University of Rome, for the help with photos of the vouchers. Work supported by Sapienza grants to VR (AR11715C7E17226C) and to MO (RM11715C818F7955).

References

Bellardi, L. (1847). Monografia delle Pleurotome fossili del Piemonte. *Memorie della Reale Accademia delle Scienze di Torino* Ser. 2 **9**, 531–650, 4 pls. [the journal issue was published in 1848 but a separate reprint was distributed in 1847; the title and pagination for the separate is: *Monografia delle Pleurotome Fossili del Piemonte*. Torino, 119 pp.].

Bethoux, J.-P. (1979). Le régime de la Méditerranée au cours de périodes glaciaires. *Il Nuovo Cimento* **2(2)**, 117–126.

Bethoux, J.-P. (1984). Paléo-hydrologie de la Méditerranée au cours des derniers 20 000 ans. *Oceanologica Acta* **7(1)**, 43–48.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., Ingram, K. K. and Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in ecology & evolution* ***22*(3)**, 148–155.

Blake, J. A., & Arnofsky, P. L. (1999). Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. *Hydrobiologia*, ***402***, 57–106. https://doi.org/10.1023/A:1003784324125

Bouchet, P. (1989). A review of poecilogony in gastropods. *Journal of Molluscan Studies*, ***55***, 67–78.

Bouchet, Philippe. (1990). Turrid genera and mode of development : the use and abuse of protoconch morphology. *Malacologia*, ***32*(1)**, 69–77.

Collin, R. (2001). The effects of mode of development on phylogeography and population structure of North Atlantic Crepidula(Gastropoda:Calyptraeidae). *Molecular Ecology*, ***10***, 2249–2262.

Collin, Rachel. (2002). Another last word on Crepidula convexa with a description of C. ustulatulina n. sp.(Gastropoda: Calyptraeidae) from the Gulf of Mexico and southern Florida. *Bulletin of Marine Science*, ***70*(1)**, 177–184.

Collin, Rachel. (2004). Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. *Evolution*, ***58*(7)**, 1488–1502.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, ***1*(1)**, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

David, A. A., Matthee, C. A., & Simon, C. A. (2014). Poecilogony in Polydora hoplura (Polychaeta: Spionidae) from commercially important molluscs in South Africa. *Mar Biol*, ***3***, 887–898. https://doi.org/10.1007/s00227-013-2388-0

Duchêne, J.-C. (2000). Effects of poecilogony and delayed larval emission on recruitment of subantarctic population of Boccardia polybranchia (Polychaeta: Spionidae). *Bulletin of Marine Science*, ***67*****(1)**, 311–319.

Ellingson, R. A., & Krug, P. J. (2006). Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus Alderia. *Evolution*, ***60*****(11)**, 2293–2310. https://doi.org/10.1111/j.0014-3820.2006.tb01866.x

Ellingson, R. A., & Krug, P. J. (2015). Reduced genetic diversity and increased reproductive isolation follow population-level loss of larval dispersal in a marine gastropod. *Evolution; International Journal of Organic Evolution*, ***70*****(I)**, 18–37. https://doi.org/10.1111/evo.12830

Fassio, G., Russini, V., Pusateri, F., Giannuzzi-Savelli, R., Høisæter, T., Puillandre, N., et al. (2019). An assessment of Raphitoma and allied genera (Gastropoda, Conoidea, Raphitomidae). *Journal of Molluscan Studies*.

Fedosov, A. E., & Puillandre, N. (2012). Systematics and Biodiversity Phylogeny and taxonomy of the Kermia – Pseudodaphnella (Mollusca : Gastropoda : Raphitomidae) genus complex : a remarkable radiation via diversification of larval development Phylogeny and taxonomy of the Kermia – Pseudodaph. *Systematics and Biodiversity*, ***10*****(4)**, 447–477.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, ***3*****(5)**, 294–299. https://doi.org/10.1371/journal.pone.0013102

Galindo, L. A., Puillandre, N., Strong, E. E., & Bouchet, P. (2014). Using microwaves to prepare gastropods for DNA barcoding. *Molecular Ecology Resources*, ***14*****(4)**, 700–705. https://doi.org/10.1111/1755-0998.12231

Galindo, Lee Ann, Puillandre, N., Utge, J., Lozouet, P., & Bouchet, P. (2016). The phylogeny and systematics of the Nassariidae revisited (Gastropoda, Buccinoidea). *Molecular Phylogenetics and Evolution*, ***99*****(June)**, 337–353. https://doi.org/10.1016/j.ympev.2016.03.019

Giannuzzi-Savelli, R., Pusateri, F., Bartolini, S., & Oliverio, M. (2017). A revision of the Mediterranean Raphitomidae (Neogastropoda, Conoidea) 4: The species of the group of Raphitoma purpurea (Montagu, 1803) with the description of a new species. *Bollettino Malacologico*, ***53***, 161–183.

Giannuzzi-Savelli, R., Pusateri, F., & Bartolini, S. (2018a). A revision of the Mediterranean Raphitomidae (Gastropoda , Conoidea), 7 : on the sibling species Raphitoma densa (Monterosato , 1884) and R . griseomaculata n . sp . *Biodiversity Journal*, ***9*(4)**, 429–440. https://doi.org/10.31396/Biodiv.Jour.2018.9.4.429.440

Giannuzzi-Savelli, R., Pusateri, F., & Bartolini, S. (2018b). A revision of the Mediterranean Raphitomidae (Gastropoda: Conoidea) 5: loss of planktotrophy and pairs of species, with the description of four new species. *Bollettino Malacologico*, ***54*(suppl.11)**, 1–77.

Haszprunar, G. (1995). On the evolution of larval development in the Gastropoda, with special reference to larval planktotrophy. *Notiz. CISMA*, ***16***, 5–13.

Haszprunar, G., Salvini-Plawen, L. v., & Rieger, R. M. (1995). Larval Planktotrophy-A Primitive Trait in the Bilateria? *Acta Zoologica*, ***76*(2)**, 141–154. https://doi.org/10.1111/j.1463-6395.1995.tb00988.x

Hayashi, S. (2014). The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives. *Molluscan Research*, ***25*(2)**, 85–98.

Hoagland, K. E., & Robertson, R. (1988). An Assessment of Poecilogony in Marine Invertebrates: Phenomenon or Fantasy? *The Biological Bulletin*, ***174*(2)**, 109–125. https://doi.org/10.2307/1541778

Hoang, D. T., Chernomor, O., Haeseler, A. Von, Minh, B. Q., & Vinh, L. S. (2017). UFBoot2 : Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, ***35*(2)**, 518–522. https://doi.org/10.5281/zenodo.854445

Jablonski, D., & Lutz, R. A. (1983). Larval ecology of marine benthic invertebrates: paleobiological implications. *Biological Reviews*, ***58*(1)**, 21–89. https://doi.org/10.1111/j.1469-185X.1983.tb00380.x

Jaeckle, W. B., & Manahan, D. T. (1989). Feeding by a “nonfeeding” larva: uptake of dissolved amino acids from seawater by lecithotrophic larvae of the gastropod Haliotis rufescens. *Marine Biology*, ***103*(1)**, 87–94. https://doi.org/10.1007/BF00391067

Katoh, K., Rozewicki, J., & Yamada, K. D. (2017). MAFFT online service : multiple sequence alignment , interactive sequence choice and visualization. *Briefings in Bioinformatics*, 1–7. https://doi.org/10.1093/bib/bbx108

Kearse, M., Moir, R., Wilson, A., Stones-havas, S., Sturrock, S., Buxton, S., et al. (2012). Geneious Basic : An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)*, ***28*(12)**, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Knott, K. E., & McHugh, D. (2012). Introduction to Symposium: Poecilogony--A Window on Larval Evolutionary Transitions in Marine Invertebrates. *Integrative and Comparative Biology*, ***52*(1)**, 120–127. https://doi.org/10.1093/icb/ics037

Krug, P J. (1998). Poecilogony in an estuarine opisthobranch : planktotrophy , lecithotrophy , and mixed clutches in a population of the ascoglossan Alderia modesta. *Marine Biology*, ***132***, 483–494.

Krug, Patrick J. (2007). Poecilogony and larval ecology in the gastropod genus Alderia*. *American Malacological Bulletin*, ***23*(1)**, 99–111. https://doi.org/10.4003/0740-2783-23.1.99

Krug, Patrick J. (2009). Not my “type”: larval dispersal dimorphisms and bet-hedging in opisthobranch life histories. *The Biological Bulletin*, ***216*(3)**, 355–72. https://doi.org/10.1086/BBLv216n3p355

Kruse, I., Reusch, T. B. H., & Schneider, M. V. (2003). Sibling species or poecilogony in the polychaete Scoloplos armiger ? *Marine Biology*, ***142***, 937–947. https://doi.org/10.1007/s00227-002-1007-2

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7 : Molecular Evolutionary Genetics Analysis Version 7 . 0 for Bigger Datasets Brief communication. *Molecular Biology and Evolution*, ***33*(7)**, 1870–1874. https://doi.org/10.1093/molbev/msw054

Kuraku, S., Zmasek, C. M., Nishimura, O., & Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research*, ***41*(May)**, 22–28. https://doi.org/10.1093/nar/gkt389

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2 : New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution*, ***34*(3)**, 772–773. https://doi.org/10.1093/molbev/msw260

Manahan, D. T. (1990). Adaptations by Invertebrate Larvae for Nutrient Acquisition from Seawater. *American Zoologist*, ***30*(1)**, 147–160. https://doi.org/10.1093/icb/30.1.147

Mcdonald, K. A., Collin, R., & Lesoway, M. P. (2014). Poecilogony in the caenogastropod Calyptraea lichen (Mollusca : Gastropoda). *Invertebrate Biology*, ***133*(3)**, 213–220. https://doi.org/10.1111/ivb.12057

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *2010 Gateway Computing Environments Workshop*

(GCE) (pp. 1–8). IEEE. https://doi.org/10.1109/GCE.2010.5676129

Modica, M. V., Russini, V., Fassio, G., & Oliverio, M. (2017). Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Marine Environmental Research*, ***127***. https://doi.org/10.1016/j.marenvres.2017.04.001

Moran, A. L., & McAlister, J. S. (2009). Egg size as a life history character of marine invertebrates: Is it all it's cracked up to be? *The Biological Bulletin*, ***216*****(3)**, 226–42. https://doi.org/10.1086/BBLv216n3p226

Morgan, T., Rogers, A., Paterson, G., Hawkins, L., & Sheader, M. (1999). Evidence for poecilogony in Pygospio elegans (Polychaeta:Spionidae). *Marine Ecology Progress Series*, ***178***, 121–132. https://doi.org/10.3354/meps178121

Nguyen, L., Schmidt, H. A., Haeseler, A. Von, & Minh, B. Q. (2014). IQ-TREE : A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution*, ***32*****(1)**, 268–274. https://doi.org/10.1093/molbev/msu300

Nutzel, A. (2014). Larval ecology and morphology in fossil gastropods. *Palaeontology*, 1–25. https://doi.org/10.1111/pala.12104

Oliverio, M. (1997). Global biodiversity and life-history evolution in prosobranchs gastropods. *Iberus*, ***16*****(1)**, 73–79.

Oliverio, M, & Mariottini, P. (2001). A molecular framework for the phylogeny of Coralliophila and related muricoids. *Journal of Molluscan Studies*, ***67*****(i)**, 215–224. https://doi.org/10.1093/mollus/67.2.215

Oliverio, Marco. (1991). Larval ecology and speciation of marine prosobranchs (Mollusca, Gastropoda). *3rd Congress Ofthe European Society for Evolutionary Biology*, ***238***.

Oliverio, Marco. (1996a). Contrasting developmental strategies and speciation in NE Atlantic prosobranchs: a preliminary analysis. *Origin and Evolutionary Radiation of the Mollusca*, ***22***, 261–266.

Oliverio, Marco. (1996b). Life-histories, speciation, and biodiversity in Mediterranean prosobranch gastropods. *Vie et Milieu*, ***46*****(2)**, 163–169.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). A Simple Fool's Guide to PCR. *University of Hawaii Press: Honolulu.*

Prowse, T. A. A., Sewell, M. A., & Byrne, M. (2008). Fuels for development: evolution of maternal provisioning in asterinid sea stars. *Marine Biology*, ***153*****(3)**, 337–349.

https://doi.org/10.1007/s00227-007-0809-7

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, ***21*****(8)**, 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Puillandre, N., Modica, M. V., Zhang, Y., Sirovich, L., & Boisselier, M. (2012). Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology*, ***21***, 2671–2691. https://doi.org/10.1111/j.1365-294X.2012.05559.x

Puillandre, Nicolas, Baylac, M., Boisselier, M., Cruaud, C., & Samadi, S. (2009). An integrative approach to species delimitation in Benthomangelia (Mollusca : Conoidea). *Biological Journal of the Linnean Society*, **(96)**, 696–708.

Puillandre, Nicolas, Stöcklin, R., Favreau, P., Bianchi, E., Perret, F., Rivasseau, A., et al. (2014). When everything converges: integrative taxonomy with shell, DNA and venomic data reveals Conus conco, a new species of cone snails (Gastropoda: Conoidea). *Molecular Phylogenetics and Evolution*, ***80***, 186–192. https://doi.org/10.1016/j.ympev.2014.06.024

Pusateri, F., Giannuzzi-Savelli, R., & Oliverio, M. (2012). A revision of the Mediterranean Raphitomidae 1: on the sibling species Raphitoma contigua Monterosato, 1884 and Raphitoma spadiana n. sp. (Gastropoda, Conoidea). *Iberus*, ***30*****(1)**, 41–52.

Pusateri, F., Giannuzzi-Savelli, R., & Oliverio, M. (2013). A revision of the Mediterranean Raphitomidae 2: On the sibling species Raphitoma lineolata (B.D.D., 1883) and Raphitoma smriglioi n. sp. (Gastropoda, Conoidea). *Iberus*, ***31*****(1)**, 11–20.

Pusateri, Francesco, Giannuzzi-Savelli, R., & Bartolini, S. (2016). A revision of the Mediterranean Raphitomidae, 3: on the Raphitoma pupoides (Monterosato, 1884) complex, with the description of a new species (Mollusca Gastropoda). *Biodiversity Journal*, ***7*****(1)**, 103–115.

Pusateri, Francesco, Giannuzzi-Savelli, R., & Bartolini, S. (2018). A revision of the Mediterranean Raphitomidae (Gastropoda Conoidea), 6 : on the Raphitoma corbis (Potiez et Michaud , 1838). *Biodiversity Journal*, ***9*****(3)**, 217–225. https://doi.org/10.31396/Biodiv.Jour.2018.9.3.217.225

Revell, L. J. (2012). phytools : an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, ***3***, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., et al. (2012). MrBayes 3 . 2 : Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large

Model Space. *Software for Systematics and Evolution Syst.*, ***61*(3)**, 539–542. https://doi.org/10.1093/sysbio/sys029

Russini, V., Fassio, G., Modica, M. V., de Maintenon, M. J., & Oliverio, M. (2017). An assessment of the genus columbella lamarck, 1799 (Gastropoda: Columbellidae) from eastern Atlantic. *Zoosystema*, ***39*(2)**. https://doi.org/10.5252/z2017n2a2

Shuto, T. (1974). Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia*, ***7***, 239–256.

Smith, H. L., & Gibson, G. D. (1999). Nurse egg origin in the polychaete Boccardia proboscidea (Spionidae). *Invertebrate Reproduction & Development*, ***35*(3)**, 177–185. https://doi.org/10.1080/07924259.1999.9652384

Strathmann, R. R. (1985). Feeding anStrathmann, R. R. (1985). Feeding and Nonfeeding Larval Development and Life-History Evolution in Marine Invertebrates. Annual Review of Ecology and Systematics, 16(1), 339–361. https://doi.org/10.1146/annurev.es.16.110185.002011d Nonfeeding Lar. *Annual Review of Ecology and Systematics*, ***16*(1)**, 339–361. https://doi.org/10.1146/annurev.es.16.110185.002011

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1 . 10. *Virus Evolution*, ***4*(1)**, 1–5. https://doi.org/10.1093/ve/vey016

Vance, R. R. (1973). More on Reproductive Strategies in Marine Benthic Invertebrates. *The American Naturalist*, ***107*(955)**, 353–361. https://doi.org/10.1086/282839

Vendetti, J. E., Trowbridge, C. D., & Krug, P. J. (2012a). Integrative and Comparative Biology Poecilogony and Population Genetic Structure in Elysia pusilla (Heterobranchia : Sacoglossa), and Reproductive Data for Five Sacoglossans that Express Dimorphisms in Larval Development. *Integrative and Comparative Biology*, ***52*(1)**, 138–150. https://doi.org/10.1093/icb/ics077

Vendetti, J. E., Trowbridge, C. D., & Krug, P. J. (2012b). Poecilogony and Population Genetic Structure in Elysia pusilla (Heterobranchia: Sacoglossa), and Reproductive Data for Five Sacoglossans that Express Dimorphisms in Larval Development. *Integrative and Comparative Biology*, ***52*(1)**, 138–150. https://doi.org/10.1093/icb/ics077

Table 1. List of the molecular samples along with vouchers registration numbers (ID: BAU: Department of Biology and Biotechnologies, 'Sapienza' University, Rome. MNHN: Muséum national d'Histoire naturelle, Paris. MT: German Centre for Marine Biodiversity Research, Senckenberg Institute, Wilhelmshaven. ZMBN: University Museum of Bergen Natural History Collections), preliminary species assignation based on morphology assessment (PSH), protoconch type (M: multispiral; P: paucispiral), collection localities, GenBank accession numbers for sequences, and relevant references.

					GenBank	**Accession**	**Numbers**		
ID code	**PSH**	**Protoconch**	**Locality**	**Coordinates**	**12S**	**16S**	**COI**	**ITS2**	**references**
ZMBN-020209-O	*Cyrillia aequalis*	M	Norway	60°13'48.0"N 5°12'00.0"E		JF834214	JF834219	X	Fassio *et al.* (2019) and this work
ZMBN-E-345-66a	*Cyrillia aequalis*	M	Norway	60°18'00.0"N 5°10'48.0"E			JF834221		Fassio *et al.* (2019) and this work
ZMBN-E-345-66b	*Cyrillia aequalis*	M	Norway	60°18'00.0"N 5°10'48.0"E			JF834225		Fassio *et al.* (2019) and this work
MT09222	*Cyrillia aequalis*	M	North Sea	55°22'15.6"N 0°12'25.2"W			KR084567		Barco *et al.* (2016)
MT09383	*Cyrillia aequalis*	M	North Sea	57°53'56.4"N 0°54'57.6"W			KR084390		Barco *et al.*, 2016
BAU-2234.1	*Cyrillia linearis*	M	Italy, Giannutri Is., 8/7/2015	42°15'10"N 011°05'32"E	MK410585	MK410605	MK410632	X	Fassio *et al.* (2019) and this work
BAU-2912.1	*Cyrillia linearis*	M	Italy, Giglio Is., Cala Cupa	42°22'06"N 10°55'12"E		MK410599	MK410623		Fassio *et al.* (2019)
ZMBN-E-37-68	*Cyrillia obesa*	M	Norway	60°18'00.0"N 5°07'48.0"E		MK410610	JF834220		Fassio *et al.* (2019)
BAU-1896.1	*Raphitoma* cf. *atropurpurea*	M	Italy, Zannone Is., 28 m, 13/6/2009	40°57'51.2"N 13°03'28.7"E			X	X	This work
BAU-2275.1	*Raphitoma* cf. *atropurpurea*	M	Croatia, Biograd, 31/5/2014	43°55'51"N 15°26'42"E			X	X	This work
BAU-3355.1	*Raphitoma atropurpurea*	M	Croatia, Cres Island, Punta Kriza	44°38'51.4"N 14°31'23.2"E			X		This work
BAU-2245.2	*Raphitoma bartolinorum*	P	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-1897.1	*Raphitoma* cf. *bicolor*	M	France, St. Maxime	43°18'49"N 6°40'22"E		MK410603	MK410630	X	Fassio *et al.* (2019) and this work
BAU-3047.1	*Raphitoma* cf. *echinata*	M	Croatia, Ciovo Is., Labadusa	43°28'44.6"N 16°14'42.0"E			X		This work
BAU-1904.1	*Raphitoma contigua*	M	Italy, Zannone Is., 13/6/2009	40°57'51.2"N 13°03'28.7"E			X	X	This work
BAU-2236.1	*Raphitoma contigua*	M	France, La Ciotat, Figuerolles, 24/7/2014	43°09'53.9"N 5°35'45.7"E			X	X	This work
BAU-2262.1	*Raphitoma cordieri*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E	MK410582	MK410595	MK410619	X	Fassio *et al.* (2019) and this work
BAU-2262.2	*Raphitoma cordieri*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			MK410625		Fassio *et al.* (2019)

BAU-1895.1	*Raphitoma densa*	M	Italy, Torre Colimena, 09/2012	40°17'39"N 17°45'17"E		MK410602	MK410629		Fassio *et al.* (2019)
BAU-2239.1	*Raphitoma densa*	M	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X		This work
BAU-2257.1	*Raphitoma densa*	M	Croatia, Sukosan, 15/2/2014	44°02'04"N 15°18'57"E	MK410581	MK410594	MK410617	X	Fassio *et al.* (2019) and this work
BAU-2260.1	*Raphitoma densa*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			X		This work
BAU-3069.1	*Raphitoma densa*	M	Croatia, Slano	42°47'00.4"N 17°52'50.7"E			X		This work
BAU-3347.1	*Raphitoma densa*	M	Canary Islands, Tenerife, Las Eras	28°11'38.1"N 16°25'11.6"W			X		This work
BAU-1900.1	*Raphitoma horrida*	P	Corsica, Tour d'Ancone, 2012	42°02'36"N 8°43'20"E		MK410604	MK410631		This work
BAU-1906.1	*Raphitoma horrida*	P	France, St. Maxime	43°18'49"N 6°40'22"E,	MK410577	MK410590	MK410612	X	Fassio *et al.* (2019) and this work
BAU-2242.1	*Raphitoma horrida*	P	Croatia, Vrsi, 8/2/2014	44°16'56"N 15°12'35"E			X		This work
BAU-2259.1	*Raphitoma horrida*	P	Croatia, Vrsi, 8-14/11/2013	44°16'56"N 15°12'35"E			X		This work
BAU-2259.2	*Raphitoma horrida*	P	Croatia, Vrsi, 8-14/11/2013	44°16'56"N 15°12'35"E			X		This work
BAU-2264.1	*Raphitoma horrida*	P	Croatia, Dugi Otok, 9/8/2014	43°59'N 15°05'34"E	MK410583	MK410596	MK410620	X	Fassio *et al.* (2019) and this work
BAU-2264.2	*Raphitoma horrida*	P	Croatia, Dugi Otok, 9/8/2014	43°59'N 15°05'34"E			X		This work
BAU-2274.1	*Raphitoma horrida*	P	Croatia, Vrsi, 19/4/2014	44°16'56"N 15°12'35"E			X		This work
BAU-3045.1	*Raphitoma horrida*	P	Greece, Agrilidi, Astipalea	36°35'02" N 026°25'24" E			X		This work
BAU-3351.1	*Raphitoma horrida*	P	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X	X	Fassio *et al.* (2019) and this work
BAU-1878.1	*Raphitoma laviae*	M	France, St. Maxime	43°18'41.2"N 6°40'19.4"E			X	X	Fassio *et al.* (2019) and this work
BAU-2243.1	*Raphitoma laviae*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			X	X	Fassio *et al.* (2019) and this work
BAU-2243.2	*Raphitoma laviae*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			X		This work
BAU-2243.3	*Raphitoma laviae*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			X		This work
BAU-2243.4	*Raphitoma laviae*	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E			X	X	This work
BAU-2246.1	*Raphitoma laviae*	M	Croatia, Zaton, 8/11/2013	44°13'07"N 15°09'41"E	MK410578	MK410591	MK410614	X	Fassio *et al.* (2019) and this work
BAU-2246.2	*Raphitoma laviae*	M	Croatia, Zaton, 8/11/2013	44°13'07"N 15°09'41"E			X		This work

