
Possible Poecilogony Due to Discontinuous Multifactorial Inheritance in Some Mediterranean Species of *Raphitoma* (Mollusca, Conoidea, Raphitomidae)

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Abstract

At least 10 pairs of similar, most probably closely related, species of *Raphitoma* are often sampled in the same Mediterranean localities. In each pair, one member bears a planktotrophic protoconch and the other a lecithotrophic one. We propose that the phenomenon may be attributed to a simple gene that functions in conjunction with others and environmental factors to exhibit a discontinuous multifactorial inheritance leading to poecilogony. Below a threshold, the animals may produce fewer and larger germ cells, giving rise to fewer and larger eggs and large lecithotrophic embryos with large paucispiral protoconch I, while above that threshold, more and smaller germ cells leading to smaller eggs and to planktotrophic larvae with small protoconch I and large multispiral protoconch II. Preliminary measurements are in support of our hypothesis. Analysis of mitochondrial DNA markers as well as interbreeding experiments could bring an end to the existing confusion.

Keywords: *Raphitoma*, protoconch, Mediterranean sea

1. Introduction

Marine shelled gastropods follow, in general, three types of larval development: (1) direct, in which embryo development is completed in the egg capsule and the juvenile is ready

to commence its benthic life immediately after hatching. (2) Lecithotrophic development in which, after hatching, the larva spends from a few minutes to several days as a veliger without feeding or growing. (3) Planktotrophic development, in which the larva hatches as a veliger and spends a few days to several months in the planktonic mode of life during which it feeds and grows [1]. In all cases, a larval shell is formed which is called protoconch.

The size of fossilized early protoconches suggests that feeding (planktotrophic) embryos first appeared at the transition from the Cambrian era to the Ordovician as Ordovician protoconches were smaller than Cambrian ones indicating smaller Ordovician eggs and offspring. It is believed that planktotrophy would prolongate their escape from benthic predators and that an increasing nutrient supply and availability of photoautotrophic plankton in the world oceans of that period have facilitated both planktotrophy and suspension feeding [2]. The eventual loss of planktotrophy in the Pliocene has been documented in some taxa of Conoidea (*Raphitoma*, *Bela*) and is believed to be irreversible [3]. The same phenomenon has occurred in the families such as Cerithiidae (*Bittium*), Turritellidae (*Turritella*), Rissoidae (*Rissoa*, *Pusillina*), and Nassariidae (*Nassarius*) [4, 5].

Planktotrophic mode of life with long-living planktonic larvae is considered advantageous in the dispersal of the larvae as they may drift considerable distances with the currents [6, 7]. In addition, it has been demonstrated that the switch in some conoidean taxa from the planktotrophic to the non-planktotrophic development has increased their adaptive radiation, especially in polar or insular region, or in groups with narrow bathymetric distribution [3, 7]. In the case of *Oenopota elongata* Bogdanov, 1989, and in the polar genus or bathyal Indo-Pacific genus *Bathytoma* Harris & Burrows, 1891, the loss of planktotrophy actually preceded adaptive radiation [8–11].

Within the Neogastropoda, Conoidea is a diverse superfamily of venomous and exclusively marine gastropods which harbors more than 300 genera, 4000 known species, and an estimated number of over 12,000 existing species [3, 12, 13]. Due to the species richness and the extensive homoplasy among shell's features and the anterior alimentary system, they have resisted repeated attempts to be permanently classified with those attempts to be hindered primarily by the absence of a stable phylogenetic framework. Rather recently, DNA analysis provided an updated classification and divided the superfamily of Conoidea into 13 families [14–16]. Among them, the position of Raphitomidae is not sufficiently secured as a clade of Conoidea and for that reason there has been an on-going attempt to clarify the phylogeny of its Mediterranean members through a greater number of taxonomic data [17, 18]. These later publications have taken into account the pioneering works of Thorson [19, 20], concerning the relationship between the morphology of the protoconch and the type of the larval development in Caenogastropoda, e.g., the dichotomy “multispiral protoconch/planktotrophic development” and “paucispiral protoconch/lecithotrophic development” that has been widely accepted [6, 21]. Although some authors have used this dichotomy to divide species into planktotrophic genera and non-planktotrophic ones [3, 22, 23], it has been clearly demonstrated that such a division based exclusively on the morphology of the protoconch produces

artificially separated polyphyletic taxa of otherwise distinct genera of closely related species. A separation, for instance, between the genus *Raphitoma* (with multispiral protoconch) and the genus *Philbertia* (with a paucispiral protoconch) is inconsistent and, therefore, must be rejected [17]. Nevertheless, this planktotrophic or lecithotrophic mode of larval life as reflected in the morphology of its protoconch comprises a useful basis for distinguishing different species in several genera in Prosobranchia and Opisthobranchia Mollusca [24–29].

Under the weight of the above unsettled situation, the aim of this study was to aid the classification of the Mediterranean *Raphitoma* species by presenting new material from the Hellenic seas, demonstrating them in detailed descriptions and coming up with new ideas on the protoconch issue. More specifically, our objective was to put to test the hypothesis that protoconch poecilogony exists within a species population, with lecithotrophic larvae being produced from fewer and larger eggs, while planktotrophic ones from more and smaller eggs that result merely by additional cleavages of the maternal stem cells.

