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ON THE EGG MASSES, EGGS AND EMBRYOS OF *NOTOCOCHLIS ISABELLEANA* (D'ORBIGNY, 1840) (GASTROPODA: NATICIDAE) FROM NORTHERN PATAGONIA

Guido Pastorino*, Andres Averbuj & Pablo E. Penchaszadeh

Museo Argentino de Ciencias Naturales – CONICET
Av. Angel Gallardo 470, 3° Piso, Lab 57, C1405 DJR Buenos Aires, Argentina

INTRODUCTION

Naticids are common predators in sandy bottoms of shallow waters around the world. Probably due to their infaunal habitat, they are hard to find alive, particularly in the Patagonian waters of Argentina. Nevertheless, their presence is confirmed by the typical predation boreholes that can be seen on their bivalve prey along the coast (e.g., Bromley, 1981; Carriker, 1981; Pastorino & Ivanov, 1996; Signorelli et al., 2006). These boreholes record information on predators without actually seeing them. Paleontologists have exploited this field, analyzing the behavior and producing models of predator-prey relationships in ancient and recent communities (Kabat, 1990, and citations herein).

Studies on the spawn of naticids are scarce in modern literature, despite its common presence in sandy bottoms. The typical sand collars were recognized several decades ago as belonging to this family. Ankel (1930) and Thorson (1935, 1940, 1946), among others, described these characteristic egg masses from different areas around the world. An accurate account of the morphology of the collar and capsules, including the way the gastropod builds them, was described by Giglioli (1955) and Ziegelmeier (1961). An interesting approach was recently published by Huelsken et al. (2008), who reviewed the naticids from Giglio, an Italian island off the coast of Tuscany. They compared mitochondrial and nuclear gene fragments (COI, 16S, H3, 18S) from adults and embryos from the egg masses, leaving no doubt about the mother species of each egg mass.

During the past few years, several papers were published in which egg capsules of various gastropods from the southwestern Atlantic were described (Pastorino & Penchaszadeh, 1999, 2002; Pastorino et al., 2007; reviewed in Gallardo & Penchaszadeh, 2001). However, there is no published information about naticids from the southwestern Atlantic. Similarly, the systematics on the group is far from complete. Pastorino (2005) mentioned 13 species of Naticidae living in the Patagonian coast and subantarctic islands. One of them, *Notocochlis isabelleana* (d'Orbigny, 1840), usually known under the genus *Natica*, is frequently found in the sandy infralittoral of northern Patagonia and southern Brazil. It actually ranges from Rio de Janeiro, Brazil, to Golfo Nuevo, Valdés Peninsula in the Argentine province of Chubut and is a typical representative of the Argentine malacological province.

