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ARTÍCULO ORIGINAL

DESCRIPTION OF EGG CAPSULES OF *VOLUTA EBRAEA* LINNAEUS,
1758 (GASTROPODA: NEOGASTROPODA)

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ABSTRACT

Voluta ebraea Linnaeus, 1758 is an endemic volutid from north and northeast Brazil. Characteristic volutid egg capsules were found attached to the alga *Udotia occidentalis* in sea grass beds. Morphology and size of those egg capsules and embryos as well as protoconch and radulae of juvenile and adults confirm that all the spawns found belong to *V. ebraea*. Egg capsules, are circular and flattened with a mean diameter of 18.2 mm, presenting analogous morphology and size to those of *Voluta musica*, endemic from Venezuela. An opening through which crawling juveniles emerge is located at the center of the egg capsules with a suture from the base to the central opening. The average number of hatchlings was three and the mean shell length of juveniles 7.8 mm. Histochemical techniques showed that egg capsules wall is composed of three layers, containing protein, carbohydrates and glycoprotein. Identifying and protecting *V. ebraea* egg capsules in the wild could help to conserve this endemic and endangered species.

KEY WORDS: Volutidae, embryonic development, endemic species, Brazil

RESUMEN

Descripción de las cápsulas ovígeras de *Voluta ebraea* Linnaeus, 1758 (Gastropoda: Neogastropoda). *Voluta ebraea* Linnaeus, 1758 es un volutido endémico del norte y nordeste de Brasil. Ovicápsulas características de la familia Volutidae fueron encontradas adheridas al alga *Udotia occidentalis* en praderas de fanerógamas marinas. La morfología y tamaño de las ovicápsulas y embriones, así como la rádula y protoconcha de juveniles y adultos confirman que las puestas encontradas corresponden a *V. ebraea*. Las ovicápsulas son circulares y aplanadas con un diámetro medio de 18,2 mm, presentando morfología y tamaños similares a las ovicápsulas de *Voluta musica*, endémica de Venezuela. Las ovicápsulas presentan una apertura en el centro (por donde emergen los juveniles) y una sutura desde ésta hasta la base. En promedio eclosionaron tres juveniles por ovicápsula, con un largo promedio de 7,8 mm. Las técnicas histoquímicas realizadas demostraron que la pared de las ovicápsulas está compuesta por tres capas, conteniendo proteínas, carbohidratos y glicoproteínas. La identificación y protección de las ovicápsulas de *V. ebraea* encontradas en el campo podría ayudar a conservar esta especie endémica y amenazada.

PALABRAS CLAVE: Volutidae, desarrollo embrionario, especies endémicas, Brasil

INTRODUCCIÓN

South American volutids lay egg capsules that are attached to hard substrata such as shells, stones or elasmobranch egg capsules (Penchaszadeh *et al.*, 1999, Penchaszadeh & Miloslavich, 2001, Bigatti *et al.*, 2010). *Adelomelon brasiliana* (Lamarck, 1811) is the only South American volutid to lay unattached egg capsules that drift on the sea bottom (Penchaszadeh & De Mahieu, 1974; Penchaszadeh *et al.*, 1999; Cledón *et al.*, 2005). A particular case is *Odontocymbiola*

magellanica (Gmelin, 1791) that lays egg capsules covered by a magnesium calcite external layer, deposited by the female pedal gland (Bigatti *et al.*, 2010). Volutid egg capsules are easily differentiated from other gastropod spawns.

The genus *Voluta* comprises three species: *V. musica* Linnaeus, 1786, *V. ebraea* Linnaeus, 1758 and *V. virescens* Solander, 1786 (Clench & Turner, 1964). Studies on egg capsules of this genus were studied by Bandel (1975; 1976) who described the egg capsules of

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V. virescens from Colombia and by Penchaszadeh & Miloslavich (2001) who studied biochemical aspects of the development of *V. musica* from Venezuela. *Voluta musica* is an endemic volutid gastropod restricted to the Venezuelan and Colombian Caribbean as well as to some Caribbean Islands (Clench & Turner, 1964). The spawn of *V. musica* consists of a single, circular and flattened egg capsule measuring 18 mm in basal mean diameter, with one to five eggs embedded in a dense, mucous liquid. At hatching, crawling juveniles measure approximately 7 mm in shell length.