BAU-2246.3	*Raphitoma laviae*	M	Croatia, Zaton, 8/11/2013	44°13'07"N 15°09'41"E			X	X	This work
BAU-2246.4	*Raphitoma laviae*	M	Croatia, Zaton, 8/11/2013	44°13'07"N 15°09'41"E			X	X	This work
BAU-2251.1	*Raphitoma laviae*	M	Croatia, Turani, 16/11/2013	43°57'48.6"N 15°23'58.3"E			X		This work
BAU-2251.2	*Raphitoma laviae*	M	Croatia, Turani, 16/11/2013	43°57'48.6"N 15°23'58.3"E			X		This work
BAU-2251.3	*Raphitoma laviae*	M	Croatia, Turani, 16/11/2013	43°57'48.6"N 15°23'58.3"E			X		This work
BAU-2253.1	*Raphitoma laviae*	M	Croatia, Telascjca, 12/8/2013	43°53'30"N 15°09'33"E	MK410579	MK410592	MK410615	X	Fassio *et al.* (2019) and this work
BAU-2270.1	*Raphitoma laviae*	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2270.2	*Raphitoma laviae*	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X		This work
BAU-2270.4	*Raphitoma laviae*	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2363.2	*Raphitoma laviae*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X		This work
BAU-3354.1	*Raphitoma laviae*	M	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X		This work
BAU-3357.2	*Raphitoma laviae*	M	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X	X	This work
BAU-3358.1	*Raphitoma laviae*	M	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X	X	This work
BAU-3358.2	*Raphitoma laviae*	M	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X	X	This work
BAU-3358.3	*Raphitoma laviae*	M	Croatia, Cres Is., Punta Kriza	44°38'51.4"N 14°31'23.2"E			X	X	This work
BAU-2248.1	*Raphitoma locardi*	M	Croatia, Vrsi, 8/11/2013	44°16'56"N 15°12'35"E			X		This work
BAU-2248.2	*Raphitoma locardi*	M	Croatia, Vrsi, 8/11/2013	44°16'56"N 15°12'35"E			X	X	This work
BAU-2248.3	*Raphitoma locardi*	M	Croatia, Vrsi, 8/11/2013	44°16'56"N 15°12'35"E			X		This work
BAU-2261.1	*Raphitoma locardi*	M	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E		X	X	X	This work
BAU-2261.2	*Raphitoma locardi*	M	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X	X	This work
ZMBN-040809_X	*Raphitoma maculosa*	M	Norway	60°18'00.0"N 5°07'48.0"E			MK410638	X	Fassio *et al.* (2019) and this work
BAU-2269.1	*Raphitoma* sp. B	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E	X		X	X	This work and Prkić *et al.* (2019)
BAU-2269.2	*Raphitoma* sp. B	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work and Prkić *et al.* (2019)
BAU-2269.3	*Raphitoma* sp. B	M	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X		This work and Prkić *et al.* (2019)

BAU-2273.1	*Raphitoma* sp. B	M	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E		X	X	X	This work and Prkić *et al.* (2019)
BAU-2273.3	*Raphitoma* sp. B	M	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X		This work and Prkić *et al.* (2019)
BAU-2273.4	*Raphitoma* sp. B	M	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X		This work and Prkić *et al.* (2019)
BAU-2256.2	*Raphitoma* sp. A	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E	X		X	X	This work and Prkić *et al.* (2019)
BAU-2256.3	*Raphitoma* sp. A	M	Croatia, Sukosan, 16/11/2013	44°02'04"N 15°18'57"E	X	X	X	X	This work and Prkić *et al.* (2019)
BAU-1888.1	*Raphitoma philberti*	P	Italy, Campomarino di Maruggio, Taranto, 10/2012	40°17'49.2"N 17°34'12.7"E			MK410611	X	Fassio *et al.* (2019) and this work
BAU-1893.1	*Raphitoma philberti*	P	Greece, Limnos Is., Koukonisi Bay, 7/2014	39°53'07"N 25°16'16"E			X	X	This work
BAU-1893.2	*Raphitoma philberti*	P	Greece, Limnos Is., Koukonisi Bay, 7/2014	39°53'07"N 25°16'16"E			X		This work
BAU-1902.1	*Raphitoma philberti*	P	Italy, Elba Is., Fetovaia bay	42°43'53.3"N 10°09'20.2"E			X		This work
BAU-1903.1	*Raphitoma philberti*	P	Italy, Elba Is., Fetovaia bay	42°43'53.3"N 10°09'20.2"E			X	X	This work
BAU-2238.1	*Raphitoma philberti*	P	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2238.2	*Raphitoma philberti*	P	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2238.3	*Raphitoma philberti*	P	Croatia, Biograd, 16/11/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2241.1	*Raphitoma philberti*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2241.2	*Raphitoma philberti*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2249.1	*Raphitoma philberti*	P	Croatia, Sukosan, 15/2/2014	44°02'04"N 15°18'57"E			X	X	This work
BAU-2249.2	*Raphitoma philberti*	P	Croatia, Sukosan, 15/2/2014	44°02'04"N 15°18'57"E			X	X	This work
BAU-2252.1	*Raphitoma philberti*	P	Croatia, Zaton, 8/11/2013	44°13'07.6"N 15°09'41.6"E			X		This work
BAU-2255.1	*Raphitoma philberti*	P	Croatia, Sabunike, 2/11/2013	44°16'08.3"N 15°10'26.3"E			X	X	This work
BAU-2258.1	*Raphitoma philberti*	P	Croatia, Vrsi, 18/4/2014	44°16'56"N 15°12'35"E			MK410618		Fassio *et al.* (2019)
BAU-2267.1	*Raphitoma philberti*	P	Croatia, Sabunike, 1/5/2014	44°16'08.3"N 15°10'26.3"E			X	X	This work
BAU-2268.1	*Raphitoma philberti*	P	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2268.2	*Raphitoma philberti*	P	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-2268.3	*Raphitoma philberti*	P	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X	X	This work

BAU-2363.1	*Raphitoma philberti*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X		This work
BAU-2365.1	*Raphitoma philberti*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E		MK410598	MK410622	X	Fassio *et al.* (2019) and this work
BAU-2365.2	*Raphitoma philberti*	P	Croatia, Biograd, 30/5/2013	43°55'51"N 15°26'42"E			X	X	This work
BAU-3046.1	*Raphitoma philberti*	P	Greece, Astipalea Is., Vai	36° 35' 13" N 26° 24' 10" E	MK410588		MK410636		Fassio *et al.* (2019) and this work
BAU-3352.1	*Raphitoma philberti*	P	Croatia, Punta Kriza, Cres Island	44°38'51.4"N 14°31'23.2"E			X	X	This work
BAU-3352.2	*Raphitoma philberti*	P	Croatia, Punta Kriza, Cres Island	44°38'51.4"N 14°31'23.2"E			X		This work
BAU-3352.3	*Raphitoma philberti*	P	Croatia, Punta Kriza, Cres Island	44°38'51.4"N 14°31'23.2"E			X		This work
BAU-3205.1	*Raphitoma pseudohystrix*	P	Malta, SW, Gnejna Bay, 22/7/2006	35°49'54.3"N 14°17'15.2"E	MK410589	MK410609	MK410637	X	Fassio *et al.* (2019) and this work
BAU-2337.1	*Raphitoma purpurea*	M	France, Ploubazlanec	48°48'5"N 3°00'10"W		MK410597	MK410621	X	Fassio *et al.* (2019) and this work
BAU-2337.2	*Raphitoma purpurea*	M	France, Ploubazlanec	48°48'5"N 3°00'10"W			MK410626		Fassio *et al.* (2019)
BAU-2337.3	*Raphitoma purpurea*	M	France, Ploubazlanec	48°48'5"N 3°00'10"W			X		This work
BAU-2337.6	*Raphitoma purpurea*	M	France, Ploubazlanec	48°48'5"N 3°00'10"W			X		This work
BAU-2338.1	*Raphitoma purpurea*	M	France, Ploubazlanec	48°48'5"N 3°00'10"W	MK410586	MK410607	MK410634		Fassio *et al.* (2019)
BAU-2539.1	*Raphitoma purpurea*	M	Spain, Malaga, Zona de Cabo Pino, Torre de Calahonda	36°28'36.0"N 4°42'30.0"W			X		This work
BAU-2247.1	*Raphitoma spadiana*	P	Croatia, Biograd, 26/10/2013	43°55'51"N 15°26'42"E			X	X	This work

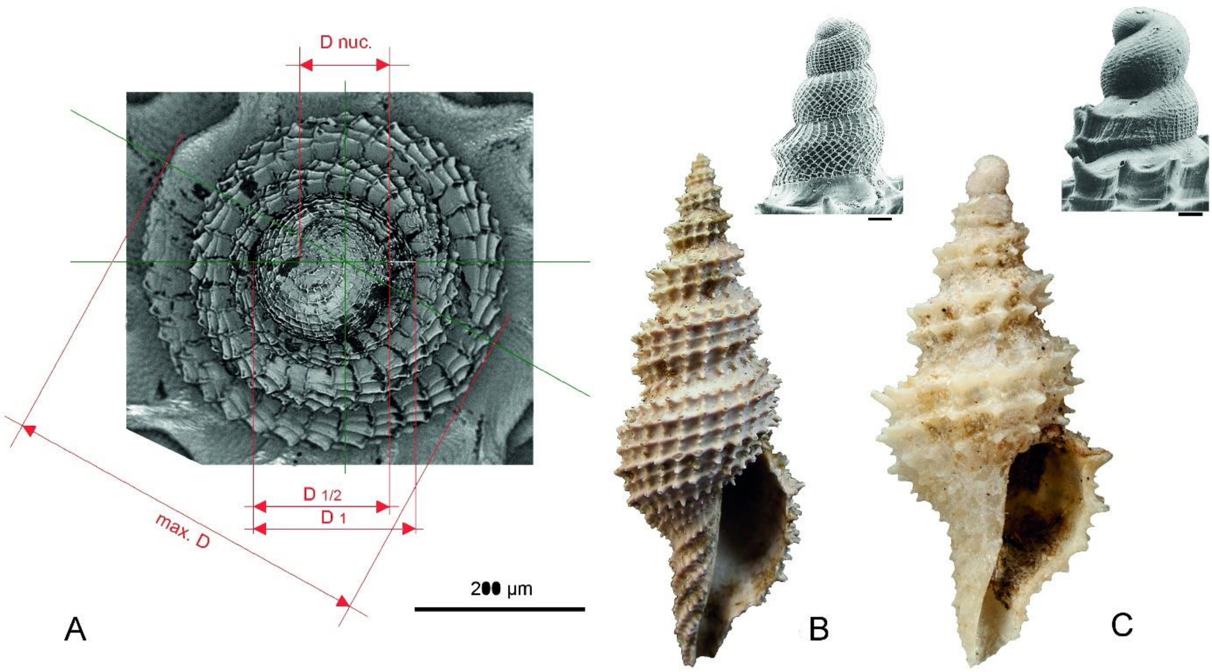


Figure 1. Larval and adult shells of *Raphitoma*. (*A*) Counting of protoconch whorls according the method of Verduin (1977). D nuc: Diameter of nucleus; D ½: diameter of first half-whorl; D 1: diameter of first whorl; max. D: maximum diameter. (*B*) *Raphitoma histrix* Bellardi, 1847; neotype, Piacentian of Colli Astesi (Pliocene), h: 17.6 mm (MRSN, Torino n. 011.16.008). (*C*) *Raphitoma pseudohystrix* (Sykes, 1906); lectotype, Adventure Bank, Sicily Channel, h: 5.1 mm (NHMUK n. 20130109).

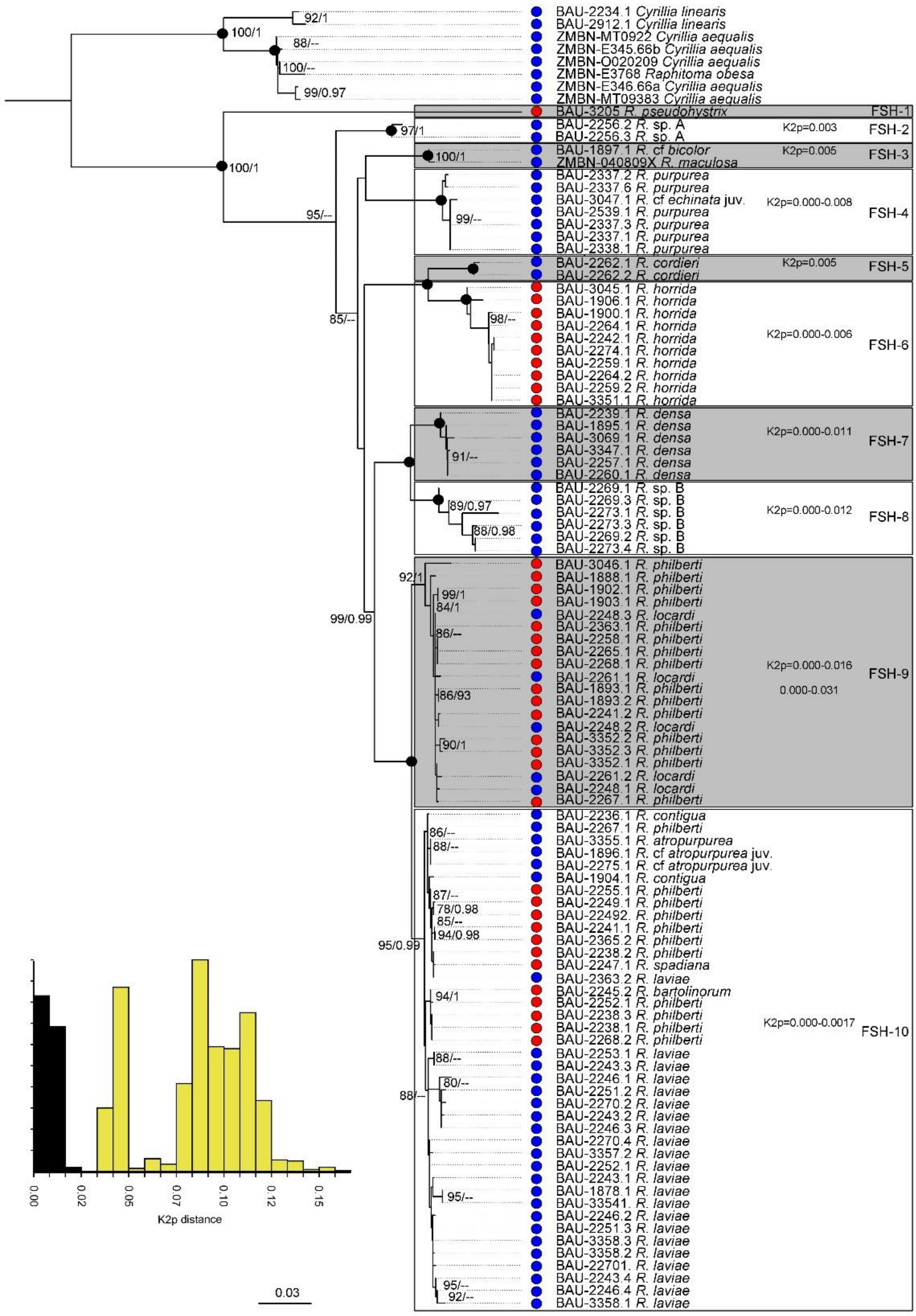


Figure 2. Phylogenetic relationships (Maximum likelihood topology, on the combined dataset) among the assayed specimens. The following models were selected by Partition Finder 2 for each partition: HKY+I+G (16S, 12S), F81 (COI position 1), SYM+G (COI position 2), GTR+G (COI position 3), K80+G (ITS2). Numbers at nodes are BS and PP supports, respectively; only values higher than 75% BS and 95% PP are reported; black dots indicate nodes supported by 100% BS and 1.0 PP. The histogram portrays the distribution of the pairwise genetic distances (K2p) among the COI sequences (black bars on the left are intraspecific comparisons, yellow bars on the right are interspecific comparisons). Blue circles indicate multispiral protoconch, and inferred planktotrophic development; red circles indicate paucispiral protoconch, and inferred non-planktotrophic development. Boxes indicate the final species hypotheses (FSH) eventually retained after the Integrative Taxonomy approach, with the ranges of genetic distance (K2p) scored in each FSH.

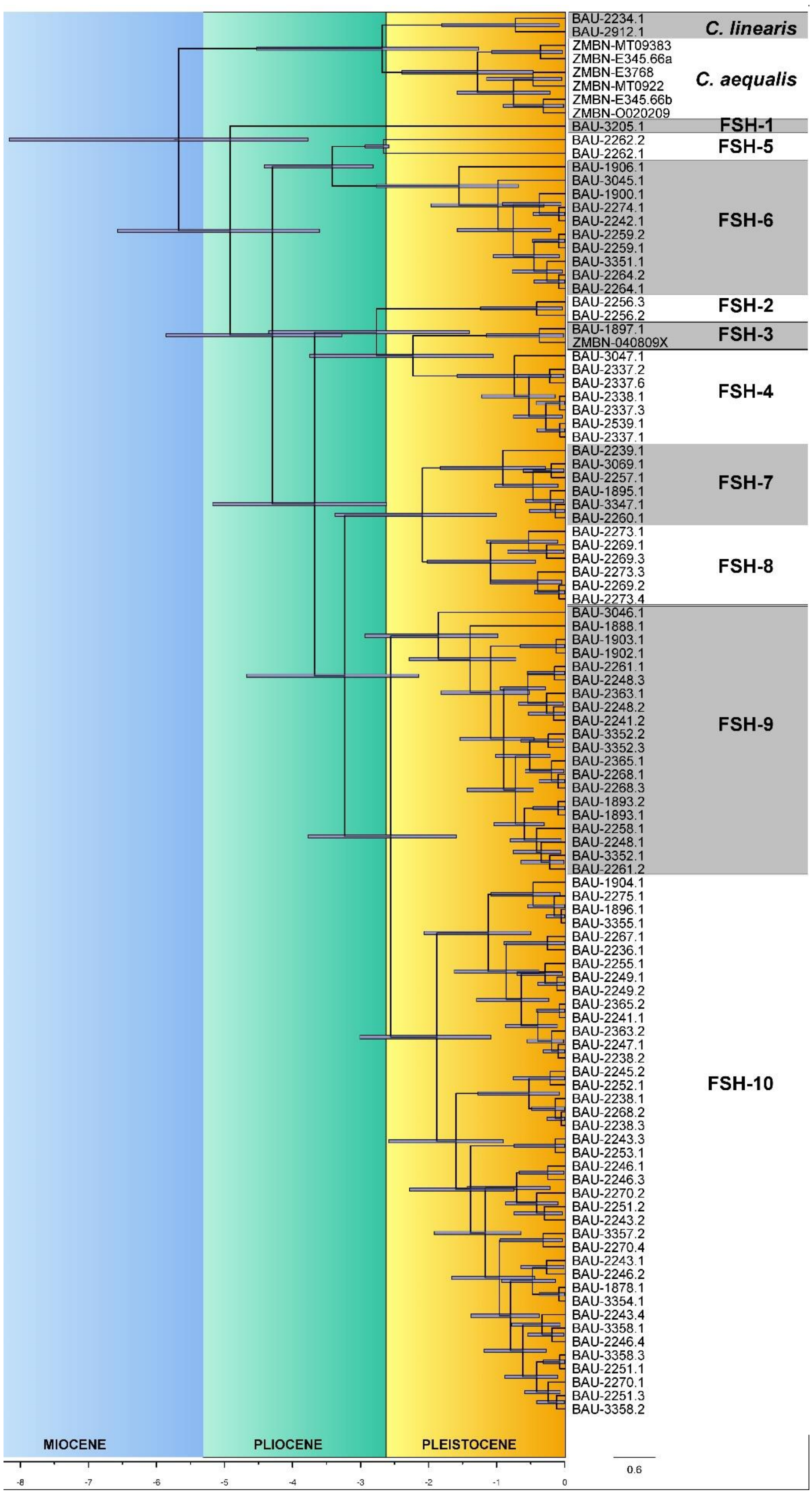


Figure 3. Evolutionary timetree of the genus *Raphitoma*, inferred from BEAST analysis of the combined molecular dataset. Coloured bars indicate 95% highest posterior density intervals for node ages of interest. White and grey boxes correspond to the Final Species Hypotheses, as in Fig. 2. Vertical banding indicates the major geological ages: Miocene (ending 5.3 mya), Pliocene (5.3-2.6 mya), Pleistocene (starting 2.6 mya).

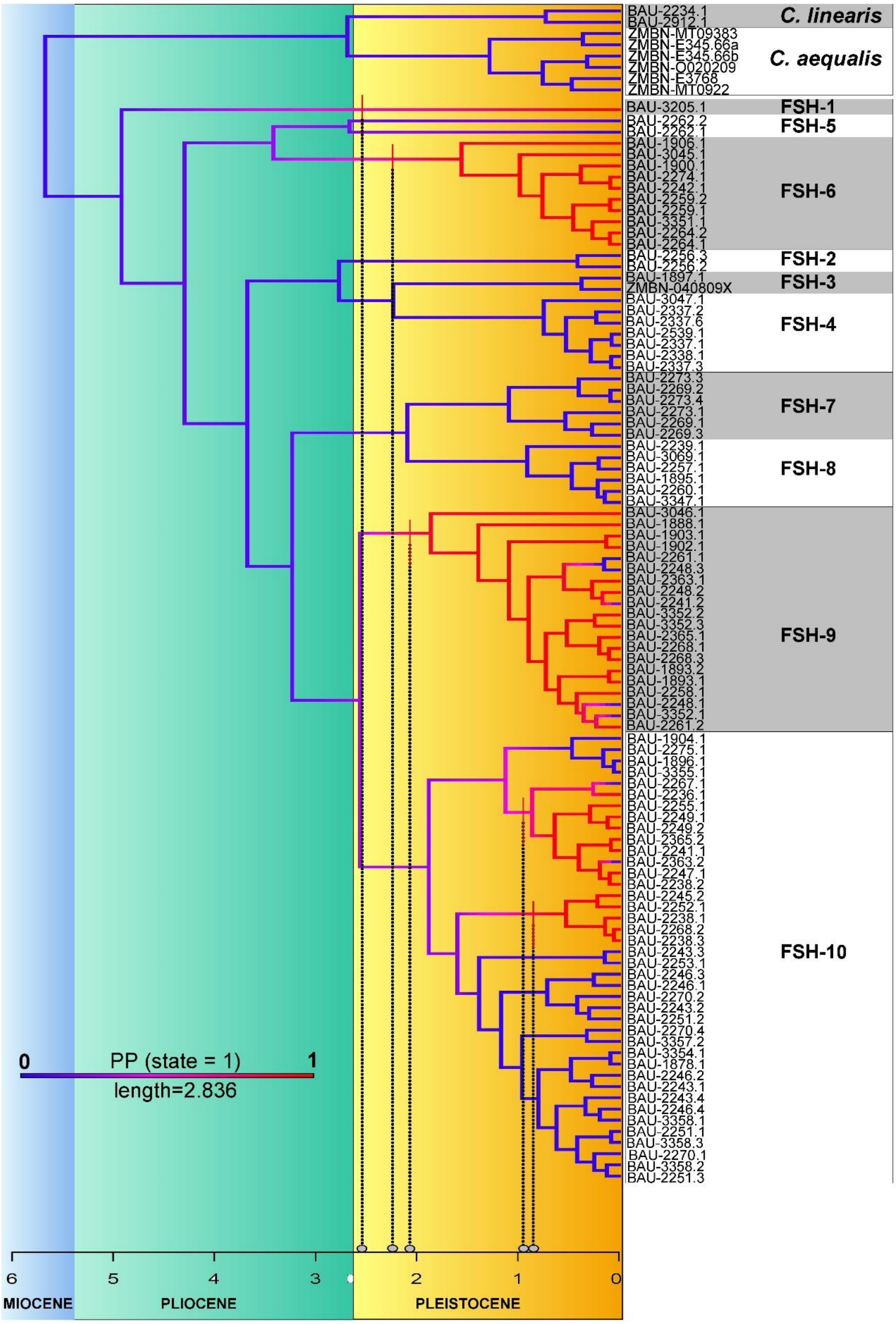


Figure 4. Stochastic character mapping for larval development along the branches of the evolutionary timetree of the genus *Raphitoma,* using the phytools package. Branch colours are the probability of the non-planktotrophic state (blue=0, planktotrophic development; red=1, non-planktotrophic development). Major changes are mapped on the timescale (mya) at the bottom. White and grey boxes correspond to the Final Species Hypotheses, as in Fig. 2. Vertical banding indicates the major geological ages: Miocene (ending 5.3 mya), Pliocene (5.3-2.6 mya), Pleistocene (starting 2.6 mya).

