Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2021) **87**: eyab027. https://doi.org/10.1093/mollus/eyab027
Published online 17 September 2021

Egg masses and larval development of the Antarctic cephalaspidean snail *Waegelea antarctica* (Cephalaspidea: Antarctophilinidae), with notes on egg masses of the related *Antarctophiline alata*

A.L. Moran, M-W.A. Toh, G.T. Lobert, T. Ely and P.B. Marko

School of Life Sciences, University of Hawai'i at Mānoa, 2538 McCarthy Mall, Edmondson 216, Honolulu, HI 96822, USA

Correspondence: A.L. Moran; e-mail: morana@hawaii.edu

(Received 22 December 2020; editorial decision 15 March 2021)

ABSTRACT

We describe, for the first time, egg masses and larval developmental mode of a recently described Antarctic philinoid snail, *Waegelea antarctica*. Egg masses resembled the gelatinous, attached masses of many temperate philinoid species and contained very large offspring that hatched as developmentally advanced veligers with many juvenile features. Like other Antarctic heterobranch egg masses, development in the masses of *W. antarctica* appeared to be largely synchronous despite low internal oxygen levels. Hatched larvae could both swim and crawl, and we did not observe metamorphosis over several days. Molecular barcoding using cytochrome *c* oxidase subunit I (COI) showed an almost perfect (<0.002% difference) match between our specimens from McMurdo Sound in the Ross Sea and a single sequence from a specimen collected >8,000 km away in the Weddell Sea, suggesting either high realized larval dispersal or a recent range expansion. We also describe the egg mass of the related *Antarctophiline alata* (identified using COI barcoding) from the Ross Sea, which differed from published descriptions in having considerably smaller embryos.

INTRODUCTION

The Southern Ocean surrounding the continent of Antarctica contains one of the least studied and most enigmatic benthic marine faunas in the world. Comparatively little is known about the reproduction or developmental biology of the majority of benthic Antarctic invertebrates (Thatje, 2012; Peck, 2018; Moran *et al.*, 2019); yet, information on life history characteristics like larval dispersal mode and length of development is increasingly important for interpreting the distribution, abundance, population structure and biogeographic history of this highly endemic fauna (Arnaud, 1977; Pearse, McClintock & Bosch, 1991; Rogers, 2007; Thatje, 2012; Moles, Avila & Malaquias, 2019; Muñoz-Ramírez *et al.*, 2020). Likewise, basic life history information is important for interpreting the dynamics and resilience of marine benthic communities on the Antarctic shelf, where climate change is predicted to increase the already frequent and often severe effects of disturbance from iceberg scour (Barnes & Conlan, 2007; Barnes, 2017).

The Antarctophilinidae Moles, Avila & Malaquias, 2019 is a family of philinoid [superfamily Philinoidea Gray, 1850 (1815)] cephalaspidean gastropods whose modern representatives are found only in Antarctic and subantarctic waters (Chaban, 2016; Moles *et al.*, 2019). There are currently seven described species in the family, including six species in the genus *Antarctophiline* Chaban, 2016 and one in the recently described, monotypic genus *Waegelea* Moles, Avila & Malaquias, 2019. The distribution of these species around the Southern Ocean is poorly known, in part because sampling has been focused on particular regions but also because philinoids often show strong morphological convergence among species (Valdés, Cadian & Gosliner, 2016; Moles *et al.*, 2019, 2021).

Philinoids were reported by some of the earliest scientific expeditions to the Southern Ocean, including the Voyage of the *Southern Cross* (1890–1900) and the Valdivia Expedition (1898–1899); at that time, most Antarctic philinoids were assigned to the genus *Philine* (family Philinidae) (Moles *et al.*, 2019). Very little is known about the reproduction or larval development of any antarctophilinids save two. Seager (1979) provided a detailed description of gametogenesis, egg masses and pre-hatching and hatching stages of *Antarctophiline gibba* (Strebel, 1908) (as *Philine gibba*) at South Georgia Island. This species had egg masses that were structurally similar to other philinoids; development was direct; metamorphosis occurred in the egg capsule; and from laying to hatching, development took *c.* 3 months (Seager, 1979). Hain (1992) and Hain & Arnaud (1992) described the egg masses of a species they identified as *A. alata* (Thiele, 1912) (as *P. alata*) from the eastern Weddell Sea and found them similar in outward appearance to *A. gibba*, but the Weddell Sea masses contained comparatively fewer and larger embryos and hatched as swimming veligers after 6–7 months. These two studies point to the presence of considerable life history diversity within the family Antarctophilinidae.

