

A Subantarctic rare gastropod reveals a new type of spawn among heterobranchs

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Spawn and specimens of *Toledonia biplicata* comb. nov. were collected from Burdwood Bank during March 2013. Species-level identification was derived from radular, shell and spawn characters. A low number of eggs laid per spawn (one to three), absence of an external gelatinous mass and a considerably thickened capsule wall (~50 µm) constitute a unique combination of spawn characters among heterobranchs. Egg capsules are ovoid in shape and measure 600–763 × 450–656 µm. They are filled with albuminous liquid, allowing complete intracapsular development of a single embryo. The developmental stages recognized are uncleaved eggs (153 µm in average diameter), veliger stages (279–378 µm in maximal length) and prehatchling juveniles (442–609 µm). *Toledonia biplicata* increases its volume 40-fold during its intracapsular development, whereas other comparable cephalaspids increase ≤ 4-fold. The role of the capsule wall as protection and an additional source of food is discussed. The adaptive value of these characters in relationship to the environmental conditions of the Magellanic region is discussed. A comparison with the spawn of other cephalaspids, nudibranchs and pleurobranchids is conducted, and the taxonomic implications are discussed. This is the first description of the spawn and developmental stages of a representative of the genus *Toledonia*.

ADDITIONAL KEYWORDS: adaptation – evolution – Mollusca – Patagonia – reproductive biology.

INTRODUCTION

The genus *Toledonia* Dall, 1902 includes 20 accepted species (WoRMS, 2019), which are mostly Subantarctic or Antarctic, but it also includes one Subarctic species (Ohnheiser & Malaquias, 2014 and references therein), one species in south-eastern Australia (Golding, 2010) and two species from the deep sea of the south-western Pacific Ocean (Valdes, 2008). Since Odhner (1914) redescribed *Toledonia* (= *Ptisanula*) *limnaeoides* (Odhner, 1913), the genus has been considered as a cephalaspid of the family Diaphanidae. Warén (1989) interpreted shell, radular and anatomical characters to allow a subfamilial rank, i.e. Toledoniinae. Oskars et al. (2015) and Moles et al. (2017c) provided a phylogenetic analysis based on molecular characters, placing *Toledonia* in the family Cylichnidae, one of the basal branches of Cephalaspidea. The last systematic account of the genus was given by Marcus (1976), and Dell (1990) provided a valuable report of Antarctic and

Magellanic species, but neither included information on the spawn or development.

In this work, we provide descriptions of the spawn and developmental stages of *Toledonia* (= *Odostomia*) *biplicata* (Strebel, 1908). The material studied here was collected from Burdwood Bank (Namuncurá), a Subantarctic area located west of at the southern tip of the Magellan Region and south of the Falkland Islands, Malvinas (Falkland).

MATERIAL AND METHODS

All specimens studied were collected from two stations in the Burdwood Bank area, i.e. 54°15.805'S, 59°59.042'W at 103 m depth and 54°14.893'S, 60°37.716'W at 117 m depth. Both stations are part of the 'Campaña Antártica de Verano' survey run in March 2013. Samples were obtained with a Rauschert sledge on board the Argentine RV Puerto Deseado. The sledge had a mouth opening of 55 cm × 15 cm and was equipped with a nylon net of 1 mm × 1 mm mesh size. The samples were manually sieved ten times,

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with the sorted material then fixed with 4% formalin in seawater and later preserved in 70% ethyl alcohol. As a result, 36 egg capsules and 16 free juveniles and adults identified as *Toledonia biplicata* were recovered.

The material here reported was deposited in the Invertebrate collection of the Museo Argentino de Ciencias Naturales (MACN) under the numbers MACN-In 42483 and 42484. Type material of *Odostomia biplicata* Strebel, 1908, housed at the Swedish Museum of Natural History (SMNH 1056), was studied. Measurements and photographs were obtained with a Zeiss Discovery V20 stereoscopic microscope and Philips XL 30 scanning electron microscope (SEM) at the MACN. Measurements are as follows: d_1 , maximal length of the capsule and d_2 , maximal width, perpendicular to d_1 ; d , egg diameter; L , maximal length of veliger, and r , radius; h and w , shell height and width, respectively. The protoconch whorls and nucleus were measured according to Bouchet & Kantor (2004). For SEM study, specimens were critical point dried (i.e. a method of dehydrating biological tissue before examination under the SEM). Radulae were obtained from whole animals dissolved in a 5% bleach solution in distilled water. The volumes (V) of eggs and hatchling specimens were obtained by: $V = 4\pi r^3/3$, considering $r = d/2$ for the spherical uncleaved eggs and $r = (h + w)/4$ for ovoid hatchling specimens.

RESULTS

IDENTIFICATION OF THE CAPSULES, EMBRYOS AND ADULTS

Adult specimens (Fig. 1A–F, H) had a conical shell, with up to five convex whorls and two strong columellar teeth. Two to six spiral rings of small pits composed a delicate pattern of ornamentation present in only the first one and three-quarters to two whorls, i.e. the protoconch (Fig. 1D–F); two of the rings were visible only in the first whorl and then hidden by the suture. These were present in the holotype of *O. biplicata* (Fig. 1B–D) and in the pre-hatchlings (Figs 1G, 2C) and recently hatched specimens studied.

