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ORIGINAL PAPER

Giant egg capsules and hatchlings in a deep-sea moon snail (Naticidae) from a southwestern Atlantic Canyon

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Abstract The globose moon snail *Bulbus carcellesi* Dell, 1990 (Naticidae), and its egg masses were collected from the Mar del Plata Submarine Canyon at depths of 201–2082 m) in August 2012 and May 2013. Embryos of this species undergo encapsulated development, and the egg capsules are the largest ever recorded for this family, 8.8–14.1 mm in diameter. The largest naticid egg capsule previously recorded was only 3 mm. Each egg capsule contains a single, 200-μm diameter egg and a considerable amount of white material (supplementary food), which allows the embryo to grow to an enormous size (up to 6.0 mm in shell diameter) before hatching as a crawling juvenile. The volume of this juvenile shell is 45 times the volume of previously reported naticid hatchlings. This great size and the number of whorls in the hatchling shell suggest a slow rate of development, akin to many other deep-sea invertebrates.

Introduction

The egg masses of the Naticidae are easily recognized, since the great majority are sand-encrusted rings or collars (Ankel 1930; Thorson 1935, 1940, 1946; Lebour 1936; Giglioli 1955; Natarajan 1957; Amio 1963; Gohar and Eisawy 1967; Bouchet and Warén 1993; Huelsken et al. 2008; Pastorino et al. 2009). The exceptions are species in the genus *Conuber* (Polinicinae) which have gelatinous egg masses, very different from the other naticids (Murray 1966). Giglioli (1955) reviewed the variability in egg masses and egg capsule spaces (the cavity or chamber where the egg capsules are laid) which Thorson (1935) called globular egg cavities.

Most naticids have a planktonic veliger stage, but in a few species hatchlings emerge as crawling juveniles (Ankel 1930; Thorson 1935, 1936, 1940, 1946; Lebour 1936; Giglioli 1955; Natarajan 1957; Amio 1963). In the naticids with encapsulated development and no pelagic larval stage, the egg capsule contains a considerable amount of white substance (supplementary food in the intracapsular fluid), with the exception of *Euspira catena* which has nurse eggs (Ankel 1930; Hertling 1932; Lebour 1936; Thorson 1946; Giglioli 1955).

In this study, we describe the egg mass of *Bulbus carcellesi* Dell, 1990, a moon snail from deep waters off Buenos Aires Province, Argentina, including a characterization of the egg mass, egg capsule spaces and embryonic development.

Materials and methods

In samples from 66 stations in the Mar del Plata Submarine Canyon area (38°S/54°W), 17 egg masses and 21 adults identified as *B. carcellesi* were collected during three cruises to the Argentine Continental Slope aboard the National Scientific and Technical Research Council's ship R/V "Puerto Deseado" (Table 1). The samples were preserved in 4 % formalin in sea water. The egg masses were examined under a stereomicroscope

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Table 1 Stations of R/V "Puerto Deseado" in Mar del Plata Submarine Canyon in 2012 and 2013 where *Bulbus carcellesi* and its egg masses were collected

Station	Latitude S	Longitude W	Depth (m)	Date	Fishing gear	# Egg masses	# Capsules egg mass^{-1}	# Adult *B. carcellesi*
1	37°58′	55°13′	201	10 Aug-12	Dredge trawl	1	2	
3	38°00′	55°13′	250	10 Aug-12	Fishing net	4	1–5–3–3	5
2	37°57′	55°11′	291	10 Aug-12	Dredge trawl	1	2	
33	37°59′	55°12′	308	17 Aug-12	Fishing net	2	4–4	
32	38°00′	55°12′	319	17 Aug-12	Dredge trawl	1	3	
8	37°58′	54°57′	647	10 Aug-12	Fishing net	5	2–1–2–1–1	3
44	37°54′	54°43′	780	26-May-13	Fishing net			1
14	38°01′	54°30′	1006	10 Aug-12	Fishing net	3	4–4–4	3
37	38°00′	54°24′	1275	25-May-13	Dredge trawl			8
21	38°08′	53°51′	2082	13 Aug-12	Fishing net			1

