



RESEARCH NOTE

THE EGG CAPSULES AND EMBRYOS OF THE PATAGONIAN GASTROPOD *TROPHON PLICATUS* (LIGHTFOOT, 1786) (CAENOGASTROPODA: TROPHONINAE) WITH REMARKS ON THE TAXONOMY OF THE SOUTHWESTERN ATLANTIC TROPHONINAE

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Two species of *Trophon* are the most common muricids in Patagonian Atlantic waters, i.e. *T. geversianus* (Pallas, 1774)—type species of the type genus of the subfamily Trophoninae—and *T. plicatus* (Lightfoot, 1786). *Trophon geversianus* lives on intertidal and subtidal banks of mytilids (mainly *Brachidontes* spp.) along the Argentine coast. It ranges from the southern coast of Buenos Aires province to Burdwood Bank in the southwestern Atlantic, and up the Pacific coast of southern Chile. It is a characteristic element of the Magellanic biogeographic province. The egg capsules are frequent and very visible because of their distinctive erect pedunculate shape and bright yellow colour. Previous authors have described and illustrated in detail the shape and size of the eggs, egg capsules and embryos of this species (Zaixso, 1973; Penchaszadeh, 1976; D'Asaro, 1991; Cumplido *et al.*, 2011). On the contrary, information about these structures in *T. plicatus* is very scanty. Pastorino (2005) revised the genus *Trophon* in South America and illustrated the variation of the adult shell, protoconch, operculum and radula of the latter species. However, no information on the capsules was available at that time. One probable reason is that this is a subtidal species only exposed during spring tides in some areas along the coast of southern Patagonia.

Here, we describe and illustrate the eggs, embryos and egg capsules of material we identified as belonging to *T. plicatus*, comparing them with adult radulae and protoconchs of morphologically similar *Trophon* species ranging in the same area.

Two complete spawns were available to study. The oldest one (of nine capsules, with eggs but no embryos) was collected on February 2000 among the roots of recently washed-up kelp *Macrocystis pyrifera* along the coast of Ushuaia, Tierra del Fuego. At that time no embryos were found on the capsules so the specific assignment was unresolved. Recent collections on board the RV *Puerto Deseado* provided a new complete spawn of 12 capsules (nine with embryos), attached to an indeterminate bryozoan colony. These capsules were collected during the expedition CONCACEN II, in the Burdwood Bank area (54°30.5676'S, 61°20.6098'W), at 128 m depth on December 11, 2009. The complete spawn was fixed in 5% formalin in seawater. The fully

developed (but still encapsulated) embryos were dissected to extract the radulae. Embryonic shells and radulae as well as the egg capsules were prepared for study under scanning electron microscope (SEM) at the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN) and the United States National Museum of Natural History, Smithsonian Institution (USNM). Radulae were cleaned with commercial bleach according to the method described by Solem (1972). In addition, adult specimens of several species of *Trophon* and *Fuegotrophon* (i.e. *T. plicatus*, *T. pelseneeri*, *T. parodizi* and *F. pallidus*) from the USNM and MACN invertebrate collections were selected in order to compare the protoconchs to confirm the specific assignment of the egg capsules described here.

The egg capsules are erect, lacking peduncle (i.e. sessile), with one side almost flat and the other curved with a blunt upper tip. A preformed subcircular plug for the exit of the embryos of *c.* 1.8 × 1.7 mm diameter is visible on the slightly oblique dorsal surface in all capsules. All capsules, open or closed, are bright yellow. The basal diameter is 4.6 mm and the total height from the base to the tip is 5.5 mm (*n* = 6). Egg diameter is *c.* 350 μm. Both studied spawns were of about the same size (Fig. 1A–D).