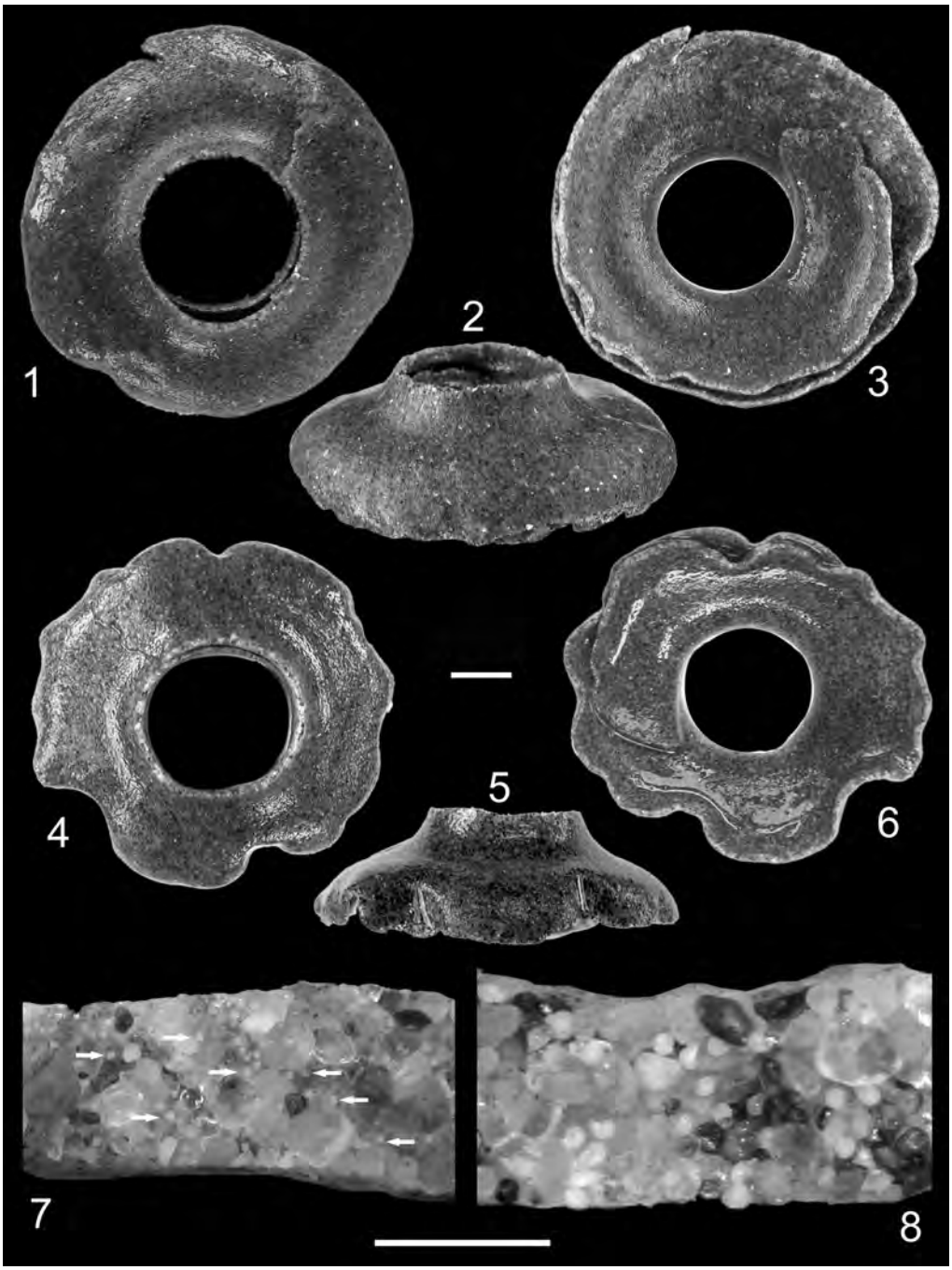
In this paper, the egg mass, egg capsules, eggs, and embryos of *Notocochlis isabelleana* are described. A comparative table with data on other naticid spawn from different geographic regions is provided.

MATERIAL AND METHODS

Thirty complete egg masses of *N. isabelleana* were collected in the subtidal sandy bottom of the following localities: Caleta Falsa, San Antonio Oeste, Rio Negro, 40°47'S, 64°50'W (April 2008); Puerto Pirámides, 42°35'S, 64°16'W (November 1992, October 1993); Punta Pardelas, 42°37'S, 64°15'W (October 2007); Punta Este and Playa Paraná, Puerto Madryn, Chubut, 42°47'S, 64°57'W (October 2000 and April 2008) by SCUBA diving in ~5–10 m of depth. Most of them were complete, although a few were crushed during transport rendering the provenance of pieces impossible to establish. Collar measurements were done on complete egg masses only. The general shape of the collars was recorded, including the margins. Apical and basal diameters and thickness were measured with calipers.

There is a reasonable doubt about the exact identification of the egg masses, because none of them were collected when the animal was