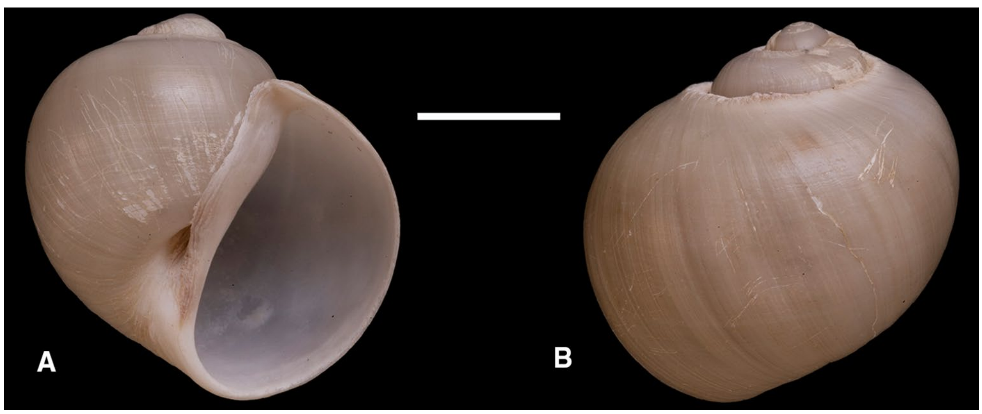

Fig. 1 *Bulbus carcellesi*: adult shell in apertural (**a**) and adapertural (**b**) view. *Scale bar* 1 cm

and measured with a caliper. Collar measurements were recorded for complete egg masses only. Embryos and shells were measured using a 0.01-mm precision ocular micrometer.

Radulae were prepared according to the method described by Pastorino (2005). Radulae of embryos were prepared by dissolving the whole individual in sodium hypochlorite and coating with gold–palladium in the usual way to be observed under a scanning electron microscope (SEM). Embryos were critical point dried and observed using a Philips XL 30 SEM at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires (MACN). The main developmental features of each stage were described. Images of the egg masses and adult shells were recorded using a digital camera Nikon D100 with a 60-mm lens. All images were digitally processed. After the study, the material was preserved in ethanol 70 %. Specimens and egg masses have been deposited in the Invertebrate collection of the MACN (MACN-In 40719; 40720; 40721; 40722).

Results

Species identification

Adult snails, *Bulbus carcellesi*, are easily recognized because, among the species of naticids living in the south-western Atlantic Ocean, it is the only one with a large, thin shell and a low spire with ~5 whorls, the last of which is globose (Fig. 1).

A comparable shell is "*Natica*" *limbata*, a shallow-water species with a calcareous operculum and a completely different radula (Pastorino 2005). The radulae extracted from the embryos and adults of *B. carcellesi* have the typical hemispherical, triangular rachidian tooth, with only one large cusp and two lateral processes and conspicuous denticles in the rachidian base (Fig. 2), which characterize the genus. The laterals have triangular cusps, and the marginals are very long; the inner is always bicuspid. The radula and corneous operculum are characteristic, leaving no doubt about its identification among the thirteen naticid species