Radulae from pre-hatchling specimens (Fig. 1I) showed the same features as those observed in adults (Fig. 1H) from the same samples that, together with the sculpture pattern, confirmed its identity. The radulae (Fig. 1H, I) had rachidian teeth, with a large central cusp and three smaller cusps on each side. The central teeth were similar to those of other species of *Toledonia*, as shown in the works of Thiele (1904, 1912), Marcus (1976), Warén (1989), Jensen (1996), Schiøtte (1998), Valdes (2008), Golding (2010) and

Ohnheiser & Malaquias (2014). According to this, *O. biplicata* should be placed in the genus *Toledonia*, and we here provide the new combination ***Toledonia biplicata* (Strebel, 1908) comb. nov.** Capsules of stages earlier than pre-hatchling were similar, but the wall was thicker (50 vs. 30–25 μm ; Fig. 2B cf. C, D).

SPAWN

The spawn was composed of one to three egg capsules laid contiguously (Fig. 2A–D). Each capsule was filled with intracapsular liquid surrounding a single egg (or embryo) and measured 600–762.5 μm \times 450–656.25 μm (mean = 689.34 μm \times 528.81 μm , $N = 27$) corresponding to d_1 and d_2 of each capsule, respectively. They were lenticular, ovoid in shape, with a flat attachment area to contiguous capsules. Most capsules were laid over hydrozoans of the genera *Grammaria* (Fig. 2A) and *Simplectoscyphus* and, in a few cases, on the bryozoan *Amastigia* sp., all of which are colonial and branched species. The capsule wall was translucent, coriaceous, considerably thick (~50 μm for capsules containing uncleaved eggs; Fig. 2B) and with a smooth surface. Recently laid capsules had strong and firm walls and contained dense intracapsular liquid (Fig. 2B), giving a yellowish colour, which became progressively less dense and was almost absent at hatching, when the capsule wall was also thinner (30–25 μm) and wrinkled (Fig. 2C, D).

DEVELOPMENTAL STAGES

A single egg ($N = 4$) or developing embryo ($N = 32$) was found inside each egg capsule. Capsules laid contiguously showed the same developmental stage (Fig. 2B, D), implying that they were probably spawned by a single animal. No free larval stages were recognized. Uncleaved eggs (Fig. 2B) were whitish, but appeared yellowish inside the capsule owing to the fluid. They measured 153 μm in diameter ($N = 2$).

Veliger stages (Fig. 2E–G) measured 279–379 μm in length (mean = 336 μm , $N = 5$). The shell had between one-half and one whorl and was thin, organic and translucent. The mantle edge, velum and foot could be recognized. Soft parts were not retracted into the shell; the operculum and eyes were not observed in the studied material.

Pre-hatchling juveniles (Figs 1G, I, 2C, D) measured 442–610 μm in height (mean = 557.3 μm , $N = 10$). The shell had between one and a quarter and two translucent whorls that became progressively more calcified and whitish (Fig. 2C cf. D). Ornamentation (Figs 1G, 2C) was composed of marked subquadrangular pits arranged in two to six spiral threads above the suture plus five or six at the shell base. Spiral threads, particularly those located at the centre of the whorls, sometimes became

Table 1. Reproductive data on cephalaspids with juveniles as the hatching stage

Current name	EGM	Capsules per spawn	Maximal capsule size (μm)	Mean egg size (μm); volume (mm^3)	Mean shell size at hatching (μm); volume (mm^3)	Locality	Source
<i>Antarctophilina gibba</i>	Yes	975–4760	549	376; 0.028	$\sim 500 \times \sim 357$; 0.041	South Georgia Islands, south-west Atlantic	Seager (1978, 1979) (as <i>Philina gibba</i>)
<i>Haminoea antillarum</i>	Yes	–	–	148; 0.002	–	South and Central Florida, north-west Atlantic	DeFreese & Clark (1983)
<i>Haminoea japonica</i>	Yes	120–1240	410 \times 330	210 \times 230; 0.006	360 \times 280; 0.017	Washington, north-east Pacific	Gibson & Chia (1989, 1991) (as <i>Haminoea callidegenita</i>)
<i>Haminoea navicula</i>	Yes	1951 \pm 1532	320 \times 292	179; 0.003	235.7 \times 205; 0.006	Alberoni, Mediterranean Sea	Schaefer (1996)
	Yes	–	–	180; 0.003	–	Alberoni, Mediterranean Sea	Schaefer (1997)
	Yes	7700–15 500	355	147; 0.002	–	Ría de Aveiro, north-east Atlantic	Malaquias & Cervera (2005)
<i>Neunesia antarctica</i>	No	1–4	6000	–	1900	Wedell Sea, Antarctic	Hain (1990); Hain & Arnaud (1992)
<i>Retusa obtusa</i>	Yes	–	–	245; 0.008	$\sim 261 \times \sim 207$; 0.007	Glamorgan, north-east Atlantic	Smith (1967)
	Yes	12–50	390	243–330; 0.008–0.019	–		Schaefer (1996)
	Yes	8–55	390	260; 0.009	–	Forth Estuary, north-east Atlantic	Berry (1989)
<i>Acteocina atrata</i>	Yes	23–148	320 \times 262	151.6; 0.002	300 \times 204; 0.008	Indian River Lagoon, north-west Atlantic	Mikkelsen & Mikkelsen (1984)
<i>Toledonia buplicata</i>	No	1–3	689 \times 529	153; 0.002	$\sim 600 \times \sim 475$; 0.081	Burdwood Bank, south-west Atlantic	Present study

All species were reported with one egg per capsule, but for *Antarctophilina gibba* there were reports of some capsules with more than one egg that does not develop. Hatching specimens of *Haminoea japonica* are mostly juveniles, with some veligers reported from the same spawn (Gibson & Chia, 1989). Measurements reported for hatching specimens of *A. gibba* are (mean \pm SD) $375 \pm 55 \mu\text{m}$ (Seager, 1979: 66), although in fig. 111 the author illustrated a specimen of $\sim 500 \mu\text{m} \times \sim 357 \mu\text{m}$; the last value is considered here to be more reliable because the egg diameter reported is $376 \mu\text{m}$. Smith (1967) did not provide measurements for hatching specimens of *Retusa obtusa*; a value of $\sim 261 \mu\text{m} \times \sim 207 \mu\text{m}$ was estimated from fig. 7A, B. Abbreviation: EGM, external gelatinous mass present in the spawn.