Figure 1E, F shows the wall ultrastructure of a recently deposited egg capsule. The outermost layer (L1 of D'Asaro, 1988; Hawkins & Hutchinson, 1988) is ridged and banded. The middle layer is thicker, fibrous with elements variously packed, and very vacuolated. The inner layer, L3, is very thin. All the embryos were contained within a thin membranous sac detached from the inner side of the capsule wall. This thin membrane is also visible in recently laid capsules, closely attached to the wall (Fig. 1C, F). This innermost layer (L4) is transparent and continuous with the plug (Fretter & Graham, 1994). The plug area is also marked in this membrane before the embryos abandon the capsule.

The embryonic shells are basically smooth; however, at high magnifications (SEM) a delicate network of weak spiral threads is visible, crossing the growth lines to form a wavy pattern. After the second whorl, the typical pattern of axials appears, first as weak varices that are replaced by the large axial lamellae that

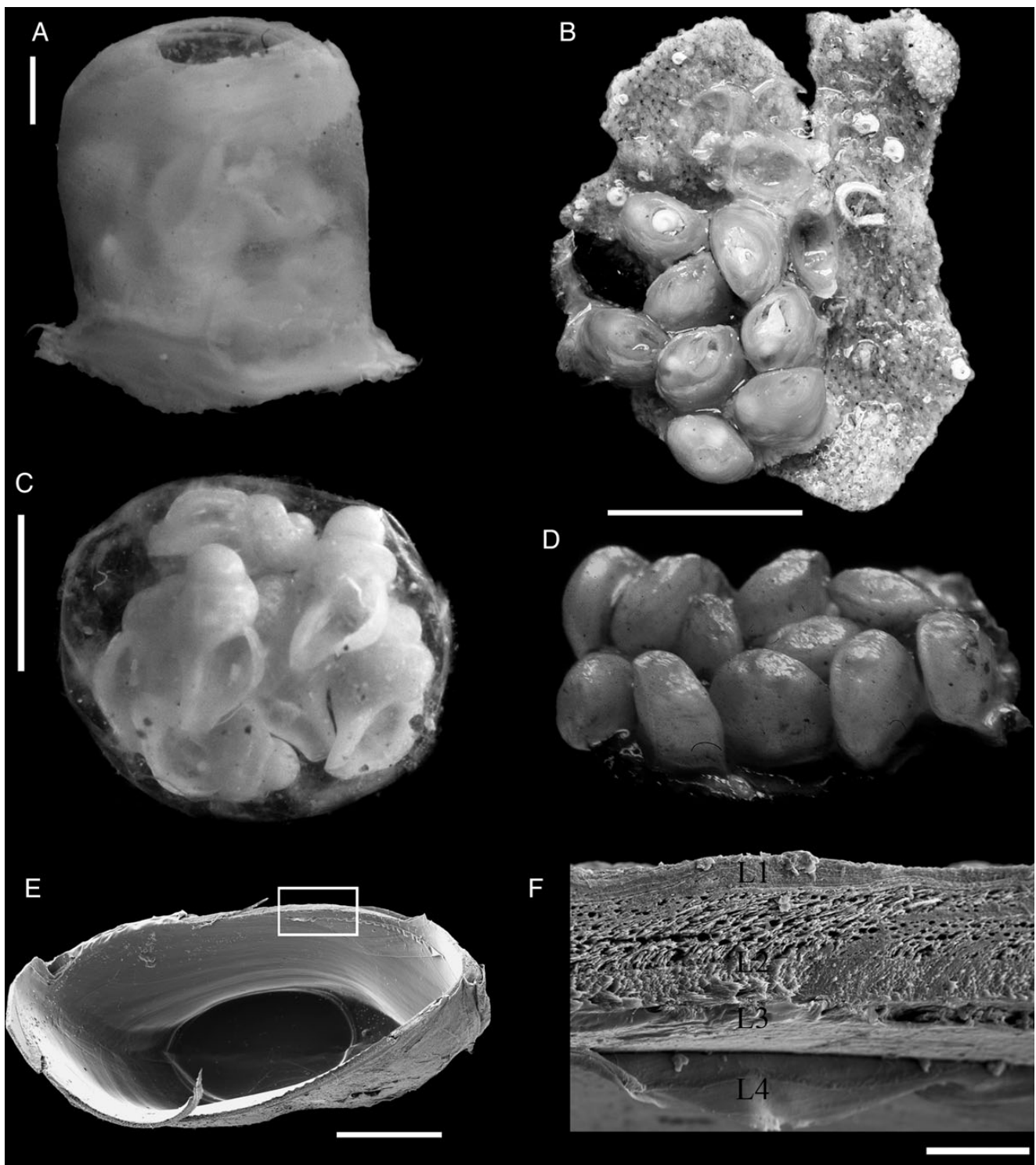