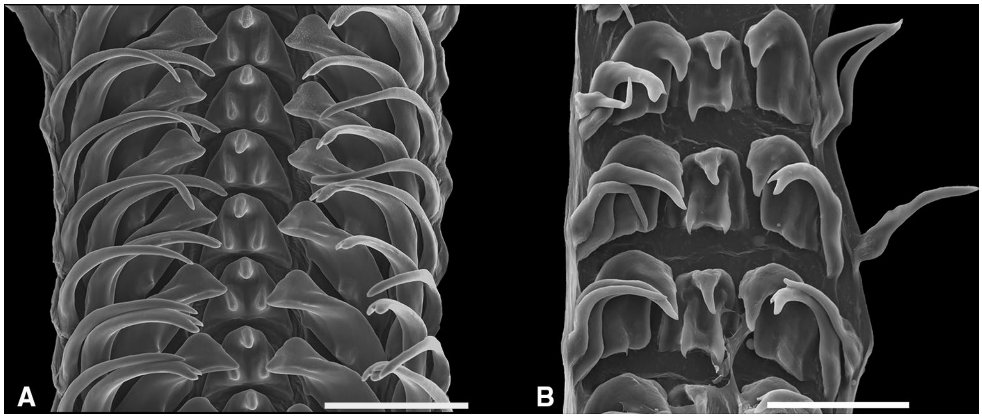

Fig. 2 *Bulbus carcellesi:* SEM photograph of radulae from adult (**a**) and embryo (**b**). *Scale bars* 200 µm (**a**), 50 µm (**b**)

recorded in the southwestern Atlantic Ocean, the adult shell of *B. carcellesi* being the largest of all the moon snails in the region. Some authors (e.g., Torigoe and Inaba 2011) have included *B. carcellesi* in the genus *Falsilunatia*.

Characteristics of the egg mass

The egg masses studied here were identified as belonging to *B. carcellesi*. Despite the small size of the early embryonic