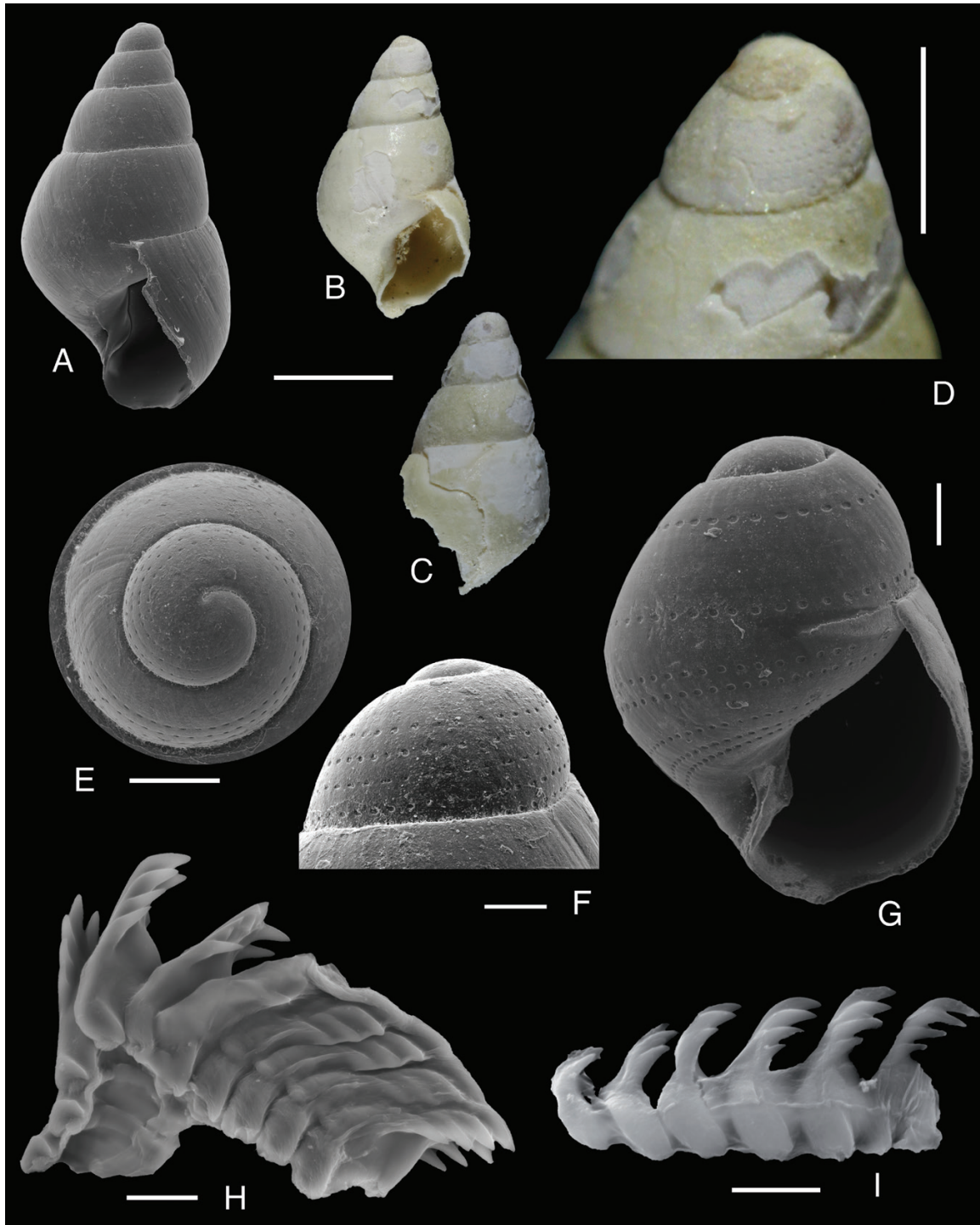


Figure 1. Prehatchlings and hatched stages of *Toledonia biplicata*. A, E, F, adult shell (five whorls), visualized by scanning electron microscopy (SEM). A, entire shell. E, F, apical and lateral views of the protoconch. B–D, holotype of *Odostomia biplicata* (SMNH 1056). B, C, two views of the shell. D, detail of the protoconch. G, I, prehatchling stage (two whorls) visualized by SEM. G, entire shell. I, complete radula. H, radula of an adult specimen visualized by SEM. Scale bars: 1 mm (A–C), 500 μ m (D), 200 μ m (E), 100 μ m (F, G), 20 μ m (H) and 10 μ m (I).