In this study, we describe the egg masses and larval developmental mode of a third philinoid from the nearshore benthos close to McMurdo Station in the Ross Sea, and we assign these masses to the antarctophilinid species *Waegelea antarctica* (E.A. Smith, 1902) based on cytochrome *c* oxidase subunit I (COI) barcoding. We also report preliminary measurements of *in situ* oxygen levels in the egg masses for comparison with oxygen levels in egg masses of other Antarctic marine animals. Lastly, we describe one additional egg mass from a locality *c.* 100 km from McMurdo Station and assign it to *A. alata*. This is the first description of development for *W. antarctica*, and the first description of an egg mass or larvae of *A. alata* that definitively identifies the egg mass to species using molecular barcoding.

MATERIALS AND METHODS

Field collection

Egg masses were gathered by divers on SCUBA between 20 and 40 m off the primary research site, the McMurdo Intake Jetty (MIJ) (77°51.069′S, 166°39.855′E) at McMurdo Station, Antarctica, in late October and November 2019. Some masses were photographed in the field and not otherwise disturbed. Others were collected by hand and transported immediately back to McMurdo Station in chilled seawater for photography and preservation. A third group of masses was used for *in situ* oxygen measurements, and then collected and brought back to the laboratory for photography and measurement. A single, similar type of egg mass was opportunistically collected by divers on SCUBA at *c.* 30 m at Explorer's Cove, New Harbor (NH) (77°34.268′S, 163°30.685′E), on the west side of McMurdo Sound. This mass was brought back to McMurdo Station in chilled seawater for photography and preservation.

At MIJ, in addition to egg masses we also collected adults that we suspected to be from the same species. Adults were collected by divers fanning the sediment to remove the top 2–3 cm, which exposed the adults. Animals were then collected by hand and transported to the Crary Laboratory at McMurdo Station in chilled water for photography and preservation.

Lab measurements and photography

To measure overall mass size, five egg masses from MIJ were measured along their longest diameter and its perpendicular using digital callipers. Eight additional masses were used for *in situ* oxygen measurements (below) and adults were photographed with an iPhone 8 (Apple Inc.). Dimensions were measured as above using a scale bar calibration in the photo with the Fiji distribution of ImageJ 1.53C (Schindelin *et al.*, 2012). Microphotography of masses and larvae was performed with either a Wild M5A or a Nikon SMZ-10 stereomicroscope with an attached GRYPHAX® SUBRA digital microscope camera (Jenoptik Inc.). The egg mass gel was not completely clear and the outside of masses was often fouled. Therefore, the gel was gently torn apart with fine forceps until individuals fell out for staging and measurement. To measure larval size, larvae lying on their sides were selected from calibrated microscope images and measured along the longest anterior–posterior axis using ImageJ. During microscopic examination and photography, egg masses, embryos and hatched larvae were kept chilled to −1 °C using a cold block (Ecotherm IC20, Torrey Pines Inc.).

The mean ± SD values are reported for all morphological measurements.

In situ field oxygen levels in egg masses

O_2 concentrations were measured by SCUBA divers in the field in eight *in situ* egg masses at MIJ at a depth of *c.* 25 m. Egg masses had been located and marked with metal stakes on a previous dive.