2. Materials and methods

Sampling of gastropods specimens was conducted from October 2008 to October 2016 in certain locations of Greece, according to Manousis and Galinou-Mitsoudi [30]. The *Raphitoma* species recognition was based on (a) systematic guides and atlases [23, 31–38], (b) faunistic and review articles [39, 40], (c) studies on the Mollusca fauna in the Hellenic seas [30, 41–47]. Information from specific websites was also taken into account. For the species nomenclature update (31 December 2016), besides the Marine Biodiversity and Ecosystem Functioning EU Network of Excellence (MarBEF, www.marbef.org) and the World Register of Marine Species (WoRMS, <http://www.marinespecies.org>), the Taxonomic on-line Database on European Marine Mollusca (CLEMAM, www.somali.asso.fr) was followed. In addition, the Ellenic Network on Aquatic Invasive Species (ELNAIS, <https://services.ath.hcmr.gr>, 31 December 2016) and the Marine Mediterranean Invasive Alien Species database (MAMIAS, <http://mamias.org/taxonomicgroup.php>) were used for the status of possible alien species in the Hellenic and Mediterranean seas. Protoconch whorls were counted according to Verduin [48]. The measurement of the protoconch maximum diameter was performed at top view according to Gofas and Oliver [49]. The shell's slenderness (h/w) was estimated including the outer lip of the aperture in the shell's width. The protoconch I maximum diameter deriving after each hypothetical additional cleavage of the stem cells was calculated by multiplying measured maximum diameter of the lecithotrophic protoconch of each, so-called, sibling species by the approximate factor of 0.7937 receiving as a fact that each stem cell is a sphere that divides into two equal spheres at cleavage.

The specimens are deposited in the premises of the Alexander Technological Educational Institute of Thessaloniki and those of Dr T. Manousis, Mr Constantinos Kontadakis, Mr George Mbazios and Mr Georgios Polyzoulis. Scientists are welcome to have access to the biological material at will.

3. Results

Among the 570 specimens collected, three pairs of 'sibling' species of *Raphitoma* appropriate for comparison were identified, which are presented in **Figures 1–4** and described below in an alphabetical order.

Raphitoma contigua (Monterosato, 1884) (**Figure 1c and d**).

Collection stations: 13 shells (5.00–10.00 mm long, 2.10–4.20 mm wide) were collected from detritus material trapped in small-scale fishing nets at 10–120 m from mixed bottoms of Central Saronikos Gulf: Pantokratoras, Preveza.

Description: The shell is hyaline, fusiform, and 2.2 times as long as wide. Its multispiral protoconch, which is approximately 390 μm (mean) wide and 490 μm (mean) high, bears a 230 μm (mean) protoconch I of slightly more than 1 whorl that is decorated with regularly placed small tubercles. Protoconch II consists of almost 1.5 diagonally cancellated and convex whorls, the last of which bears a weak keel with erasures before the onset of the teleoconch. The teleoconch consists of five convex whorls separated by a deep suture. The body whorl occupies almost 65% of the total length and bears 15–16 orthocline axial ribs with interspaces approximately 1.5 times wider than the ribs themselves and 17–18 spiral cords slightly thinner than the ribs, six of which are situated above the aperture and the rest 12 below the extension of the suture. The spiral cords in their intersections with the axial ribs form erasures in the form of small elongated rectangular tubercles. The tubercles on the first two adapical cords are spiny and close to each other forming a subsutural ramp. The shell's inner wall viewed through the aperture exhibits a transparency. The aperture occupies approximately 45% of the shells length and exhibits a smooth and sigmoid columella, angled at its upper part. The anterior siphonal canal is short and wide, while the posterior one is deep and narrow. The color is uniformly light yellowish tan and in certain shells darker with irregularly situated areas of cream-white and a white subsutural band by the ramp and one more as an extension of the suture on the body whorl. Protoconch I is white and the rest is light yellowish tan with white erasures.

Similar species: *R. contigua* superficially resembles a number of congeneric Mediterranean *Raphitoma* species, but it is different from: *R. alternans* (Monterosato, 1884), in which, the latter has a paucispiral protoconch, a more elongated shell, and a different color pattern; *R. atropurpurea* in its color, which is honey-red instead of purple-brown in *R. atropurpurea* and in its less slender spire than *R. atropurpurea*; *R. densa* (Monterosato, 1884) in the color and the paucispiral protoconch of the late; *Raphitoma lineolata* (Bucquoy, Dautzenberg and Dollfus, 1883) in its more inflated profile, its more robust shell, in the presence of a narrow subsutural ramp in *R. contigua*, in its wider aperture, and in the absence in *R. contigua* of a subsutural white cord present in *R. lineolata*; *R. oblonga* (Jeffreys, 1867) in its wider aperture and the different color pattern; *R. spadiana* mainly because the latter bears a paucispiral protoconch [17].

Habitat and distribution: Infralittoral, on sandy and muddy bottoms, also under stones and in the holes of the rocks [38]. Central Mediterranean Sea [17, 36, 38].