Voluta ebraea, an endemic volutid snail restricted to north and northeast Brazil, is distributed from Pará to Bahia states (Clench & Turner, 1964; Rios, 1970; Cosel, 1976). Matthews (1969) found that *V. ebraea* was more abundant at 25 to 40 meters depth. Other authors, however have found this species in the shallow waters of Ceará state, living on corals, rocks or sandy bottoms (Rios, 1994; Cordeiro, 1999; Dias, 2008).

Limited research has been published about *V. ebraea*, except for anatomical, imposex incidence and feeding studies. Clench & Turner (1964) and Rios (1970) state that *V. ebraea* exhibits external sexual dimorphism, the female shell width being greater than that of the male. Fertilization in *V. ebraea* is internal as in all neogastropods with females laying external egg capsules, which have not been known until now. *Voluta ebraea* is captured and sold for ornamental purposes in northeast Brazil (Alves *et al.*, 2006) and is being consumed by some coastal communities (Farias & Barreira, 2007). Population declines have occurred on coastal reefs or in shallow waters where this species was previously abundant (Dias, 2008). Imposex incidence in *V. ebraea* was reported by de Castro *et al.* (2008) from marine traffic zones in Ceará state.

The study of the spawn, egg capsules and embryos of gastropods is important not only for studies of reproductive biology in general, but also for its implication for dispersal, biogeography and for taxonomy (Pastorino & Penchaszadeh, 2009). In this paper we describe for the first time veliger embryos and hatchlings of *V. ebraea* from the shallow waters of Ceará state, Brazil, as well as capsular morphology and wall composition.

MATERIALS AND METHODS

Sampling

Egg capsules and adults of *V. ebraea* were collected by snorkeling at Paracurú beach (3° 23' 53" S - 39° 00' 58,8" W), Ceará state, Brazil during low tides (0.5 to 3 m depth) in December 2005 and October 2006. Paracurú beach is located in western Ceará State, 90 km from the capital city, Fortaleza. After

collecting, material was maintained in sea water and transported to the laboratory at the Universidade Federal do Ceará. Alive egg capsules were kept in 20 l aquaria with aerated sea water and maintained at room temperature (approximately 24 °C). Voucher material was deposited in the Malacological collection "Professor Henry Ramos Matthews" Labomar, Universidade Federal do Ceará (PHRM-LABOMAR, UFC N.3286-3896).

Egg capsule and embryo morphology

The diameter and height of the egg capsules were measured with a caliper. Development was registered until hatching when possible by keeping egg capsules in aquaria and recording hatching with a video camera. Protoconchs of hatchlings were observed using scanning electronic microscopy (SEM, Philips XL 30) at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". The veliger and pre-hatching developmental stages were measured and photographed alive with a light microscope.

Egg capsule wall composition

Pieces of egg capsule walls were fixed in Bouin's saline solution for eight hours, dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. The capsule walls were sectioned at 7 µm thickness and stained with Harris' hematoxylin followed by 1% aqueous eosin for morphological analysis. To assess whether protein was present within the egg capsule wall, the bromophenol blue technique was used. Periodic Acid-Schiff reaction (PAS) and Alcian blue/PAS staining were used to detect the presence of acidic mucopolysaccharides and carbohydrates in the egg capsule wall (Bayne, 1968). Measurements of the egg capsule wall width and the different layers were made using a light microscope at 40X magnification.

RESULTS

A total of 40 egg capsules of *V. ebraea* were found attached to the green calcareous alga *Udotia occidentalis* A.Gepp and E.S. Gepp (Fig. 1 A and B) in the same place that *V. ebraea* adults were found (1 to 2 meters depth in sea grass beds off Paracurú beach, Ceará state, Brasil).