Figure 1. Spawn of *Trophon plicatus*. **A.** Single capsule in lateral view from the spawn in **B.** **B.** Complete spawn; 54°30.5676'S, 61°20.6098'W, Burdwood Bank area, at 128 m (MACN-In 39493). **C.** Group of embryos still inside of the membrane taken from a closed egg capsule from the spawn in **B.** **D.** Complete spawn collected at Ushuaia on holdfast of kelp (MACN-In 39494). **E.** Interior view of the top half part of one capsule of **D**, with the plug still closed at the bottom. **F.** Detail of the wall of the capsule from **E**. Scale bars, **A** = 1 mm, **B, D** = 1 cm; **C** = 2 mm; **E** = 1 cm; **D** = 200 μ m.

characterize the adults (Fig. 2A–D). Juveniles also show a delicate spiral pattern, better observed on the lower half of the last whorl; this is absent at later growth stages and also in the embryos (Fig. 2E, F).

Protoconchs of the species living in the same area as *T. plicatus* are depicted in Figure 2. Figure 2H shows *T. parodizi* Pastorino,

2005, a very rare species known from two specimens, with a radula morphologically close to *T. plicatus*. However, the protoconch is twice as large confirming a clear difference. *Trophon pel-seneeri* E. A. Smith, 1915 (Fig. 2I) is also similar. Very few details are known about this species, including protoconch variation. According to the few complete specimens examined, the