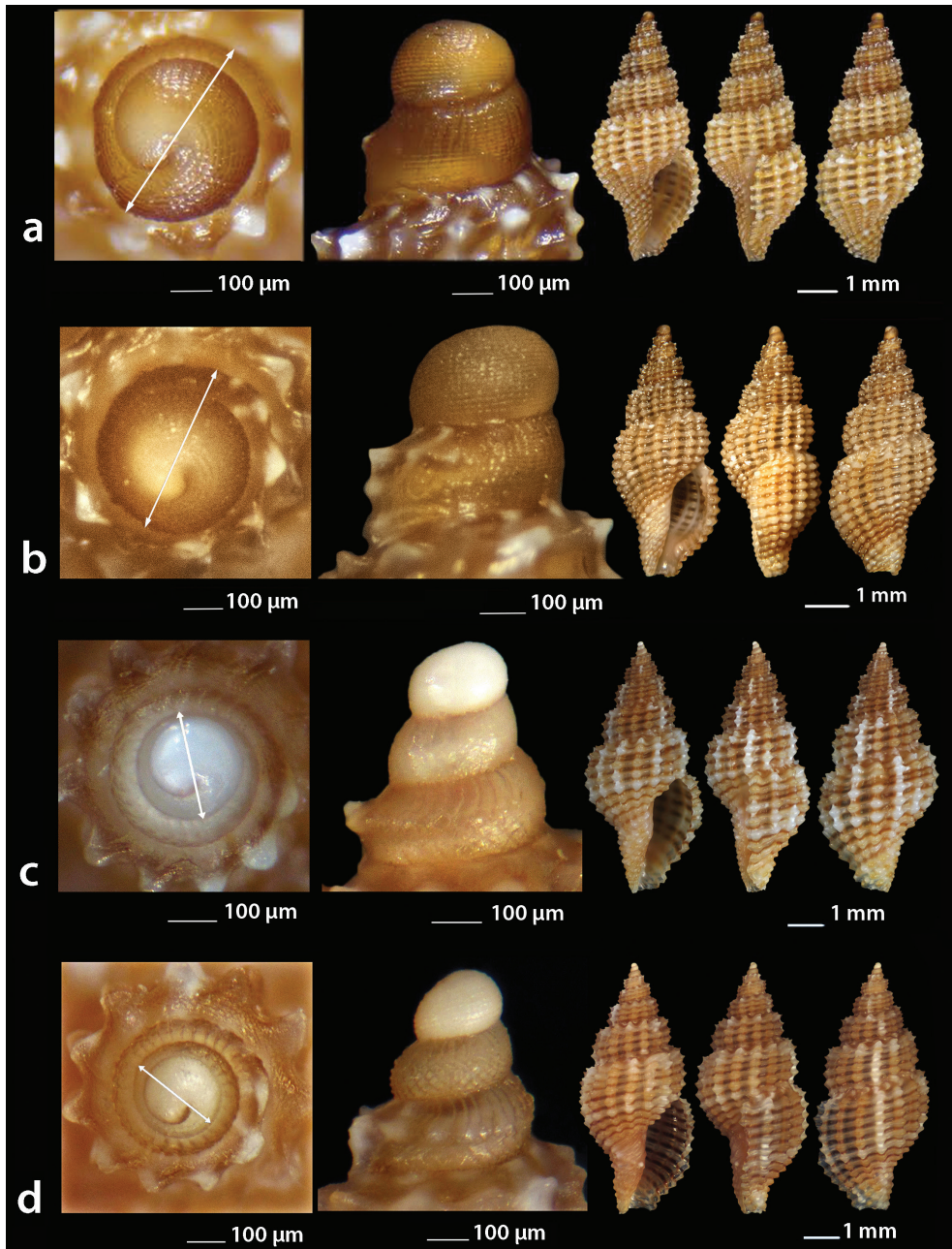


Figure 1. (a and b): *R. spadiana*, (c and d): *R. contigua*.

Status: Uncommon [34]. First record for the Greek seas.

Raphitoma lineolata (Bucquoy, Dollfus & Dautzenberg, 1883) (**Figure 2c and d**).

Collection stations: 44 shells (4.00–8.00 mm long, 1.40–2.80 mm wide) were collected from detritus material trapped in small-scale fishing nets at 10–40 m depth from mixed bottoms of Palioura, Epanomi; Pyrgadikia, Chalkidiki; Psakoudia, Chalkidiki; Anavyssos, Attiki; Legrena, Attiki; Central Saronikos Gulf; Syros Island; and Pantokratoras, Preveza.

Description: The slim, small, thin but solid and fusiform shell is almost 2.5 times as long as wide. Its multispiral protoconch, which is 410 μm (mean) wide and 510 μm (mean) high, bears a protoconch I of 226 μm (mean) and consists of 2.7 convex whorls with a white nucleus decorated with diagonally cancellated striae and the last whorl with a weak keel before the onset of the teleoconch. The later consists of five moderately convex whorls separated by a deep and canaliculated suture with a narrow ramp. The body whorl occupies in certain shells almost 75% and in others almost 60% of the total length, bearing 17–18 orthocone axial ribs with interspaces approximately as wide as the ribs and 16–17 spiral cords slightly thinner than the ribs, six of which are situated above the aperture and the rest below. The spiral cords in their intersections with the axial ribs exhibit mammiliform tubercles. The tubercles on the first two adapical cords are spiny and close to each other. The shell's inner wall viewed through the aperture exhibits a transparency. The aperture occupies something more than 40% of the shells length and exhibits a smooth and S-shaped columella angled at its upper part. The anterior siphonal canal is short and wide, while the posterior one is conspicuous. The outer lip bears 9–10 strong teeth with the first one delimiting the posterior canal and the last the anterior. The whole shell exhibits either a lemon-yellow or a honey-red background color with beige or honey-yellow the tubercles, respectively, and irregularly placed white highlights all over and an interrupted white band as a prolongation of the suture on the body whorl.