Egg capsule morphology

Egg capsules are circular and flattened (Fig. 1 A and B). The diameter of the egg capsule (N=40) averaged 18.2 ± 2.63 mm (range 15.1-29.5 mm). The height of the egg capsule averaged 0.73 ± 0.27 mm (range 0.5-1.27 mm). An opening was observed in the center of the capsules as well as a suture from the base to the opening. Eighty two percent of the egg capsules were found fixed to the concave side of the alga, while

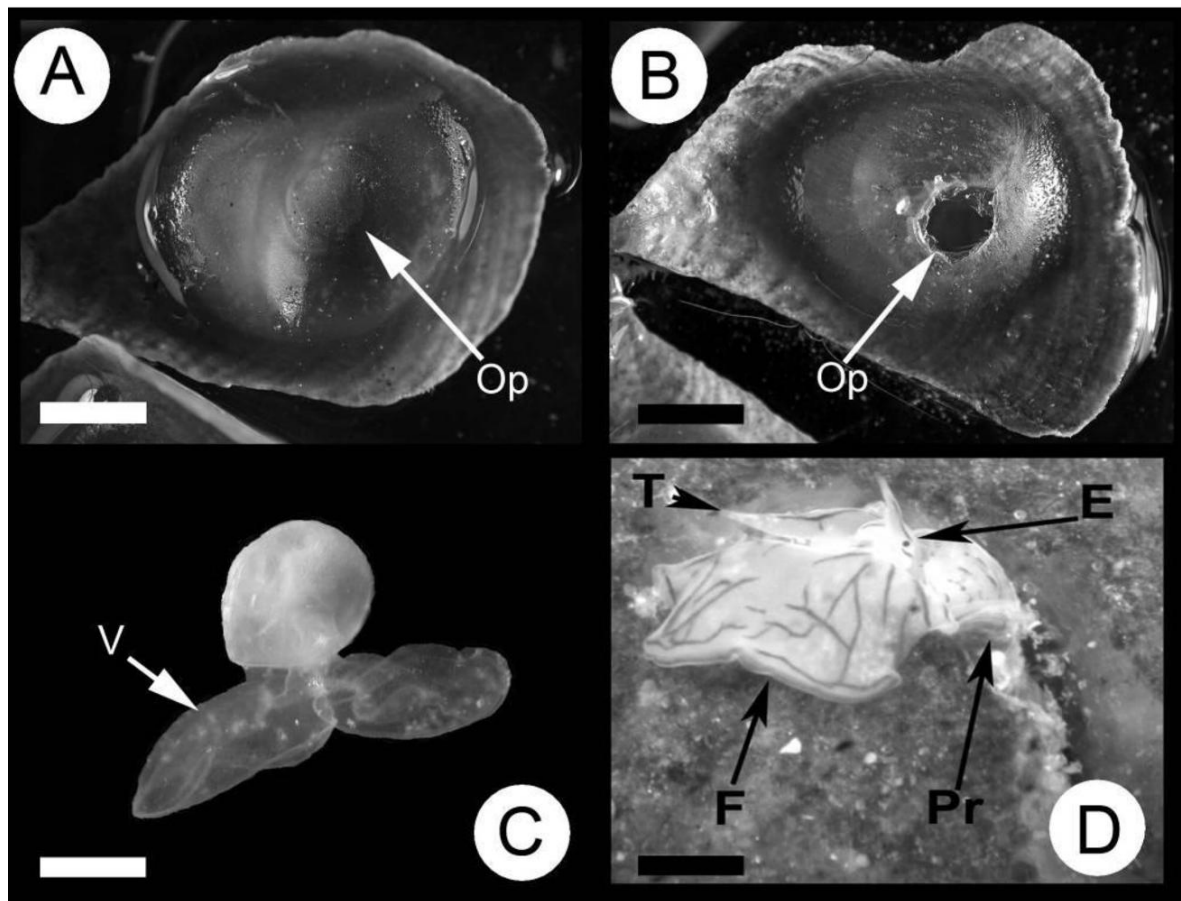


Figure 1: A: egg capsule of *Voluta ebraea* recently laid and attached to the calcareous green alga *Udotia occidentalis*, B: hatched egg capsules with broken opening, C: intracapsular “veliger” stage with a large velum, D: juvenile hatching from the opening (note the foot pattern coloration, typical of *V. ebraea*). Abbreviations: E: eye, F: foot, Op: opening, Pr: protoconch, T: tentacle, V: velum, Scale bars: A and B= 3cm, C and D=3mm.

18% were fixed to the convex side. On the other hand, 86% of the algae examined had only one egg capsule attached, 9% presented two and 5% presented three egg capsules attached to both sides of the alga.