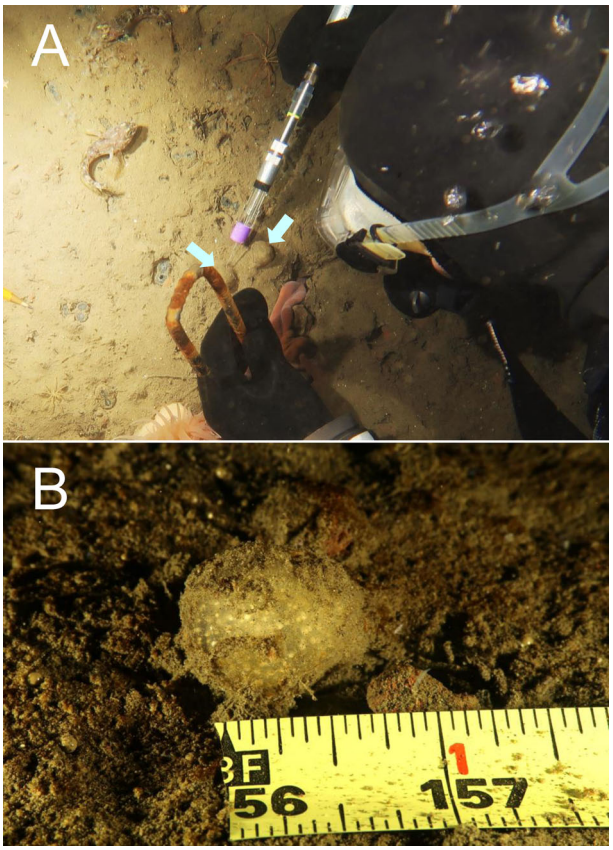

Figure 1. A. SCUBA diver using a Clark-style microelectrode to measure oxygen concentration inside egg masses. Two egg masses are visible in the picture, indicated by blue arrows; the probe is inserted in the mass that is slightly obscured by the metal stake. **B.** Egg mass photographed *in situ*. Embryos are visible as yellow dots inside the mass. Images by S. Rupp.

Measurements were made using an underwater picoammeter connected to a Clark-style microelectrode with a 50-μm tip (UWMeter, Unisense, Inc., Denmark). Before deployment, the meter and probe were calibrated to surface oxygen tension by cross-validation with a handheld oxygen meter (YSI 550A, YSI Inc., Yellow Springs, OH). Underwater measurements were performed by a team of two divers, one of whom read the meter and recorded data, and the other of whom handled the probe. Prior to each mass measurement, two baseline O_2 measurements were recorded in the water column *c.* 10 and 1 cm above the mass. Then, the tip of the probe was pushed by hand slowly through the egg mass until it was as close to the centre as possible (Fig. 1A); readings were allowed to stabilize (*c.* 10–30 s) and then the O_2 level was recorded. The probe was then removed and the process was repeated for a total of three replicate internal measurements for each mass. The mean ± SD values of the three internal readings are reported for each mass.

DNA extraction, amplification and sequencing

To identify the egg masses and adults to species, and to confirm that the MIJ adults and masses were conspecific, egg masses and adults were preserved in 95% ethanol and shipped chilled to the University of Hawai'i at Mānoa for analysis. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's protocol, except that DNA was eluted in a final volume of 50 μl. Three adults and four egg masses were analysed. For the adult specimens, tissue was taken from a small section of the foot. For the egg masses, three embryos were isolated from each mass and rinsed with 70% ethanol to remove debris and potential inhibitors. A *c.* 710-bp region of COI was amplified using the primer pair