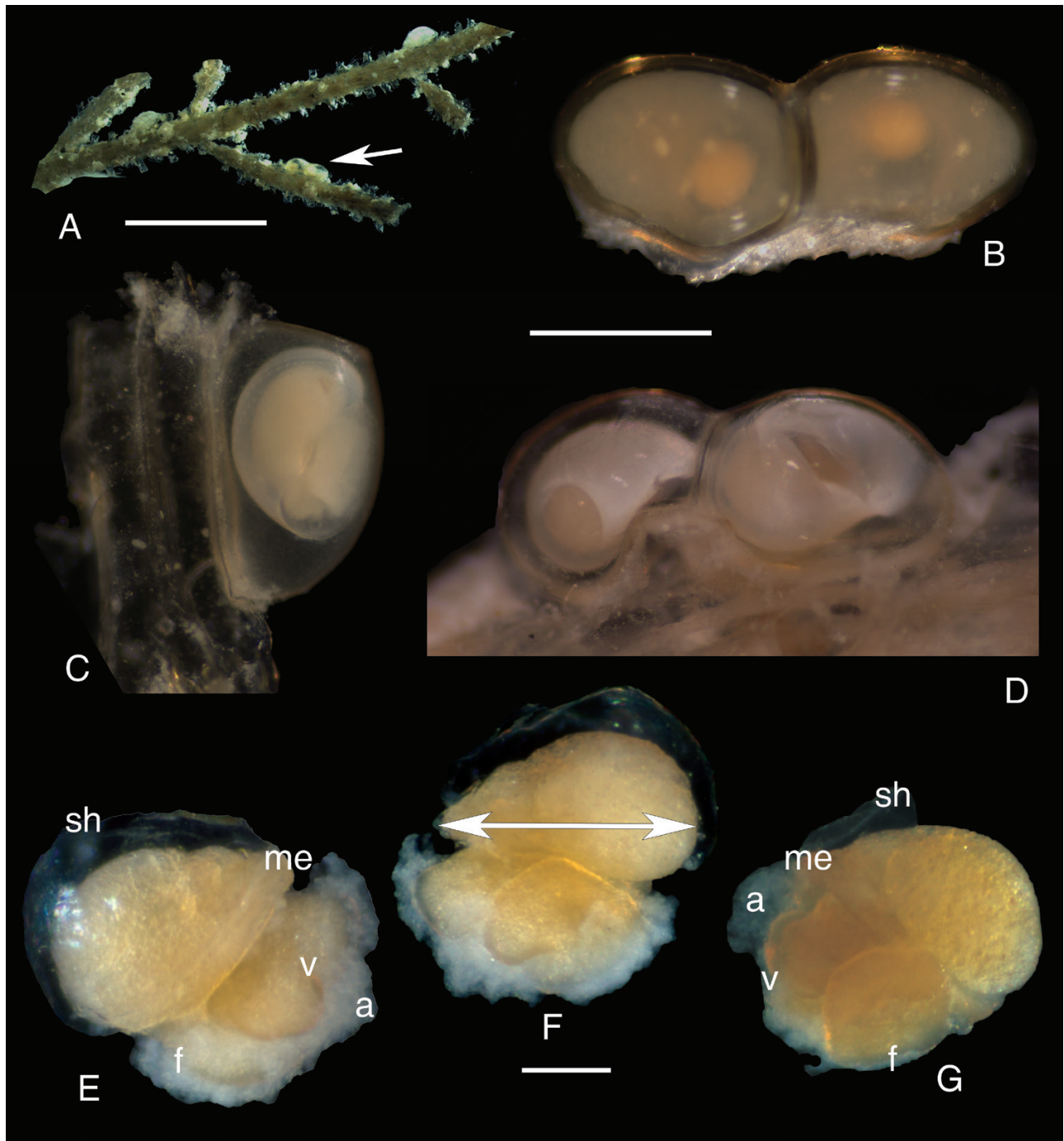


Figure 2. Developmental stages of *Toledonia biplicata*. A, two capsules contiguously placed on the hydrozoan *Grammaria* sp.; arrow indicates position of capsules. B, capsules with uncles eggs. C, capsule with early pre-hatching juvenile. D, capsules with late pre-hatching juveniles. E–G, three views of veliger stages; double-headed arrow in F indicates the maximal length (L) measured. Scale bars: 50 mm (A), 500 μ m (B–D) and 100 μ m (E–G). Abbreviations: a, albumin; f, foot; me, mantle edge; sh, shell traces; v, velum.

progressively less evident (Fig. 2G). Lines were weak in the last part of the final whorl of specimens with two whorls. A short foot, a head with two rounded lobes and subepithelial eyes were visible. Heterostrophy, a

common character among heterobranchs represented by several whorls, was weakly insinuated by about a half whorl and a subtly sunken and twisted apex (Fig. 1F).

Juvenile and adult specimens (Fig. 1A–F, H) had two to five whorls ($h_{\max} = 4.1$ mm), the shell surface was pinkish, sometimes with faint spiral threads visible only by SEM in whorls two to five. The nucleus of pre-hatchlings and hatched specimens measured 173–231 μm (mean = 213 μm , $N = 7$), and the protoconch was 593–725 μm (mean = 647 μm , $N = 7$) wide.

Table 1 provides a comparison of the results from this study with previously reported Cephalaspidea species that have direct development.

DISCUSSION

SPAWN CHARACTERS AMONG HETEROBRANCHIA

Spawn of *T. biplicata* (here described) is mainly characterized by the absence of an external, surrounding gelatinous mass, extremely small spawn with a low number of capsules and eggs or embryos (one to three; Table 1) and the presence of substantially thick capsule walls in early developmental stages (~50 μm). These features constitute a unique combination among the currently known Cephalaspidea and even Heterobranchia spawns (Hurst, 1967; Fernández-Ovies, 1981; Klussmann-Kolb & Wägele, 2001).