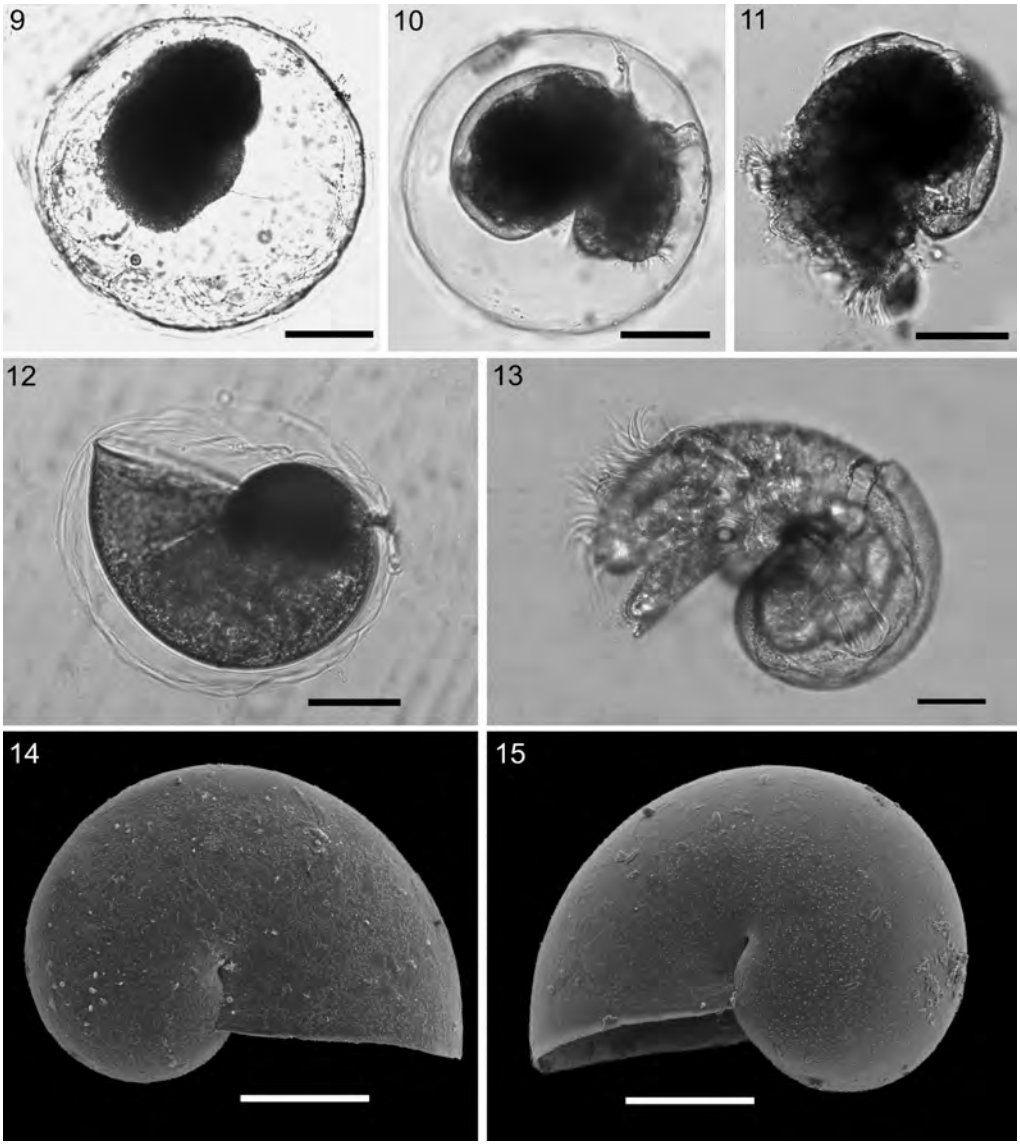
*Corresponding author: gpastorino@macn.gov.ar



FIGS. 1–8. Egg masses of *Notocochlis isabelleana*. FIGS. 1–6: Apical, basal and lateral view of two egg masses of *Notocochlis isabelleana* (d'Orbigny, 1840); FIG. 7: Section of the collar showing the arrangement of embryos (arrows) among the sand grains; FIG. 8: Section of the collar with embryos with shell developed. Scale bars = 1 cm.

actually spawning. However, *N. isabelleana* is the only naticid living in the area and particularly at the depth where the material was collected. Similarity in the morphology and size of the egg masses, eggs, and embryos confirmed that all the spawns here studied belongs to this species.

Egg capsules, eggs, and embryos from all spawns representing different stages of development were measured under a microscope, with a 0.01 mm precision ocular micrometer and a Zeiss Axio Imager Z.1 microscope. Scanning electronic microscope images of the embryo shells were taken at the Museo Argentino de Ciencias Naturales (MACN) with a Philips LX 30. The main developmental features of each stage were described.



FIGS. 9–15. Embryonic stages. FIG. 9: Morulae; FIG. 10: Early “veliger” (intracapsular); FIG. 11: Late “veliger”; FIG. 12: Embryonic shell at hatching stage, FIG. 13: Veliger hatched larvae; FIG. 14: SEM of an embryonic shell, apical view; FIG. 15: SEM of another embryonic shell, basal view. Scale bars = 50 µm.

TABLE 1. Egg capsules, egg and embryo sizes of *Notocochlis isabelleana* (d'Orbigny, 1840) in µm.