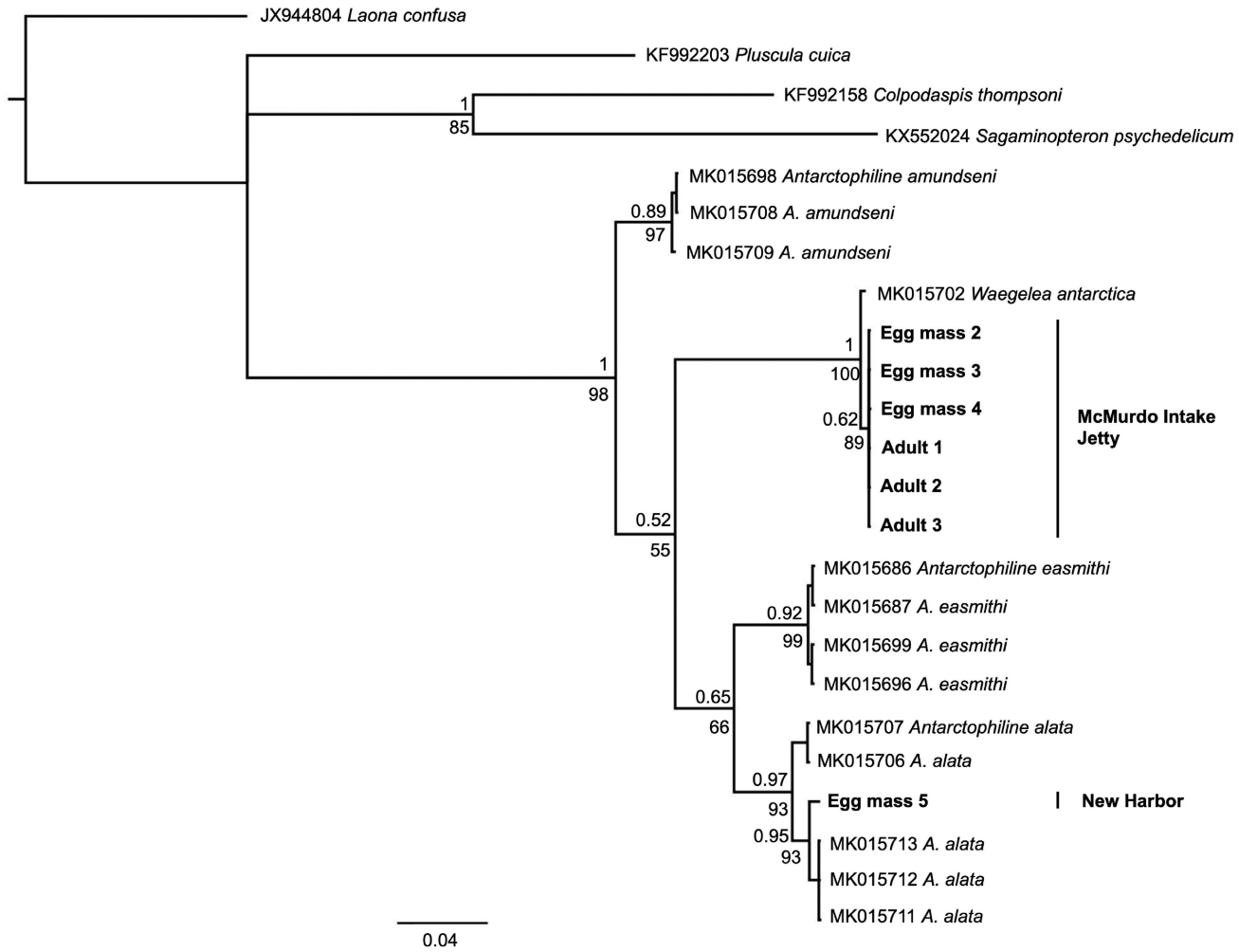

Figure 2. Phylogenetic tree inferred from Bayesian analysis using mitochondrial cytochrome *c* oxidase subunit I sequences. Support values (posterior probabilities >0.50 and bootstrap support values >50% above and below internal branches, respectively) are given for clades shared with the maximum likelihood topology. Acc. nos are included for all individuals retrieved from GenBank. Samples from this study are in bold with sample location to the right of each group. Scale bar indicates number of substitutions per site.

jgLCOI490 and jgHCO2198 (Geller *et al.*, 2013). Each reaction included 6.75 μl Taq 2× Ready Master Mix (Bioline, Inc.), 0.25 μl of 10-μm primer, 0.25–0.75 μl of bovine serum albumin (20 mg/ml), 1 μl DNA template and enough nuclease-free water to reach 12 μl total volume. The thermal profile for the adult specimens consisted of an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 40 °C for 1 min and a slow ramp (1 °C/s) up to 72 °C for 2 min. The thermal profile concluded with a final extension at 72 °C for 10 min. The thermal profile for the egg mass specimens was the same except that it included 35 cycles instead of 30 cycles. A negative control was added to each round of amplifications. Amplicon quality and lack of contamination in negative controls were assessed by gel electrophoresis. Samples were sequenced in both directions on an Applied Biosystems 3730XL DNA Analyzer at the Advanced Studies in Genomics, Proteomics, and Bioinformatics facility, University of Hawai'i at Mānoa.