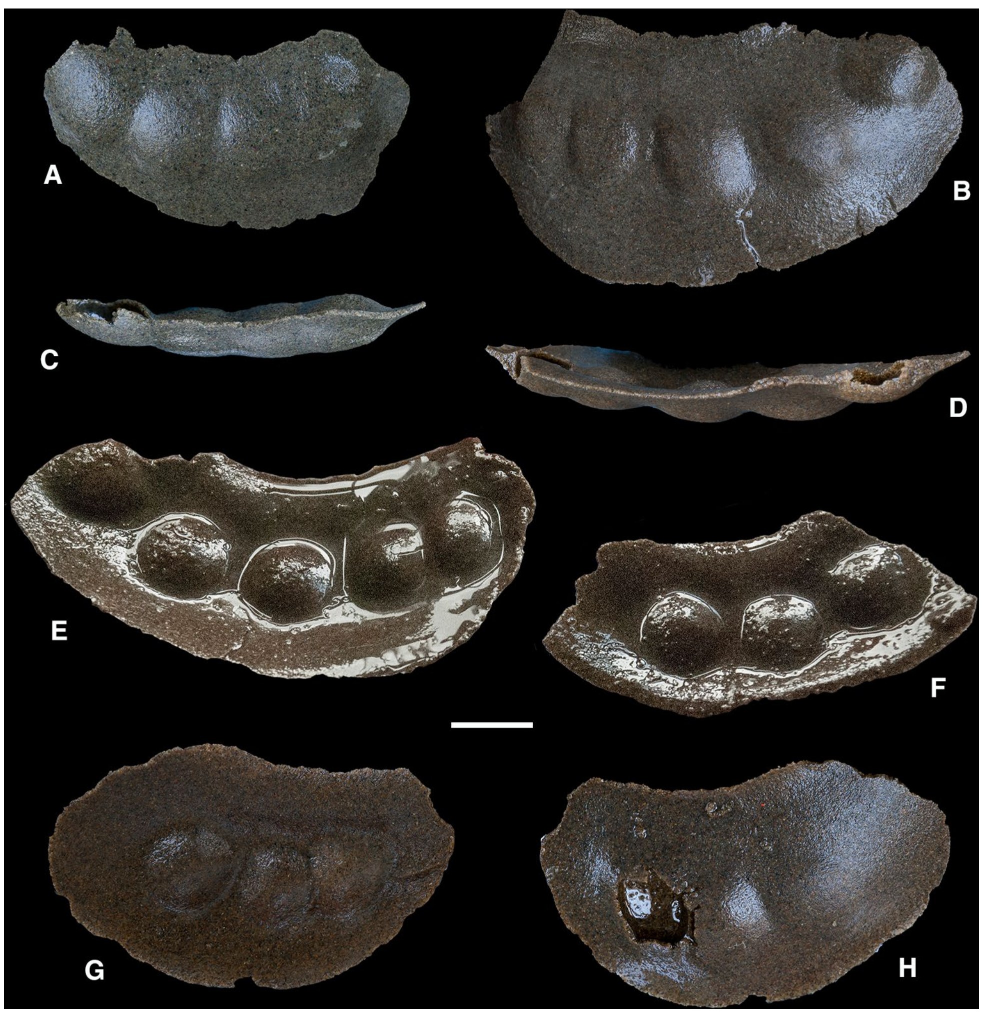

Fig. 3 *Bulbus carcellesi*: egg mass. Dorsal (**a**, **b**, **h**), lateral (**c**, **d**) and basal (**e**, **f**, **g**) view. *Scale bar* 1 cm

Fig. 4 *Bulbus carcellesi*: egg mass containing a growing/pre-hatching embryo. *Scale bar* 3 mm

shell, it is large enough to recognize a quite similar morphology to that of the adults. Also of note is the fact that the other species of Naticidae that live in proximity to *B. carcellesi* (or were collected in the same trawl) never reach a large enough size to produce the collar described here. This is the only species in the genus inhabiting this particular area and depth range.

Bulbus carcellesi produces a flat, semicircular egg mass, with non-undulating margins and sand grains embedded in their gelatinous walls. The sand grains were 62–268 μm in diameter. Egg masses ($n = 17$) were 19.1–31.0 mm wide and 30.0–62.2 mm long. Within the rigid walls, the egg mass contains 1–5 egg capsule spaces (8.8–14.1 mm diameter) in a single row. The egg capsules were visible to the naked eye (Figs. 3, 4). They are globular chambers made of sand grains glued together, which bulge prominently on the external face of the egg mass. Each egg capsule space contains a single egg capsule with only one embryo, which is immersed in a dense white substance completely filling the capsule early in development. The capsule wall is thin, 7–8 μm, and no nurse eggs or sibling embryos were found.

General features of the developmental stages

The earliest embryos had a maximum diameter of ~198 μm, a stage corresponding to early cell division, ectoderm formation, and gastrulation (Fig. 5). Several micromeres and four macromeres could be distinguished at this early stage, when the "white" or capsular jelly was dense and abundant. The next observed stage was the "early veliger", characterized by movement of the ciliated mouth or stomodeum to an anterior ventral position. The anterior part of the embryo was divided into a head and a foot by a ciliated incipient velum (425–450 μm). In the subsequent "veliger stage", an incipient spiral structure was observed in the embryo and the beginning of the formation of the organic matrix of the future shell. At this stage, the embryo (700–873 μm) was more elongated, and the bi-lobed velum had increased in size, with enlarged cilia (Fig. 6). In the next stage, the pediveliger, a developed foot could be distinguished. There was