Newnesia antarctica Smith, 1902, a cephalaspidean species (Table 1), has a comparable spawn (*sensu* Hain, 1990: pl. 9, fig. 1i). It has no external, surrounding gelatinous mass and is composed of one to four capsules disposed in line, similar to what is described here for *T. biplicata*. Both spawns constitute an exception to the observations of Ghiselin (1966) and Klussmann-Kolb & Wägele (2001), who pointed out that all Heterobranchia egg masses are formed by a gelatinous external mass. In addition, thick capsule walls were reported by Gibson *et al.* (1970), Wägele (1989, 1996) and Moles *et al.* (2017b) for the nudibranchs *Bathydoris hodgsoni* Eliot, 1907, *Bathydoris clavigera* Thiele, 1912 and *Doris kerguelenensis* (Bergh, 1884) and the pleurobranchid *Bathyberthella antarctica* Willan & Bertsch, 1987, all of which are Antarctic species.

DIRECT DEVELOPMENTAL STAGES AMONG CEPHALASPIDEA

Development of *T. biplicata* occurs completely inside the egg capsule, where a single embryo develops, feeding in the intracapsular liquid. Schaefer (1996) reviewed information on the spawn and development of ~70 species of Cephalaspidea worldwide, which represents ~15% of the 634 accepted species (*sensu* Oskars *et al.*, 2015), and pointed out that most species

have indirect development. This general review and additional works (Hain, 1990; Hain & Arnaud, 1992; Schaefer, 1997; Malaquias & Cervera, 2005; present study) include reports of only eight species (10%) with direct development [i.e. *Acteocina atrata* Mikkelsen & Mikkelsen, 1984, *Antarctophilina gibba* (Strebel, 1908), *Haminoea antillarum* (d'Orbigny, 1841), *Haminoea japonica* Pilsbry, 1895, *Haminoea navicula* (da Costa, 1778), *N. antarctica*, *Retusa obtusa* (Montagu, 1803) and *T. biplicata*; Table 1]. Cephalaspidea species with direct development have large eggs (> 100 μm in diameter) in lower number per spawn (between one and 4760) compared with the species that have indirect development (< 100 μm , 50–60 000; Schaefer, 1996). These results are supported by the statement by Picken (1979) that prosobranch species with direct development produce fewer and larger eggs than species with indirect development. Also, Thompson (1967) correlated direct development with eggs measuring 110–250 μm .

The veliger stages of *T. biplicata*, in which eyes and an operculum were not observed, are comparable to those of *A. gibba* (Seager, 1979: fig. 11F–H). Seager (1979) reported the inability of the veliger to retract its soft parts into the shell, a behavioural feature that we think is also present in *T. biplicata*. Although only preserved veligers were studied, all had exposed soft parts, in contrast to all pre-hatchling and hatchling specimens, which had their soft parts retracted into the shell. Hatchling specimens were large in comparison to other species of cephalaspids with direct development (Table 1; i.e. ~600 μm , compared with ~500 μm in *A. gibba*, with the exception of *N. antarctica*, which is a considerably larger species at adult size, ≤ 38 mm; Moles *et al.*, 2017a).

The characters studied here fit the report by Shuto (1974) for prosobranch gastropods with direct development. Although *T. biplicata* is a heterobranch gastropod, it has a globose protoconch, with few whorls (up to two whorls, 647 μm) and with a large apex (213 μm). Also, a large egg size (153 μm) is in accord with the report by Shuto (1974).

SPAWN CHARACTERS AND THEIR POSSIBLE ADAPTIVE VALUE

The absence of an external gelatinous mass, as protection for the embryos (Klussmann-Kolb & Wägele, 2001), could be interpreted as a trade-off with the thickness of the capsule wall. Given that *T. biplicata* is a cold-water species, low metabolism and slow growth of the embryos are expected, resulting in considerable exposure to potential predators during intracapsular development (Wägele, 1996). Therefore, effective protection, such as a thicker wall, is expected for the eggs to survive. Wägele (1989, 1996) and Moles *et al.* (2017b) reported the capsule wall to be a possible additional source of food for the embryos of Antarctic

unshelled heterobranchs. Despite the nutritional role of the intracapsular liquid, which should be essential, the thick capsule wall of *T. biplicata* could also play an important role; a considerable reduction in the thickness of the wall was seen throughout the development of the embryo (from 50 to 30–35 µm). Moreover, comparison with the other Cephalaspidea species with direct development (Table 1) supports this hypothesis, because *T. biplicata* almost quadruples its length during the intracapsular development (from 153 to ~600 µm), whereas the other species only double in length (from 151.6 to ~300 µm in *A. atrata*). The large difference in size is even more evident in terms of volume: the internal volume in *T. biplicata* increases ~40-fold (from 0.002 to 0.081 mm³), whereas for other species the internal volume increases ~4-fold (from 0.002 to 0.008 mm³ in *A. atrata*). Comparable ‘cold-water’ species (such as *A. gibba*, *N. antarctica* and *R. obtusa*) exhibit an increase in length of ~1.3-fold (from 376 to ~500 µm) and 1.5-fold in volume (from 0.028 to 0.041 mm³).