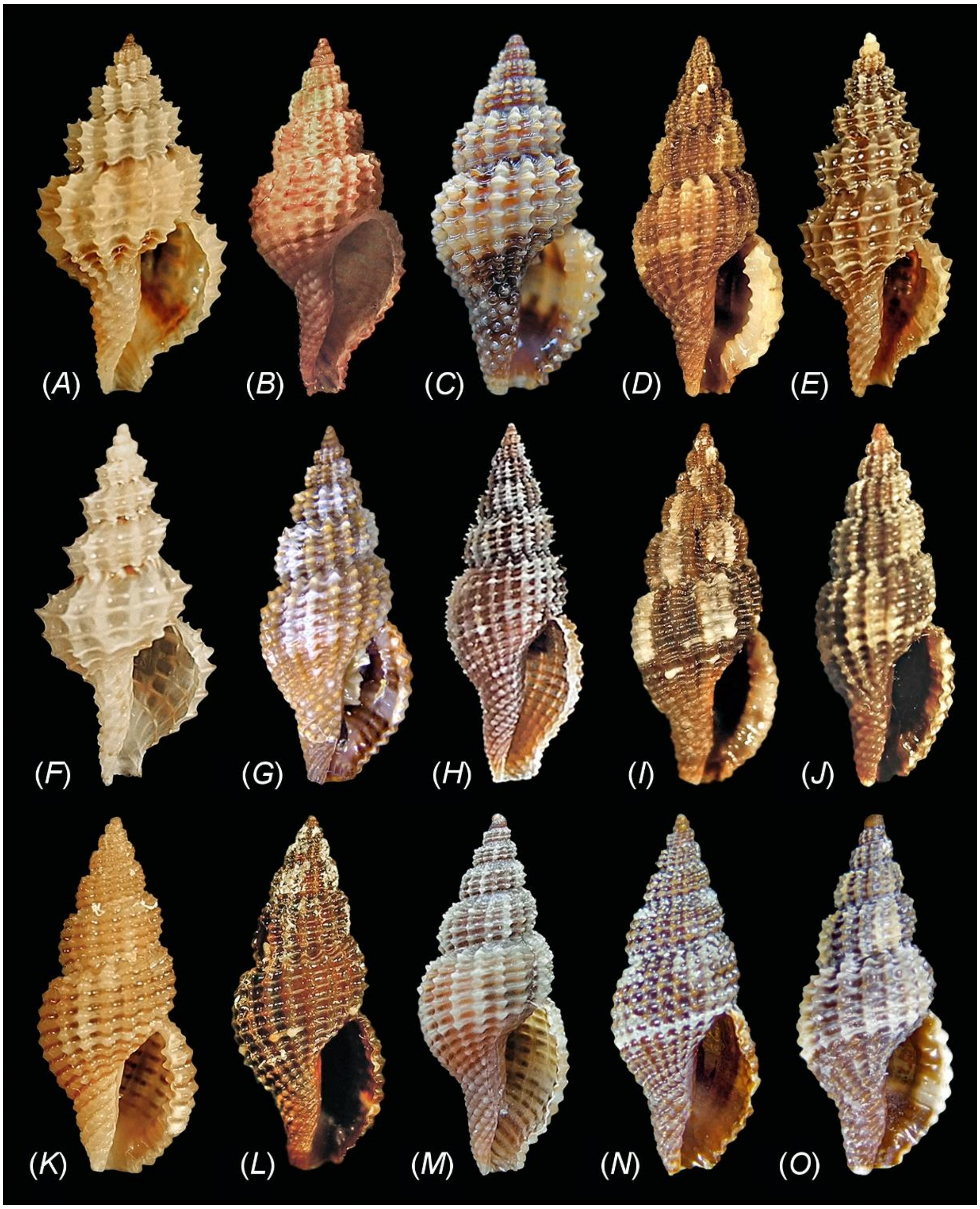


Figure 5. Voucher shells of *Raphitoma* spp. used in this work, with their preliminary morphological identification. (*A*) *Raphitoma* sp. A, Sukosan (Croatia), BAU-2256.2 h: mm 10.2. (*B*) *Raphitoma maculosa* Høisater, 2016, Liholmsrennen, Raunefjorden, 60°18′N, 05°09′E, 70–90 m. (Norway), holotype ZMBN 107134, h: 7.2 mm. (*C*) *Raphitoma* cf *bicolor* (Risso, 1826), Saint Maxime (France), BAU-1897.1, h: 9.3 mm. (*D*) *Raphitoma purpurea* (Montagu, 1803), Ploubazlanec, Bretagne (France), BAU-2337.6, h: 14.9 mm. (*E*) *Raphitoma cordieri* (Payraudeau, 1826), Sukosan (Croatia), BAU-2262.2, h: mm 11.9. (*F*) *Raphitoma horrida* (Monterosato, 1884), Agrilidi, Astypalea Is. (Greece), BAU-3045.1, h: 10.5 mm. (*G*) *Raphitoma densa* (Monterosato, 1884), Slano, Dubrovnik (Croatia), BAU-3069.1, h: 7.8 mm. (*H*) *Raphitoma* sp. B, Biograd, Croatia, BAU-2273.3, h: 9.5 mm. (*I*) *Raphitoma philberti* (Michaud, 1829), Campomarino, Taranto (Italy), BAU-1888.1, h: 11.6. (*J*) *Raphitoma locardi*, Pusateri and Giannuzzi-Savelli, 2013, Biograd (Croatia), BAU-2261.1, h: 8.8 mm. (*K*) *Raphitoma contigua* (Monterosato, 1884), Figuerolles, La Ciotat (France), BAU-2236.1, h: 11.3. (*L*) *Raphitoma atropurpurpurea* (Locard and Caziot, 1899), Punta Kriza, Cres Is. (Croatia), BAU 3355.1, h: 12.1 mm. (*M*) *Raphitoma spadiana* Pusateri and Giannuzzi-Savelli, 2012, Biograd (Croatia), BAU 2247.1, h: 6.4 mm. (*N*) *Raphitoma laviae* (Philippi, 1836), Sukosan (Croatia), BAU 2243.2, h: 7 mm. (*O*) *Raphitoma philberti*, Sukosan (Croatia), BAU 2249.1, h: 6.6 mm.

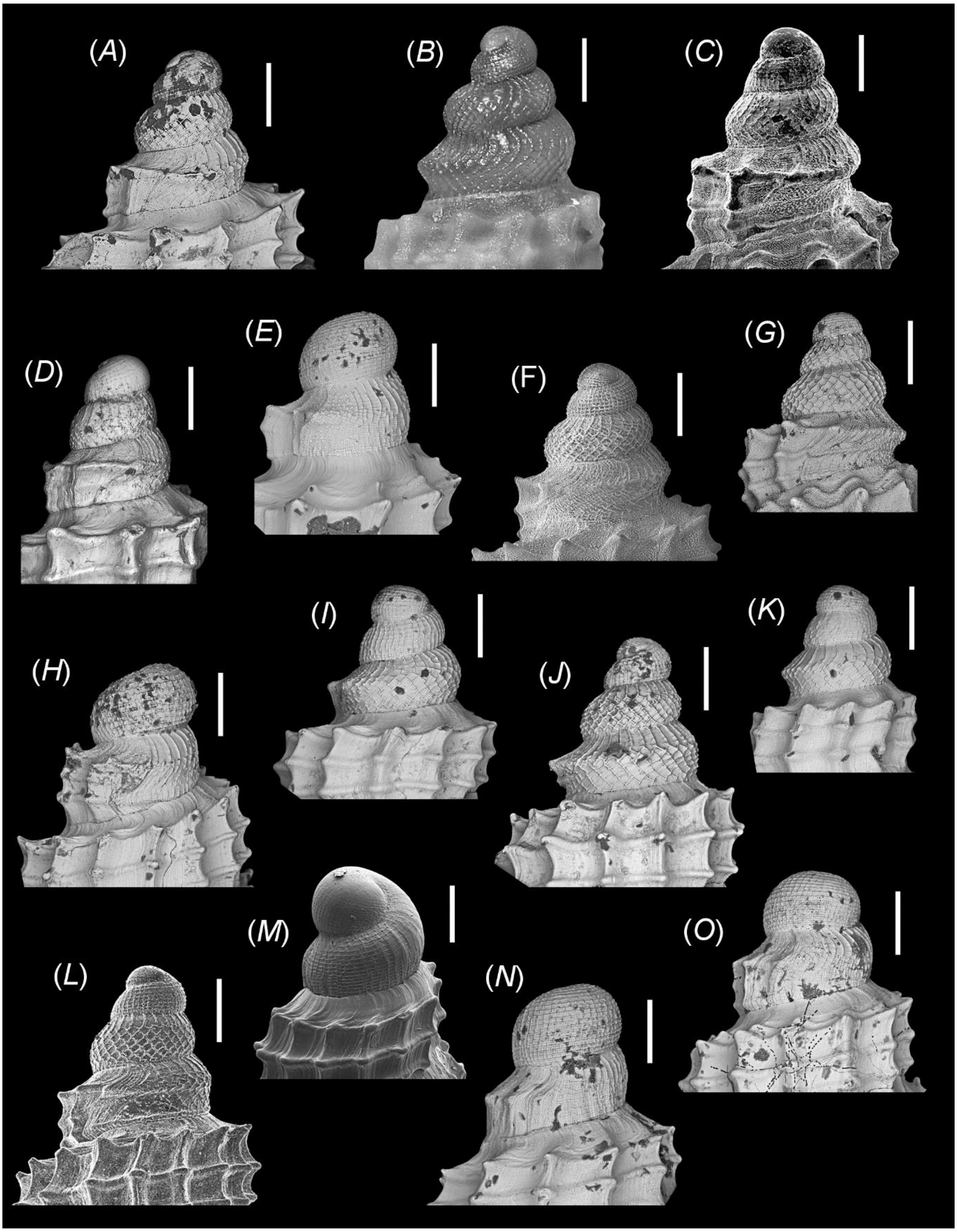


Figure 6. Protoconchs of *Raphitoma* spp. *(A) R.* sp. A, Brač Is. (Croatia). *(B) R. maculosa* Høisater, 2016, Liholmsrennen, Raunefjorden (Norway), holotype ZMBN. *(C) R. purpurea* (Montagu, 1803), Ploubazlanec (France). *(D) R. cordieri* (Payraudeau, 1826), Biograd (Croatia). *(E) R. horrida* (Monterosato, 1884), Agrilidi, Astypalea Is. (Greece), BAU-3045.1. *(F) R. densa* (Monterosato, 1884), Isola delle Femmine, Palermo (Italy). *(G) R.* sp. B, Stari Trogir (Croatia). *(H) R. philberti* (Michaud, 1829), Fetovaia bay, Elba Is. (Italy), BAU-1903.1. *(I) R. locardi*, Pusateri and Giannuzzi-Savelli, 2013, Biograd (Croatia). *(J) R. contigua* (Monterosato, 1884), Dugi Otok Is. (Croatia). *(K) R. laviae* (Philippi, 1836), Sukošan (Croatia), BAU-2243.2. *(L) R. atropurpurpurea* (Locard and Caziot, 1899), Gulf of Napoli (Italy). *(M) R. spadiana* Pusateri and Giannuzzi-Savelli, 2012, Scilla, Reggio Calabria (Italy). *(N) R. bartolinorum* Pusateri and Giannuzzi-Savelli, 2018, Biograd (Croatia), BAU-2245.2. *(O) R. philberti* (Michaud, 1829), Biograd (Croatia), BAU-2365.2. Scale bar: 200 µm.

Chapter III

- Biogeographic analysis of loss of planktotrophy in the mud whelks (Gastropoda, Nassariidae)

Biogeographic analysis of loss of planktotrophy in the mud whelks (Gastropoda, Nassariidae)

Valeria Russini[1], Lee Ann Galindo[2], Roberto Bonanni[3], Giulia Fassio[1], Maria Vittoria Modica[4], Marco Oliverio[1]

[1] Department of Biology and Biotechnology "C. Darwin", Università La Sapienza di Roma;

[2] Institut de Systématique, Evolution, Biodiversité ISYEB – UMR7205 – CNRS, MNHN, UPMC, EPHE, Muséum National d'Histoire Naturelle, Sorbonne Universités Paris;

[3] Via Giuseppe Donati 32, 00159 Rome, Italy;

[4] Department of Integrative Marine Ecology, Stazione Zoologica "Anton Dohrn" di Napoli

Short communication

Introduction

In marine benthic invertebrates the larval development is a key feature for evolution and ecology of species (Cowen & Sponaugle, 2009; Levin, 2006). Marine gastropods have two major type of larval development (Thorson, 1950). In planktotrophic development (P) larvae spend from a few days up to one year in the plankton feeding actively and have a relatively high dispersion ability. The larvae are strictly dependent to the environmental trophic availability. In non-planktotrophic development (NP) larvae spend very little or no time in the plankton feeding almost exclusively on yolk supplies and have small dispersion ability. This kind of larvae are isolated from environmental trophic availability and advantage in areas whit not constant trophic provision (Poulin et al., 2002).

In marine shelled gastropods the larval development is a very variable feature (Collin, 2004; Collin et al., 2007; Duda & Palumbi, 1999; Houart, 2013), nevertheless it reflected on morphology of larval shell (protoconch) and is easy to identify also in extinct species.

In the Caenogastropoda (Gastropoda), non-planktotrophic development is mostly considered as a derived condition that arises in response to environmental conditions that counterselect planktotrophy, allowing independence from trophic availability. Planktotrophy is considered the hypothetical ancestral condition and very difficult to reacquire due to the loss of peculiar feeding structure in larvae (Haszprunar, 1995; Haszprunar et al., 1995; Oliverio, 1996b).

The major aim of this study is to try to understand if an evolutionary pattern can be found in a big group of marine Caenogastropoda like the family Nassariidae Iredale, 1916 (1835). Thanks to the recent complete revision of this family (Galindo et al., 2016) is known that within Nassariidae are described the presence of both types larval development. A fossil records dataset was used as calibration point to date the phylogeny. The known of larval development for each species of the family, allowed to perform evolutionary analysis of larval development and dating in geological time the changes events. Some evolutionary question about this particular issue arose. Since the larval development is supposed to be link to environment, we wanted to verify if the event of loss of planktotrophy (LOP) can be connected to some particular geological event occurred in the past or to some particular biogeographic regions of origin. Furthermore, we want to investigate if a common pattern of evolution is present trough the phylogeny. We formulated two hypotheses to be verified. The first hypothesis, that we named Climate Change Hypothesis (CCH), links the change in larval development with paleogeological events occurred trough geological history. We know that in different ages worldwide environment conditions change and those maybe could favour the non-planktotrophic development instead the planktotrophic one. The second hypothesis not exclusive, mention as Geographic Confinement Hypothesis (GCH), associates the loss of planktotrophy (LOP) with biogeographic regions origins. The non-planktotrophic development could be favoured in delimited geographic area, where a wide dispersal does not necessary to provide high dispersion pattern.

We have calculated the frequency of loss-of-planktotrophy events through the lineages of a robust nassariid phylogeny, testing unequal occurrence across geological epochs (CCH) and biogeographic regions (GCH).

Materials and methods

The dataset was retrieved from the work of Galindo et al., 2016 and contained sequences for 229 samples of which 7 represented outgroup of the family Nassariidae (Table 1). Five molecular markers have been used: two nuclear markers, the 354 bp fragment of histone H3 (with primers H3R1-H3F1: Colgan et al., 1998), and 779 bp of the rDNA 28S (with primers C1'-D2: Chisholm et al., 2001); three mitochondrial markers, the 658 bp barcode fragment of the cytochrome c oxidase subunit I (COI, with primers LCO1490 - HCO 2198: Folmer et al., 1994; or 5COIF -492COIR/492COIRD: Galindo et al., 2016), a 641 bp fragment of the 12S rDNA (with primers 12S I - 12S III: Simon et al., 1991) and a 565 bp fragment of the 16S rDNA (with primers 16SA - 16SB: Palumbi et al., 1991).

(Table 1)

Ancestral state reconstruction and evolution of larval development

The analysis was performed on a calibrated phylogeny using BEAST 1.8 (Drummond et al., 2012) based on a set of twelve calibration points across the tree. We retrieved part of fossil information from the work of Galindo et al. (2016) and six additional calibration points were added to the phylogeny to date the origin of family and refine youngest nodes (Table 2).

The first appearance of the family Nassariidae occurred during the Coniacian stage (Cretaceous) dated at 86.3-89.8 mya (Tracey et al., 1993). In the genus *Tritia* we identified the first appearance of five extant species included in the phylogeny: *Tritia reticulata* appeared in the early Pliocene (5.3-3.6 mya) (Gili & Martinell, 1994); *T. neritea* during the Pleistocene (2.58-0.78 mya) (Gili & Martinell, 1999); *T. pellucida* in the lower Pliocene (5.3-3.6 mya) (Gili & Martinell, 1999); *T. mutabilis* in the middle Pleistocene (1.8-0.12 mya) (Van Dingenen et al., 2015); *T. incrassata* probably in the lower Pliocene (5.3-3.6 mya) (MHNH Collection). Regarding the fossil data retrieved from Galindo et al. (2016), the oldest known Nassariinae is *Buccitriton* from the Ypresian (Eocene, 56-47.8 mya) (Tracey et al., 1993); the first occurrence of *Buccinanops* has also been dated during the Ypresian (56-47.8 MYA) (Allmon, 1990); *Tritiaria* appeared during the Lutetian (41-34 MYA) (MacNeil & Dockery, 1984); *Dorsanum* s.l. is known (*D. gaasensis*) from the early Oligocene (32 MYA) of Europe (Lozouet, 1999); the oldest *Cyllene* species are known from the Chattian (28-23 MYA) and the first appearance of the genus *Tritia* is also dated in this stage (28-23 MYA) (Lozouet, 1999).

Planktotrophic development is the most common strategy in extant Nassariidae but loss of planktotrophy occurred many times in the history of the family. Modern *Buccinanops*, *Engoniophos*, *Bullia*, *Nassaria* and some *Cyllene* have independently acquired a non-planktotrophic mode, while in their fossil record most species are planktotrophic (Allmon, 1990; Woodring, 1928). Planktotrophy was set as ancestral state of the family according to the fossil record and to the assumption that planktotrophy is the ancestral state of caenogastropod lineages (Haszprunar, 1995; Haszprunar et al., 1995; Oliverio, 1996a).

(Table 2)

We have used two methods to investigate the evolution of larval development in the family. In the first method, we have considered the change of larval development as having occurred at the nodes that lead to species with different larval development type. We assigned each node to five time-category (Paleogene and older, Lower Miocene, Upper Miocene, Pliocene, Quaternary) and to four biogeographic-category (Indo-Pacific, Caribbean, Mediterranean and Atlantic, South America) showed in Table 3.

(Table3)

However, the very important assumption, that the events of change in larval development occurred at nodes, may be unrealistic. For this reason, we have also employed a second method for dating these events. We have performed an ancestral state reconstruction (with the package phytools in R: Revell, 2012). This R tool allowed to estimate the distribution of changes modelled by a continuous time Markov chain approach to sample character histories from their posterior probability distribution, called stochastic character mapping (Huelsenbeck et al., 2003) on the dated phylogenetic tree. In this method the branches were split into the five time-category and assigned to the four biogeographic-category when possible. In addition, as in the previous method, test for differences in the frequency of LOP events among the time-category and among biogeographic-categories were performed whit the Fischer exact test (Uitenbroek, 1997).

Results

The calibrated phylogeny (Figure 1A and 1B) set the origin of the family at 86.5 MYA (95% HPD 86.02,88.2) during Coniacian (Upper Cretaceous), and as having probably occurred in the centre of the ancient Atlantic Ocean. The two subfamilies Buccinanopsinae and Cylleninae are estimated to have originated 56 MYA (95% HPD 55,43-58,59) and 55 MYA (95% HPD 40,1-70,46), respectively, during the Ypresian stage (Early Eocene). The subfamily Photinae is estimated to have originated 36.94 MYA during the Priabonian stage in the Upper Eocene (95% HPD 34.41-41.05), whereas the origin of the large subfamily Nassariinae is dated at 48.7 MYA (95% HPD 49,94-62,35) during the Ypresian stage (Early Eocene). The five genera of the subfamily Nassariinae, are all estimated to have originated in the late Paleogene (23-37.8): *Naytia, Reticunassa*, *Phrontis* and *Nassarius* during the Rupelian stage (Oligocene), and the genus *Tritia* in the Chattian stage (Oligocene). Within the genus *Nassarius,* the major diversification occurred rapidly during the Miocene (23-5.3), as also suggested by paleontological data (Haasl, 2000; Lozouet and Galindo, 2015).

(Figure 1A, 1B)

With the first methods of dating the events of LOP (events constrained at the relevant nodes), we have considered only the nodes with posterior probability higher than 0.7. The analyses showed that there was no significant different in the frequency of events across the different epochs (Fischer exact test, p-value>0.05). Conversely, the frequency varied significantly across the different biogeographic categories (Fischer exact test, p-value= 0.006), with the highest frequency in the South Atlantic (33.3%), then the Caribbean area (26.3%), and the Mediterranean-Atlantic area (24%).

The second method used showed similar results. The ancestral state reconstruction analysis (Figure 2A and 2B) pointed out that 28 events of LOP, within 13 genera. As the previous analysis, the frequency of events varied significantly between biogeographic categories (Fischer exact test, p-value= 0.00417), and the highest relative frequency occurred in the South America (28.6%), then Mediterranean-Atlantic area (11.9%) and Caribbean area (11.8%). No significant variation of frequency was detected among different epochs category (Fischer exact test, p-value>0.05).

(Figure 2A and 2B)

Discussion

The new dating of the phylogenetic framework of the Nassariidae showed some differences from the previous estimates (Galindo et al., 2016), in particular concerning the range of HPD. The origin of the family has been estimated at 86.5 MYA (95% HPD 86–88.2), more recent than the previous 120 MYA (95% HPD 80.3–140.4) estimate. Other remarkable differences were the origin of the subfamily Photinae estimated at 36.94 MYA (95% HPD 34.5–41.5) v. the previous dating at 70.2 MYA with a large range (46.1–92.33), and of the subfamily Cylleninae estimated at 54.9 MYA (95% HPD 40.7–71.1) v. the previous dating at 93 MYA (65–120).

Both methods used to set in a temporal framework the evolution of larval development, showed similar results, addressing to significant biogeographical variation in the frequency of LOP events. The differences in LOP frequency between epochs, instead, were not statistically significant. The ancestral state reconstruction analysis yielded a more accurate dating of events of LOP due to the computation of posterior probability of each event along the branches. Although there was not a statistically significant difference among epochs, a remarkable concentration of LOP events occurred during the Miocene and the Pleistocene, with 32.14% and 42.85% of the total events, respectively. During the Miocene the drop of the average bottom water temperature by 4°C to 6°C, and the closure of three important oceanic gateways severely affected the circulation of deep water in the global ocean and global climate (Potter & Szatmari, 2009). The closure of the Isthmus of Panama in the Caribbean area (Coates & Obando, 1996; Duque-Caro, 1990), that began 13 MYA and was completed 3.5 MYA in the Pliocene, disrupted the global equatorial flow and initiated the inception of the Gulf Stream as we know it today. Restriction of the Indonesian Gateway between Borneo and New Guinea connecting the Pacific to the Indian Ocean, began in the latest Oligocene 25 MYA, hampering until block the deep flow by the late Early Miocene (Kuhnt et al., 2004). The third key closure concerned the Tethys Sea and the formation of Mediterranean region: in the Middle Miocene the connection between the Atlantic-Mediterranean area and the Indian Ocean became intermittent, and a final closure occurred in the early Late Miocene about 10–11 MYA (Rögl, 1999); at the very end of the Miocene (Hsü, 1983; Krijgsman et al., 1999), the Messinian salinity crisis was caused by the closure of the connection with the Atlantic, the Mediterranean Sea becoming an evaporation basin (with evaporites accumulating on the bottom and marginal canyons both on and offshore).