	Egg capsule diameter	Uncleaved egg diameter	2-cell stage	8-cell stage	Morula	Early "veliger"	Shelled hatchling
Mean Size	185.2	97.6	148.3	132.4	123.4	133.3	151.5
SD	15.1	10.2	9.6	9.4	5.4	8.3	9.6
Range	153.7–235.3	73.5–114.4	119.1–162.9	109.2–153.4	112.4–136.4	118–150	137.7–167.7
n	202	24	38	61	55	32	24

RESULTS

Spawn consists of a 1–1.5 whorl spiral collar made of sand grains of two different diameters, the basal larger than the apical (Figs. 1–6). Compared to other members of the family, the collar is large. Basal diameter ranges between 35–67 mm, with an average of 45 mm (n = 23). Apical diameter is 14–28 mm, with and average of 19 mm. Thickness in its middle part never exceeds 2.5 mm (X = 1.9). The basal margin is always plicated and the apical one smooth.

The egg capsules are small and thin, of 185.21 ± 15.07 µm (X ± SD; N = 202) in maximum diameter, suboval in shape. The distribution on the collar around sand grains apparently never follows a specific pattern. Nevertheless, the egg capsules seem to be situated in the middle of the sand ribbon, between two layers of sand grains that keep them somewhat isolated from the outside (Figs. 7, 8). Each egg capsule hosts a unique round whitish egg. The diameter of the uncleaved egg measures 97.57 ± 10.2 µm (X ± SD; n = 24). We found the following stages according to the number of cells developed: two cells (148.34 ± 9.55 µm; n = 38), eight cells (132.4 ± 9.35 µm; n = 61), morulae (123.35 ± 5.43 µm; n = 55) (Fig. 9), and early "veliger" (133.28 ± 8.31 µm; n = 32) (Figs. 11, 12).

At hatching, a free swimming planktotrophic larva emerges, with one pair of eye spots, as well as statoliths, a bilobed unpigmented velum, and a corneous operculum (Fig. 13). The larval shell measured 151.48 ± 9.60 µm (X ± SD; n=24) maximum length (measurements in Table 1).

DISCUSSION

Giglioli (1955) proposed a classification of several naticid egg masses, with the aim of recognizing them in the absence of the spawning female. Classification by external morphology resulted in two large groups: thick (more than 1.5 mm thick) with rigid walls, and thin (less than 1.5 mm thick) with flexible walls. The first group has collars of 60 mm maximum height and the second of more than 85 mm. Each group includes two other divisions according to the morphology of the basal margin, smooth or plicated.

Gohar & Eisawy (1967) studied the egg masses of two naticids from the Red Sea, *Polinices mammilla* (Linnaeus, 1758) (as *Natica (Mamma) mamilla*) and *Mammilla melanostoma* (Gmelin, 1791) (as *Natica*). They criticized Giglioli's classification of the collars, because it is not completely applicable to the latter species. Essentially these authors agree with the external classification and disagree with the internal arrangement of the masses.