The low number of eggs per spawn in *T. biplicata* could be related to the expected relative high production cost of the thick capsule walls. A small adult size (≤ 4.1 mm for *T. biplicata*) represents a particular limitation to the energy available for reproduction (Ramírez-Llorda, 2002; Ituarte & Presta, 2017). However, comparable larger species with direct development, such as the previously mentioned *N. antarctica* and *B. hogdsoni* (≤ 87 mm; Valdes, 2011), lay few eggs (up to four in both cases; Hain, 1990 and Wagele, 1996, respectively). The narrow branches of hydrozoan and bryozoan species where the spawns are laid constitute a small area, unsuitable for larger spawn. This could represent a benefit for the populations of *T. biplicata*, because large potential benthic predators are unable to access the spawn. Direct development allows the embryos to feed on the intracapsular liquid without requiring external, planktonic sources of food, which results in avoidance of predation and dispersion to unfavourable areas that could occur in the water mass. Therefore, it allows hatchling juveniles to live in the same environment as the adults (Smith, 1967; Pechenik, 1979). Moreover, the place where most heterobranchs live is the same as where they feed and breed, and it also provides excellent opportunities to find partners; hermaphroditism is typical in heterobranchs (Rudman & Willan, 1998).

DIRECT DEVELOPMENT AS A BIOGEOGRAPHICAL PATTERN IN THE SOUTHERN OCEAN

Antarctic and Subantarctic marine environments, such as the Magellan Region, are well known because of the high incidence of direct development in several benthic taxa (e.g. Thomson, 1876; Picken, 1979; Hain

& Arnaud, 1992; Poulin & Féral, 1996; Gallardo & Penchaszadeh, 2001; Lockhart, 2006; Pearse *et al.*, 2009). Related to environmental conditions such as the presence of a low, almost constant water temperature and markedly seasonal productivity (Clarke *et al.*, 2004), there are other biological adaptations that also have a high incidence in these areas, such as comparatively lower growth rates, which contribute to longevity and later sexual maturity (Hoegh-Guldberg & Pearse, 1995; Peck *et al.*, 2006; Moles *et al.*, 2017b; among others). Most gastropods show comparatively larger spawn, with fewer eggs and capsules laid, that involve considerably longer periods of development (Picken, 1979; Hain & Arnaud, 1992; Wagele, 1996; Peck *et al.*, 2006; Moles *et al.*, 2017b; among others). Pearse *et al.* (2009) suggested that the high incidence of direct development would be not only a consequence of adaptation to the environmental conditions of such areas. These authors suggested that this type of development was acquired independently by many taxa of invertebrates, in other environmental conditions before the Pliocene–Pleistocene Antarctic glaciations, and remains as the dominant mode of reproduction today because of the selective extinction of most of the species with indirect development, produced by the reduction in primary production of food for the larvae. Occasional transport, mainly via the Antarctic Circumpolar Current between glacial periods, would allow dispersion and establishment of isolated populations around Antarctic and Subantarctic environments that might result in new species. Evidence of isolation was found by Hoffman *et al.* (2011), who reported genetically more structured populations in a species with direct development with respect to a comparable indirect development. Moreover, several reports (e.g. Poulin & Féral, 1996; Gallardo & Penchaszadeh, 2001; Pearse & Lockhart, 2004; Pearse *et al.*, 2009) have found direct development in Antarctic and Magellan areas restricted to certain clades of invertebrate taxa.

In this scenario, the similarities of the spawn of *T. biplicata* and *N. antarctica* (i.e. a small number of capsules, not included in an external, surrounding gelatinous mass), suggest a common origin. Also, Oskars *et al.* (2015) and Moles *et al.* (2017c), based on molecular characters, reported Newnesiidae and *Toledonia* as basal branches of Cephalaspidea. Radular and/or anatomical characters also support a close relationship of *Newnesia* and *Toledonia* species (Thiele, 1904, 1912; Marcus, 1976; Warén, 1989; Hain, 1990; Jensen, 1996; Schjötte, 1998; Valdes, 2008; Golding, 2010; Ohnheiser & Malaquias, 2014; Moles *et al.*, 2017a, c; present study). The mainly circum-Antarctic distribution of most *Toledonia* and all Newnesiidae species (see

Dell, 1990, and Moles *et al.*, 2017a, c, respectively) could be related to the dispersal potential of the Antarctic Circumpolar Current (Beu *et al.*, 1997; Pearse *et al.*, 2009). It applies to the Burdwood Bank because the northern branches of the Antarctic Circumpolar Current run northwards to the west and east of this area (Guerrero *et al.*, 1999).