During the Pleistocene the Earth's climate was strongly influenced by more than 11 major glacial cycles, along with several minor glacial events (Richmond & Fullerton, 1986). The statistical analyses showed that in the Caribbean, the South America, and the Mediterranean-Atlantic units, shifts in larval development (P→NP) occurred more frequently compared to the Indo Pacific. These areas were undergoing major oceanographic events that may have influenced the marine biota. In the geologically very instable Caribbean region, beside the closure of the Isthmus of Panama, three main species extinctions (Middle to Late Eocene, Late Oligocene to Early Miocene, and Plio-Pleistocene) (Budd, 2000) coincided with large-scale environmental perturbations. During the Early Miocene, increased upwelling and associated turbidity and cooling have been inferred for the Caribbean (Edinger & Risk, 1994, 1995). During the Late Pliocene, drops in sea surface temperature associated with the onset of the northern hemisphere glaciation cycles affected many marine organisms (including molluscs and bryozoans) in the Caribbean (Jackson et al., 1993, 1996; Jackson & Budd, 1996; Stanley, 1986). Regarding the South America area, it has been strictly correlated with the Antarctica region, that could influence the composition of biota.

The results of this work show that the frequencies of LOP event significantly vary between biogeographic regions. As mentioned, geological history from Neogene of these biogeographic regions suggest that these areas might have undergone events of instability promoting geographic confinement of species and strengthens our second hypothesis (GCH). Semi-closed or closed basins like the Caribbean area and the Mediterranean Sea have probably been areas where the loss of planktotrophy has been particularly promoted. Even if no statistically evidence has been detected, we do not exclude that different geological epochs could influence the larval development strategy, however the role still remains unclear.

Aknowledgment

We thank Prof. Gabriele Gentile (Univesity of Tor Vergata) and Prof. Giorgio Bertorelle (University of Ferrara) for providing fundamental advice and criticism. Work partly supported by Regione Lazio through "Torno Subito" programme (0031101/15, to VR).

References

Allmon, W. (1990). Review of the Bullia Group (Gastropoda: Nassariidae) with comments on its evolution, biogeography, and phylogeny. *Paleontological Research Institution*, ***99***, 1–179.

Budd, A. F. (2000). Diversity and extinction in the Cenozoic history of Caribbean reefs. *Coral Reefs*, ***19*(1)**, 25–35. https://doi.org/10.1007/s003380050222

Chisholm, L. A., Morgan, J. A. T., Adlard, R. D., & Whittington, I. D. (2001). Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. *International Journal for Parasitology*, ***31*(11)**, 1253–1263. https://doi.org/10.1016/S0020-7519(01)00223-5

Coates, A., & Obando, J. (1996). The geologic evolution of the Central American Isthmus. *Evolution and Environment in Tropical America*, ***21*(56)**.

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., et al. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, ***46*(5)**, 419. https://doi.org/10.1071/ZO98048

Collin, R. (2004). Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. *Evolution*, ***58*(7)**, 1488–1502.

Collin, R., Chaparro, O. R., Winkler, F., & Véliz, D. (2007). Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. *Biological Bulletin*, ***212*(2)**, 83–92.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, ***1*(1)**, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Van Dingenen, F., Ceulemans, L., Landau, B. M., & Marques da Silva, C. (2015). The family Nassariidae (Gastropoda : Buccinoidea) from the late Neogene of northwestern France. *Cainozoic Research*, ***15*(1–2)**, 75–122.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, ***29*(8)**, 1969–1973. https://doi.org/10.1093/molbev/mss075

Duda, T. F., & Palumbi, S. R. (1999). Developmental shifts and species selection in gastropods. *Proceedings of the National Academy of Sciences*, ***96*(18)**, 10272–10277. https://doi.org/10.1073/pnas.96.18.10272

Duque-Caro, H. (1990). Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama seaway. *Palaeogeography, Palaeoclimatology, Palaeoecology*, ***77*(3–4)**, 203–234. https://doi.org/10.1016/0031-0182(90)90178-A

Edinger, E. N., & Risk, M. J. (1994). Oligocene-Miocene Extinction and Geographic Restriction of Caribbean Corals: Roles of Turbidity, Temperature, and Nutrients. *PALAIOS*, ***9*(6)**, 576. https://doi.org/10.2307/3515129

Edinger, E. N., & Risk, M. J. (1995). Preferential survivorship of brooding corals in a regional extinction. *Paleobiology*, ***21*(2)**, 200–219. https://doi.org/10.1017/S0094837300013208

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.

Molecular Marine Biology and Biotechnology, ***3*(5)**, 294–299. https://doi.org/10.1371/journal.pone.0013102

Galindo, L. A., Puillandre, N., Utge, J., Lozouet, P., & Bouchet, P. (2016). The phylogeny and systematics of the Nassariidae revisited (Gastropoda, Buccinoidea). *Molecular Phylogenetics and Evolution*, ***99*(June)**, 337–353. https://doi.org/10.1016/j.ympev.2016.03.019

Gili, C., & Martinell, J. (1994). Relationship between species longevity and larval ecology in nassariid gastropods. *Lethaia*, ***27***, 291–299.

Gili, C., & Martinell, J. (1999). Revision del genero Cyclope Risso, 1826 (Gasteropoda:Nassariidae) en el Plioceno Mediterraneo. *Revista Espanola de Paleontologia*, ***14*(1)**, 147–156.

Haszprunar, G. (1995). On the evolution of larval development in the Gastropoda, with special reference to larval planktotrophy. *Notiz. CISMA*, ***16***, 5–13.

Haszprunar, G., Salvini-Plawen, L. v., & Rieger, R. M. (1995). Larval Planktotrophy-A Primitive Trait in the Bilateria? *Acta Zoologica*, ***76*(2)**, 141–154. https://doi.org/10.1111/j.1463-6395.1995.tb00988.x

Houart, R. (2013). Description of two new Chicomurex species (Gastropoda: Muricidae) from the Philippine Islands novapex. *Novapex*, ***14*(3)**, 69–75.

Hsü, K. (1983). The Mediterranean was a desert: A voyage of the Glomar Challenger. Princeton University Press, Princeton.

Huelsenbeck, J. P., Nielsen, R., & Bollback, J. P. (2003). Stochastic Mapping of Morphological Characters. *Systematic Biology*, ***52*(2)**, 131–158. https://doi.org/10.1080/10635150390192780

Jackson, J., Budd, A., & Pandolfi, J. (1996). The shifting balance of natural communities. *Evolutionary Paleobiology.*, 89–122.

Jackson, J. B. C., & Budd, A. F. (1996). Evolution and environment: introduction and overview. In *Evolution & Environment in Tropical America* (pp. 1–20). University of Chicago Press.

Jackson, J. B. C., Jung, P., Coates, A. G., & Collins, L. S. (1993). Diversity and Extinction of Tropical American Mollusks and Emergence of the Isthmus of Panama. *Science* , ***260*(5114)**. https://doi.org/10.1126/science.217.4557.350

Krijgsman, W., Hilgen, F. J., Raffi, I., Sierro, F. J., & Wilson, D. S. (1999). Chronology, causes and progression of the Messinian salinity crisis. *Nature*, ***400***, 652–655. https://doi.org/10.1038/23231

Kuhnt, W., Holbourn, A., Hall, R., Zuvela, M., & Käse, R. (2004). Neogene History of the Indonesian Throughflow. *Geophysical Monograph Series*, ***149***, 299–320. https://doi.org/10.1029/149GM16

Levin, L. A. (2006). Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, ***46*(3)**, 282–297. https://doi.org/10.1093/icb/icj024

Lozouet, P. (1999). Nouvelles espèces de gastéropodes (Mollusca : Gastropoda) de l ' Oligocène et du Miocène inférieur de l ' Aquitaine (Sud-Ouest de la France). Partie 2. *Cossmanniana*, ***6*(1–2)**, 1–68.

MacNeil, F. S., & Dockery, D. T. (1984). Lower Oligocene Gastropoda, Scaphopoda, and

Cephalopoda of the Vicksburg group in Mississippi. Mississippi Department of Natural Resources, Bureau of Geology.

Oliverio, M. (1996a). Contrasting developmental strategies and speciation in NE Atlantic prosobranchs: a preliminary analysis. *Origin and Evolutionary Radiation of the Mollusca*, ***22***, 261–266.

Oliverio, M. (1996b). Life-histories, speciation, and biodiversity in Mediterranean prosobranch gastropods. *Vie et Milieu*, ***46*****(2)**, 163–169.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). A Simple Fool's Guide to PCR. *University of Hawaii Press: Honolulu.*

Potter, P. E., & Szatmari, P. (2009). Global Miocene tectonics and the modern world. *Earth-Science Reviews*, ***96*****(4)**, 279–295. https://doi.org/10.1016/j.earscirev.2009.07.003

Poulin, E., Palma, A. T., & Féral, J. P. (2002). Evolutionary versus ecological success in Antarctic benthic invertebrates. *Trends in Ecology and Evolution*, ***17*****(5)**, 218–222. https://doi.org/10.1016/S0169-5347(02)02493-X

Revell, L. J. (2012). phytools : an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, ***3***, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Richmond, G. M., & Fullerton, D. S. (1986). Summation of quaternary glaciations in the United States of America. *Quaternary Science Reviews*, ***5***, 183–196. https://doi.org/10.1016/0277-3791(86)90184-8

Rögl, F. (1999). Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). *Geologica Carpathica*, ***50*****(4)**, 339–349.

Simon, C., Franke, A., & Martin, A. (1991). The Polymerase Chain Reaction: DNA Extraction and Amplification. In *Molecular Techniques in Taxonomy* (pp. 329–355). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-83962-7_22

Stanley, S. M. (1986). Anatomy of a Regional Mass Extinction: Plio-Pleistocene Decimation of the Western Atlantic Bivalve Fauna. *PALAIOS*, ***1*****(1)**, 17–36. https://doi.org/10.2307/3514456

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, ***25*****(1)**, 1–45. https://doi.org/10.1111/j.1469-185X.1950.tb00585.x

Tracey, S., Todd, J. A., & Erwin, D. (1993). Mollusca: Gastropoda. *Fossil Record 2*, 131–167.

Uitenbroek, D. G. (1997). SISA-Binomial. Retrieved from https://www.quantitativeskills.com/sisa/distributions/binomial.htm.

Woodring, W. (1928). Miocene mollusks from Bowden, Jamaica. Part II. Gastropods and discussion of results, Contributions to the Geology and Paleontology of the West Indies. Carnegie Institution of Washington, Washington.

Table and Figure

Table 1. List of the molecular samples with vouchers registration numbers, protoconch type (M: multispiral; P: paucispiral), collection localities, GenBank accession numbers for sequences.

Genus	***Species***	**Country**	**development**	**ID**	**Number**	**COI**	**16S**	**12S**	**28S**	**H3**
Amiantofusus	*sp*			MNHN	IM-2007-34648	JQ950210	JQ950144.2		JQ950166	
Anentome	*helena*	Vietnam		MNHN	IM-2009-29658	KY451412	KY488922	KY489121	KY489289	KY489374
Antillophos	*beauii*	Guadeloupe		MNHN	IM-2013-8364	KY451406	KY488916			KY489371
Antillophos	*candeanus*	Guadeloupe		MNHN	IM-2013-8450	KY451407	KY488917		KY489286	KY489372
Antillophos	*chazaliei*	Guadeloupe		MNHN	IM-2013-20358	KY488915		KY489116	KY489285	KY489370
Buccinanops	*cochlidium*	Brazil		MZUSP	80628	KY451219				KY489293
Buccinanops	*globulosus*	Argentina		MNHN	IM-2009-24004	KY451220	KY488730	KY488927	KY489125	KY489294
Buccinanops	*gradatus*	Brazil		MZUSP	108269	KY451221	KY488731	KY488928	KY489126	KY489295
Buccinanops	*monilifer*	Argentina		MZUSP	28084	KY451222			KY489127	KY489296
Buccinum	*undatum*					FN677402	FN677455	FN677400	FN677456	
Bullia	*cataphracta*	Mozambique		MNHN	IM-2009-22716	KY451223	KY488732	KY488929		KY489297
Bullia	*diluta*	Mozambique		MNHN	IM-2009-22535	KY451224	KY488733	KY488930		KY489298
Bullia	*natalensis*	Mozambique		MNHN	IM-2009-22718	KY451226	KY488735	KY488932		
Bullia	*perlucida*	Madagascar		MNHN	IM-2009-12299	KY451227	KY488736	KY488933		KY489300
Bullia	*sp. 607*	Madagascar		MNHN	IM-2009-12887	KY451228	KY488737	KY488934		KY489301
Bullia	*sp. 608*	Mozambique		MNHN	IM-2009-22679	KY451229	KY488738	KY488935		KY489302
Bullia	*sp. 611*	Madagascar		MNHN	IM-2009-12884	KY451230	KY488739	KY488936		KY489303
Bullia	*sp. 612*	Madagascar		MNHN	IM-2009-12886	KY451231		KY488937		KY489304
Bullia	*sp. 613*	Madagascar		MNHN	IM-2009-12877	KY451232	KY488740	KY488938		KY489305
Cyllene	*lamarcki*	Republic of the Congo		MNHN	IM-2009-23725	KY451235			KY489130	KY489307
Cyllene	*owenii*	Republic of the Congo		MNHN	IM-2009-23727	KY451236		KY488941	KY489131	KY489308
Cyllene	*parvula*	Madagascar		MNHN	IM-2009-12765	KY451237	KY488742	KY488942	KY489132	KY489309
Cyllene	*pulchella*	Vanuatu		MNHN	IM-2007-31755	KY451238	KY488743	KY488943	KY489133	KY489310
Dorsanum	*miran*	Mauritania		MNHN	IM-2013-52428	KY451239	KY488744	KY488944	KY489134	KY489311
Dorsanum	*miran*	Mauritania		MNHN	IM-2013-52431	KY489461			KY489489	
Engina	*fusiformis*			MNHN	IM-2007-32845	JQ950200	JQ950141		JQ950156	
Engoniophos	*unicinctus*	Guadeloupe		MNHN	IM-2009-24414	KY451413	KY488923	KY489122		KY489375
Fusinus	*colus*			LSGB	2341301	HQ834100	HQ833955	HQ833907		HQ834178
Mitrella	*bicincta*			LSGB	231022	JN052989	JN052928			
Nassaria	*magnifica*	Japan				FJ712703	AB044264		FJ710100	
Nassaria	*sp*	New Caledonia		MNHN	IM-2007-17856	KY451415			KY489291	KY489377
Nassaria	*sp*	Papua New Guinea		MNHN	IM-2009-13155	KY451414	KY488924	KY489123	KY489290	KY489376
Nassarius	*acuminatus*	Papua New Guinea		MNHN	IM-2009-13116	KY451253	KY488759	KY488961	KY489149	
Nassarius	*acuticostus*	Vanuatu		MNHN	IM-2007-31698	KY451254	KY488760	KY488962		
Nassarius	*agapetus*	Vanuatu		MNHN	IM-2007-31774	KY451255	KY488761	KY488963	KY489150	
Nassarius	*alfuricus*	Philippines		MNHN	IM-2007-35602		KY488764	KY488966	KY489153	
Nassarius	*arcularia*	Philippines		MNHN	IM-2007-31898	KY451259	KY488766	KY488968	KY489155	KY489317
Nassarius	*arcus*	New Caledonia		MNHN	IM-2007-36798	KY451260	KY488767	KY488969	KY489156	
Nassarius	*babylonicus*	Papua New Guinea		MNHN	IM-2009-22686	KY451261	KY488768	KY488970	KY489157	KY489318
Nassarius	*barsdelli*	New Caledonia		MNHN	IM-2007-34770	KY451262	KY488769	KY488971	KY489158	
Nassarius	*bellulus*	Philippines		MNHN	IM-2007-31903	KY451263	KY488770	KY488972	KY489159	
Nassarius	*bicallosus auct*	Madagascar		MNHN	IM-2007-36697	KY451264	KY488771	KY488973	KY489160	
Nassarius	*bimaculosus*	Philippines		MNHN	IM-2007-31867	KY451265		KY488974		KY489319
Nassarius	*boucheti*	New Caledonia		MNHN	IM-2009-21554	KY451266	KY488772	KY488975	KY489161	
Nassarius	*callospira*	Vanuatu		MNHN	IM-2007-31770	KY451267	KY488774	KY488977		
Nassarius	*camelus*	Philippines		MNHN	IM-2007-31934	KY451268	KY488775	KY488978	KY489163	
Nassarius	*cf. comptus*	Vietnam		MNHN	IM-2009-29669	KY451271		KY488981	KY489166	KY489320
Nassarius	*cf. crematus*	Philippines		MNHN	IM-2007-34484	KY451272	KY488778	KY488982	KY489167	

Nassarius	*cf. dijki*	Papua New Guinea	MNHN	IM-2009-13177	KY451273	KY488779	KY488983	KY489168	
Nassarius	*cf. hansenae*	Philippines	MNHN	IM-2007-31931	KY451274	KY488780	KY488984		
Nassarius	*cf. noguchii*	Papua New Guinea	MNHN	IM-2009-13170	KY451276	KY488782	KY488986	KY489170	KY489321
Nassarius	*cf. pumillio*	Senegal	MNHN	IM-2009-12313	KY451278	KY488784			
Nassarius	*cinctellus*	Vanuatu	MNHN	IM-2007-31764	KY451279	KY488785	KY488988	KY489172	
Nassarius	*cinnamomea*	French Polynesia	MNHN	IM-2009-21733	KY451280	KY488786	KY488989	KY489173	
Nassarius	*concinnus*	Philippines	MNHN	IM-2007-31852	KY451283	KY488789		KY489175	
Nassarius	*conoidalis*	Vietnam	MNHN	IM-2009-29668	KY451284	KY488790	KY488992	KY489176	
Nassarius	*coronatus*	Mozambique	MNHN	IM-2009-22308	KY489176	KY488793	KY488995	KY489178	
Nassarius	*crematus*	Vanuatu	MNHN	IM-2007-31702	KY451288	KY488794	KY488996	KY489179	
Nassarius	*crenoliratus*	Vanuatu	MNHN	IM-2007-31668	KY451289	KY488795	KY488997		
Nassarius	*dijki*	Papua New Guinea	MNHN	IM-2009-13145	KY451293	KY488799	KY489001	KY489182	
Nassarius	*disparilis*	Philippines	MNHN	IM-2007-31886	KY451294			KY489183	KY489325
Nassarius	*distortus*	Vanuatu	MNHN	IM-2009-20637	KY451295	KY488800	KY489002	KY489184	
Nassarius	*dorsuosus*	Philippines	MNHN	IM-2007-31890	KY451296	KY488801	KY489003		
Nassarius	*ecstilbus*	Vanuatu	MNHN	IM-2007-31751	KY451297	KY488802	KY489004		
Nassarius	*euglyptus*	Solomon Islands	MNHN	IM-2007-32393	KY451299	KY488804	KY489006		KY489327
Nassarius	*eximius*	Philippines	MNHN	IM-2007-31944	KY451300	KY488805	KY489007		KY489328
Nassarius	*fenistratus*	Madagascar	MNHN	IM-2009-12791	KY451301	KY488806	KY489008	KY489186	
Nassarius	*filosus*	Madagascar	MNHN	IM-2009-12324	KY451302	KY488807	KY489009	KY489187	
Nassarius	*fraudulentus*	French Polynesia	MNHN	IM-2007-39380	KY451303	KY488808	KY489010	KY489188	KY489329
Nassarius	*fretorum*	Philippines	MNHN	IM-2007-31936	KY451304	KY488809	KY489011		
Nassarius	*gaudiosus*	French Polynesia	MNHN	IM-2009-21719	KY451305	KY488810	KY489012	KY489189	KY489330
Nassarius	*gibbosuloideus*	Vanuatu	MNHN	IM-2007-31678	KY451306	KY488811	KY489013	KY489190	KY489331
Nassarius	*glans*	Madagascar	MNHN	IM-2009-12809	KY451308	KY488813	KY489015	KY489192	
Nassarius	*globosus*	Vanuatu	MNHN	IM-2007-31703	KY451309	KY488814	KY489016		
Nassarius	*graniferus*	Vanuatu	MNHN	IM-2007-31676	KY451311	KY488816	KY489018	KY489194	
Nassarius	*haldemani*	Vanuatu	MNHN	IM-2007-31662	KY451313	KY488818	KY489020	KY489196	
Nassarius	*hepaticus*	China	LSGB	2340302	HQ834075	HQ833945	HQ833897		HQ834168
Nassarius	*herosae*	French Polynesia	MNHN	IM-2007-39259	KY451314	KY488819	KY489021	KY489197	KY489334
Nassarius	*horridus*	Madagascar	MNHN	IM-2009-12852	KY451240	KY488745	KY488945	KY489135	KY489312
Nassarius	*houbricki*	Solomon Islands	MNHN	IM-2007-36143		KY488822	KY489024	KY489200	
Nassarius	*idyllius*	Philippines	MNHN	IM-2007-31927	KY451345	KY488852	KY489053	KY489228	KY489347
Nassarius	*interliratus*	Philippines	MNHN	IM-2007-31925	KY451316	KY488824	KY489026		
Nassarius	*irus*	Madagascar	MNHN	IM-2009-12797	KY451317		KY489027	KY489202	KY489336
Nassarius	*javanus*	Philippines	MNHN	IM-2007-31862	KY451318	KY488825	KY489028	KY489203	
Nassarius	*kooli*	Philippines	MNHN	IM-2007-31661	KY451321	KY488827	KY489030	KY489206	
Nassarius	*kraussianus*	Madagascar	MNHN	IM-2009-12883	KY451322	KY488828	KY489031	KY489207	
Nassarius	*labordei*	Mozambique	MNHN	IM-2009-22325	KY451323	KY488829	KY489032	KY489208	KY489337
Nassarius	*leptospirus*	Philippines	MNHN	IM-2007-31868	KY451324	KY488830		KY489209	
Nassarius	*limnaeiformis*	New Caledonia	MNHN	IM-201016549	KY451325	KY488831	KY489033		
Nassarius	*lochi*	Vanuatu	MNHN	IM-2007-31728	KY451326	KY488832	KY489034	KY489210	
Nassarius	*luridus*	Vanuatu	MNHN	IM-2007-31716	KY451327	KY488833	KY489035	KY489211	
Nassarius	*margaritifer*	Philippines	MNHN	IM-2007-31858	KY451329	KY488835	KY489037	KY489213	
Nassarius	*martensi*	Madagascar	MNHN	IM-2007-38227		KY488836	KY489038	KY489214	
Nassarius	*martinezi*	New Caledonia	MNHN	IM-2007-34768		KY488837	KY489039	KY489215	
Nassarius	*moolenbeeki*	Vanuatu	MNHN	IM-2007-31680	KY451332	KY488839	KY489041	KY489217	KY489339
Nassarius	*multicostatus*	Vanuatu	MNHN	IM-2007-31710	KY451333	KY488840	KY489042	KY489218	
Nassarius	*multipunctatus*	Mozambique	MNHN	IM-2009-7418	KY451334	KY488841	KY489043		
Nassarius	*nigrus*	Vanuatu	MNHN	IM-2007-31730	KY451241	KY488746	KY488946	KY489136	KY489313
Nassarius	*noguchii*	Philippines	MNHN	IM-2007-31664	KY451338	KY488845	KY489047	KY489221	KY489343
Nassarius	*novaezelandiae*	Vanuatu	MNHN	IM-2007-30294	KY451339	KY488846	KY489048	KY489222	KY489344
Nassarius	*obvelatus*	Mozambique	MNHN	IM-2009-22307		KY488847	KY489049	KY489223	KY489345
Nassarius	*ocellatus*	Philippines	MNHN	IM-2007-31906	KY451341	KY488848	KY489050	KY489224	
Nassarius	*olivaceus*	Philippines	MNHN	IM-2007-31897	KY451342	KY488849	KY489051	KY489225	KY489346
Nassarius	*olomea*	New Caledonia	MNHN	IM-2009-20620	KY451343	KY488850	KY489052	KY489226	