Similar species: *R. lineolata* superficially resembles *R. contigua* (Monterosato, 1884), from which it differs in that *R. lineolata* is slender, less robust, bears a narrow subsutural ramp, and has a smaller aperture and only a narrow white band on its body whorl [17, 18].

Habitat and distribution: Infralittoral, on sandy and muddy bottoms. During the day, also under stones and crevices. It can be found live among the rhizomes of *Posidonia oceanica* [38]. Mediterranean Sea [[18, 34, 38], <http://www.conchigliedelmediterraneo.it>].

Status: Uncommon [34, <http://www.conchigliedelmediterraneo.it>]. First record for the Greek seas.

Raphitoma locardi Pusateri & Giannuzzi 2013 (**Figures 3c and d, and 4**).

Collection stations: Two shells (7.90 and 8.00 mm long, 2.90 and 2.95 mm wide, respectively) were collected from detritus material trapped in small-scale fishing nets at about 60 m depth from mixed bottoms of Central Saronikos Gulf.

Description: The solid and fusiform shell is almost 2.3 times as long as wide. Its multispiral protoconch is 400 μm (mean) wide and 440 μm (mean) high, bears a protoconch I of 221 μm (mean)

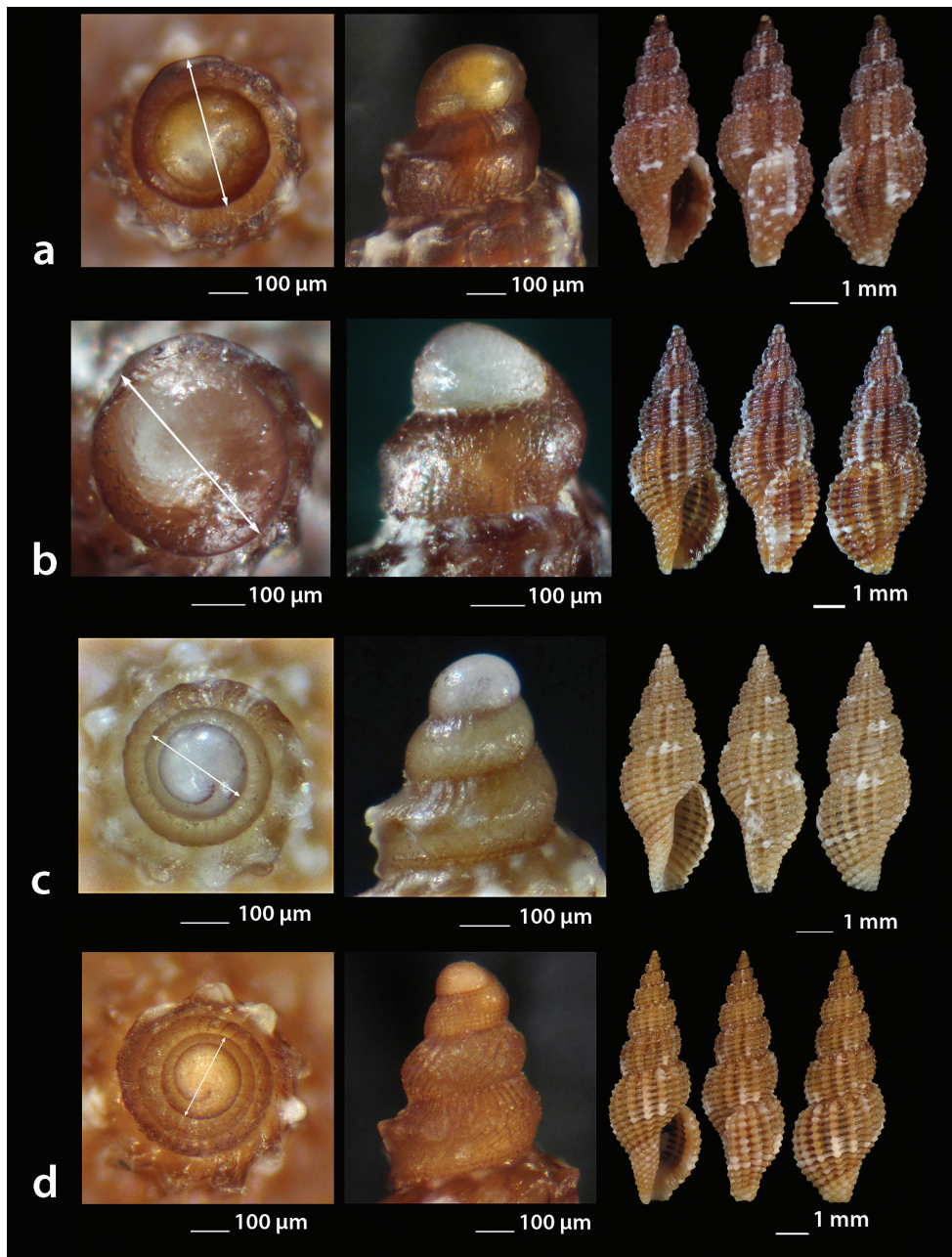


Figure 2. (a and b): *R. smriglioi*, (c and d): *R. lineolata*.