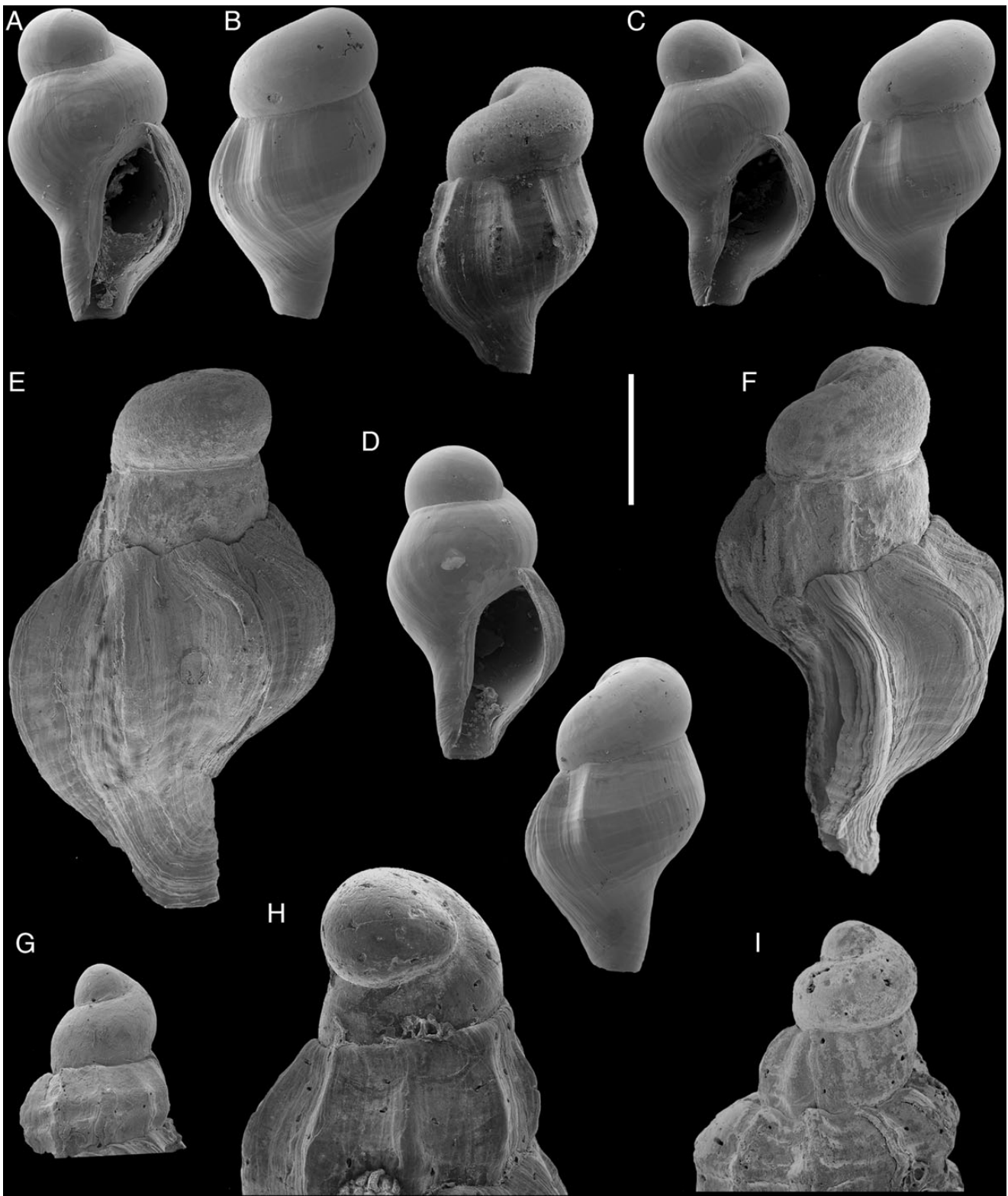


Figure 2. *Trophon* embryos and protoconchs. **A–D.** *T. plicatus*; four different embryos from the same egg capsule, 54°30.5676'S, 61°20.6098'W, Burdwood Bank area, at 128 m (MACN-In 39495). **E, F.** Two views of *T. plicatus* juvenile from same locality (MACN-In 39495). **G.** Protoconch of *Fuegotrophon pallidus* from same locality (MACN-In 39496). **H.** Protoconch of *T. parodizi* (USNM 896397, holotype). **I.** Protoconch of *T. pelseneeri* (USNM 846550). Scale bar = 1 mm.

protoconch morphology appears to be slightly different from that of the embryos of *T. plicatus*, as no ornamentation is present at the transition to teleoconch. Also, *T. pelseneeri* is currently

recorded from southern Brazil to offshore Buenos Aires province (Pastorino, 2005: 69). This area lies further north than the locality where the two spawns described herein were collected.