Molecular identification

Chromatograms were visualized, trimmed and assembled to form contigs in Geneious Prime 2020.0.5 (Kearse *et al.*, 2012). The consensus sequence for each amplicon was compared to existing sequences in GenBank using BLAST. All of the top matches were sequences from a recent phylogenetic analysis of Antarctic philinoids (Moles *et al.*, 2019). Therefore, we electronically retrieved representative sequences from that study from GenBank and imported them into Geneious Prime. Sequences from all samples (adults and egg masses) in this study and the reference sequences from GenBank were aligned with Clustal Omega v. 1.2.3 (http://www.clustal.org/omega/). This alignment was used to create a bootstrapped maximum likelihood (ML) tree (GTR GAMMA, 500 bootstrap replicates) with the RAxML plugin (Stamatakis, 2006) and a Bayesian tree (HKY85+γ, 10,000,000 steps sampling every 5,000 with a 10% burn-in) with the MrBayes plug-in (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), both using *Laona confusa* as an outgroup (after Moles *et al.*, 2019). Figure 2 shows the Bayesian tree, and nodes were labelled with posterior probabilities (PP) of >0.5 and bootstrap support (BS) values of >50% for nodes shared between the Bayesian and ML topologies.

RESULTS

Field observations

McMurdo Intake Jetty: The first mass at MIJ was found on 21 October 2019, and masses were continuously seen from that date until diving ceased in late January 2020. Masses and adults were found at

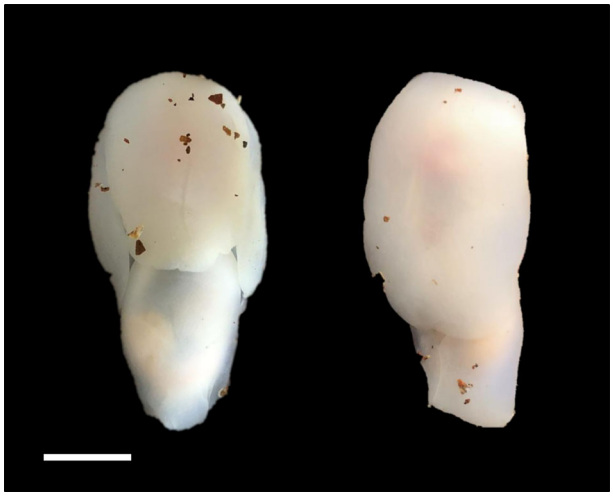

Figure 3. Living adult of *Waegelea antarctica* from the McMurdo Intake Jetty site. Left, dorsal view; right, ventral view. Scale bar = 1 cm.

all depths between 20 and 40 m (searches were depth limited at the upper end by the large boulders forming the base of the Intake Jetty, which ended the soft substrate, and at the low end by depth restrictions on scientific diving with the United States Antarctic Program). Densities and distributions were not quantified, but both masses and adults were common enough that divers could easily find several of each in 10 min of swimming along the bottom.

Egg masses were always found attached at the surface, on soft sediments. Masses were globular to oblong, gelatinous, and the gel was mostly transparent; pale yellow unhatched larvae could be seen inside some masses in the field but not others, because the majority of each mass was coated with a layer of flocculent material that adhered to the outer surface of the gel (Fig. 1B). This coating made them visually difficult to distinguish from small, mud-coated rocks. Each mass was tethered to the substrate by a long, stretchy, but tough gelatinous string.

Adults (Fig. 3) were white, translucent and *c.* 4 cm long. Adults were buried in the substrate *c.* 1–3 cm deep. Divers also occasionally observed adults with small parts of their bodies visible above the surface, apparently engaged in behaviours such as mating or, once, extruding a mucous ball as though laying an egg mass. We never unequivocally observed an adult laying an egg mass. In the lab, adults burrowed when provided with gravel or muddy substrate, and created mucous-lined tubes in the sediment as they crawled through it.

New Harbor: The single NH egg mass was found at *c.* 30-m depth on sandy/muddy bottoms, resting on the surface and tethered to the substrate as above. To the naked eye, the NH egg mass appeared similar to the MIJ masses except that the NH embryos were smaller and darker yellow.

Laboratory observations

McMurdo Intake Jetty: MIJ egg masses were gelatinous, globular to oblong and measured 30.6 ± 2.8 mm across the longest diameter and 24.6 ± 2.5 mm across the shortest diameter ($n = 13$). Embryos were arranged in a single line in a membranous string that was spirally wound within the mass (Fig. 4A). The number of individuals per mass was not quantified but varied considerably between masses; in some masses, embryos were spread throughout the gel (Fig. 4A), while in others embryos were only in one area and the rest of the mass was only gel (Fig. 4B). Of the 14 masses examined under the microscope, one contained individuals at the four-cell stage, and the rest contained well-developed veligers, each contained singly in an ellipsoid egg envelope that was slightly larger than the veliger (Fig. 5A). Veligers had a bilobed, ciliated velum behind the developing cephalic shield [termed the "cephalic disc" by Seager (1979)], a large foot and well-developed parapodial lobes that extended up to the dorsal side (Fig. 5B, C). The large majority of veligers also had a well-developed shell and a prominent, yellow larval kidney (Fig. 5B, C). Eyespots were visible behind the cephalic disc on some individuals (Fig. 5A) though they were not conspicuous. Some veligers appeared to have significant shell overgrowth by the mantle (Fig. 5C).