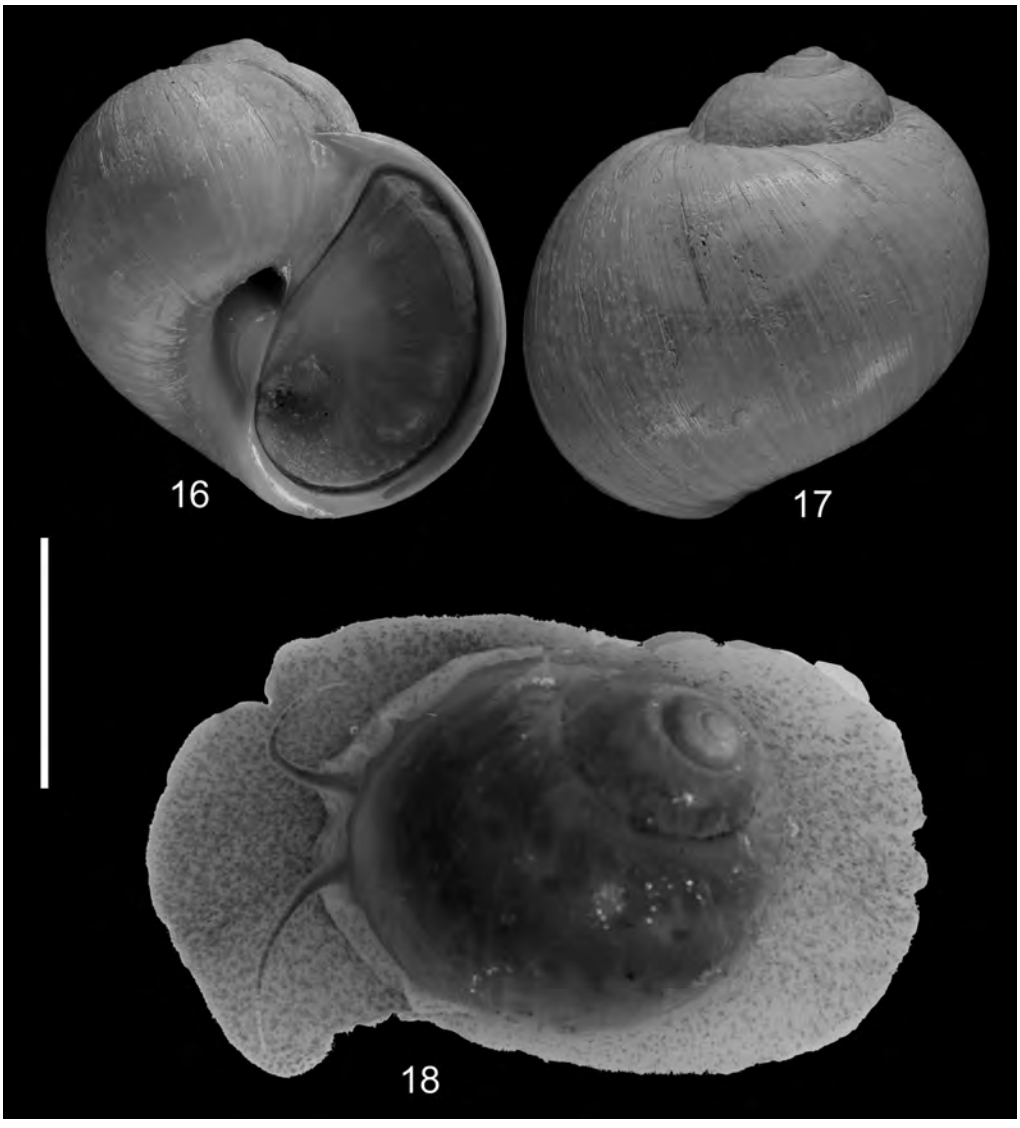
According to Giglioli's (1955) classification of the egg masses, *N. isabelleana* belongs in the "thin" group, with walls generally less than 2 mm of thickness.

There is only one egg of approximately 100 microns of maximum diameter in each capsule. No extraembrionic food and no nurse eggs were recorded. The egg capsules are very small, with little, completely translucent liquid. The embryo hatches as a planktotrophic veliger larvae with a maximum shell length of 185 microns. The size of the uncleaved egg diameter and therefore the small size of the free larvae could result in an extended period in the plankton. In fact, this contrasts with species of this family from other latitudes where either complete intracapsular development with large egg diameters (e.g., *Cryptonatica clausa*, a circumboreal species) have been described, or else with nurse eggs (e.g., *Euspira catena* from the North Sea and Mediterranean). As most of other studied naticid species (Thorson, 1940; Giglioli, 1955; Table 2), only one embryo per capsule develops in *N. isabelleana*.

According to Pedersen & Page (2000), naticids hatch in three ways: two with swimming larvae, planktotrophic and lecithotrophic, and

TABLE 2. Comparison of spawn, egg capsules and hatchling shells measurements of naticids. Sources are: Thorson, 1935[1], 1940[2]; Giglioli, 1949[3], 1955[4]; Natarajan, 1957[5]; Fioroni, 1966[6]; Gohar & Eisawy, 1967[7]; Ziegelmeier, 1961[8]; Bandel, 1975[9], 1976[10]; Pedersen & Page, 2000[11]; Kingsley-Smith et al., 2005[12]; Huelsken et al., 2008[13].