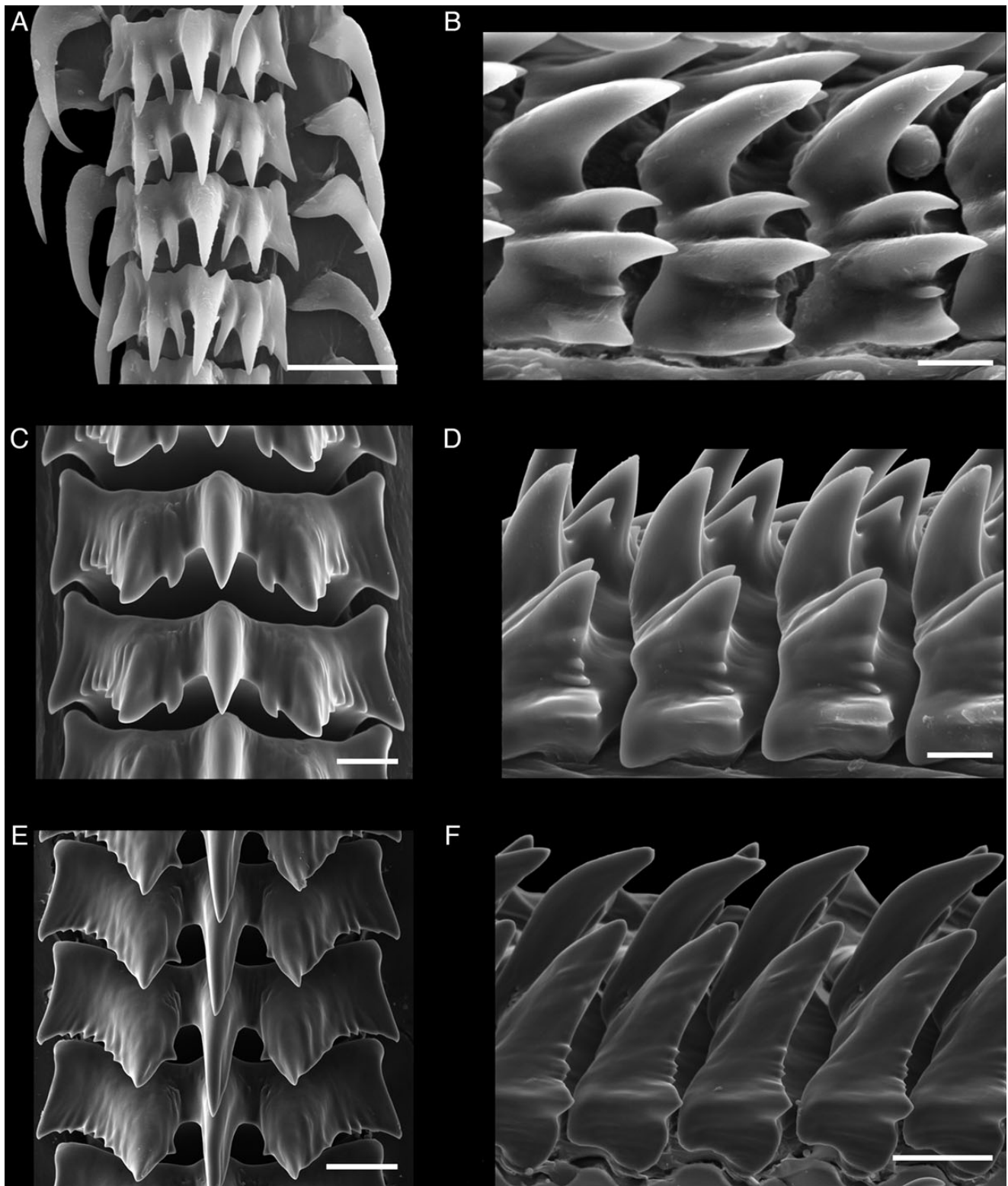


Figure 3. Radulae of embryo, juvenile and adult of *Trophon plicatus*. **A.** Embryo, dorsal view. **B.** Embryo, lateral view. **C.** Juvenile, dorsal view. **D.** Juvenile, lateral view. **E.** Adult, dorsal view. **F.** Adult, lateral view. Scale bars: **A** = 10 μm ; **B** = 4 μm ; **C** = 20 μm ; **D** = 10 μm ; **E** = 40 μm ; **F** = 50 μm .

Finally, Figure 2G shows the protoconch of *Fuegotrophon pallidus* (Broderip, 1833), also a comparable and sympatric species, but which is smaller, particularly in the first protoconch whorl. In

addition, the adult fimbriated ornamentation pattern that characterizes the genus *Fuegotrophon* appears soon enough to be recognized at the end of the last whorl of its embryos.

Table 1. Morphology of the egg capsules, protoconchs and radulae of embryos of southwestern Atlantic Trophoninae and some Pagodulinae.