Embryo morphology

We found egg capsules at different stages of development from early veligers to juveniles. Capsules containing juveniles lacked nurse eggs, and embryos were surrounded by intracapsular fluid. Embryos at the “veliger” stage (Fig. 1C) had a large bilobed velum that could be easily recognized. The velum generates a current that allow it to ingest the intracapsular fluid. At this stage the embryo body (without velum) measures 3.84 mm (SD = 0.34).

In the aquarium, between two and five (mean = 3.0, SD = 0.8) crawling juveniles hatched from the opening of each egg capsule (Fig. 1D). Juveniles had the same pattern of coloration in the foot as the adults (Fig. 2 A and B), and the same external morphology, i.e the typical operculum of the species, shell shape, etc. The mean shell length of hatching juveniles was 7.8

mm (SD = 0.5) and the mean height of the shell was 5.1 mm (SD = 0.8). Egg capsules hatched can be identified in the field by a broken opening (Fig. 1B).

SEM pictures showed that the protoconchs of juveniles had 2.25 whorls (Fig. 2C), the same as the adults (Fig. 2D). The teleoconch has parallel plicae (Fig. 2 C and D) as in the adult shell, but without the spines characteristic of larger specimens.

Egg capsule wall composition

Bromophenol Blue and H-E staining showed that the egg capsule wall is composed by three protein layers (Fig. 3). The external layer (L1) is dense, homogeneous and amorphous, and is composed only by proteins. Its width varies from 250 μm to 300 μm . The medium layer (L2) is also composed by protein fibers orientated in either a circular or longitudinal direction. Its width ranges from 32.5 μm to 72.5 μm . Layers L1 and L2 are strongly attached together. The internal layer (L3) is a very thin protein layer (25 μm width) that is not attached to the other layers, and is visibly separated when fresh egg capsules are opened. The alcian