Species and source	Collar size (in mm)		Egg capsules				Larval shell length (in µm)
	Basal diameter (apical)	Thickness	Diameter (in µm)	Embryos per capsule	Nurse cells	Emergent form	
Amauropsis islandica (Gmelin, 1791)[1]	?		1,500–1,750	1	No	Crawling juvenile?	750
Cryptonatica clausa (Broderip & G. B. Sowerby I, 1829)[1]	40		2,250	1	No	Crawling juvenile	1,500
Euspira catena (da Costa, 1778)[3], [6]	73 (47)	1.5–2.2	1,425–1,925	2–4	Yes	Crawling juvenile	800
Euspira heros (Say, 1822)[3]	25–73	0.9–1.1	630–850	4–84	No	Veliger	130–150
Euspira lewisii (Gould, 1847)[4], [11]	195 (75)	2.0–2.3	190–260	1	No	Veliger	180
Euspira nitida (Donovan, 1803)[8], [12]	30		208–0.254	1	No	Veliger	190–200
Euspira pallida (Broderip & G. B. Sowerby I, 1829), as *E. groenlandica* (Möller, 1842)[1]	45 (12–13)		2,500–3,000	1	No	Crawling juvenile	1,500–1,750
Euspira triseriata (Say, 1826)[3], [4]		1	850–1150	1–3	No	Crawling juvenile	500
Glossaulax didyma (Röding, 1798), as *G. ampla* (Philippi, 1848)[2]		1		1		Crawling juvenile	600–700
Mammilla melanostoma (Gmelin, 1791)[7]	70	0.6–1.6				Veliger	200
Natica livida Pfeiffer, 1840[9], [10]	15–17	0.7		3–6		Veliger	
Natica marochiensis (Gmelin, 1791)[5]	35–50	0.45–0.52	183–300	3–7	No	Veliger	100–116
Natica tigrina (Röding, 1798)[5]	56–79	0.8–1.1	251–284	1		Veliger	200–250
Natica trailli Reeve, 1855[2]		1		1		Veliger	310
Natica vitellus (Linnaeus, 1758), as *N. rufa* (Born, 1778)[2]		0.5–1		1	No	Crawling juvenile	800
Naticarius canrena (Linnaeus, 1758)[9], [10]	80–120	1.0	400	1		Veliger	560
Neverita duplicata (Say, 1822)[4]		1.0–1.2	190–250	1	No	Veliger?	180
Neverita josephinia Risso, 1826[13]	51–59			1		Crawling juvenile	780
Notocochlis isabelleana (d'Orbigny, 1840)	45 (19)	< 2.5	185	1	No	Veliger	151
Polinices hepaticus (Röding, 1798)[9], [10]	40–45		200–300	1		Veliger	280
Polinices lacteus (Guilding, 1834)[9], [10]	23–43 (37–40)		100	1		Veliger	400
Polinices mammilla (Linnaeus, 1758)[7]	36–80	1		1		veliger	130
Tectonatica sagraiana (d'Orbigny, 1842)[13]	24–40			1		Veliger	300



FIGS. 16–18. Adult of *Notocochlis isabelleana* (d'Orbigny, 1840). FIGS. 16, 17: Two views of a shell; FIG. 18: Living adult with the foot extended. Scale bar = 1 cm.

crawling juveniles. *Notocochlis isabelleana* hatches as planktotrophic larvae. This could explain, at least in part, the wide geographic distribution of the species along the southwestern Atlantic coast, although Johannesson's (1988) study proved at least in part that transport of benthic stages other than planktonic free larvae could be a dispersal factor.

Gallardo & Penchaszadeh (2001) postulated that sand environments in the southwestern Atlantic have a large percentage of species with an encapsulated complete development, and hatchlings are crawling juveniles. *Notocochlis isabelleana* (Figs. 16–18) constitutes an exception to this pattern, despite its clear association to soft bottoms.

The morphology of the egg masses of *N. isabelleana*, as in all Naticidae, shows a peculiar adaptation to soft bottoms. The shape of the collar fixes the whole spawn to the bottom, allowing, however, good aeration. In addition, the wavy basal margin is apparently a very effective deterrent for the accumulation of sand that could hamper water circulation and ventilation of embryos and eggs during development (Gohar & Eisawy, 1967).

As far as we can see in sections of the sand collar, there is a selection of the place where most of the egg capsules are settled. Thus, the capsules in the middle of the sand grains gain some protection.

Table 2 shows different spawn measurements of naticid species from several regions. The problem comparing these species is the unresolved taxonomy. Studies on the phylogeny of the family are needed, and the relationship among genera is far from understood. *Notocochlis isabelleana* has an extremely small veliger shell, only comparable to several species of *Natica* s. l. (i.e., *N. marochiensis*), *Neverita*, *Polinices,* and *Euspira* (i.e., *Neverita duplicata*, *Polinices mammilla* and *Euspira lewisii*), despite the fact that these genera belong in different subfamilies. In addition, the same table also shows that all species with a crawling juvenile have a larval shell length larger than 310 microns, and those with free veliger larvae are smaller. As can be seen in the literature, nurse eggs are not the rule among the naticids or at least they are not as common as in the Muricidae. Most of the known species have only one embryo per capsule, however some showed a very variable number (i.e., *E. catena*, *E. heros* and *N. marochiensis*).

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