Nassarius	*oneratus*	Mozambique	MNHN	IM-2009-22704	KY451344	KY488851		KY489227	
Nassarius	*papillosus*	Mozambique	MNHN	IM-2009-22320	KY451347	KY488854	KY489055	KY489230	KY489348
Nassarius	*pauperatus*	Australia	MNHN	IM-2009-22739		KY488855	KY489056	KY489231	
Nassarius	*poupini*	French Polynesia	MNHN	IM-2007-38554	KY451351	KY488859	KY489060	KY489235	KY489351
Nassarius	*pullus*	Philippines	MNHN	IM-2007-41498	KY451353	KY488861	KY489062	KY489237	
Nassarius	*pyrrhus*	Australia	MNHN	IM-2009-23718		KY488863	KY489064	KY489239	
Nassarius	*radians*	Vanuatu	MNHN	IM-2007-31729		KY488864	KY489065	KY489240	KY489353
Nassarius	*reeveanus*	Philippines	MNHN	IM-2007-31895	KY451355		KY489066	KY489241	
Nassarius	*rufus*	Saudi Arabia	MNHN	IM-2009-24002	KY451358	KY488867	KY489069	KY489244	
Nassarius	*samiae*	Philippines	MNHN	IM-2007-31663	KY451359	KY488868	KY489070	KY489245	
Nassarius	*semisulcatus*	Philippines	MNHN	IM-2007-31891	KY451360	KY488869	KY489071		KY489355
Nassarius	*sinusigerus*	Philippines	MNHN	IM-2007-31916	KY451363	KY488873	KY489074	KY489248	
Nassarius	*siquijorensis*	China	LSGB	23404	HQ834076	HQ833946	HQ833898		HQ834169
Nassarius	*smithii*	Philippines	MNHN	IM-2007-31885		KY488874	KY489075		
Nassarius	*sp. 13*	Papua New Guinea	MNHN	IM-2009-13103	KY451366	KY488876	KY489077	KY489250	KY489358
Nassarius	*sp. 205*	New Caledonia	MNHN	IM-2009-24001	KY451367	KY488877	KY489078	KY489251	
Nassarius	*sp. 213*	New Caledonia	MNHN	IM-2007-36788	KY451368	KY488878	KY489079	KY489252	KY489359
Nassarius	*sp. 221*	New Caledonia	MNHN	IM-2009-21556	KY451369	KY488879	KY489080	KY489253	
Nassarius	*sp. 268*	Philippines	MNHN	IM-2007-31857	KY451370	KY488880	KY489081	KY489254	
Nassarius	*sp. 279*	Vanuatu	MNHN	IM-2007-31682	KY451371	KY488881	KY489082	KY489255	KY489360
Nassarius	*sp. 301*	Madagascar	MNHN	IM-2009-12330	KY451372		KY489083	KY489256	
Nassarius	*sp. 303*	Madagascar	MNHN	IM-2007-38011	KY451374	KY488883	KY489085	KY489258	KY489362
Nassarius	*sp. 307*	New Caledonia	MNHN	IM-2009-20628	KY451375	KY488884	KY489086	KY489259	KY489363
Nassarius	*sp. 404*	Philippines	MNHN	IM-2007-31894	KY451376	KY488885	KY489087	KY489260	
Nassarius	*sp. 405*	Philippines	MNHN	IM-2009-12240	KY451377	KY488886	KY489088	KY489261	
Nassarius	*sp. 408*	Philippines	MNHN	IM-2007-35600	KY451378	KY488887	KY489089	KY489262	
Nassarius	*sp. 418*	Guadeloupe	MNHN	IM-2009-24470	KY451379	KY488888	KY489090	KY489263	
Nassarius	*sp. 427*	Guadeloupe	MNHN	IM-2009-24284	KY451380	KY488889	KY489091	KY489264	
Nassarius	*sp. 502*	Philippines	MNHN	IM-2007-31939	KY451382	KY488891	KY489093		
Nassarius	*sp. 61*	Madagascar	MNHN	IM-2007-36666	KY451383	KY488892	KY489094	KY489266	
Nassarius	*sp. 64*	Papua New Guinea	MNHN	IM-2009-13092	KY451384	KY488893	KY489095	KY489267	KY489364
Nassarius	*sp. 655*	New Caledonia	MNHN	IM-2009-22544	KY451385	KY488894	KY489096		
Nassarius	*sp. 733*	Guadeloupe	MNHN	IM-2009-24306	KY451386	KY488895	KY489097	KY489268	
Nassarius	*sp. 784*	New Caledonia	MNHN	IM-2007-34769	KY451387	KY488896	KY489098	KY489269	
Nassarius	*sp. 918*	Guadeloupe	MNHN	IM-2009-24462	KY451388	KY488897		KY489270	
Nassarius	*sp. A10*	Philippines	MNHN	IM-2007-34474	KY451389	KY488898	KY489099		
Nassarius	*sp. A9*	New Caledonia	MNHN	IM-2007-32388	KY451390	KY488899	KY489100	KY489271	KY489365
Nassarius	*sp. FP5622*	French Polynesia	MNHN	IM-2009-21750	KY451391	KY488900	KY489101	KY489272	
Nassarius	*sp*	Vanuatu	MNHN	IM-2007-31724	KY451242		KY488947	KY489137	
Nassarius	*sp*	Philippines	MNHN	IM-2007-31902	KY451245	KY488749	KY488950	KY489140	
Nassarius	*sp*	Philippines	MNHN	IM-2007-31941	KY451246		KY488951	KY489141	
Nassarius	*sp*	Philippines	MNHN	IM-2007-35267	KY451247	KY488750	KY488952		
Nassarius	*sp*	Philippines	MNHN	IM-2007-35597	KY451248	KY488751	KY488953	KY489142	
Nassarius	*sp*	Philippines	MNHN	IM-2007-35738	KY451249	KY488752	KY488954		
Nassarius	*sp*	Philippines	MNHN	IM-2009-12246	KY451250	KY488753	KY488955	KY489143	
Nassarius	*sp*	Papua New Guinea	MNHN	IM-2009-13129		KY488754	KY488956	KY489144	
Nassarius	*sp*	French Polynesia	MNHN	IM-2009-23705		KY488755	KY488957	KY489145	
Nassarius	*sp*	New Caledonia	MNHN	IM-2009-23990	KY451252	KY488757	KY488959	KY489147	
Nassarius	*splendidulus*	Vanuatu	MNHN	IM-2007-33019	KY451365	KY488875	KY489076	KY489249	
Nassarius	*stigmarius*	Mozambique	MNHN	IM-2009-22672	KY451392	KY488901	KY489102	KY489273	
Nassarius	*subspinosus*	Vanuatu	MNHN	IM-2007-31677	KY451243	KY488747	KY488948	KY489138	KY489314
Nassarius	*subtranslucidus*	Mozambique	MNHN	IM-2009-22673	KY451393	KY488902	KY489103	KY489274	
Nassarius	*succinctus*	China	LSGB	2340501	HQ834078	HQ833948	HQ833900		HQ834171
Nassarius	*tabescens*	Vanuatu	MNHN	IM-2007-31712	KY451394	KY488903	KY489104	KY489275	

Nassarius	*thachi*	Philippines	MNHN	IM-2007-34482	KY451395	KY488904	KY489105	KY489276	
Nassarius	*vanpeli*	New Caledonia	MNHN	IM-2009-20606	KY451399	KY488907	KY489108	KY489279	
Nassarius	*vanuatuensis*	Vanuatu	MNHN	IM-2007-31786		KY488908	KY489109	KY489280	
Nassarius	*venustus*	Philippines	MNHN	IM-2007-31865	KY451400	KY488909	KY489110		
Nassarius	*vitiensis*	Madagascar	MNHN	IM-2007-36838	KY451403	KY488912	KY489113	KY489282	
Nassodonta	*dorri*	Vietnam	MNHN	IM-2009-20649	KY451404	KY488913	KY489114	KY489283	KY489369
Naytia	*glabrata*	Republic of the Congo	MNHN	IM-2009-23946	KY451307	KY488812	KY489014	KY489191	KY489332
Naytia	*granulosa*	Republic of the Congo	MNHN	IM-2009-23948	KY451225	KY488734	KY488931	KY489128	KY489299
Naytia	*johni*	Morocco	MNHN	IM-2009-22574	KY451319		KY489029	KY489204	
Naytia	*priscardi*	Madagascar	MNHN	IM-2009-12870	KY451352	KY488860	KY489061	KY489236	KY489352
Naytia	*sp*	Republic of the Congo	MNHN	IM-2009-23951	KY451251	KY488756	KY488958	KY489146	
Phos	*alabastrum*	New Caledonia	MNHN	IM-2009-20613	KY451405	KY488914	KY489115	KY489284	
Phos	*cf. hirasei*	Papua New Guinea	MNHN	IM-2009-13144	KY451408	KY488918	KY489117	KY489287	
Phos	*cf. roseatus*	New Caledonia	MNHN	IM-2009-20623	KY451409	KY488919	KY489118		
Phos	*hirasei*	Papua New Guinea	MNHN	IM-2009-13112	KY451410	KY488920	KY489119	KY489288	KY489373
Phos	*senticosus*	China	LSGB	232091	JN053008	JN052944			
Phos	*sp*	New Caledonia	MNHN	IM-2009-20608	KY451411	KY488921	KY489120		
Photinae	*sp*	Papua New Guinea	MNHN	IM-2009-13141	KY451416	KY488925	KY489124	KY489292	
Phrontis	*alba auct*	Guadeloupe	MNHN	IM-2009-24340		KY488763	KY488965	KY489152	
Phrontis	*alba*	Guadeloupe	MNHN	IM-2009-24295	KY451256	KY488762	KY488964	KY489151	
Phrontis	*antillarum*	Guadeloupe	MNHN	IM-2009-24320	KY451258	KY488765	KY488967	KY489154	KY489316
Phrontis	*candidissima*	Guadeloupe	MNHN	IM-2009-24297	KY451269	KY488776	KY488979	KY489164	
Phrontis	*cf. alba*	Guadeloupe	MNHN	IM-2009-24316	KY451270	KY488777	KY488980	KY489165	
Phrontis	*compacta*	Panama	MNHN	IM-2009-22344	KY451281	KY488787	KY488990	KY489174	KY489322
Phrontis	*complanata*	Panama	MNHN	IM-2009-22345	KY451282	KY488788	KY488991		
Phrontis	*hotessieriana*	Guadeloupe	MNHN	IM-2009-24317	KY451315	KY488821	KY489023	KY489199	
Phrontis	*karinae*	Guadeloupe	MNHN	IM-2009-24296	KY451320	KY488826		KY489205	
Phrontis	*luteostoma*	Panama	MNHN	IM-2009-21715	KY451328	KY488834	KY489036	KY489212	
Phrontis	*nassiformis*	Panama	MNHN	IM-2009-24034	KY451336	KY488843	KY489045		KY489341
Phrontis	*pagoda*	Panama	MZUR	BAU00237	FM999173	FM999125	FM999094		
Phrontis	*polygonata*	Guadeloupe	MNHN	IM-2009-24329	KY451350	KY488858	KY489059	KY489234	KY489350
Phrontis	*sp*	Guadeloupe	MNHN	IM-2009-24289		KY488758	KY488960	KY489148	
Phrontis	*versicolor*	Panama	MNHN	IM-2009-24032	KY451401	KY488910	KY489111		KY489368
Phrontis	*vibex*	Guadeloupe	MNHN	IM-2009-24334	KY451402	KY488911	KY489112	KY489281	
Pisania	*striata*		MZUR	BAU00698	FM999175	FM999128	FM999097		
Reticunassa	*annabolteae*	Madagascar	MNHN	IM-2009-12862	KY451373	KY488882	KY489084	KY489257	KY489361
Reticunassa	*cf. neoproducta*	Mozambique	MNHN	IM-2009-22676	KY451275	KY488781	KY488985	KY489169	
Reticunassa	*cf. paupera*	Vanuatu	MNHN	IM-2007-31779	KY451277	KY488783	KY488987	KY489171	
Reticunassa	*crenulicostata*	Philippines	MNHN	IM-2007-31900	KY451290	KY488796	KY488998		
Reticunassa	*festiva*	China	LSGB	23401A2	JQ975433	JQ975569			
Reticunassa	*neoproducta*	Madagascar	MNHN	IM-2009-12896	KY451337	KY488844	KY489046	KY489220	KY489342
Reticunassa	*paupera*	Vanuatu	MNHN	IM-2007-31778	KY451348	KY488856	KY489057	KY489232	KY489349
Reticunassa	*rotunda*	Vanuatu	MNHN	IM-2007-31783	KY451357	KY488866	KY489068	KY489243	
Reticunassa	*silvardi*	French Polynesia	MNHN	IM-2009-23955		KY489072	KY489072	KY489247	KY489357
Reticunassa	*simoni*	Madagascar	MNHN	IM-2009-13086	KY451362	KY488872	KY489073		
Reticunassa	*tringa*	Vanuatu	MNHN	IM-2007-31753	KY451397	KY488906	KY489107		KY489367
Tomlinia	*frausseni*	Vietnam	MNHN	IM-2013-52188	KY451417	KY488926			KY489378
Tritia	*burchardi*	Australia	MNHN	IM-2009-23746		KY488773	KY488976	KY489162	
Tritia	*conspersa*	Spain	MNHN	IM-2009-22353	KY451285	KY488791	KY488993		
Tritia	*cuvierii*	Spain	MNHN	IM-2009-5378	KY451291	KY488797	KY488999	KY489180	KY489324
Tritia	*denticulata*	Spain	MNHN	IM-2009-21546	KY451292	KY488798	KY489000	KY489181	
Tritia	*ephamilla*	New Zealand	MNHN	IM-2009-24014	KY451298	KY488803	KY489005	KY489185	KY489326
Tritia	*goreensis*	Senegal	MNHN	IM-2009-12296	KY451310	KY488815	KY489017	KY489193	
Tritia	*grana*	Spain	MNHN	IM-2009-22546	KY451312	KY488817	KY489019	KY489195	KY489333
Tritia	*heynemanni*	Senegal	MNHN	IM-2009-12304		KY488820	KY489022	KY489198	
Tritia	*incrassata*	Spain	MNHN	IM-2009-21589		KY488823	KY489025	KY489201	KY489335

Tritia	*lanceolata*	Tunisia	MNHN	IM-2013-32028				KY489278	
Tritia	*miga*	Senegal	MNHN	IM-2009-12309	KY451331	KY488838	KY489040	KY489216	KY489338
Tritia	*mutabilis*	France	MNHN	IM-2009-29683	KY451335	KY488842	KY489044	KY489219	KY489340
Tritia	*neritea*	Tunisia	MNHN	IM-2009-30508	KY451233		KY488939	KY489129	KY489306
Tritia	*obsoleta*	USA	MNHN	IM-2009-21755	KY451244	KY488748	KY488949	KY489139	KY489315
Tritia	*ovoidea*	Spain	MNHN	IM-2009-21580	KY451346	KY488853	KY489054	KY489229	
Tritia	*pallaryana*	Tunisia	MNHN	IM-2013-31770	KY451286	KY488792	KY488994	KY489177	KY489323
Tritia	*pellucida*	Spain	MNHN	IM-2009-5374	KY451234	KY488741	KY488940		
Tritia	*pfeifferi*	Morocco	MNHN	IM-2009-22558	KY451349	KY488857	KY489058	KY489233	
Tritia	*pygmaea*	Spain	MNHN	IM-2009-21586	KY451354	KY488862	KY489063	KY489238	
Tritia	*reticulata*	Spain	MNHN	IM-2009-22330	KY451356	KY488865	KY489067	KY489242	KY489354
Tritia	*senegalensis*	Senegal	MNHN	IM-2009-12284	KY451361	KY488870		KY489246	KY489356
Tritia	*sp. 500*	Senegal	MNHN	IM-2009-12300	KY451381	KY488890	KY489092	KY489265	
Tritia	*tingitana*	Spain	MNHN	IM-2009-24094	KY451396	KY488905	KY489106	KY489277	KY489366
Volutharpa	*perryi*		LSGB	232042	JN053003	JN052938			

Table 2. Fossil record used as calibration point.

Node	Calibration reference
Nassariidae	Coniacian, Cretaceus (89.9-86.3) (Tracey et al., 1993)
Tritia reticulata	Early Pliocene (5.3-3.6 mya) (Gili and Martinell, 1994)
T. neritea	Pleistocene (2.58-0.78 mya) (Gili and Martinell, 1999)
T. pellucida	Lower Pliocene (5.3-3.6 mya) (Gili and Martinell, 1999)
Tritia mutabilis	Middle Pleistocene (1.8-0.12 mya) (Van Dingenen et al., 2015)
T. incrassata	Lower Pliocene (5.3-3.6 mya) (MHNH Collection)
Dorsanum	Early Oligocene (32 MYA) (Lozouet, 1999)
Tritia	*T. pygmaeus* (Schlotheim 1820), 28–23 (Lozouet, 1999)
Buccinanops	B. calli (Aldrich 1886), 57 Ma (Allmon, 1990);
Cyllene	C. desnoyersi (Basterot 1825), 28–23
Nassariinae	Buccitriton sp., 56–47.8 Ma (Tracey et al., 1993)
Photinae	Tritaria sp., 41–34 Ma (MacNeil and Dockery, 1984)

Table 3. The four biogeographic categories assigned and their relative marine biogeographic region.

Biogeographic category	Marine Biogeographic region	
Indo-Pacific	IPW, IPC, Aus, IPE	Indo-Pacific East, Indo-Pacific Central, Temperate Australian, Indo-Pacific West
Caribbean	TrAW	Tropical West Atlantic
Mediterranean & Atlantic	TAE, TrAE	Temperate East Atlantic, Tropical East Atlantic
South America	MAG, TSAE, TESP	Magellanic, Temperate East South America, Temperate Estern-South Pacific

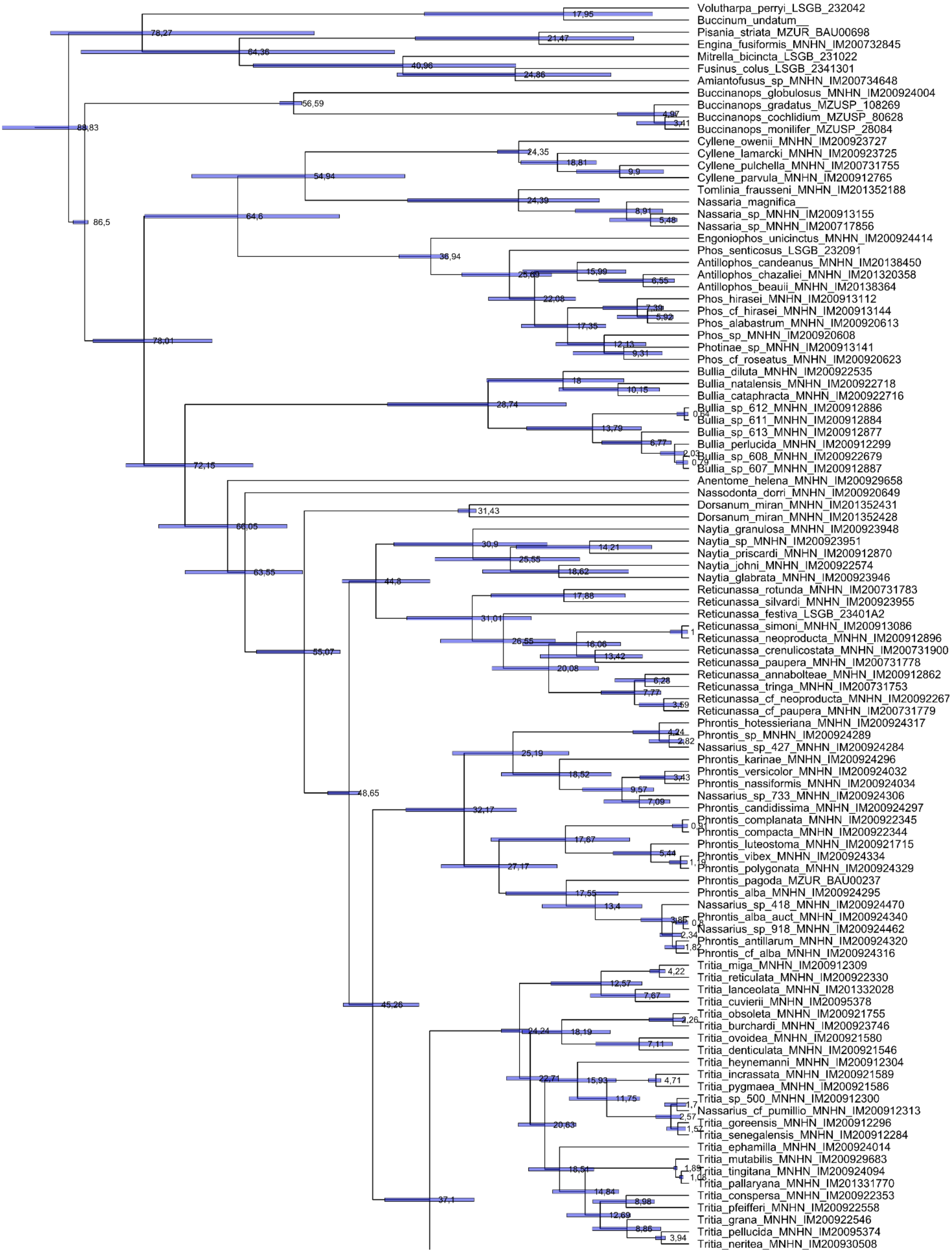


Figure 1A. Calibrated phylogeny (BEAST). First part.

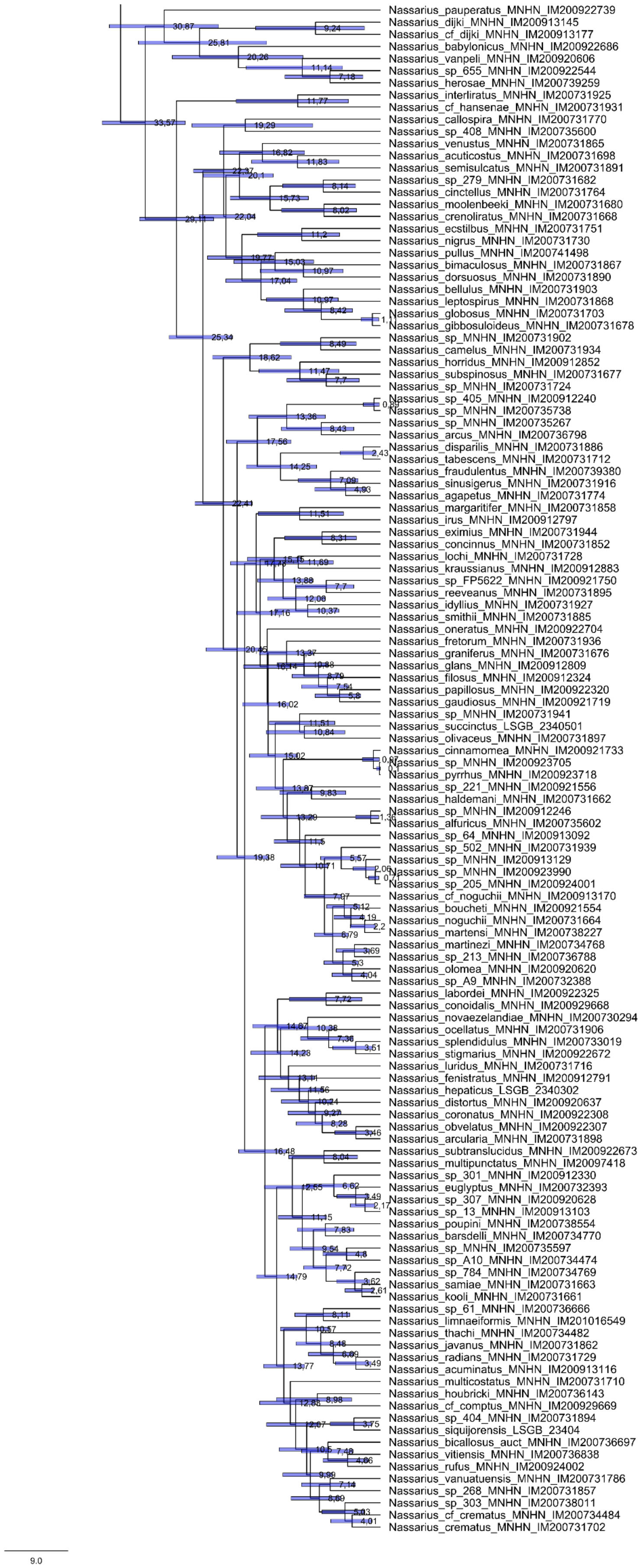


Figure 1B. Calibrated phylogeny (BEAST). Second part.