and consists of 3.0 convex whorls with nucleus decorated with diagonally cancellated striae and the last whorl with a weak keel before the onset of the teleoconch. The latter consists of five moderately convex whorls separated by a deep and canalculated suture with a ramp. The inflated body whorl occupies almost 65% of the total length, is rounded at its base, and bears 17–18 opisthocline axial ribs with slightly wider interspaces and 19–20 spiral cords slightly thinner than the ribs, seven of which are situated above the aperture and the rest below. The spiral cords in their intersections with the axial ribs exhibit pointed tubercles that lend a rough appearance. The tubercles on the first two adapical cords are particularly spiky. The shell's inner wall viewed through the aperture exhibits a transparency. The aperture occupies some 44% of the shells length and exhibits a smooth and S-shaped columella, angled at its upper part. The anterior siphonal canal is rather long and wide, while the posterior one is narrow and shallow. The outer lip bears 10–11 strong teeth with the first one delimiting the posterior canal and the last the anterior. The whole shell exhibits a chestnut-purple background color with beige or honey-yellow irregularly placed highlights all over and an interrupted white band as a prolongation of the suture on the body whorl, while the aperture is lilac and the apex dark chestnut-purple with irregularly placed yellow spotlets. The animal's body is gray turning lighter towards the foot, the foot and the antennas are off white with white speckles and there is a white area around the eyes. The siphon is dark gray with irregularly placed white speckles but its white edge.

Similar species: *R. locardi* superficially resembles *R. contigua* (Monterosato, 1884), from which it differs in that *R. locardi* is slender, less robust, bearing a narrow subsutural ramp, and has a smaller aperture and only a narrow white band on its body whorl [17].

Habitat and distribution: Mediterranean Sea [[18, 34], <http://www.conchigliedelmediterraneo.it>].

Status: Uncommon ([34], <http://www.conchigliedelmediterraneo.it>). First record for the Greek seas.

Raphitoma philberti (Michaud, 1829) (**Figure 3a and b**).

Collection stations: 35 shells (8.90–9.15 mm long, 3.70–3.80 mm wide) were collected from *Zostera* bed, 0.2 m, Cape, Epanomi; several depths of Psakoudia, Chalkidiki; Nea Roda, Chalkidiki; Alonissos Island; Amorgos Island; Elaphonisos, Lakonia; Siros Island; Elaphonisi, Crete; Karpathos Island.

Description: Shell solid, fusiform, and almost 2.4 times as long as wide. Its paucispiral protoconch is 358 μm (mean) wide and 420 μm (mean) high and consists of approximately 1.25 convex whorls, a white nucleus decorated with diagonally cancellated striae and bears a weak keel before the onset of the teleoconch. The later consists of five moderately convex whorls separated by a deep and canalculated suture with a ramp. The inflated body whorl occupies almost 65% of the total length, is rounded at its base, and bears 17–18 slightly opisthocline axial ribs with slightly wider interspaces and 17–19 spiral cords slightly thinner than the ribs, 6–7 of which are situated above the aperture and the rest below. The spiral cords in their intersections with the axial ribs exhibit pointed tubercles lending a rough appearance. The tubercles