Veligers in the MIJ masses measured 913.5 ± 65.4 μm in longest length (grand mean and SD for $n = 6$ masses; with five larvae measured per mass; all masses at hatching or close to hatching stage). Shell lengths from the same individuals were 591.3 ± 25.0 μm. Prior to hatching, veligers either had their velum extended with cilia beating or were actively crawling with their foot on the inner surface of their egg envelopes. In two masses, hatchlings that had emerged on their own from their egg envelopes could be seen crawling on the surface of the egg mass gel or on the surface of the glass dishes that masses were kept in (Fig. 6A, Supplementary Material Video S1). Hatchling size matched the size of the pre-hatched veligers. The entire hatchling shell was clearly visible and the mantle did not appear to be overgrowing the shell. The velum was still prominent, although when hatchlings were crawling, the velum was fully retracted behind the cephalic shield and hard to see. Periodically, crawling hatchlings would stop, extend the velum and swim with the velum up (Fig. 6B, Supplementary Material Video S2). Hatchlings produced copious mucous, as did the adults.

New Harbor: The single mass collected at NH was globular to oblong and measured 20.6 mm × 17.4 mm (Fig. 4C). Larvae were densely packed in the mass (more so than the MIJ masses), each in its own egg envelope, and were arranged single file in a long mucinous string that was wound throughout the mass. Larvae were at the veliger stage with a well-defined velum and foot with parapodial lobes, shell and a yellow visceral mass that appeared very yolky (Fig. 7). Embryos averaged 549.9 ± 27.5 μm in longest length ($n = 12$).

Field oxygen measurements

Oxygen saturation in the water column above the MIJ masses averaged 86.5% and did not differ between the far (20 cm) and close (1 cm) measurements taken above the surface of each egg mass (one-way repeated ANOVA: $n = 8$ pairs, $P = 0.322$). Oxygen measurements inside the egg masses were always lower than those in the surrounding water, sometimes considerably so; mean % saturation in the eight masses ranged from a high of 49.6 ± 0.01 to a low of 13.8 ± 0.05. For the eight masses combined, mean central % saturation was 32.8 ± 0.046.

Molecular identification

We obtained COI sequences from three egg masses and three adults collected at MIJ. All six sequences were identical. The longest (627 bp) differed at only one third-codon position (<0.002%) from the single sequence in GenBank obtained from *Waegelea antarctica* (acc. no. MK015702). All six sequences were placed in a strongly supported monophyletic clade with *W. antarctica*, in both the ML and Bayesian trees (Fig. 2).

The COI sequence from the single egg mass collected at NH was *c.* 10% divergent from the sequences obtained from the MIJ masses. The best match in GenBank for the NH sequence was *Antarctophiline alata* (acc. no. MK015710), differing at only four third-codon positions (out of 615 bp). In both the Bayesian and ML trees, the NH mass was placed in a well-supported (PP = 0.95, BS = 93%) clade of sequences from *A. alata* (Fig. 2).

Both ML and Bayesian trees nested the cluster of *W. antarctica* COI sequences within the genus *Antarctophiline* (Fig. 2). This topology contrasted with Moles *et al.* (2019), who found a more strongly