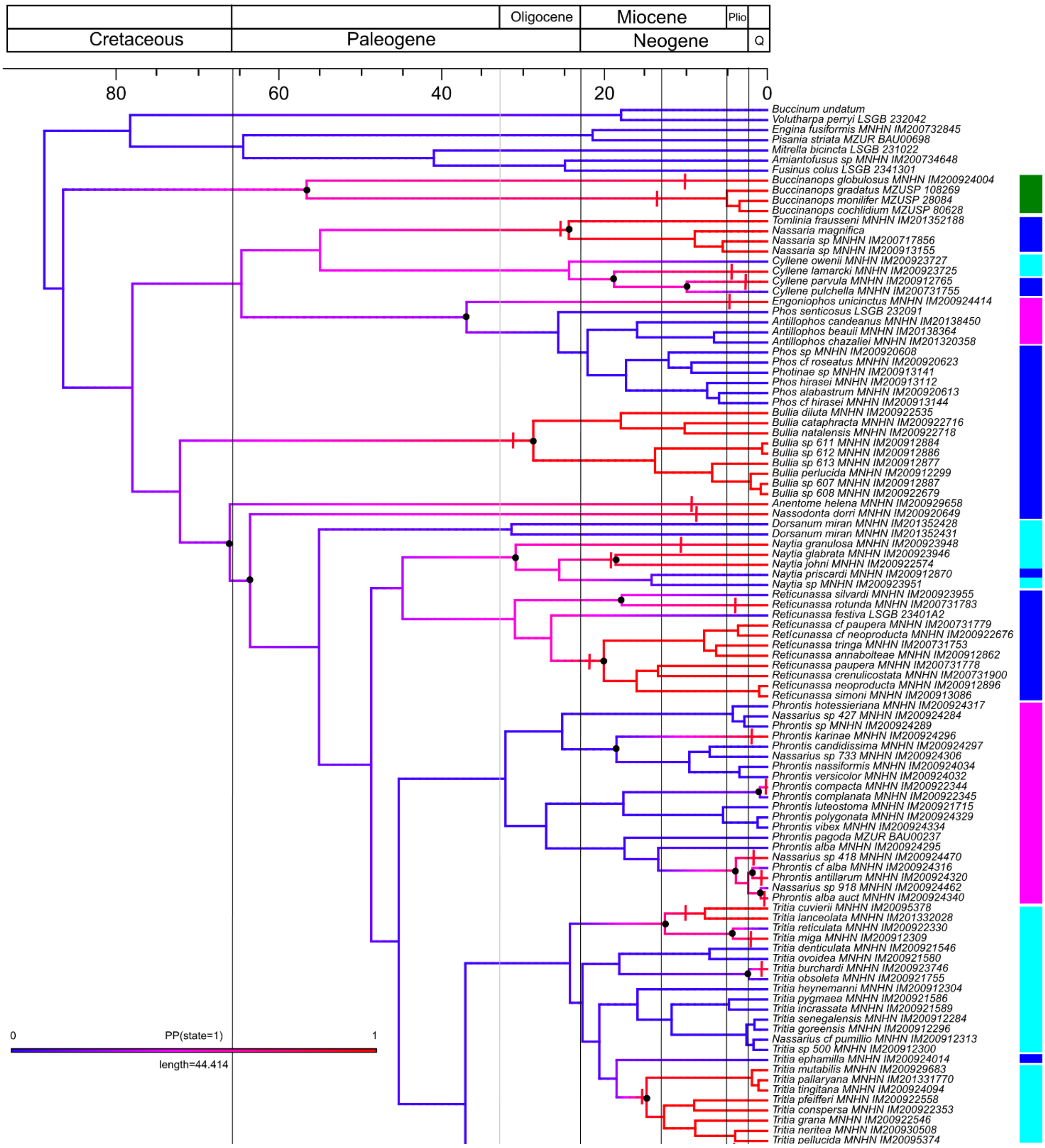


Figure 2A. Ancestral state reconstruction. In red high posterior probability of Non-Planktotrophy state. The nodes in black were considered where change of larval development occurred. Biogeographic region: Blu (Indo-Pacific), Pink (Carribean), Cyan (Mediterranean & Atlantic), Green (South America). First part.

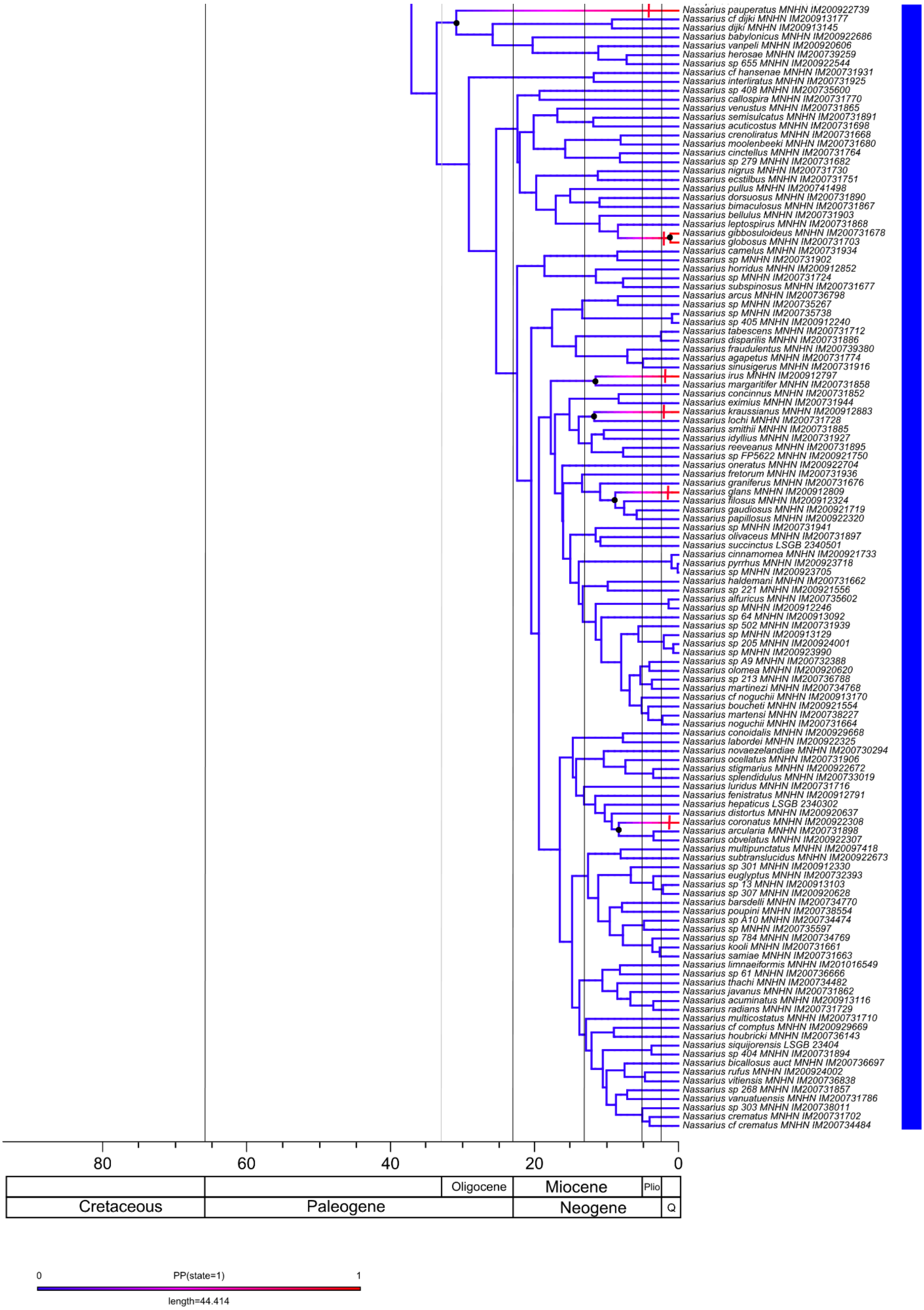


Figure 2B. Ancestral state reconstruction. Second part.

Chapter IV

- Whelks, rock-snails and allied: the evolution of larval development within a new phylogenetic framework for the family Muricidae (Mollusca: Gastropoda)

Whelks, rock-snails and allied: the evolution of larval development within a new phylogenetic framework for the family Muricidae (Mollusca: Gastropoda)

Russini V[1]., Houart R.[2], Barco A.[3], Puillandre N.[4], Lozouet P.[5], Oliverio M.[1]

[1] Department of Biology and Biotechnologies “C. Darwin”, Sapienza University of Rome, Italy
[2] Royal Belgian Institute of Natural Sciences, Brussels, Belgium
[3] Deutsches Zentrum für Marine Biodiversitätsforschung, Wilhelmshaven, Germany
[4] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE CP 26, Paris, France.
[5] Muséum National d’Histoire Naturelle, Paris, France

Introduction

Given the small dispersal ability of benthic marine organism, larval development is a key feature bearing on population connectivity, range, and genetic structure (Cowen & Sponaugle, 2009; Modica et al., 2017).

In marine gastropods larval development is an important character due to the severely reduced mobility of the adult, compared to the potential dispersal by larvae (Cowen & Sponaugle, 2009; Ellingson & Krug, 2015). Larval development can be divided in two major types (Thorson, 1950): Planktotrophic (P) with larvae feeding actively on phytoplankton, spending a relatively long time in the pelagic phase, reflected in a multispiral larval shell (protoconch) and high dispersion ability; Non Planktotrophic (NP), with lecithotrophic larvae that may spend little time in the pelagic phase, or even complete their development within the egg-capsule (intracapsular development), reflected in a paucispiral protoconch and a relatively low dispersal ability.

Larval development in gastropods is a rather plastic feature, with the frequent occurrence of sibling species originated by switch in their larval development (loss of planktotrophy) (Oliverio, 1996; Collin, 2004; Collin et al., 2007; Duda and Palumbi, 1999; Houart, 2013). Poecilogony (intraspecific variation in the mode of larval development) is very rare in Caenogastropoda (P Bouchet, 1989; Mcdonald et al., 2014; Russini et al., 2019) allowing for the use of larval shell characters (the protoconch is very frequently retained at the apex of the adult shells) to diagnose sibling species, morphologically very similar, but differing in their larval development. However, this morphological peculiarity of many conchiferan molluscs (characters of larval life-history still readable in the adults) offers the unique occasion to study the evolution of larval developmental strategies across a phylogenetic framework when this is available.

In this work we investigated the evolution of larval development in the phylogenetic lineages of the family Muricidae. The neogastropod family Muricidae is one of the most species-rich family of Gastropoda, with an estimated 1800+ extant species of whelks, rock-shells, murex-shells, drill-shells, coral-shells (WoRMS, Appeltans et al., 2012). The family has a worldwide distribution, from shallow water down to more than 3000 m (Aldea & Troncoso, 2010), all carnivore predators, from generalists to highly specialized. Muricids are known since ancient times, with Mediterranean species used by Phoenicians to produce their Tyrian purple dye, and Greeks, Arabians and Chinese using them for pharmacological use (Benkendorff et al., 2015). Nowadays, some rock shells have economic relevance being either consumed as food (e.g. *Murex, Concholepas, Trunculariopsis, Bolinus, Chicoreus*) or a pest of commercial oysters (Buhle & Ruesink, 2009). The classification of the family in the last century was repeatedly revised based on morphological features of Recent and fossil taxa (Bouchet and Rocroi, 2005; Vokes, 1996; Ponder and Waren, 1988; Radwin and D'Attilio, 1971; Keen, 1971; Thiele, 1929; Cossmann, 1903) and a single comprehensive attempt at building a molecular phylogenetic framework has been recently performed (A Barco et al., 2010) with a few other work at the subfamily level (A Barco et al., 2015; Andrea Barco et al., 2012; Claremont et al., 2008, 2013; M Oliverio & Mariottini, 2001).

In the present study we aimed at extending the analysis of the family Muricidae based on a larger dataset, including all the recognised subfamilies to produce a solid phylogenetic framework.

Based on this, the ancestral state reconstruction on a calibrated tree will show the evolution of larval development along the family evolutionary history.

Materials and methods

Dataset

The analysis was based on four molecular markers: one nuclear marker (the 28S rDNA of 1417 bp) and three mitochondrial markers (the barcode fragment of 658 bp of the cytochrome c oxidase subunit I, COI, a 455 bp fragment of the 12S rDNA and a 649 bp fragment of the 16S rDNA) (Table 1). DNA was extracted from tissue samples at the Service de Systématique Moléculaire of the Muséum National d'Histoire Naturelle (MNHN, Paris) and at the Department of Biology and Biotechnologies "Charles Darwin" of Sapienza University of Rome (BAU), with either the 6100 Nucleic Acid Prepstation system (Applied Biosystems), a standard DMSO protocol, or a standard phenol/chloroform protocol. Primers used to amplify the selected markers are reported in Table 1.

(Table 1)

The starting dataset was composed by merging all published sequences of Muricidae and 604 original sequences. Taxonomic identification of every specimen was assessed by the examination of the original specimens were available, or by cross-checking sequences in single gene alignments (especially the barcode COI) with pedigreed vouchers in publicly available collections. Thereafter, the taxa for the final dataset were selected in order to maximize the taxonomic coverage of the family and the available sequences per taxon: samples with at least the barcoding sequence of COI or at least two of the other three markers (12S, 16S and 28S).

The sequences were aligned with Geneious R7 (Kearse et al., 2012) (COI) and with the software MAFFT (Katoh et al., 2017; Kuraku et al., 2013) choosing the Q-INS-I algorithm (12S, 16S and 28S). The hypervariable regions of alignment of 12S, 16S and 28S were excluded in the analysis after selection by the software Gblocks (v. 0.91b, Castresana, 2000), setting all the options for a less stringent selection. The concatenated dataset was assembled with SequenceMatrix (Vaidya et al., 2011). Single gene alignments were used to check for potential contaminants, wrong sequences and redundant identical sequences that were all eliminated, in order to have a single or few representatives to each species, yielding a final dataset containing 418 muricid specimens represented 382 species.

Phylogenetic reconstruction

Phylogenetic analyses were performed by Maximum likelihood (ML) and Bayesian inference (BI) on the concatenated dataset. ML analyses were performed with the software IQ-TREE (Nguyen et al., 2014) on 10000 bootstrap replicates (with ultrafast bootstrap, UFBoot: Hoang et al., 2017). BI analysis was performed using Beast 1.8.0 (Drummond et al., 2012) running two Markov chain Monte Carlo (MCMC) analyses in parallel for 10^8 generations, with a 25% burn-in and sampling every 10000 steps. Using TRACER 1.6 (Rambaut et al., 2018), chains convergence was assumed when the effective sample size values (ESS) were >200. All the phylogenetic trees were visualised with FigTree (v 1.4.4).

The substitution model for each partition (12S, 16s, 28s and positions 1^{st}, 2^{nd} and 3^{rd} of COI) was chosen with Partition finder 2 (Lanfear et al., 2016).

We used as outgroup the conoidean *Conus judaeus* Bergh, 1895 (Conidae), and species of Buccinoidea, related to the Muricoidea (Marco Oliverio & Modica, 2010): *Buccinum undatum* Linnaeus, 1758, *Kelletia lischkei* Kuroda, 1938, *Penion ormesi* (Powell, 1927), *Serratifusus lineatus* Harasewych, 1991.

Nodes with Bootstraps support (BS) of 70-90% and Posterior Probabilities (PP) of 0.90-0.95 have been considered as moderately supported; those with BS > 90% and PP > 0.95 have been consider as highly supported.

Temporal calibration of the phylogenetic framework

We identified 12 calibration points that we used to calibrate the tree of Muricidae (Table 2).

(1) The first appearance of the family is probably witnessed in the Upper Cretaceous of Texas (70 - 112 mya) (Merle et al., 2011) with the earliest known species attributed to **Muricidae**, the fossil *Paziella (Flexopteron) cretacea* (Garvie, 1991). The family was certainly not present before the Albian (Lower Cretaceous, 112 Mya), which was set as the lower bound (Andrea Barco et al., 2012).

(2) The Middle Eocene *Coralliophila (Timotia) aldrichi* (Cossmann, 1903) is the earliest known species of **Coralliophilinae** (Clairbonian of Mississippi and Louisiana, approx. 40 Mya; Dockery 1980). Congruently, the lower bound was defined at 65.5 Mya, in agreement with the estimate that the diversification of the muricid subfamilies probably occurred during the Paleocene and Eocene (Lozouet & Renard, 1998; M Oliverio, 2008).

(3) The fossil record of **Typhinae** dates the first certain appearance of the subfamily in the Lower Eocene (Ypresian) (MHNH collection) based on the occurrence of *Typhis tubifer* (Bruguière, 1792).

(4) Fossils belonging to the subfamily **Ocenebrinae** are common in the lower Miocene, and probably the first appearance of the subfamily was during the Lower Oligocene (Lozouet, 2012). (5) The genus ***Nucella*** (Ocenebrinae), has the first documented record in the lower Miocene (Aquitanian) c. 22.5 mya (Collins et al., 1996).

(6) The first fossil sample identified as **Rapaninae** was in the lower Oligocene (Lozouet, 2012).

(7) The first appearance of the subfamily **Ergalataxinae** matches with the fossil record of the genus *Lindapterys* in the lower Oligocene (MNHN collection).

Concerning the subfamily Muricinae, (8) the oldest known record of the genus ***Chicoreus*** is from the Piacenzian (2.5 mya) (Merle et al., 2011). For the genus *Murex*, fossil records of both (9) ***M. trapa*** and (10) ***M. tenuirostrum*** appeared during the Pliocene of Java (W. Ponder & Vokes, 1988). (11) The oldest fossil record for the genus ***Poirieria*** is from the lower Eocene (Ypresian) (Merle & Pacaud, 2002). (12) The genus ***Timbellus*** has the first documented appearance at least in the lower Eocene (47.8–56 mya) (Cossmann, 1923).

(Table 2)

The combined dataset was used to create a calibrated tree to estimate the node ages of each clade of the family Muricidae with the software Beast 1.8.0 (Drummond et al., 2012). The heterogeneity of the mutation rate across lineages was set under uncorrelated lognormal distributed relaxed clocks for the three partitions found (see below), and the Yule process (Gernhard, 2008) was chosen. All other priors were set with default values. The twelve calibration points were set under exponential prior (Ho & Phillips, 2009), with the major distributions within the boundaries of the relative stage age of identification. We performed two runs of 10^8 generations, sampled every 10,000 steps, results were analysed with Tracer 1.6 (Rambaut et al., 2018) and all runs were pooled together and re-sampled using LogCombiner 1.8.0, after 25% samples were discarded as a burn-in. Then, the maximum clade credibility tree was estimated with TreeAnnotator 1.8.0.

Ancestral state reconstruction and evolution of larval development

The mode of larval development of 278 species represented in the phylogeny was inferred by the direct examination of the larval shell morphology of each assayed specimen, or of conspecific specimens genetically analysed. To investigate the evolution of larval development through the different lineages and within each subfamily, we performed an ancestral state reconstruction (package phytools in R: Revell, 2012) on an calibrated ultrametric tree, generated with the software BEAST (v. 1.8.0) (Suchard et al., 2018). We used the concatenated alignment of a reduced dataset including only the species with a known larval development. The 12 calibration points were used to estimate the nodes ages of the tree and the ages of the character changes, the planktotrophy was assumed to be the ancestral state in the lineages (Haszprunar, 1995; Marco Oliverio, 1996).

Results

We retrieved from the GenBank c. 800 sequences for the molecular markers 12S, 16S and 28S, and 3980 sequences of the barcode marker COI. After analysing all sequences, checking for consistency and redundancy, and assessing the taxonomic ID of each sequence, we eventually selected the sequences in order to maximize the number of represented species. The final dataset was composed of sequence of 418 individuals. The combined alignment was 3179 bp long, of which 455 bp for the 12S, 649 bp for the 16S, 1417 bp for the 28S and 658 bp for the COI.

Phylogenetic reconstruction

The substitution models found by Partition Finder 2 for each partition of our dataset are shown in Table 3.

(Table 3)

The ML and BI analyses yielded trees with very similar topologies, with only different support value for some of the major nodes (Figure 1A and 1B).

The monophyly of the family Muricidae was confirmed (BS 98%; PP 1). The monophyly was also supported for the subfamilies Ergalataxinae (BS 100%; PP 1), Coralliophilinae (BS 100%; PP 1), Rapaninae (BS 98%; PP 1), Ocenebrinae (BS 88%; PP 0.49), Pagodulinae (BS 76%; PP 1), Haustrinae (BS 100%; PP 1) and Typhinae (BS 91%; PP 1). The subfamily Trophoninae (clade E, Figure 1A) comprised paraphyletic lineages (genera *Trophon* and *Leptotrophon)* but not confirmed due to a very low support. In all the supported subfamilies, few genera appeared to be monophyletic.

The subfamilies Muricinae and Muricopsinae were not monophyletic and were splitted in several lineages.

It is possible to distinguish a large monophyletic clade (BS 98%; PP 1) **Muricinae** (s.s., clade A, Figure 1A), that included species of the genera *Murex, Chicoreus*, *Muricantus, Haustellum, Hexaplex, Naquetia, Chicomurex, Phyllonotus, Siratus, Bolinus, Vokesimurex.* However, the genera included in this clade did not always form distinct groups; species of *Naquetia* and *Chicomurex* were intermixed within a monophyletic clade (BS 82%; PP 1), suggesting an artificial division of genera; species of *Chicoreus* were in a monophyletic clade (BS 66%; PP 0.94) together with *Muricanthus radix* and *Monstrotyphis montfortii* suggesting a revision of the latter two species; the genus *Murex,* with the exception of *Murex occa,* was monophyletic (BS 94%; PP 0.94).

The subfamily **Muricopsinae** as traditionally conceived did not form a monophyletic clade due to the inclusion of the genera *Attiliosa, Aspella* and *Dermomurex* (formerly Muricinae or Aspellinae), and *Tripterotyphis triangularis* (formerly Tripterotyphinae). However, the support of this mixed clade was very high (BS 99%; PP 0.99), but the genus *Vitularia* (traditionally considered a muricopsine) was not included in the clade. We consider the supported clade B in figure 1A as Muricopsinae s.s.

The genera *Timbellus* (BS 100%; PP 1) and *Pterynotus* (BS 100%; PP 1) traditionally in Muricinae, resulted in two well distinct and monophyletic clades (clade C e D, Figure 1A). Moreover, *Homalocantha pele Flexopteron poppei*, *Ponderia magna* and *Daphnellopsis lamellosa* settled as independents linaeges.