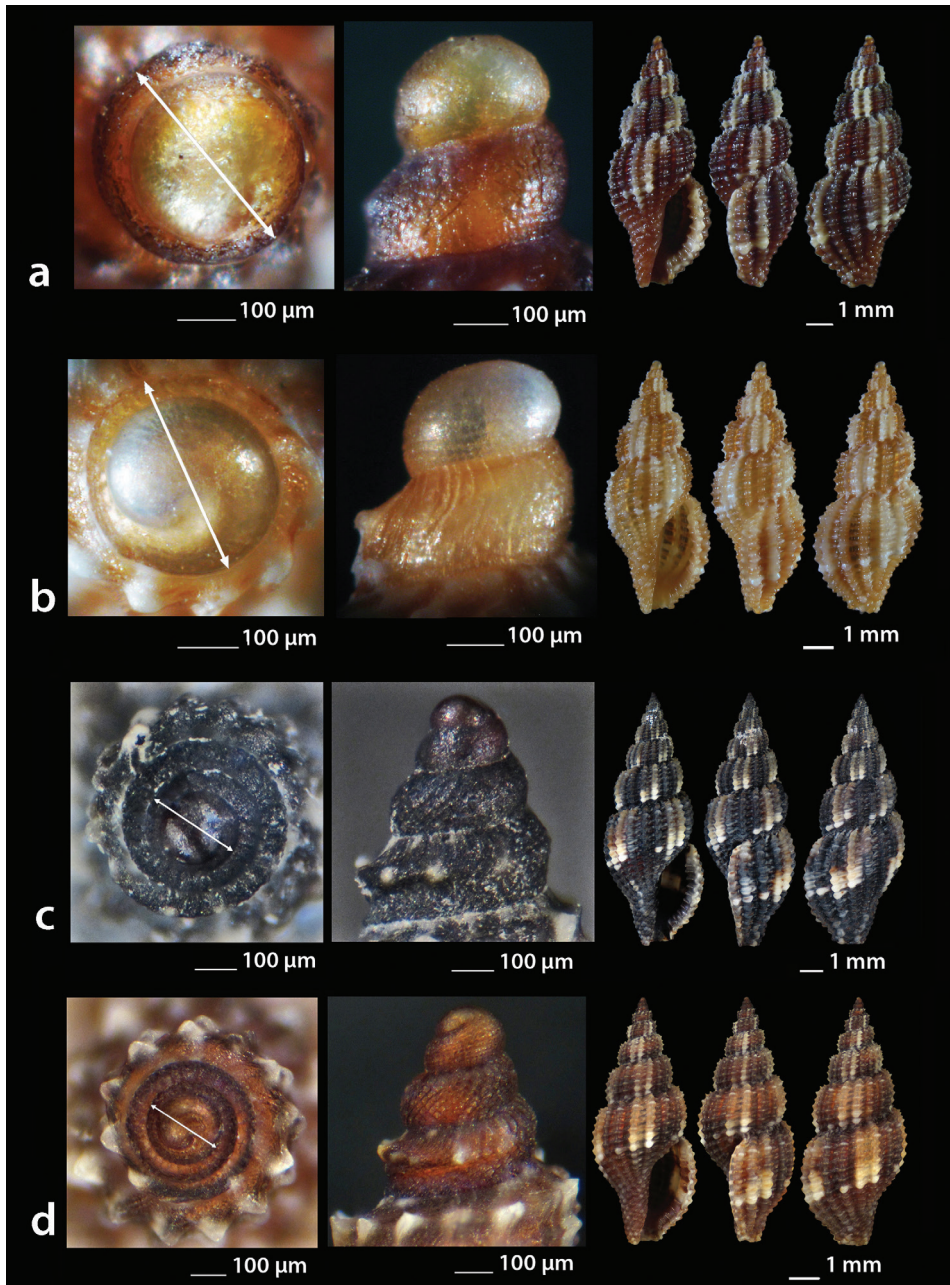


Figure 3. (a and b): *R. philberti*, (c and d) *R. locardi*.



Figure 4. *R. locardi* live individual.

on the first two adapical cords are particularly spiky. The shell's inner wall viewed through the aperture exhibits a transparency. The aperture occupies some 40% of the shells length and exhibits a smooth and S-shaped columella angled at its upper part. The anterior siphonal canal is rather long and wide while the posterior one is narrow and shallow. The outer lip bears 9–11 strong teeth with the first one delimiting the posterior canal and the last the anterior. The whole shell exhibits a chestnut-purple or, rarely, a honey-yellow background color with white or light beige irregularly placed highlights all over and an interrupted white band as a prolongation of the suture on the body whorl, while the aperture is lilac and the apex dark chestnut-purple with irregularly placed yellow spotlets.

Similar species: *R. philberti* superficially resembles *R. contigua* (Monterosato, 1884), from which it differs in that *R. philberti* is slender, less robust, and has a smaller aperture. It differs from *R. locardi*, in which it bears a lecithotrophic protoconch while *R. locardi* bears a planktotrophic one [17].

Habitat and distribution: Mediterranean Sea [18, 34, <http://www.conchigliedelmediterraneo.it>], Central and Eastern Mediterranean Sea [18].

Status: Uncommon [34, <http://www.conchigliedelmediterraneo.it>). Species already reported for the Greek seas.

Raphitoma smriglioi Pusateri & Giannuzzi-Savelli, 2013 (**Figure 2a and b**).

Collection stations: Nine shells (3.75–9.70 mm long, 2.15–3.80 mm wide) were collected from detritus material trapped in small-scale fishing nets at about 50 m depth from mixed bottoms of Central Saronikos Gulf; found beached in Legrena, Attiki; mixed bottom of Pantokratoras, Preveza; *Zostera* bed, 0.2 m, Cape, Epanomi.

Description: The slim, small, thin but solid and fusiform shell is almost 2.5 times as long as wide. Its paucispiral protoconch, which is 375 μm (mean) wide and 390 μm (mean) high, consists of approximately 1.25 convex whorls, bears a white nucleus, and is decorated with irregularly cancellated fine striae. The mature teleoconch consists of five moderately convex whorls separated by a deep and canalculated suture. The body whorl occupies some 70% of the total length and bears 17–18 orthocone axial ribs with interspaces almost twice as wide as the ribs. Spiral decoration of 17–18 spiral cords is slightly thinner than the ribs, six or seven of which are situated above the aperture and the rest below. The spiral cords in their intersections with the axial ribs exhibit small and elongated tubercles. The first two adapical cords are vestigial, spiky and close to each other. The shell's inner wall viewed through the aperture exhibits a transparency. The aperture occupies some 44 % of the shell length and exhibits a smooth and S-shaped columella angled at its upper part. The anterior siphonal canal is short and wide while the posterior one (anal) is conspicuous. The whole shell exhibits a variety of shades from lemon-yellow to honey-red background color with slightly lighter the cords, and irregularly placed white highlights all over.

Similar species: *R. smriglioi* differs from *R. contigua* (Monterosato, 1884) in its paucispiral protoconch and the lack of a subsutural ramp; *R. lineolata* (Bucquoy, Dollfus & Dautzenberg, 1883) in its paucispiral protoconch; from *R. philberti* (Michaud, 1829) in its white and smaller nucleus of the protoconch and its relatively smaller body whorl; from *R. spadiana* Pusateri & Giannuzzi-Savelli, 2012 in the lack of a subsutural ramp and in the protoconch which is slightly smaller and slender in *smriglioi* (395 $\mu\text{m} \times 400 \mu\text{m}$) while almost 10% larger in *R. spadiana* (425 $\mu\text{m} \times 450 \mu\text{m}$) [17, 18].