Species	Subfamily	Morphology of protoconch/transition to teleoconch	Morphology of the egg capsule	Radula type (embryo/adult)	Source
<i>Trophon geversianus</i>	Trophoninae	Paucispiral, smooth, asymmetrical (2 whorls)/conspicuous	Erected with peduncle	Ocenebrine-Muricine (3D/Flattened)	Penchaszadeh, 1976; Herbert <i>et al.</i> , 2007; Pio, Herbert & Pastorino, 2014
<i>Trophon plicatus</i>	Trophoninae	Paucispiral, smooth (2.5 whorls)/conspicuous	Erected, no peduncle	Ocenebrine-Muricine (3D/Flattened)	this paper
<i>Coronium acanthodes</i>	Trophoninae	Paucispiral, smooth (~4 whorls)/conspicuous	Lenticular	Ocenebrine-Muricine (3D/Flattened)	Pastorino & Penchaszadeh, 2009
<i>Coronium coronatum</i>	Trophoninae	Paucispiral, smooth (3.5 whorls)/conspicuous	Lenticular	Ocenebrine-Muricine (3D/Flattened)	Pastorino <i>et al.</i> , 2007
<i>Xymenopsis muriciformis</i>	Pagodulinae	Paucispiral, smooth (3 whorls)/conspicuous	Lenticular, with central subovate depression	?/Muricine (?/flattened)	Pastorino & Harasewych, 2000
<i>Trophonella scotiana</i>	Pagodulinae	Paucispiral (1.5–2 whorls), symmetrical; fine, spiral threads/conspicuous	Lenticular	?/Muricine (?/flattened)	Hain & Arnaud, 1992; Harasewych & Pastorino, 2010
<i>Fuegotrophon pallidus</i>	Trophoninae?	Paucispiral, smooth (1.5–2 whorls), conspicuous	?	?	

The variation of the radula of *T. plicatus* was observed in different ontogenetic stages (Fig. 3A–F). This radula presents a different *bauplan* at each stage. Herbert, Merle & Gallardo, (2007) called the type of adult radula “flattened” in which all cusps lie in the same plane and the central cusp is longer than the laterals. In contrast the “3D” radula is one in which the central cusp is curved and shorter than the laterals. The radula of the embryos of *T. plicatus* is of the 3D type, while the flattened type occurs in the adult. In the embryo, the rachidian tooth is narrow, and the central and lateral cusps project in different planes. The central cusp is thin and long. The lateral cusps, slightly shorter than the central one, show a large inner denticle and a small one projecting from the outer margin of the lateral cusp (Fig. 3A, B). The marginal area has a single cusp and the base appears to be still in the process of formation (Fig. 3B). The position and relative size of cusps and denticles in this stage are important because of the difference with juveniles and adults (Fig. 3C, E).

Rachidian teeth in an intermediate growth stage have a short, curved central cusp, wider and shorter lateral cusps and the denticles of the inner margin of the laterals appear shorter than in larval specimens (Fig. 3C). The outer denticles of the laterals are conspicuous in this growth stage (Fig. 3C, D). The marginal area is characterized by a single cusp and a deep base, where the teeth attach to the radular membrane (Fig. 3D).

The adult rachidian tooth is wide and shows a low projection of the central cusp and lateral from the base (Fig. 3E). Lateral cusps are wider than in any earlier stage and have a short denticle at the inner margin and numerous denticles at the outer margin (Fig. 3E, F). The marginal area has a single cusp at either side of the conspicuous rachidian base (Fig. 3F).

Table 1 summarizes the available data on egg capsules and embryos of the subfamily Trophoninae from the southwestern Atlantic. Thorson (1940, 1946) suggested that different morphology patterns in egg capsules should be in agreement with taxonomic affinities. South American muricid egg capsules follow two typical patterns, i.e. one flat, sessile, bulliform and hemispherical in shape and the other erect, sometimes with a peduncle. *Trophon geversianus* has an erect capsule with a flattened peduncle (Zaixso, 1973; Penchaszadeh, 1976; D’Asaro, 1991) of the second type. The egg capsules of *T. plicatus* described here have no peduncle; however, they are erect as in *T. geversianus*.