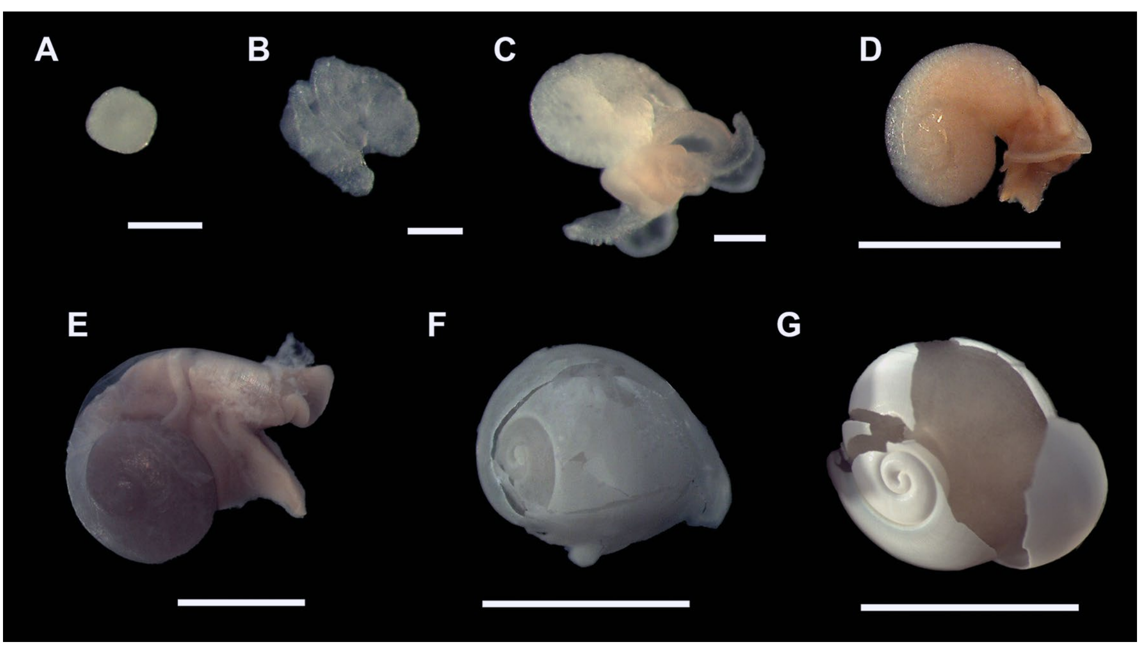

Fig. 5 *Bulbus carcellesi*: developmental stages. Roundish embryo with observable micromeres and macromeres (**a**), early veliger (**b**), veliger (**c**), pre-hatching (**d**, **e**) and hatching stage (**f**, **g**). *Scale bars* 200 μm (**a**–**c**), 4 mm (**d**–**g**)