Habitat and distribution: Mediterranean Sea [18, 34, <http://www.conchigliedelmediterraneo.it>], Central and Eastern Mediterranean Sea [18].

Status: Uncommon ([35]; <http://www.conchigliedelmediterraneo.it>). First record for the Greek seas.

Raphitoma spadiana Pusateri & Giannuzzi-Savelli, 2012 (**Figure 1a and b**).

Collection stations: Two live specimens and three shells (2.00–9.80 mm long, 1.05–4.00 mm wide) were collected: detritus material trapped in small-scale fishing nets at 60–80 m depth from mixed bottoms of Kardamili, Messinia and South Saronic Gulf.

Description: The shell is hyaline, fusiform and almost 2.3 times as long as wide. Its paucispiral protoconch, which is approximately 457 μm (mean) wide and 470 μm (mean) high, consists of

almost 1.35 irregularly cancellated and convex whorl, the first of which is decorated only with fine spiral striae. The teleoconch consists of five convex whorls separated by a deep suture. The body whorl occupies almost 65% of the total length and bears 16–17 orthoconical axial ribs with interspaces approximately two times wider than the ribs themselves and 19–20 spiral cords thinner than the ribs, six of which are situated above the aperture and the rest 14 below the aperture. The spiral cords in their intersections with the axial ribs form erasures in the form of small elongated rectangular tubercles. The tubercles on the first two adapical cords are spiky and close to each other. The shell's inner wall viewed through the aperture exhibits a transparency. A narrow ramp is evident immediately below the suture, formed by the vestigial first two spiral cords and the much prominent and spiky third cord. The aperture occupies approximately 45% of the shell's length and exhibits a smooth and slightly sinuous columella in its lower part, angled at its upper part. The anterior siphonal canal is short and wide, while the posterior one is deep and narrow. The outer lip bears 11 strong teeth with the first one delimiting the posterior canal and the last the anterior. The shells are of yellow-beige background color, while the tubercles and some irregularly situated areas or isolated tubercles are of lighter color. The body whorl usually bears at its middle a lighter color spiral band as a prolongation of the suture.

Similar species: *R. spadiana* is different from: *R. alternans* (Monterosato, 1884), in which the latter is slender and with a different color pattern; *R. atropurpurea* in its color which is light red-brown instead of purple-brown in *R. atropurpurea* and in its more inflated spire; *R. contigua*, in which the latter bears a multispiral protoconch, is larger, of lighter color, and more robust; *R. densa* (Monterosato, 1884) in the color and the less dense sculpture; *R. lineolata* (Bucquoy, Dautzenberg and Dollfus, 1883), in which *R. lineolata* has a less robust shell, a more narrow aperture, and a more narrow subsutural ramp; *R. oblonga* (Jeffreys, 1867) in its wider aperture and the different color pattern [17, 18].

Habitat and distribution: Whole Mediterranean Sea [17].

Status: Uncommon [17]. First record for the Greek seas.

4. Discussion

It is generally accepted that the morphology of the gastropod protoconch determines the mode of development and the duration of larval stage in the ontogenesis [3, 6, 11]. We are of the opinion that such a change in the larval morphology in members of different genetically isolated molluscan lower taxa must have taken place independently and must be due to a simple genetic change involved in the cell's contingency. Otherwise, it would be rather improbable for the same, yet complex, genetic changes to have taken place simultaneously in different individuals belonging to different taxa. Point mutations or even reverse mutations could easily occur leading also to switches. Such multiple switches in the mode of protoconch development are shown to have occurred in the evolutionary history of the Indo-Pacific *Kermia*–*Pseudodaphnella* complex and the diversity of protoconch morphologies exhibited in this group points to a high developmental and evolutionary plasticity [10].

Both, planktotrophy and lecithotrophy commence with an initial short lecithotrophic stage equipped with the larval shell I. The initial difference between the two types of larval shells I lies in their size which, in turn, is associated with the size of the egg. Larval shell I, leading to multispiral protoconch II, is smaller in width and accounts for the planktotrophic developmental mode, whereas larval shell I, directly linked to lecithotrophy (paucispiral), is larger. In the case of the Mediterranean pair of the so-called sibling species, *R. spadiana* and *R. contigua* (**Figure 1**) were examined in this work; the first bears a larger lecithotrophic larval shell (protoconch I), while the second bears a smaller protoconch I (of its planktotrophic larval shell) of a diameter that corresponds to 3 additional cell cycles (**Table 1**) of the stem cells. In the case of *R. smriglioi* and *R. lineolata* (**Figure 2**), the size of the protoconch I of the latter corresponds to 2 additional cell cycles (**Table 1**), while in that of *R. philberti* and *R. locardi* (**Figure 3**) also to 2 (**Table 1**). This kind of difference in the size of protoconch I could be attributed simply to a single gene intervening in the control of the germ line cell cycle in the gonads, functioning either in favor of a few additional mitoses and thus to a larger number and smaller germ cells and eggs (reduced parental investment per offspring) and eventually smaller in diameter (by a factor of approximately 0.7937 per cell cycle) stage I embryos, or in favor of no further mitoses and thus to a smaller number of larger germ cells, eggs and embryos (increased parental investment per offspring). At the same time, the reproductive output per adult biomass (total energy expenditure or reproductive effort) in those alternative gene actions would not constrain any of the two types of larval development; otherwise, all mollusks should tend to have the same mode of development if that mode gave higher returns per effort [50].