(Figure 1A and 1B)

Calibration phylogeny

The calibrated phylogeny estimated the origin of the family **Muricidae** at 74.53 mya (95% HPD 70.6–82.73) during the upper Cretaceus (Campanian). The clade of **Muricinae** s.s., was dated at 29.49 mya (95% HPD 22.86–39.27) between Oligocene and early Miocene. The estimated origin of the genus *Chicoreus* at 12.41 mya (95% HPD 9.89–15.4) during the Middle-Upper Miocene seems to predate the known fossil record. The origin of the **Muricopsinae** s.s. (as here conceived) was dated during the Middle Eocene (Ypresian) at 50.13 mya (95% HPD 41.64–59.85); in this clade, the origin of genus *Favartia* s.s. is dated at 34.33 mya (95% HPD 23.16–44.11) during early Oligocene. The subfamily **Pagodulinae**, due to the first appearance of the genus *Poirieria*, is estimated as having originated 52.34 mya (95% HPD 49.65–55.62) during the Ypresian (Middle Eocene); meanwhile the other genus of the subfamily seems to be more recent, 23.64 mya (95% HPD 17.1–31.64) during the Late Oligocene. The subfamily **Haustrinae** is estimated to have arisen 33.29 mya (95% HPD 16.04–48.55) in the Early Oligocene. In the subfamily **Trophoninae**, that in the time-calibrated analysis was monophyletic, the origin is dated at 31.26 mya (95% HPD 19.01–48.41) in the lower Oligocene. The **Ergalataxinae** were estimated to have arisen in the middle-upper Eocene, with the node dated at 36.55 mya (95% HPD 29.55–44.82), and its genus *Drupella* originated at 15.4 mya (95% HPD 10.06–20.52) during the middle Miocene. The **Coralliophilinae** are suggested to have originated 51.84 mya (95% HPD 44.73–59.93) during the Ypresian (middle Eocene) in agreement with the fossil record. The **Ocenebrinae** are estimated to have originated during the lower Oligocene at 30.77 mya (95% HPD 28.22–34.63), in agreement with the very rich fossil record of the subfamily in Lower Miocene, while the genus *Ocenebra* was dated at 13.17 mya (95% HPD 7.93–19.33). The origin of the **Rapaninae** was estimated at 52.34 mya (95% HPD 44.64–61.16) during the Ypresian (middle Eocene). The origin of the **Typhinae** was dated at 49.44 mya (95% HPD 48–53.73) during the Ypresian (Early Eocene).

(Figure 2)

Ancestral state reconstruction and evolution of larval development

The ancestral state reconstruction was performed on a reduced dataset of the family Muricidae (Figure 3A and 3B). Despite the planktotrophy as ancestral state, the non planktotrophy larval development appear early in the family lineages (Early Paleocene) and formed a clade where this kind of development is the ancestral character and more common than the other. The origin of the clade Muricinae s.s. is uncertain, probably planktotrophy. These two clades contain the subfamilies of Muricinae s.s., Muricopsinae s.s, Thyphinae, Timbellus, Haustrinae, Pagodulinae and Ocenebrinae. A total of nine change of larval development from NP to P occurred, until now this reversal events were generally excluded due to the difficult reacquire of feeding structure of the larvae. In subfamily Muricinae larval development appeared to be a very plastic features, and there are nine change from P to NP and three change from NP to P.

The second clade has planktotrophy as ancestral condition, and contain the subfamilies Rapaninae, Coralliiophilinae and Ergalataxinae. In this lineage only six change form P to NP occurred, and here the larval development is a more conservative feature.

Only one event of loss of planktotrophy is more ancient that others and occurred in Early Paleocene, meanwhile the rest of events occurring from Oligocene, in particular one event occurred in Oligocene, five during Miocene, seven loss of planktotrophy occurred during Pliocene, and thirteen during last 2.5 million years in Pleistocene.

(Figure 3A and 3B)

Discussion

The Muricidae family is one of the largest groups of marine gastropods, and their phylogenetic systematics was always controversial (A Barco et al., 2010). In this work we have gathered all available information to build a solid phylogenetic reconstruction to use as a framework for investigating the evolution of larval development.

The phylogenetic hypothesis confirmed the monophyly of several major clades to be ranked as subfamilies: Ergalataxinae, Haustrinae Coralliophilinae were highly supported, Ocenebrinae, Typhinae and Pagodulinae were moderately supported. We propose here to rank as subfamily Muricinae s.s. the clade A (highly supported, BS 100%; PP 1), excluding from the subfamily, as suggested already by Barco et al. 2010, the genera *Dermomurex, Timbellus, Flexopteron, Ponderia* and *Pterynotus.*

A revision of the scope of the subfamily Muricopsinae is urged. We detected a monophyletic clade that can be proposed as Muricopsinae s.s. (Clade B, BS 99%; PP 0.99), which also includes the former aspellines *Aspella* and *Dermomurex,* the genus *Attiliosa* (formerly Muricinae) and *Tripterotyphis triangularis* (formerly Tripterotyphinae).Conversely the traditionally considered muricopsine genera *Homalocantha* and *Vitularia* were excluded from Muricopsinae s.s.

Concerning the subfamily Trophoninae, the monophyly can be confirmed just for the genera *Scabrotrophon* and *Nippotrophon* (100/1). The position of genera *Trophon* and *Leptotrophon* respect to the clade is low supported by our analyses, but the monophyly of the subfamily was confirmed in a previous study (Andrea Barco et al., 2012).

Despite the bias due to the impossibility nowadays to include all the known species of the family because lack of molecular information, a completest evolutionary reconstruction of change of larval strategy was performed. Larval development is described as a rather plastic feature in the family Muricidae, in particular in the subfamilies Muricinae s.s. and Muricopsinae s.s.. In the Muricinae the analysis scored a total of twelve changes in larval development: nine events we losses of planktotrophy, the other three were reacquisitions of planktotrophy. In the Muricopsinae two losses of planktotrophy and three reversal to planktotrophy were scored. The secondary acquisition of a planktotrophic larval development is considered a very rare phenomenon. Larval planktotrophy requires a suite of alimentary features that are very unlikely to re-evolve if definitively lost. It is commonly assumed that if loss of planktotrophy involves the loss of such anatomical characters then, the event is irreversible. This is probably why in marine invertebrates only a few cases of secondary reacquisition of planktotrophy are consistently reported: in Polychaeta (Rouse, 2000) and in three families of Caenogastropoda, Littorinidae (Reid, 1989), Calyptraeidae (Collin et al., 2007) and in the Muricidae (Pappalardo et al., 2014). Hookham and Page (2016) suggested that retention of a larval esophagus and a full complement of velar ciliary tracts needed for particle capture and ingestion observed in non-planktotrophic larvae of some muricids may help explain how larval planktotrophy re-emerged within this clade. No information is available on the veliger morphology of the secondarily reacquired planktotrophic larvae.

The ancestral condition for the clade of Muricinae is uncertain, whereas non planktotrophy is the ancestral state for the clade that contain the Typhinae, Muricopsinae, Pagodulinae, Haustrinae, Trophoninae, Ocenebrinae, *Timbellus* and *Vitularia,* that all show a large predominance of non planktotrophy larval development. Also, here we found five events of reversal, from NP to P.

The second large group comprises the subfamilies Coralliophilinae, Rapapniane and Ergalataxinae, all with a largely dominant planktotrophic developmen, which is reflected in the ancestral condition (planktotrophic), with a total of five losses of planktotrophy along lineages of the three subfamilies.

The ancestral state reconstruction showed as the larval development evolved differently in two major groups of muricids. In one group it seems more stable and larval planktotrophy is largely preserved, whereas in the other it has changed very frequently and in both directions, thus confirming that in muricids larval planktotrophy can be reacquired secondarily (Pappalardo et al. 2014). As suggested by Hookham and Page (2016), the incomplete loss of feeding structures in lecithtrophic larvae may be a prerequisite for the reacquisition of larval planktotrophy.

Aknowledgment

Work partly supported by the Doctorate School in Evolutionary and Environmental Biology of “Sapienza” University and by “Progetti per Avvio alla Ricerca 2018 - Tipo 1” n. AR118164367D5FC0

References

Aldea, C., & Troncoso, J. S. (2010). Remarks on the genus Trophon (s .l.) montfort , 1810 (Mollusca: Gastropoda: Muricidae) in the southern ocean and adjacent areas. *Thalassas*, ***26*****(2)**, 47–73.

Appeltans, W., Bouchet, P., Boxshall, G., & Fauchald, K. (2012). World register of marine species. Retrieved from http://www.marinespecies.org/index.php

Barco, A, Claremont, M., Reid, D. G., Houart, R., Bouchet, P., Williams, S. T., et al. (2010). Molecular Phylogenetics and Evolution A molecular phylogenetic framework for the Muricidae , a diverse family of carnivorous gastropods. *Molecular Phylogenetics and Evolution*, ***56*****(3)**, 1025–1039. https://doi.org/10.1016/j.ympev.2010.03.008

Barco, A, Marshall, B., Houart, R., & Oliverio, M. (2015). Molecular phylogenetics of Haustrinae and Pagodulinae (Neogastropoda: Muricidae) with a focus on New Zealand species. *Journal of Molluscan Studies*, ***81***, 476–488. https://doi.org/10.1093/mollus/eyv020

Barco, Andrea, Schiaparelli, S., Houart, R., & Oliverio, M. (2012). Cenozoic evolution of Muricidae (Mollusca, Neogastropoda) in the Southern Ocean, with the description of a new subfamily. *Zoologica Scripta*, ***41*****(6)**, 596–616. https://doi.org/10.1111/j.1463-6409.2012.00554.x

Benkendorff, K., Rudd, D., Nongmaithem, B. D., Liu, L., Young, F., Edwards, V., et al. (2015). Are the Traditional Medical Uses of Muricidae Molluscs Substantiated by Their Pharmacological Properties and Bioactive Compounds? *Marine Drugs*, ***13***, 5237–5275. https://doi.org/10.3390/md13085237

Bouchet, P. (1989). A review of poecilogony in gastropods. *Journal of Molluscan Studies*, ***55***, 67–78.

Bouchet, Philippe, & Rocroi, J.-P. (2005). Classification and nomenclator of gastropod families. *Malacologia*, ***47***, 1–397. Retrieved from http://malacologia.fmnh.org

Buhle, E. R., & Ruesink, J. L. (2009). Impacts of invasive oyster drills on Olympia oyster (Ostrea lurida Carpenter 1864) recovery in Willapa Bay, Washington, United States. *Journal of Shellfish Research*, ***28*****(1)**, 87–96.

Castresana, J. (2000). Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Molecular Biology and Evolution*, ***17*****(4)**, 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Claremont, M., Reid, D. G., & Williams, S. T. (2008). A molecular phylogeny of the Rapaninae and Ergalataxinae (Neogastropoda: Muricidae). *Journal of Molluscan Studies*, ***74*****(3)**, 215–221. https://doi.org/10.1093/mollus/eyn005

Claremont, M., Houart, R., Williams, S. T., & Reid, D. G. (2013). A molecular phylogenetic framework for the Ergalataxinae (Neogastropoda: Muricidae). *Journal of Molluscan Studies*, ***79*****(1)**, 19–29. https://doi.org/10.1093/mollus/eys028

Collin, R. (2004). Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. *Evolution*, ***58*****(7)**, 1488–1502.

Collin, R., Chaparro, O. R., Winkler, F., & Véliz, D. (2007). Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. *Biological Bulletin*, ***212*****(2)**, 83–92.

Collins, T. M., Frazer, K., Palmer, A. R., Vermeij, G. J., & Brown, W. M. (1996). Evolutionary History of Northern Hemisphere Nucella (Gastropoda, Muricidae): Molecular, Morphological, Ecological, and Paleontological Evidence. *Evolution*, ***50*****(6)**, 2287–2304. https://doi.org/10.2307/2410698

Cossmann, M. (1903). *Essais de Paleoconchologie Comparée* (Vol. 5). Paris.

Cossmann, M. (1923). Description des mollusques. G. O'Gorman, Le gisement Cuisien de Gan (Basses-Pyrénées). Cossmann et O'Gorman, Pau, 188.

Cowen, R. K., & Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science*, ***1*****(1)**, 443–466. https://doi.org/10.1146/annurev.marine.010908.163757

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, ***29*****(8)**, 1969–1973. https://doi.org/10.1093/molbev/mss075

Duda, T. F., & Palumbi, S. R. (1999). Developmental shifts and species selection in gastropods. *Proceedings of the National Academy of Sciences*, ***96*****(18)**, 10272–10277. https://doi.org/10.1073/pnas.96.18.10272

Ellingson, R. A., & Krug, P. J. (2015). Reduced genetic diversity and increased reproductive isolation follow population-level loss of larval dispersal in a marine gastropod. *Evolution; International Journal of Organic Evolution*, ***70*****(I)**, 18–37. https://doi.org/10.1111/evo.12830

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, ***3*****(5)**, 294–299. https://doi.org/10.1371/journal.pone.0013102

Gernhard, T. (2008). The conditioned reconstructed process. *ArXiv E-Prints*.

Haszprunar, G. (1995). On the evolution of larval development in the Gastropoda, with special reference to larval planktotrophy. *Notiz. CISMA*, ***16***, 5–13.

Ho, S. Y. W., & Phillips, M. J. (2009). Accounting for Calibration Uncertainty in Phylogenetic Estimation of Evolutionary Divergence Times. *Systematic Biology*, ***58*****(3)**, 367–380. https://doi.org/10.1093/sysbio/syp035

Hoang, D. T., Chernomor, O., Haeseler, A. Von, Minh, B. Q., & Vinh, L. S. (2017). UFBoot2 : Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, ***35*****(2)**, 518–522. https://doi.org/10.5281/zenodo.854445

Hookham, B., & Page, L. R. (2016). How did phytoplankton-feeding larvae re-evolve within muricid gastropods? A view from developmental morphology. *Marine Biology*, ***163***, 1–11. https://doi.org/10.1007/s00227-015-2784-8

Houart, R. (2013). Description of two new Chicomurex species (Gastropoda: Muricidae) from the Philippine Islands novapex. *Novapex*, ***14*****(3)**, 69–75.

Katoh, K., Rozewicki, J., & Yamada, K. D. (2017). MAFFT online service : multiple sequence alignment , interactive sequence choice and visualization. *Briefings in Bioinformatics*, 1–7. https://doi.org/10.1093/bib/bbx108

Kearse, M., Moir, R., Wilson, A., Stones-havas, S., Sturrock, S., Buxton, S., et al. (2012). Geneious Basic : An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)*, ***28*****(12)**, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Keen, A. M. (1971). A review of the Muricacea. *The Echo*, ***4***, 35–36.

Kuraku, S., Zmasek, C. M., Nishimura, O., & Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research*, ***41*****(May)**, 22–28. https://doi.org/10.1093/nar/gkt389

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2 : New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution*, ***34*****(3)**, 772–773. https://doi.org/10.1093/molbev/msw260

Lozouet, P. (2012). *Stratotype Stampien . In Muséum national d' Histoire naturelle, Paris; Biotope, Mèze; (Patrimoine géologique; 4).*

Lozouet, P., & Renard, P. (1998). The Coralliophilidae, Gastropoda from the Oligocene and Lower

Miocene of the Aquitaine (Southwestern France): systematics and details of coral hosts. *Geobios*, ***31*(2)**, 171–185. https://doi.org/10.1016/S0016-6995(98)80037-5

Mcdonald, K. A., Collin, R., & Lesoway, M. P. (2014). Poecilogony in the caenogastropod Calyptraea lichen (Mollusca : Gastropoda). *Invertebrate Biology*, ***133*(3)**, 213–220. https://doi.org/10.1111/ivb.12057

Merle, D., & Pacaud, J.-M. (2002). First record of Poirieria subcristata (d'Orbigny, 1850) (Muricidae: Muricinae) in the early Cuisian of the Paris Basin (Celles-sur-Aisne, Aizy Formation), with comments on the sculptural evolution of some Palaeocene and Eocene Poirieria and Paziella. *Tertiary Research*, ***21*(1–4)**, 19–27.

Merle, D., Garrigues, B., & Pointier, J.-P. (2011). *Fossil and Recent Muricidae of the World. Part Muricinae*.

Modica, M. V., Russini, V., Fassio, G., & Oliverio, M. (2017). Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. *Marine Environmental Research*, ***127***. https://doi.org/10.1016/j.marenvres.2017.04.001

Nguyen, L., Schmidt, H. A., Haeseler, A. Von, & Minh, B. Q. (2014). IQ-TREE : A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution*, ***32*(1)**, 268–274. https://doi.org/10.1093/molbev/msu300

Oliverio, M. (2008). Coralliophilinae (Neogastropoda: Muricidae) from the southwest Pacific. In R. H. C. & P. B. V. Heros (Ed.), *Tropical Deep-Sea Benthos 25, du Muséum National d'Histoire Naturelle 196* (Editions d, pp. 481–585).

Oliverio, M, & Mariottini, P. (2001). A molecular framework for the phylogeny of Coralliophila and related muricoids. *Journal of Molluscan Studies*, ***67*(i)**, 215–224. https://doi.org/10.1093/mollus/67.2.215

Oliverio, Marco. (1996). Life-histories, speciation, and biodiversity in Mediterranean prosobranch gastropods. *Vie et Milieu*, ***46*(2)**, 163–169.

Oliverio, Marco, & Modica, M. V. (2010). Relationships of the haematophagous marine snail Colubraria (Rachiglossa: Colubrariidae), within the neogastropod phylogenetic framework. *Zoological Journal of the Linnean Society*, ***158*(4)**, 779–800. https://doi.org/10.1111/j.1096-3642.2009.00568.x

Pappalardo, P., Rodriguez-Serrano, E., & Fernàndez, M. (2014). Correlated Evolution between Mode of Larval Development and Habitat in Muricid Gastropods. *PLoS ONE*, ***9*(4)**.

https://doi.org/10.1371/journal.pone.0094104

Ponder, W., & Vokes, E. (1988). *A revision of the Indo-West Pacific fossil and recent species of Murex ss and Haustellum (Mollusca: Gastropoda: Muricidae)*. (Australian Museum, Ed.).

Ponder, W. F., & Waren, A. (1988). Classification of the Caenogastropoda and Heterostropha - A list of the family-group names and higher taxa. *Prosobranch Phylogeny Malacological Review*, ***4***, 288–326.

Radwin, G. E., & D'Attilio, A. (1971). Muricacean supraspecific taxonomy based on the shell and radula. *The Echo*, ***4***, 55–67.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, ***67*****(5)**, 901–904. https://doi.org/10.1093/sysbio/syy032

Reid, D. G. (1989). The comparative morphology, phylogeny and evolution of the gastropod family littorinidae. *Philosophical Transactions of the Royal Society B: Biological Sciences*, ***324*****(1220)**, 1–110. https://doi.org/10.1098/rstb.1989.0040

Revell, L. J. (2012). phytools : an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, ***3***, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rouse, G. W. (2000). Polychaetes have evolved feeding larvae numerous times. *Bulletin of Marine Science*, ***67*****(1)**, 391–409.

Russini, V., Giannuzzi-Savelli, R., Pusateri, F., Prkić, J., Fassio, G., Modica, M. V, & Oliverio, M. (2019). *Genetic evidence of poecilogony in the genus Raphitoma Bellardi, 1847*.

Thiele, J. (1929). *Handbuch der systematischen Weichtierkunde*.

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, ***25*****(1)**, 1–45. https://doi.org/10.1111/j.1469-185X.1950.tb00585.x

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, ***27*****(2)**, 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Vokes, E. H. (1996). One last look at the Muricidae. *American Conchologist*, ***24*****(2)**, 4–6.

Tables and Figures

Table 1. List of primer for each molecular marker

		Sequence primer 5'-3'	
12S	12SI-12SIII	TGCCAGCAGCCGCGGTTA- GAGCGACGGGCGRTTWGTAC	Oliverio & Mariottini, 2001
16S	16SA-CgLE^{UUR}	CGCCTGTTTATCAAAAACAT- TATTTAGGGCTTAAACCTAATGCAC	Palumbi, 1996; Hayashi, 2003
28S	LSU5'-ECD2S 900F-LSU1600	TAGGTCGACCCGCTGAAYTTAAGCA- CTTGGTCCGTGTTTCAAGACGG CCGTCTTGAAACACGGACCAAG- AGCGCCATCCATTTTCAGG	Littlewood et al., 2000; Williams et al., 2003
COI	LCO1490-HCO2198	GGTCAACAAATCATAAAGATATTGG- TTAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994

Table 2. Fossil record used as calibration point and 95% of highest posterior probability.

	Node	Calibration reference	95% HPD
1	Muricidae	70 - 112 mya (Merle et al., 2011)	70.6-79.64
2	Coralliophilinae	40 - 65 mya (Dockery 1980, Lozouet & Renard, 1998; Oliverio 2008)	40.42-54.29
3	Typhinae	Lower Eocene, Ypresian (MNHN Collection)	48-53.25
4	Ocenebrinae	Lower oligocene (Lozouet, 2012)	22.81-28.06
5	*Nucella*	22.5 (Collins, 1996)	13.77-22.07
6	Rapaninae	Lower Oligocene (Lozouet, 2012)	35.11-50.65
7	*Lindapterys* (Ergalataxinae)	Lower Oligocene (MNHN collection)	31.62-46.01
8	*Chicoreus*	Piacenziano (Merle et al., 2011)	10.05-15.72
9	*Murex trapa*	Plio-Pleistocene (MNHN collection)	0.11-1.11
10	*Murex tenuirostrum*	Plio-Pleistocene (Ponder & Vokes, 1988)	1.91-6.16
11	*Poirieria*	Ypresian, Lower Eocene (Merle & Pacaud 2002)	48-50.07
12	*Timbellus*	Lower Eocene (MNHN collection)	11.02-39.31

Table 3. Substitution models found for each partition.

Partition	Substitution model	Base pairs
12S	GTR+I+G	455
16S	GTR+I+G	649
28S	GTR+I+G	1417
COI position cod1	GTR+I+G	219
COI position cod2	SYM+I+G	219
COI position cod3	HKY+G	220

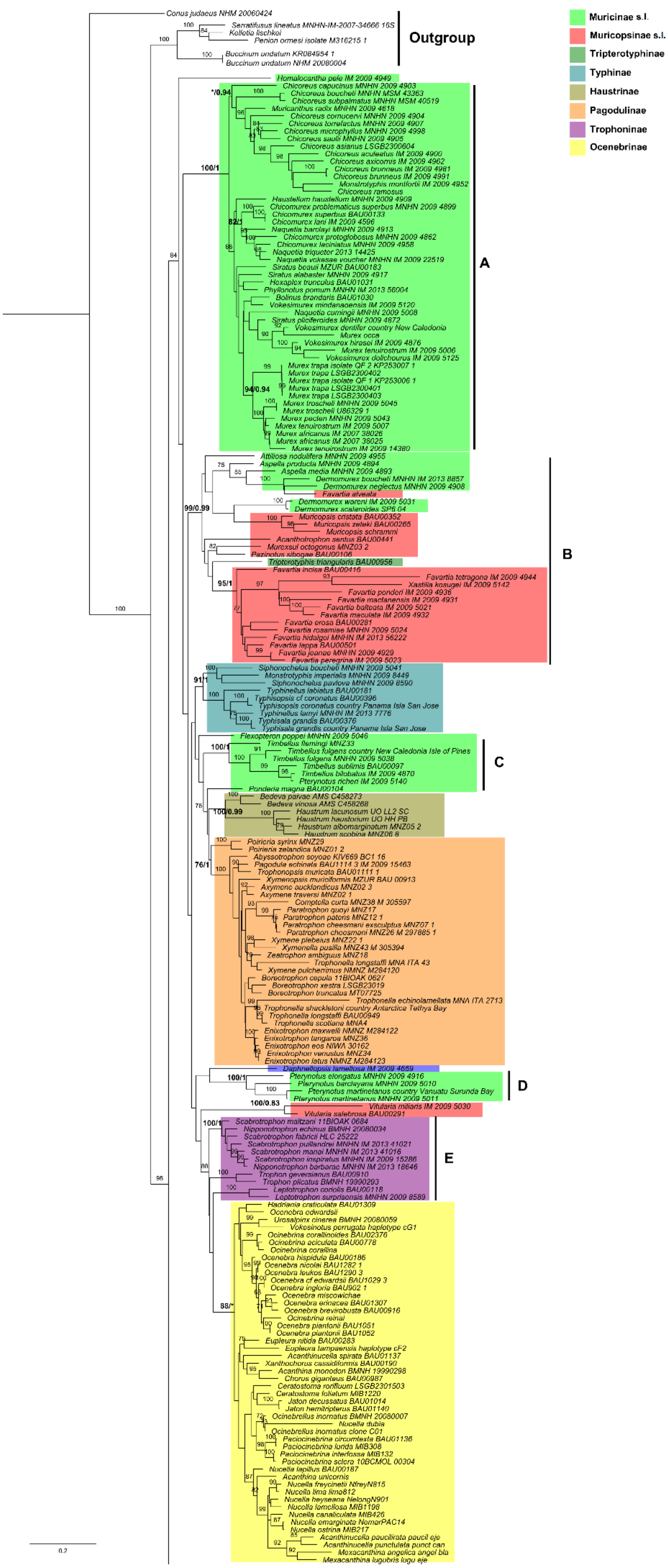


Figure 1A. Phylogenetic reconstruction of the family Muricidae. The topology is retrieved by the analysis of ML and reported all the bootstrap values over 70. In bold were reported both value, bootstrap value and posterior probability. First part. Clade A: Muricinae s.s., Clade B: Muricopsinae s.s., Clade C: *Timbellus* lineage, Clade D: *Vitularia* and *Pterynotus* lineage, Clade E: Trophoninae.