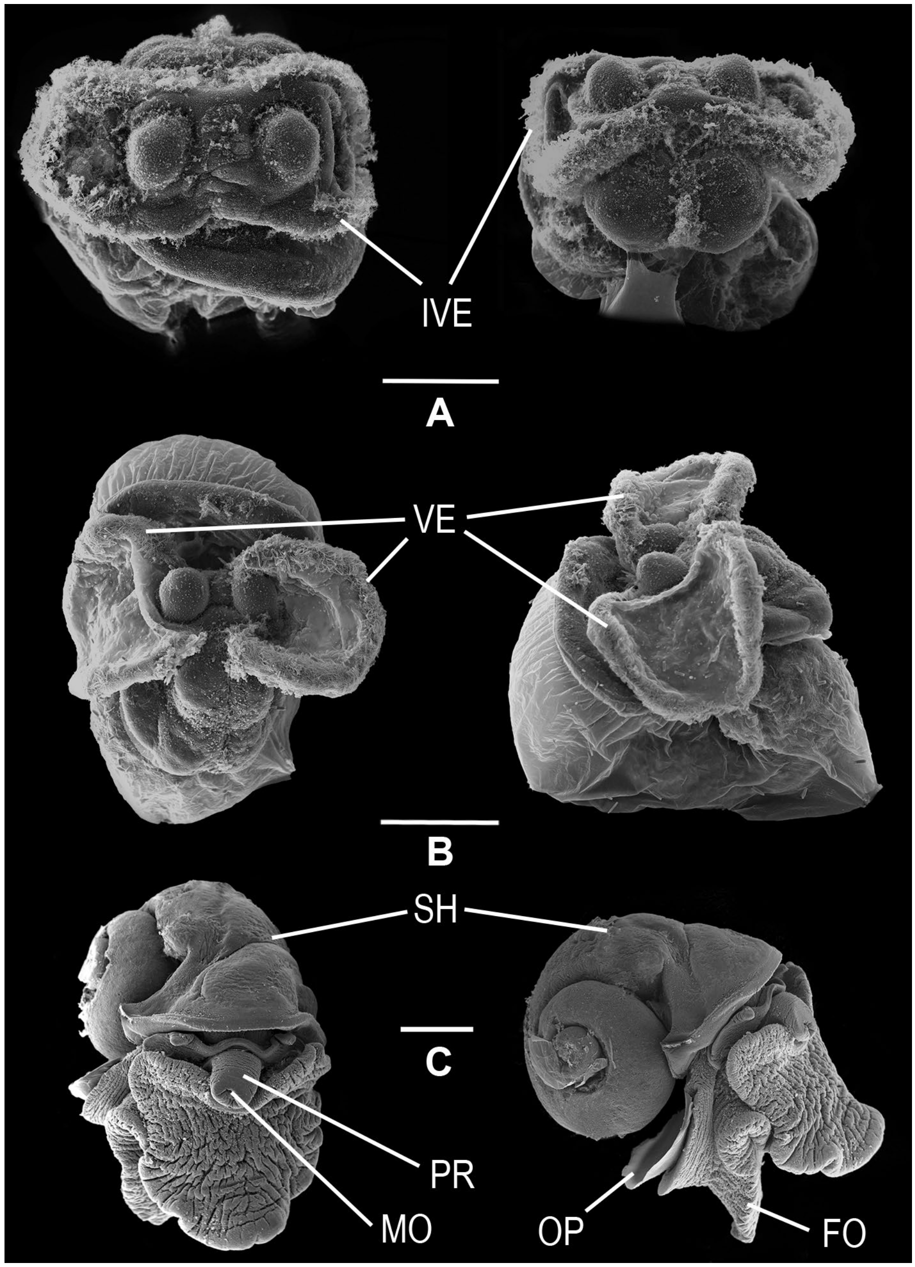

Fig. 6 *Bulbus carcellesi*: SEM photographs of three different developmental stages. Early veliger (**a**), veliger (**b**), and pre-hatching stage (**c**). *Scale bars* 100 µm (**a**), 200 µm (**b**), 1000 µm (**c**). *IVE* incipient velum, *VE* velum, *SH* shell, *PR* proboscis, *MO* mouth, *OP* operculum, *FO* foot

some resorption of the velum, but it was still visible, with enlarged cilia (Fig. 6). The embryo had now consumed the intracapsular material and had begun to increase in size. These embryos had a shell diameter of 0.9–1.5 mm. The "growing/pre-hatching" stage was characterized by the presence of a developed foot, and shells in different stages of calcification with up to 2½ whorls. The velum was completely reabsorbed. Embryos in this stage were 1.4–5.9 mm in shell diameter. Finally, the most developed embryo found, the "hatching" stage was 4.3–6.0 mm shell diameter and had a calcified, globose shell (Fig. 5). Table 2 shows the distinctive characteristics of each embryonic stage. Just before hatching, the embryonic shell was mostly smooth and lightly calcified with about three well-developed whorls. The nature of the shell makes it difficult to observe under SEM. Some wrinkles were visible in the suture of the first whorl.

Discussion

When compared with other members of the family, the egg mass of *B. carcellesi* is unique in shape and the developmental characteristics of its embryos. The reproductive mode of about 30 species of naticids is known, and in the majority hatching takes place as swimming veligers

Table 2 Distinctive features of embryonic stages of *Bulbus carcellesi*

Stage	Description	Mean (SD) embryo (μm) or shell diameter (mm)	Range in embryo (μm) or shell diameter (mm)	Whorls	*n* (egg masses)
Early embryo	Roundish embryo with observable micromeres and macromeres	197.5 ± 0.5 μm	197–198 μm	–	2 (1)
Early veliger	Early veliger stage with incipient velum, stomodeum toward front ventral section	437.3 ± 12.5 μm	425–450 μm	–	2 (1)
Veliger stage	Embryo with bi-lobed and ciliated velum	782.6 ± 87.5 μm	700–875 μm	1	2 (2)
Pediveliger stage	Foot developed, partially reabsorbed velum	1.4 ± 0.5 mm	0.952–1.496 mm	$1^{1/2}$–$1^{3/4}$	4 (4)
Growing/Pre-Hatching	Foot greatly developed, Velum completely reabsorbed	4.3 ± 0.8 mm	1.3–5.9 mm	2–$2^{1/2}$	9 (7)
Hatching	Embryo with calcified shell	4.5 ± 0.3 mm	4.2–6.0 mm	$3^{1/4}$	2 (2)