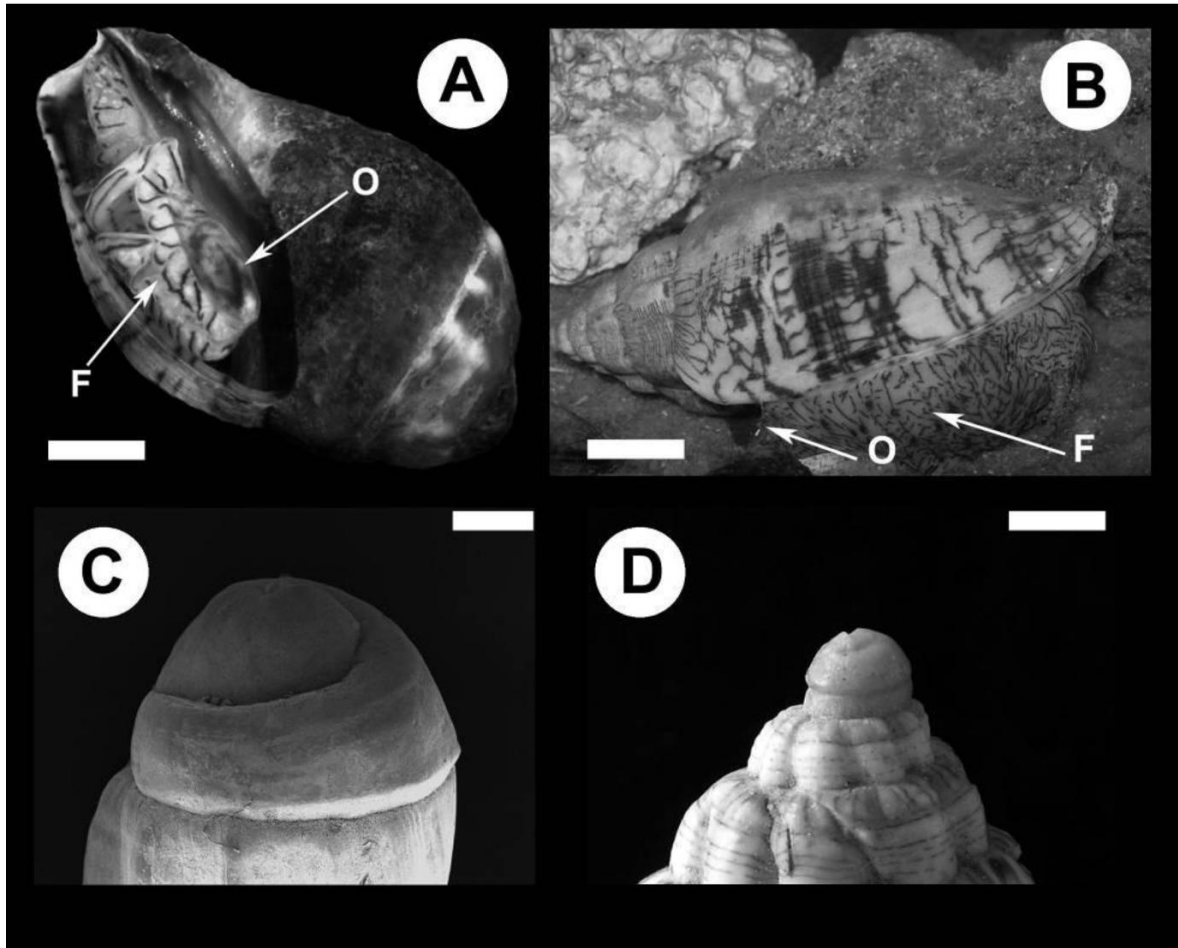


Figure 2: A: juvenile after hatching (note the operculum and pattern coloration of foot, similar to adults) B: adult specimen showing the same pattern coloration than juvenile, C: SEM of juvenile protoconch, D: adult protoconch. Abbreviations: F: foot, O: operculum. Scale bars: A= 1mm, B= 2cm, C= 1mm, D=4mm.

blue/PAS technique confirmed the presence of carbohydrates and glycoproteins in L3. This layer is composed of at least five sub-layers, showing an internal protein layer followed by an internal carbohydrate zone. The latter is preceded by a protein layer, another thin carbohydrate layer and another protein layer, located in the inner side of the capsule. The L3 layer remains in contact with the intracapsular fluid.

All egg capsule walls studied corresponds to “veliger” stage embryos, so we assume they had been spawned some time ago. Because of that, epibiotic algae grew on the external face of the egg capsule, so L1 was externally PAS positive and had an irregular surface.

DISCUSSION

All known volutid egg capsules are similar in shape and easily distinguishable from spawns of other gastropod families. The morphology of the egg capsules of *V. ebraea* found in this work is remarkably similar to

those of *V. musica* from Venezuelan shallow waters. The spawn of *V. ebraea* was unknown until now and together with *V. musica* egg capsules are the smallest registered for South American volutes. *Voluta musica* egg capsules are hemispheric and measure approximately 18 mm in basal diameter (Clench & Turner 1964, Cosel 1976), similar to the size of *V. ebraea* (18.2 mm), suggesting that the size and shape is a characteristic feature of the genus *Voluta*.

The occurrence of volutid egg capsules attached to algae was not reported previously. *Udotia occidentalis* is a calcareous alga from shallow waters with hard consistency and similar shape to bivalve empty shells, the typical substrate for volutid egg capsule laying (Penchaszadeh *et al.*, 1999; Bigatti *et al.*, 2008, Bigatti *et al.*, 2010). Possibly, deeper populations of *V. ebraea* could utilize bivalve shells as spawn substrate as other South American volutes do. The habitat where the egg capsules and adults were found in this work is the same as that described for *V. ebraea* in other zones in northeast Brazil by Dias