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REFERENCES

- Berry AJ. 1989.** Spawning season and egg production in Forth Estuary *Retusa obtusa* (Montagu) (Gastropoda: Opisthobranchia). *Journal of Molluscan Studies* **55**: 455–459.
- Beu AG, Griffin M, Maxwell PA. 1997.** Opening of Drake Passage gateway and Late Miocene to Pleistocene cooling reflected in Southern Ocean molluscan dispersal: evidence from New Zealand and Argentina. *Tectonophysics* **281**: 83–97.
- Bouchet P, Kantor Y. 2004.** New Caledonia: the major centre of biodiversity for volutomitrid molluscs (Mollusca: Neogastropoda: Volutomitridae). *Systematics and Biodiversity* **1**: 467–502.
- Clarke A, Aronson RB, Crame JA, Gili J, Blake DB. 2004.** Evolution and diversity of the benthic fauna of the Southern Ocean continental shelf. *Antarctic Science* **16**: 559–568.
- DeFreese DE, Clark KB. 1983.** Analysis of reproductive energetics of Florida Opisthobranchia (Mollusca: Gastropoda). *International Journal of Invertebrate Reproduction* **6**: 1–10.
- Dell RK. 1990.** Antarctic Mollusca with special reference to the fauna of the Ross Sea. *Bulletin of the Royal Society of New Zealand* **27**: 1–311.
- Fernández-Ovies CL. 1981.** Contribución a la clasificación morfológica de las puestas de los opisthobranchios (Mollusca: Gastropoda). *Boletín de Ciencias de la Naturaleza I. D. E. A.* **28**: 3–12.
- Gallardo CS, Penchaszadeh PE. 2001.** Hatching mode and latitude in marine gastropods: revisiting Thorson's paradigm in the southern hemisphere. *Marine Biology* **138**: 547–552.
- Ghiselin MT. 1966.** Reproductive function and the phylogeny of opisthobranch gastropods. *Malacologia* **3**: 327–378.
- Gibson GD, Chia F. 1989.** Developmental variability (pelagic and benthic) in *Haminoea callidegenita* (Opisthobranchia: Cephalaspidea) is influenced by egg mass jelly. *Biological Bulletin* **176**: 103–110.
- Gibson GD, Chia F. 1991.** Contrasting reproductive modes in two sympatric species of *Haminoea* (Opisthobranchia: Cephalaspidea). *Journal of Molluscan Studies* **57**: 49–60.
- Gibson R, Thompson TE, Robilliard GA. 1970.** Structure of the spawn of an Antarctic dorid nudibranch *Austrodoris macmurdensis* Odhner. *Journal of Molluscan Studies* **39**: 221–225.
- Golding R. 2010.** Anatomy in *Toledonia warenella* n. sp. (Gastropoda: Opisthobranchia: Diaphanidae) visualized by three-dimensional resolution. *Invertebrate Biology* **129**: 151–164.
- Guerrero RA, Baldoni A, Benavides H. 1999.** Oceanographic conditions at the southern end of the Argentine continental slope. *INIDEP Documento Científico* **5**: 7–22.
- Hain S. 1990.** Die beschalteten benthischen Mollusken (Gastropoda und Bivalvia) des Weddellmeeres. *Antarktisk, Berichte zur Polarforschung* **70**: 1–181.
- Hain S, Arnaud PM. 1992.** Notes on the reproduction of high-Antarctic molluscs from the Weddell Sea. *Polar Biology* **12**: 303–312.
- Hoegh-Guldberg O, Pearse JS. 1995.** Temperature, food availability, and the development of marine invertebrate larvae. *Integrative and Comparative Biology* **35**: 415–425.
- Hoffman JI, Clarke A, Linse K, Peck LS. 2011.** Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. *Marine Biology* **158**: 287–296.
- Hurst A. 1967.** The egg masses and veligers of thirty northeast Pacific opisthobranchs. *The Veliger* **9**: 255–288, pls. 26–38.
- Ituarte C, Presta ML. 2017.** Fecundity and timing of oogenesis at high latitudes: reproductive traits in the brooding bivalve *Neolepton cobbi* (Cyamioidea: Neoleptonidae). *Journal of Molluscan Studies* **83**: 340–350.
- Jensen K. 1996.** The Diaphanidae as a possible sister group of the Sacoglossa (Gastropoda, Opisthobranchia). In: Taylor JD, ed. *Origin and evolutionary radiation of the Mollusca*. New York: Oxford University Press, 231–247.