Therefore they can also be classified in the second type, in agreement with other members of the subfamily Trophoninae.

Recently Barco *et al.* (2012) described the new subfamily Pagodulinae based on molecular and morphological data. They included the genera *Xymenopsis* Powell, 1951, from southern South America and *Trophonella* Harasewych & Pastorino, 2011 from Antarctica. Both genera were previously included in Trophoninae. Hain (1990), Hain & Arnaud (1992) and Harasewych & Pastorino (2010) reported *Trophonella scotiana* (Powell, 1951), type species of the genus *Trophonella* (not *T. rugosolamellata* Harasewych & Pastorino, 2010 in error in Barco *et al.*, 2012) from Antarctic waters. According to them, this species has flat lenticular egg capsules like those of *Xymenopsis muriciformis* (King, 1832) (type species of *Xymenopsis*), which supports the inclusion of these genera in the subfamily Pagodulinae (Barco *et al.*, 2012). The New Zealand genera *Xymene* Iredale, 1915 and *Zeatrophon* Finlay, 1927 (the latter doubtfully) were also included in Pagodulinae by Barco *et al.* (2012). The type species of these genera, *X. plebeius* (Hutton, 1873) (see Pastorino & Harasewych, 2000) and *Z. ambiguus* (Philippi, 1844) (see D’Asaro, 1991), have the flat and hemispherical type of capsules justifying this taxonomic position. The morphology of the radulae of the genera *Xymenopsis* and *Trophonella*, as illustrated by Pastorino & Harasewych (2000) and Harasewych & Pastorino (2010), is also in agreement with this taxonomic placement.

Coronium Simone, 1996, was included in Trophoninae but appears to be the exception to this rule, because the egg capsules do not follow the expected pattern. *Coronium coronatum* (type species of the genus) and *C. acanthodes* both have the first type of egg capsules, i.e. flat and hemispherical, with minor differences between them (Pastorino, Penchaszadeh & Scarabino, 2007; Pastorino & Penchaszadeh, 2009). In addition, the distinctive protoconch of *Coronium* is very different from those known in Trophoninae and Pagodulinae. It has a multispiral somewhat enlarged protoconch, with a very acute first whorl. The radula seems to be closer to Trophoninae, with the intermediate cusps of the rachidian rising from the side of the lateral cusps, but its subfamilial assignment remains uncertain.

The egg capsules of *Fuegotrophon*, considered *incertae sedis* by Barco *et al.* (2012), are unknown, but the radula and protoconch are consistent with Trophoninae.

In general terms, the layers described by previous authors (i.e. Tamarin & Carriker, 1967; D'Asaro, 1988; Hawkins & Hutchinson, 1988) for the capsules of other muricids were also observed in those of *T. plicatus*. However layer L4 is completely detached from the rest of the layers in a way that has not been described before. According to D'Asaro (1988), this layer (called the “albumen retaining layer”) is usually neglected because of its thinness and because it is “bound tightly to other components”. This L4 layer was observed completely attached to the wall while embryos were not developed. However, it was free and surrounding the grown up embryos just before hatching. The function of this unusual feature is unknown.

Penchaszadeh (1976) described the eggs and egg capsules of what was identified at that time as *T. laciniatus* and *T. varians*. According to the drawings, measurements and localities where the specimens were collected they probably belong to *T. geversianus* or *T. patagonicus*. *Trophon laciniatus* is a junior synonym of *T. plicatus*, a name that has been used wrongly for specimens of *T. patagonicus* from Buenos Aires province. *Trophon plicatus* has never been collected offshore in Buenos Aires province (Pastorino, 2005).

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