Table 3 Comparison of naticid masses with direct development

Species	Capsules egg mass^{-1}	Range in capsule diameter (µm)	Embryos capsule^{-1}	Egg diameter (µm)	Embryo nutrition	Hatchling diameter (µm)	Whorls at hatching	Source
Euspira catena	20	1200–2000	2–8		"Nurse eggs"	800	1.5	Ankel (1930), Thorson (1946)
Euspira pallida, as E. groenlandica	13–38	2500–3000	1	2250	"White"	1500–1700	1.5–2	Thorson (1935)
Cryptonatica clausa	13–24	2000–2250	1		"White"	1500	1.75–2	Thorson (1935)
Amauropsis islandica	150–200	1500–1750	1	1500	"White"	750	1	Thorson (1935, 1946)
Natica vitellus, as *N. rufa*	~1000		1	1000	"Albumen-like substance"	800		Thorson (1940)
Glossaulax didyma, as *G. ampla*	5800–6000		1			600–700		Thorson (1940)
Neverita Josephinia		1000	1			780	1.5	Giglioli (1955)
Euspira triseriata		850–1150	1–3	350		520	1.5	Giglioli (1955)
Glossaulax vesicalis		1300–1450	1	480		1000		Amio (1963)
Bulbus carcellesi	1–6	8780–14,140	1	<200	White	4250–6000	$3^{1/4}$	Present study

(Table 3) (Ankel 1930; Thorson 1935, 1940, 1946; Lebour 1936; Giglioli 1955; Natarajan 1957; Amio 1963; Gohar and Eisawy 1967; Huelsken et al. 2008; Pastorino et al. 2009). In about ten species, complete intra-capsular development was recorded and eclosion takes place as crawling juveniles. Among the species that hatch as juveniles, *Euspira pallida* (as *Natica groenlandica* in Thorson 1935) and *Cryptonatica affinis* (as *Cryptonatica clausa* in Thorson 1935) from North Atlantic cold waters have comparable semi-circular egg masses with non-undulating margins

(Thorson 1935; Giglioli 1955). When compared with other previously described naticids, *E. pallida* and *C. affinis* have the largest egg capsules: *E. pallida* has 13–38 capsules, each 2.5–3.0 mm diameter, and *C. affinis* has 13–24 capsules, each 2.0–2.3 mm diameter (Thorson 1935). Those of *B. carcellesi* were 8.8–14.1 mm, and each egg mass contained only 1–6 egg capsule spaces. Since many of the egg masses with 1 or 2 egg capsule spaces seemed to be fragments, it is very likely that the egg masses would normally contain 3–6 egg capsule spaces. *Euspira pallida* has an irregular arrangement of the egg capsule spaces, generally in at least three rows (Thorson 1935), and *C. affinis* generally has its egg spaces arranged in two offset rows (Thorson 1935). In contrast, the egg capsules of *B. carcellesi* are arranged in a single row along the egg mass.

Bulbus carcellesi, *E. pallida* and *C. affinis* have egg capsule spaces with only a single embryo which, in the early stages of development, is surrounded by a dense mass of albumen-like substance (Thorson 1935). Thus, the three species provide a great amount of supplementary food for their embryos. In fact, all the species with direct development possess this type of embryonic nutrition, with the exception of *Euspira catena*, which, to our knowledge, is the only naticid that has nurse eggs (Ankel 1930; Hertling 1932; Lebour 1936; Thorson 1946; Giglioli 1955).