- Klussmann-Kolb A, Wägele H. 2001.** On the fine structure of Opisthobranch egg masses (Mollusca, Gastropoda). *Zoologischer Anzeiger* **240**: 101–118.
- Lockhart SJ. 2006.** *Molecular evolution, phylogenetics, and parasitism in Antarctic cidaroid echinoids*. Unpublished D. Phil. Thesis, University of California, Santa Cruz.
- Malaquias MA, Cervera JL. 2005.** The genus *Haminoea* (Gastropoda: Cephalaspidea) in Portugal, with a review of the European species. *Journal of Molluscan Studies* **72**: 89–103.
- Marcus E. 1976.** A taxonomic survey of the genus *Toledonia* Dall, 1902 (Opisthobranchia, Diaphanidae). *Zoologica Scripta* **5**: 25–33.
- Mikkelsen PS, Mikkelsen PM. 1984.** Comparison of *Acteocina canaliculata* (Say, 1826), *A. candei* (d'Orbigny, 1841), and *A. atrata* spec. nov. (Gastropoda: Cephalaspidea). *The Veliger* **27**: 164–192.
- Moles J, Ávila C, Malaquias MA. 2017a.** Systematic revision of the Antarctic gastropod family Newnesiidae (Heterobranchia: Cephalaspidea) with the description of a new genus and a new abyssal species. *Zoological Journal of the Linnean Society* **183**: 763–775.
- Moles J, Wägele H, Cutignano A, Fontana A, Ballesteros M, Avila C. 2017b.** Giant embryos and hatchlings of Antarctic nudibranchs (Mollusca: Gastropoda: Heterobranchia). *Marine Biology* **164**: 1–13.
- Moles J, Wägele H, Schrödl M, Avila C. 2017c.** A new Antarctic heterobranch clade is sister to all other Cephalaspidea (Mollusca: Gastropoda). *Zoologica Scripta* **46**: 127–137.
- Odhner NH. 1914.** *Ptisanula limnaeoides*, a new arctic opisthobranchiate mollusc, its anatomy and affinities. *Arkiv för Zoologi* **8**: 1–18, pl. 1.
- Ohnheiser LT, Malaquias MA. 2014.** The family Diaphanidae (Gastropoda: Heterobranchia: Cephalaspidea) in Europe, with a redescription of the enigmatic species *Colobocephalus costellatus* M. Sars, 1870. *Zootaxa* **3774**: 501–522.
- Oskars TR, Bouchet P, Malaquias MA. 2015.** A new phylogeny of the Cephalaspidea (Gastropoda: Heterobranchia) based on expanded taxon sampling and gene markers. *Molecular Phylogenetics and Evolution* **89**: 130–150.
- Pearse JS, Lockhart SJ. 2004.** Reproduction in cold water: paradigm changes in the 20th century and a role for cidaroid sea urchins. *Deep-Sea Research II* **51**: 1533–1549.
- Pearse JS, Mooi R, Lockhart SJ, Brandt A. 2009.** Brooding and species diversity in the Southern Ocean: selection for brooders or speciation within brooding clades. In: Krupnik I, Lang MA, Miller S, eds. *Smithsonian at the poles: contributions to International Polar Year Science*. Washington: Smithsonian Institution, 181–196.
- Pechenik JA. 1979.** Role of encapsulation in invertebrate life histories. *The American Naturalist* **114**: 859–870.
- Peck LS, Clarke A, Chapman AL. 2006.** Metabolism and development of pelagic larvae of Antarctic gastropods with mixed reproductive strategies. *Marine Ecology Progress Series* **318**: 213–220.
- Picken GB. 1979.** Non-pelagic reproduction of some Antarctic prosobranch gastropods from Signy Island, South Orkney Islands. *Malacologia* **19**: 109–128.
- Poulin E, Féral J. 1996.** Why are there so many species of brooding Antarctic echinoids? *Evolution* **50**: 820–830.
- Ramírez-Llorda E. 2002.** Fecundity and life-history strategies in marine invertebrates. *Advances in Marine Biology* **43**: 87–170.
- Rudman WB, Willan RC. 1998.** Opisthobranchia introduction. In: Beesley PL, Ross GJB, Wells A, eds. *Mollusca: the southern synthesis. Fauna of Australia 5, part B*. Melbourne: CSIRO Publishing, 915–942.
- Schaefer K. 1996.** Review of data on cephalaspid reproduction, with special reference to the genus *Haminaea* (Gastropoda, Opisthobranchia). *Ophelia* **45**: 17–37.
- Schaefer K. 1997.** Early development and morphogenesis of the intracapsular veliger of *Haminaea navicula* (Gastropoda: Opisthobranchia: Bullomorpha). *Invertebrate Reproduction and Development* **32**: 89–105.
- Schiøtte TA. 1998.** A taxonomic revision of the genus *Diaphana* Brown, 1827, including a discussion of the phylogeny and zoogeography of the genus (Mollusca: Opisthobranchia). *Steenstrupia* **24**: 77–140.
- Seager JR. 1978.** A redescription of the Antarctic opisthobranch *Philine gibba* Strebel, 1908, from the type locality, South Georgia, South Atlantic. *Journal of Molluscan Studies* **44**: 171–179.
- Seager JR. 1979.** Reproductive biology of the Antarctic Opisthobranch *Philine gibba* Strebel. *Journal of Experimental Marine Biology and Ecology* **41**: 51–74.
- Shuto T. 1974.** Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia* **7**: 239–256.
- Smith ST. 1967.** The development of *Retusa obtusa* (Montagu) (Gastropoda, Opisthobranchia). *Canadian Journal of Zoology* **45**: 737–764.
- Strebel H. 1908.** Die Gastropoden (mit Ausnahme der nackten Opisthobranchier). *Wissenschaftliche Ergebnisse der Schwedischen Südpolar-Expedition 1901–1903* **6**: 1–112, pls. 1–6.
- Thiele J. 1904.** *Die beschalten Gastropoden der deutschen Tiefsee-Expedition 1898–1899. B. Anatomisch-systematische Untersuchungen einiger Gastropoden. Wissenschaftliche Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899, Vol. 7*. Jena: Verlag von Gustav Fischer, 147–174.
- Thiele J. 1912.** *Die Antarktischen Schnecken und Muscheln. Deutsche Südpolar-Expedition (1901–1903) Im auftrage des Reichsamtes des Innern Herausgegeben von Erich von Drygalski Leiter der Expedition, Vol. 13*. Zoologie **5**. Berlin: Druck und Verlag von Georg Reimer, 183–286.
- Thomson CW. 1876.** Notice of some peculiarities in the mode of propagation of certain Echinoderms of the

- Southern Sea. *Zoological Journal of the Linnean Society of London* **13**: 55–79.
- Thompson TE. 1967.** Direct development in a nudibranch, *Cadlina laevis*, with a discussion of developmental processes in Opisthobranchia. *Journal of the Marine Biological Association of the United Kingdom* **47**: 1–22.
- Valdes A. 2008.** Deep sea “cephalaspidean” heterobranchs (Gastropoda) from the tropical southwest Pacific. In: Héros V, Cowie RH, Bouchet P, eds. *Tropical deep-sea benthos 25. Mémoires du Muséum National d’Histoire Naturelle*, Vol. **196**. 587–792.
- Valdes A. 2011.** Phylogenetic systematics of “*Bathydoris*” s.l. Bergh, 1884 (Mollusca, Nudibranchia), with the description of a new species from New Caledonian deep waters. *Canadian Journal of Zoology* **80**: 1084–1099.
- Wägele H. 1989.** Über die Morphologie und Feinstruktur einiger Eigelege antarktischer Nudibranchia (Gastropoda). *Zoologischer Anzeiger* **222**: 225–243.
- Wägele H. 1996.** On egg clutches of some Antarctic Opisthobranchia. *Malacological Review Suppl. 6 Molluscan Reproduction*: 21–30.
- Warén A. 1989.** New and little known Mollusca from Iceland. *Sarsia* **74**: 1–28.
- WoRMS Editorial Board. 2019.** *World register of marine species*. Available at: <http://www.marinespecies.org> at VLIZ. Accessed 29 March 2019. doi: 10.14284/170.