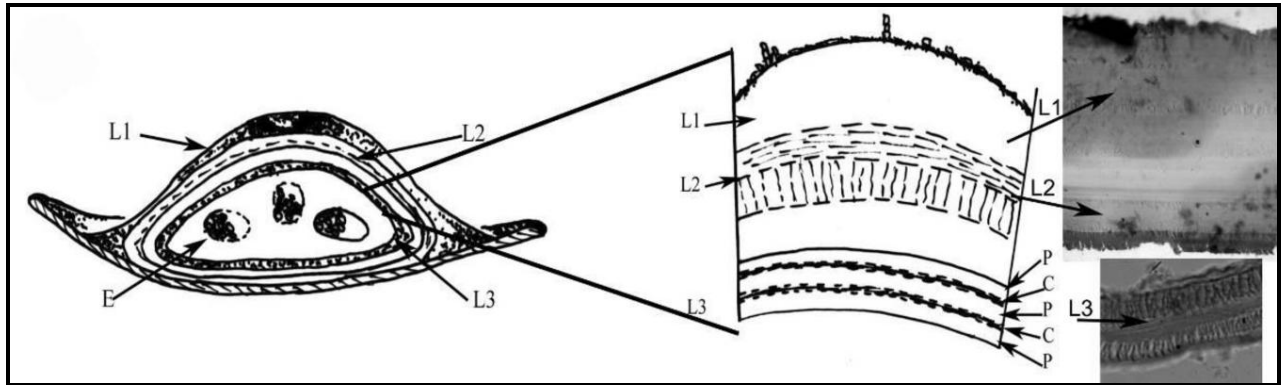


Figure 3: Diagram and histological sections of egg capsules wall (transversal cut), showing the composition of the 3 layers (L1 and L2: bromophenol blue staining; L3: PAS staining). P=protein composition, C=carbohydrates (PAS +) composition.

(2008), and similar to the habitat of *V. musica* from Venezuela (Penchaszadeh & Miloslavich, 2001; Peralta *et al.*, 2008). The egg capsules of *V. musica* from Venezuela were found attached to hard substrata, usually to the internal side of empty bivalve shells (Penchaszadeh & Miloslavich, 2001; Peralta *et al.*, 2008).

In other volutids studied in South Atlantic waters, the egg capsules also contain few eggs and the embryos feed on substances contained in the intracapsular fluid. In these, development is direct and hatchlings may have a shell length of more than 8 mm (de Mahieu *et al.*, 1974; Penchaszadeh *et al.*, 1999; Bigatti, 2005). The exception to this rule is *V. virescens* whose egg capsules contain about 200 eggs of which only one or two develop ingesting the rest as nurse eggs (Bandel, 1976). Gibson-Smith (1973) reported three or four embryos inside each egg capsule of *V. musica*, all of them developing to the hatching stage. In the case of *V. ebraea* three embryos developed on average probably after they consumed the intracapsular fluid. The internal egg capsule layer (L3), which contains carbohydrates and glycoproteins is a potential source of food, but it was not registered in this work.

The histochemical composition of the egg capsule revealed a protein-carbohydrates composition. Miloslavich (1996) reported the biochemical composition of some prosobranch egg capsules, founding protein, glycogen and lipids potentially available for the embryos to feed on. Penchaszadeh *et al.* (1999) reported a protein egg capsule wall for members of the genus *Adelomelon*. In *Odontocymbiola magellanica* the egg capsule wall is also composed of three protein layers and has an ultrastructure of protein fibers attached to globular proteins constituting a semi-permeable membrane that allow the passage of gases, water and probably small

molecules (Bigatti *et al.*, 2010). The existence of permeable protein layers has been reported in other neogastropods (D'Asaro, 1986; Hawkins and Hutchinson, 1988). Although we did not study the permeability of the egg capsule wall of *V. ebraea*, the composition is similar to other volutids studied and semi-permeability for small molecules is predictable.

An outstanding characteristic during the embryonic development of *V. musica* is the presence of a large velum (Penchaszadeh & Miloslavich, 2001) as reported for *V. ebraea*. The velum may have several functions in species with intracapsular metamorphosis. The first is for handling and ingesting nurse eggs (Fioroni & Schmekel, 1976) or the ingestion of intracapsular fluid as observed in this work. Other volutids studied present smaller velum during development (De Mahieu *et al.*, 1974; Bigatti *et al.*, 2005) and also use it to feed on intracapsular fluids. When comparing protoconch whorls of *V. ebraea*, we registered the same as in adult specimens (2.25 whorls). Clench & Turner (1964) reported two whorls in protoconchs probably due to intraspecific variations (these authors observed only two individuals).

All Volutidae members fix and shape their capsules with a pedal (or cementing) gland, and all the genera studied up to now have a different kind of egg capsule. A comparative study on functional anatomy of pedal glands within the Volutidae should be undertaken in order to determine whether differences in egg capsule shape are correlated or not with differences in the pedal gland microstructure.

Although further studies regarding reproduction and development of *V. ebraea* are necessary, information and protection of their egg capsules, particularly in human accessible beaches or fisheries zones, could allow the conservation and recuperation of this endemic and endangered species.

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