Hain (1990) reported two egg masses from Antarctica (74°41′S, 35°04′W and 74°30′S, 26°24′W at a depth of 500 m and 483 m, respectively). He referred to the naticid "*Amauropsis*" *grisea,* described as *Kerguelenatica bioperculata* by Dell (1990), as a problematic species with a wide distribution and unresolved taxonomic classification. The published figure (plate III, Fig. 6b) resembles the egg mass of *B. carcellesi* here described. Hain (1990) found a single embryo in each chamber, of 1–2 mm shell diameter, at the veliger stage, surrounded by white material. The illustration of the shell of the embryo (plate III, Fig. 6a) has a very different profile from *Bulbus carcellesi.*

The earliest stage of development found for *B. carcellesi* was ~198 μm, and corresponded to early cell division. It is likely that the uncleaved egg is <200 μm in diameter. Other naticids with eggs of a similar size are *Natica buriasiensis* (as *N. trailli* in Thorson 1940), with eggs measuring 200 μm, and *Natica maculosa*, 170 μm (Amio 1963). Both species hatch as a planktotrophic veliger (Thorson 1940; Amio 1963). However, there is a great variation in the size of the uncleaved egg in the Naticidae family. The smallest egg known belongs to *Notocochlis gualtieriana* (as *Natica marochiensis* in Natarajan 1957), 66–83 μm in diameter, while *Natica vitellus* (as *N. rufa* in Thorson 1940) probably has the largest egg, 1000 μm in diameter. Thorson (1935) described an even larger egg (2.2 mm) for *E. pallida* but, given his description of a smaller emerging juvenile (1500–1700 μm), he was probably referring to the egg capsule, not the uncleaved egg. The hatchlings of the species with direct development are larger than the planktonic larvae of the species with indirect development (Pastorino et al. 2009). The hatchlings of *E. pallida* and *C. affinis* were the largest recorded for naticids prior to the present study (1.5–1.7 mm and 1.5 mm, respectively) (Thorson 1935), but the hatchlings of *B. carcellesi* grow within the egg space to a remarkable 4.8–6.0 mm diameter, ~45 times the volume of *E. pallida*. *Bulbus carcellesi* also has the greatest number of shell whorls at hatching, with up to 31/4, while *E. pallida* and *C. affinis* only have two at this stage.

The deep sea is often considered a constant environment, presenting almost no seasonal variation (Tyler and Young 1992). This stability enables the different species to reproduce asynchronously or continuously throughout the year (Rokop 1974; Giese and Kanatani 1987; Tyler and Young 1992). The finding of all intracapsular stages of development in the same expedition at the same date (including early stages and hatchlings as enormous crawling juveniles) may suggest a long reproductive season for *B. carcellesi*. This has been observed for various deep-sea gastropods, for example the Rissoidae, *Benthonella tenella* (Rex et al. 1979), the Buccinidae, *Colus jeffreysianus* (Colman et al. 1986) and the Calliotropidae, *Calliotropis ottoi* (Colman and Tyler 1988).

The deep sea is a marine environment where metabolic processes are usually slow (Robison et al. 2014). Specifically, embryonic development rates are among the slowest of all animal life, and they generally include direct development without a larval stage (Robison et al. 2014). In fact, the development can last up to 53 months in the deep-sea octopus *Graneledone boreopacifica* (Robison et al. 2014), and up to 20 months in the bathypelagic mysid crustacean *Neognathophausia ingens* (formerly *Gnathophausia*) (Childress and Price 1978). In most marine ectotherms, embryonic development takes longer as environmental temperature decreases (Childress and Price 1978; Robison et al. 2014). The number of whorls in the hatchling juvenile shell and the size they attain could be indicative of a long period of embryonic development in *Bulbus carcellesi*.

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Compliance with ethical standards

Conflict of interest Pablo E. Penchaszadeh, Melina Atencio, Mariano I. Martinez and Guido Pastorino declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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