Larger embryos (lecithotrophic) leave the water column early, while smaller ones (planktotrophic) later and thus continue with the development of protoconch II. In spite that time latency, both types of larvae eventually lose their buoyancy and sink. There seems to be no

| Pair of 'sibling' species | Measured lecithotrophic protoconch I maximum diameter (μm) (mean) | Expected planktotrophic protoconch I maximum diameter (μm) after one additional cell cycle | Expected planktotrophic protoconch I maximum diameter (μm) after two additional cell cycles | Expected planktotrophic protoconch I maximum diameter (μm) after three additional cell cycles | Measured planktotrophic protoconch I maximum diameter (μm) (mean) | Corresponding additional cell cycles |
|---|--|---|--|--|--|--------------------------------------|
| <i>R. spadiana</i> – <i>R. contigua</i> | <i>R. spadiana</i> 457 | <i>R. contigua</i> 363 | <i>R. contigua</i> 88 | <i>R. contigua</i> 228 | <i>R. contigua</i> 230 | 3 |
| <i>R. smriglioi</i> – <i>R. lineolata</i> | <i>R. smriglioi</i> 375 | <i>R. lineolata</i> 297 | <i>R. lineolata</i> 235 | <i>R. lineolata</i> 186 | <i>R. lineolata</i> 226 | 2 |
| <i>R. philberti</i> – <i>R. locardi</i> | <i>R. philberti</i> 358 | <i>R. locardi</i> 284 | <i>R. locardi</i> 225 | <i>R. locardi</i> 179 | <i>R. locardi</i> 221 | 2 |

Table 1. Expected and measured maximum diameter of protoconch I in planktotrophic sibling species after hypothetical additional cell cycles. Bold characters indicate concurrence between expected and measured protoconch I maximum diameter.

reason why, at least some of those two different types of larvae, not to find themselves in the same locality and, as the initial mutation responsible for the differentiation of the larval mode of life has not led to the establishment of a genetic barrier, when maturation is reached, to interbreed. There are no publications on interbreeding of Mediterranean 'sibling' species so that one can draw conclusions on the existence of a genetic barrier and thus to a confirmation that loss of either planktotrophy or lecithotrophy in the past has eventually led to speciation.

If there is no genetic barrier, then a rising question is associated with the type of inheritance imposed by the initial mutation on that gene controlling the germ cell cycle prior to meiosis. If it displayed a Mendelian inheritance, we would expect also the production of heterozygotes exhibiting a kind of semi-planktotrophic mode of life of shorter duration and presumably protoconches with fewer whorls. In conclusion, someone would expect to find in the same morphological species all three types of protoconches, e.g., paucispiral, multispiral, and intermediate. As this is not the case, at least in Mediterranean *Raphitoma* species [17, 18], we are inclined to propose that the mutated gene cooperates in conjunction with other genes and environmental factors in a discontinuous multifactorial inheritance in which environmental or even population factors also effect a threshold. *Ceteris paribus*, below that threshold, the animals would produce fewer and larger germ cells giving rise through meiosis to fewer and larger eggs that after fertilization produce large lecithotrophic embryos with large paucispiral protoconch I, while, above that threshold, more and smaller germ cells would be produced leading after meiosis to smaller eggs which eventually give rise to planktotrophic larvae with small protoconch I and large multispiral protoconch II. Those two possibilities would jointly constitute protoconch-related poecilogony, a phenomenon already known in some sacoglossan mollusks [51].

In support to the above hypothesis, there are at least 10 pairs of similar, most probably closely related species of *Raphitoma*, one of which bears planktotrophic protoconch and the other lecithotrophic, often sampled in the same localities [17, 18]. That implies the same mode of adulthood life supported by a common gene pool maintained by free gene exchange. Populations of such pairs would employ simultaneously (under different environmental conditions) different dispersal strategies that might reduce interspecific competition. Apparently, long-living planktotrophic larvae maintain a wide geographic range of a species and high genetic integrity between distant subpopulations [11]. Comprehensive accounts on the benefits of these strategies are already given [10, 50]. At the same time, it is generally accepted that, in shelled molluscs, the presence or the absence of any nutritional resource during development affects egg size, which, in turn, affects the size, the number of whorls, and the morphology of the protoconch [1, 52].

Apart from the loss of planktotrophy (in our view, in some members of the same population, as mutations are random phenomena) in Raphitomidae, there is also a well-documented tendency for repeated loss of other conoidean important foregut structures such as radula, proboscis, and venom gland without alteration of the teleoconch morphology [12, 53–55]. Nevertheless, it is worth noting that the loss of planktotrophy in some turrids, like *Raphitoma*, is not necessarily related to simplification in shell morphology [56] which means that the teleoconch morphology could remain unaltered in a species population consisted of individuals with either lecithotrophic or planktotrophic protoconch. We are of the opinion that at least

some of the Mediterranean *Haedropleura*, *Mangelia*, and *Bela* species also fall in the same case as *Raphitoma*, but we conservatively refrain from formally proposing that possibility pending the mitochondrial DNA markers analysis and employment of interbreeding experiments that could answer the questions raised and solve existing paradoxes.

Finally, through the present work the Raphitomid fauna of the Hellenic sea has been enriched by five new members as the search targeted appropriate environments such as biogenic backgrounds, maerl beds, and deeper waters.

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