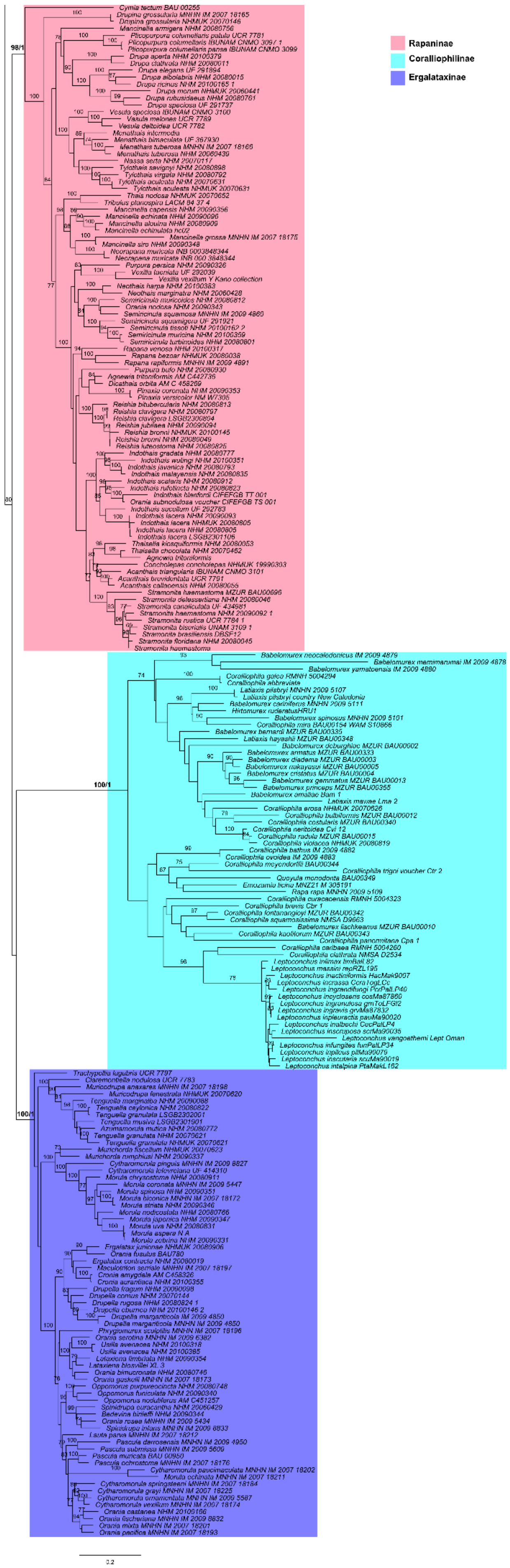


Figure 1B. Phylogenetic reconstruction of the family Muricidae. Second part.

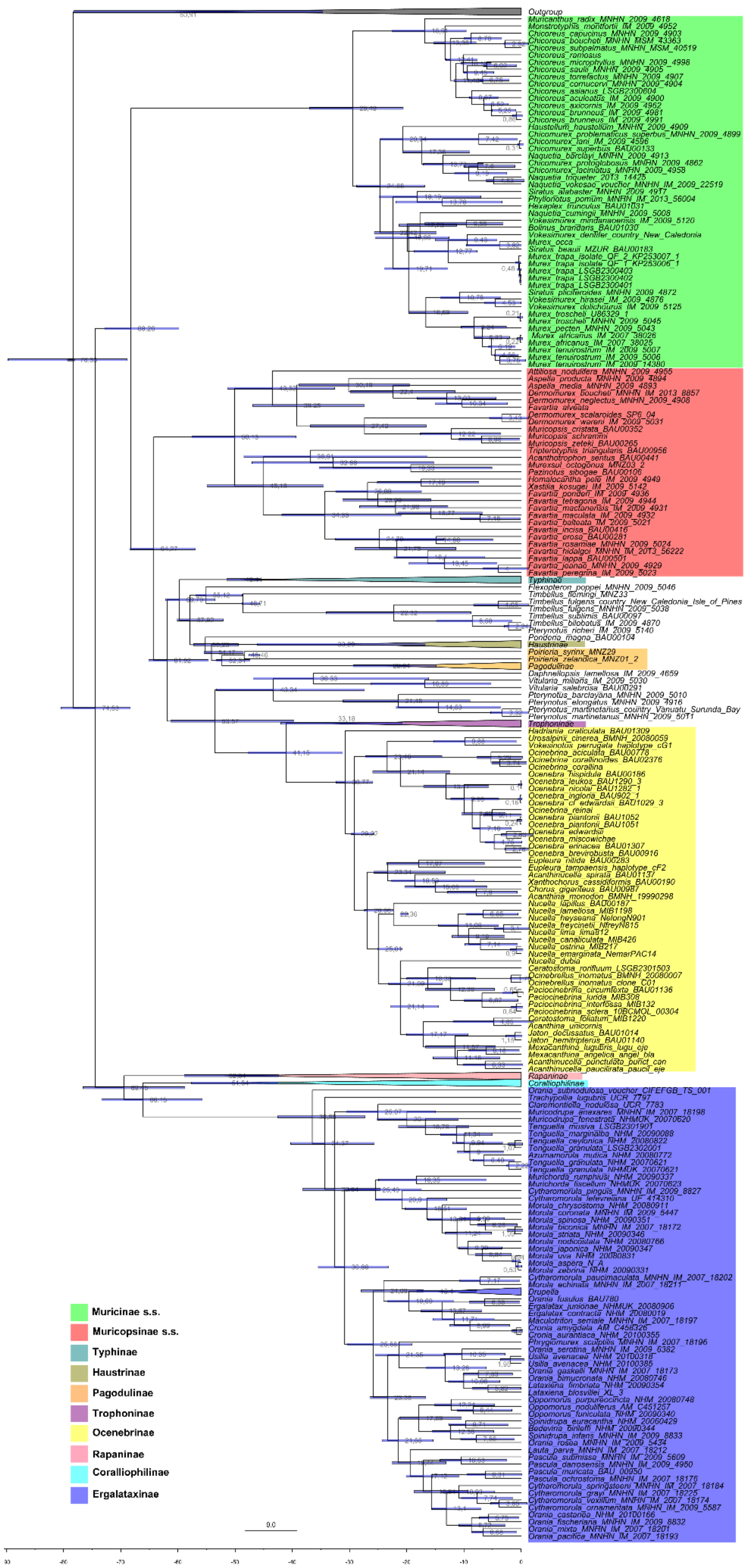


Figure 2. Calibrated tree. The node bars represent the 95% HPD.

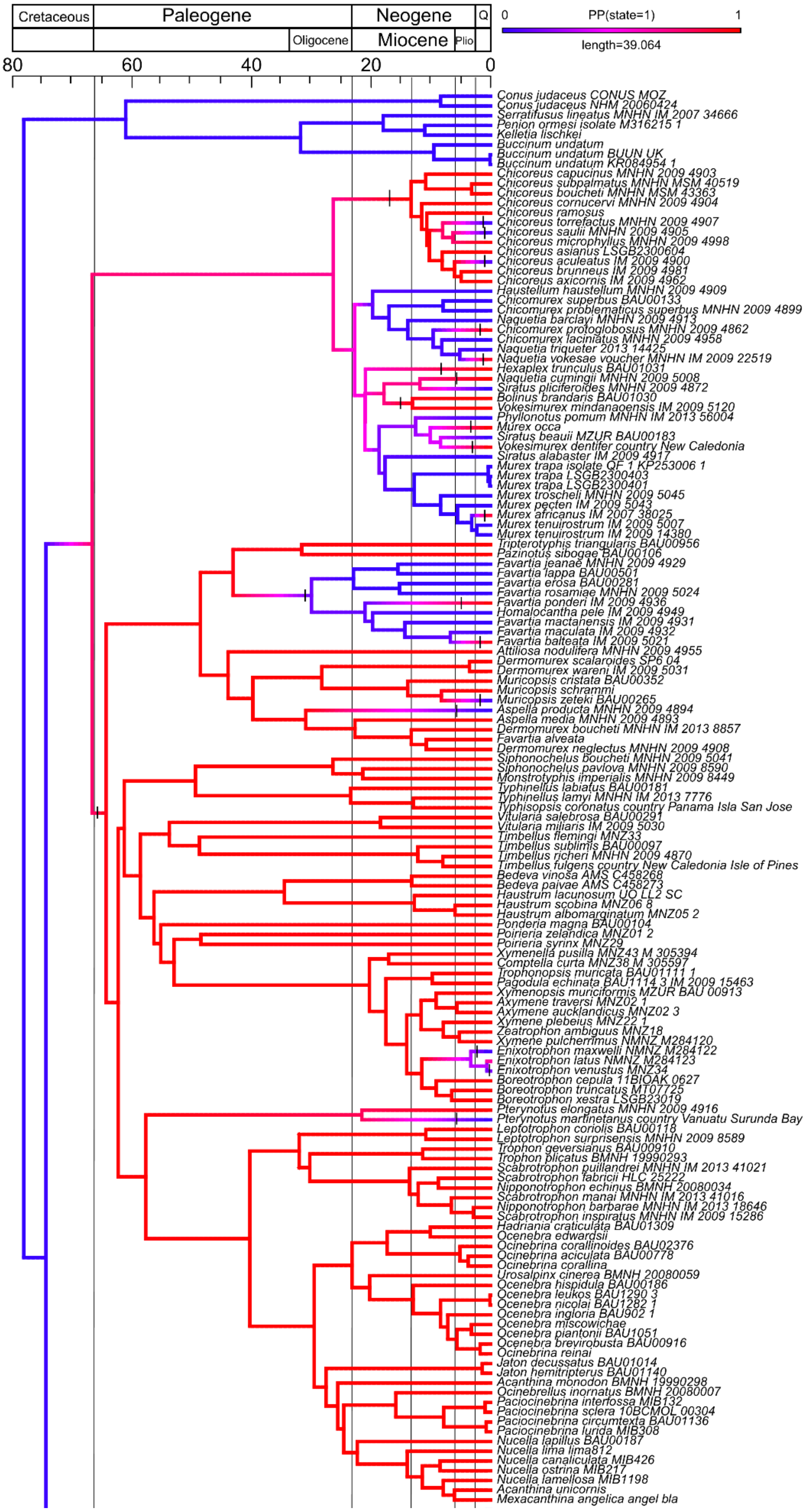


Figure 3A. Ancestral state reconstruction. In red high posterior probability of Non-Planktotrophy state. First part.

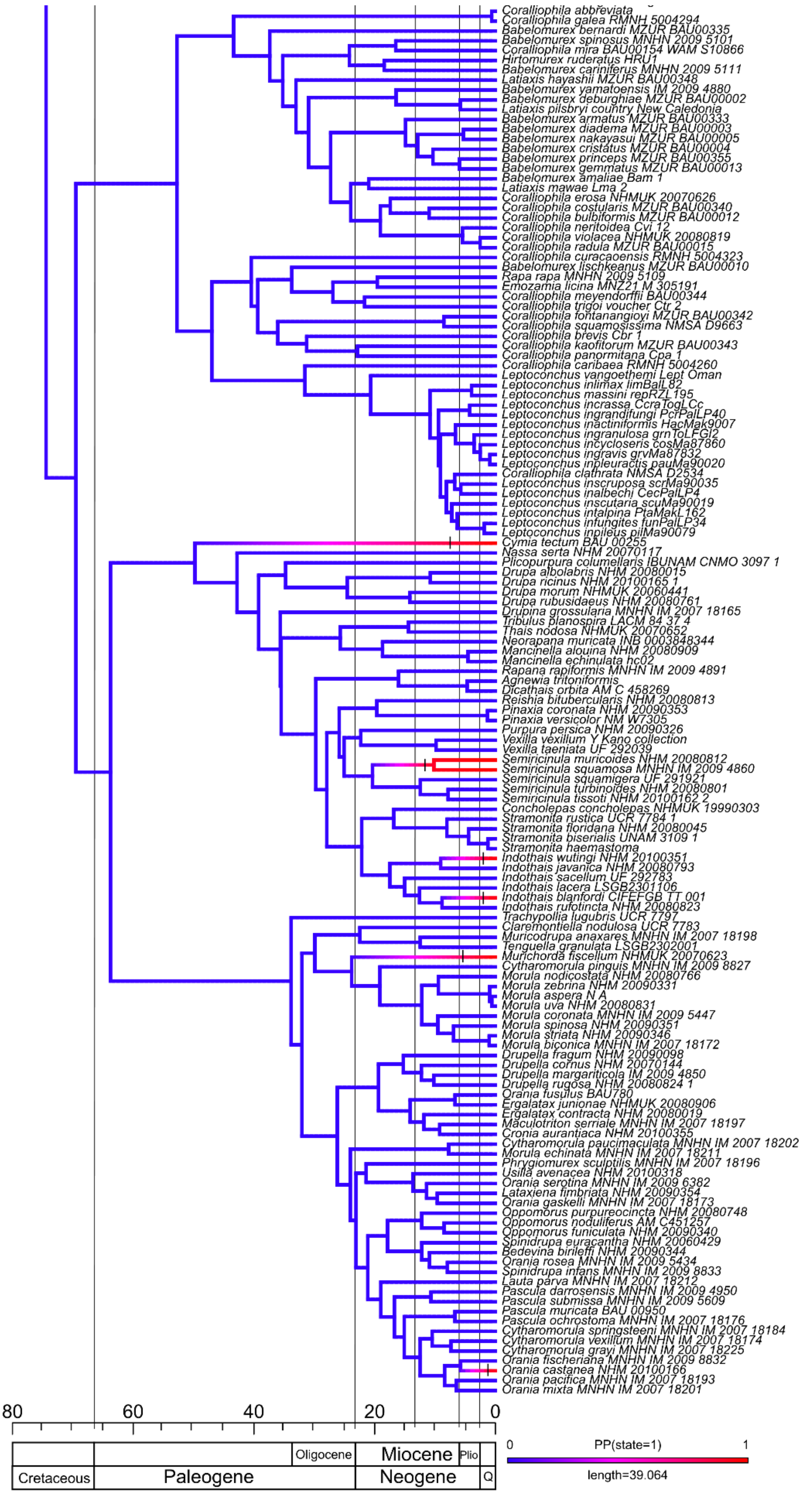


Figure 3B. Ancestral state reconstruction. Second part.

Conclusion

In this thesis I have investigated several aspects of the evolution of larval development in Caenogastropoda, the largest extant radiation of gastropod molluscs, probably also the largest radiation of extant marine invertebrates. Within marine invertebrates, the gastropods provide unique tools to perform studies on the evolution of larval development: important aspects of larval development are reflected in the morphology of their embryonic/larval shell, the protoconch, which is very often retained at the apex of the adult shell, allowing for inference on larval ecology of the organisms by the study of the adults, also fossil. This work is aimed at shedding light on two of the most controversial issues about larval development evolution: poecilogony and secondary reacquisition of planktotrophy.

With my research I have confirmed the crucial role of larval strategies in the evolutionary history of gastropod species. The different larval developmental strategies influence the real duration of the pelagic larval phase that in turns affects the dispersal ability of propagules throughout the marine environment. The dispersal ability, investigated in the first chapter, has turned out to be an important driver of the genetic structure of population in different species with several wide implications for the population connectivity. Low *vs* high connectivity species may react differently to environmental and climate changes: for example, water temperature seems to be crucial to trigger the duration and success of larval stage (Rombough, 1997). It may be argued that the better the spatial genetic structure of a species and the underlying mechanisms are known, the better the population response to the global/local changes can be predicted. Different larval ecology may affect the success likelihood of invasive alien species, not necessarily favouring planktotrophic developers (Chemello & Oliviero, 1997). Therefore, while designing networks of marine protected areas, the knowledge of the ecological attributes of the communities will become crucial, also in terms of the variation in larval ecology of the species involved.

I have then investigated (second chapter) the phenomenon of sibling species in Caenogastropoda, differing in their contrasting larval strategies. I have built a new solid phylogenetic framework for the large conoidean family Raphitomidae with particular attention to delimit the actual scope of the genus *Raphitoma* (separated from the related but distinct genera *Leufroyia* and *Cyrillia*). Several sibling species were described in this taxon, suggesting a special plasticity of the character within the group. The study confirmed the existence of at least one pair of sibling species, very similar in their adult morphology, but with distinct larval strategies. However, more important, the genetic evidence of poecilogony, the intraspecific variation of larval strategy, in at least two species of *Raphitoma* has been gathered. This case represents the first documented case of poecilogony in the Neogastropoda, the second within the subclass Caenogastropoda, and one of the very few among the invertebrates. There is a long list of gastropod species described only or mostly based on the morphology of the larval shell, under the assumption that poecilogony was not present in caenogastropods. Although a wide screening will be necessary to asses every single case by genetic data in extant groups, the new evidence of the presence of poecilogony in caenogastropods raises issues about the identification of sibling species based on different protoconch shape, questioning the larval shell features as a taxonomic character. Poecilogony has always been a controversial issue, but despite its rarity, poecilogonous species can provide a unique model to understand the mechanisms underlying the evolution of larval development.

Finally, I have attempted at studying the evolution of larval development in a high rank taxonomic group (family), across the temporal dimension, using two robust phylogenies with the nodes dated. To calibrate the phylogenetic trees, I have used several fossils record retrieved form the literature and from the malacological fossil collections at the Muséum National d'Histoire Naturelle of Paris. By this approach, I have studied the evolution of larval development and the temporal distribution of changes of state of the characters across dated phylogenies of the families Nassariidae and Muricidae.

The phylogeny reconstruction of the family Muricidae represented the first complete phylogenetic study for the family, after several works were based on specific subfamilies in the last decades. Merging published data with new sequences produced for the occasion, yielded an unprecedented dataset, fundamental for the resolution of the phylogenetic framework of this large family of gastropods. Combining the calibrated phylogeny with the phylogenetic R tools “phytools” I have found some cases of reversal, the secondary acquisition of planktotrophy, in the family Muricidae. In this caenogastropods family cases of reversal were detected in the two major subfamilies, Muricinae and Muricopsinae. It is commonly assumed that if loss of planktotrophy involves the loss of anatomical characters then, the event is irreversible. This is probably why in marine invertebrates only a few cases of secondary reacquisition of planktotrophy are consistently reported: in Polychaeta (Rouse, 2000) and in three families of Caenogastropoda, Littorinidae (Reid, 1989), Calyptraeidae (Collin et al., 2007) and in the Muricidae (Pappalardo et al., 2014).

A similar analysis was performed on the Nassariidae, another large family of neogastropods. No case of reversal was found in this family, where the plesiomorphic state of the character (planktotrophic) was lost at least 28 times.

I have detected no significant temporal asymmetry in the distribution of the loss of planktotrophy events, but rather the change in larval strategy seems to be biogeographically biased. Change frequency is linked with the geographic region of origins of species, addressing to the geographic confinement hypothesis, where the closure of oceanographic basins may have promoted the loss of planktotrophy due to a restricted suitable environment for the pelagic larval life. Semi-closed or closed basins like the Caribbean area and the Mediterranean Sea have probably been areas where the loss of planktotrophy has been particularly promoted.

References

Chemello, R., & Oliviero, M. (1997). Lessepsian migrations: a theoretical "island-jumping" model. *Biologia Marina Mediterranea*, *3*(1), 444–446. Retrieved from https://www.cabi.org/isc/abstract/20087206285

Collin, R., Chaparro, O. R., Winkler, F., & Véliz, D. (2007). Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. *Biological Bulletin*, *212*(2), 83–92.

Pappalardo, P., Rodriguez-Serrano, E., & Fernàndez, M. (2014). Correlated Evolution between Mode of Larval Development and Habitat in Muricid Gastropods. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0094104

Reid, D. G. (1989). The comparative morphology, phylogeny and evolution of the gastropod family littorinidae. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *324*(1220), 1–110. https://doi.org/10.1098/rstb.1989.0040

Rombough, P. J. (1997). The effects of temperature on embryonic and larval development. In C. M. Wood & D. G. McDonald (Eds.), *Global Warming: Implications for Freshwater and Marine Fish* (pp. 177–224). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511983375.009

Rouse, G. W. (2000). Polychaetes have evolved feeding larvae numerous times. *Bulletin of Marine Science*, *67*(1), 391–409.

Other publications

Prkić, J., Giannuzzi-Savelli, R., Pusateri, F., **Russini, V.**, Fassio, G. & Oliverio M. (2019). Three new species of *Raphitoma* (Mollusca, Gastropoda, Raphitomidae) from the Croatian waters (NE Adriatic Sea). Zoosystema. SUBMITTED

Fochetti, R., Oliverio, M., **Russini, V**., Tapia, G. & Tierno de Figueroa, J.M. (2019) Molecular identity of *Nemoura lacustris* throughout its distributional range. Zootaxa. IN PRESS

Centorame, M., Moschella, F., **Russini, V.** & Fanfani, A. (2018). DNA-barcoding of the Italian members of the *Aphaenogaster testaceopilosa*-group (Hymenoptera: Formicidae). Hybridization and biogeographic hypothesis. Zoologischer Anzeiger, 277, 121-130, ISSN: 0044-5231, doi: 10.1016/j.jcz.2018.09.003

Ringraziamenti

Prima di tutto, ringrazio il Prof. Marco Oliverio e la Dott.ssa Maria Vittoria Modica per avermi dato l'opportunità di svolgere questa esperienza nel loro gruppo di ricerca. Ringrazio tutto il gruppo del laboratorio di Malacologia, anche chi solo di passaggio, e tutti i coautori degli articoli del mio progetto. Ringrazio con affetto Barbara Buge, Marie Hennion, Virginie Héros, Philippe Maestrati e Pierre Lozouet per aver reso indimenticabile la mia esperienza al Museo di Storia Naturale di Parigi (MNHN).

Vorrei ringraziare inoltre tutte le persone che mi sono state vicino in questi anni, pieni di gioie e di grandi sfide.

Ringrazio tutta la mia famiglia, mia sorella e il mio compagno per aver creduto in me e per avermi supportato nei momenti più difficili.
Ringrazio Giulia, semplicemente indispensabile durante tutto questo percorso, e i miei colleghi e amici, Massimiliano, Fausto e Giovanna per esserci sempre stati, soprattutto nei momenti più duri. Spero che questi anni pieni di risate e discorsi profondi, passati fianco a fianco, nonostante tutto, siano solo l'inizio di questa sincera amicizia.
Ringrazio Alice per la complicità e l'empatia che ci siamo scambiate a vicenda mentre vivevamo le stesse difficoltà, superate tutte alla grande.
Con grande affetto ringrazio il Prof. Alberto Fanfani per essere stato un punto di riferimento, che va al di là dell'accademia, mostrandoci il lato buono e sincero della vita.
Ringrazio Antonietta, in lei ho sempre trovato un'alleata combattiva, pronta a difenderci dalle mille ingiustizie.
Ringrazio Domenico Davolos da sempre sostenitore di noi giovani dottorandi, che con la sua spontaneità ha reso spesso le giornate più leggere.
Ringrazio tutti i colleghi dottorandi e assegnisti di Zoologia per i pranzi spensierati che hanno (ri)animato i corridoi del nostro amato istituto.