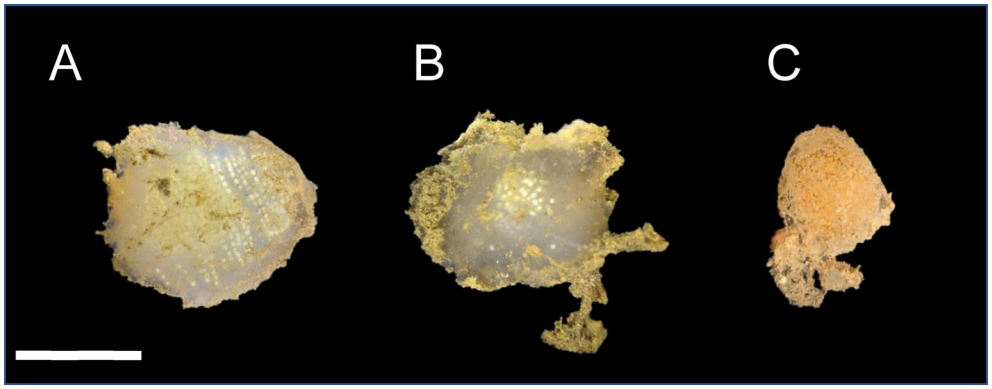

Figure 4. A. Egg mass of *Waegelea antarctica*, showing embryos (yellow dots) in a spiral arrangement throughout the mass. **B.** Different egg mass of *W. antarctica*, showing embryos concentrated in a smaller area. **C**. Egg mass of *Antarctophiline alata*. Scale bar = 2 cm.

supported sister relationship between *Waegelea* and *Antarctophiline*. This discrepancy is likely due to Moles *et al.*'s (2019) much larger sequence dataset, which presumably provided more power to delineate deeper relationships.

DISCUSSION

This report describes the egg masses, late-stage encapsulated and hatched veligers, and developmental mode of *Waegelea antarctica* for the first time and, also for the first time, describes the egg masses of the related species *Antarctophiline alata* (identified by COI sequencing). Of the seven described species of antarctophilinids, egg masses were previously known only from the description by Seager (1979) of *A. gibba* and a briefer description of *A. alata* by Hain & Arnaud (1992). Like *A. gibba* (Seager, 1979), *W. antarctica* and *A. alata* (this study) both have a "type C" egg mass typical of many cephalaspideans (Hurst, 1967; Schaefer, 1996) with embryos encapsulated singly and embedded in spiral strings in a gelatinous mass, tethered at its base to the substrate.

Hatchlings of *W. antarctica* emerged at a very advanced veliger stage capable of both crawling and swimming, and were quite large; total length of hatching *W. antarctica* was almost 1 mm, and hatching shell length was *c.* 590 μm. For comparison, temperate eastern Pacific cephalaspideans have hatching shell lengths that range from 101 to 360 μm with a mean of *c.* 165 μm (n = 13 species; Goddard, 2004; Goddard & Hermosillo, 2008). For *A. alata*, hatching lengths (total or shell) could not be determined, but at *c.* 550 μm total length, larvae of *A. alata* were also large compared to temperate relatives. These data match a biogeographic pattern of Antarctic "embryo gigantism" (Moran & Woods, 2012; Woods & Moran, 2020), in which Antarctic marine organisms, including other heterobranchs (Woods & Moran, 2008; Moles *et al.*, 2017), tend to have larger eggs and offspring than their warmer-water counterparts (reviewed in Peck, 2018). The underlying evolutionary mechanisms driving embryo gigantism in the Antarctic are not well understood, but may be related to cold-temperature-mediated release from size constraints on embryos and juveniles in polar waters (Moran & Woods, 2012; Woods & Moran, 2020).

Our size measurements of embryos of *A. alata* conflict with an earlier description (Hain & Arnaud, 1992: fig. 5) that showed a hatched larva of "*Philine alata*" from the eastern Weddell Sea that, at >1 mm total length, was almost twice as long as the embryos we observed. Given the large amount of taxonomic uncertainty surrounding Antarctic philinoids prior to genetic methods of species identification (Moles *et al.*, 2019), we do not know whether this discrepancy represents true life history variation within *A. alata*, or a possible misidentification. The larva pictured in Hain & Arnaud (1992) is very similar in size and shape to our observations of *W. antarctica*, but they seem unlikely to be conspecific because the adults that produced Hain & Arnaud's (1992) masses were 15 mm long (Hain, 1992), less than half the length of adults of *W. antarctica*. The adults that produced Hain & Arnaud's (1992) larva were more consistent in size with *A. easmithi* and *A. amundseni*, two species that occur in the Weddell Sea (Moles *et al.*, 2019). However, until sampling of this region is improved and expanded to include molecular data for identification and additional descriptions of larval development, this question cannot be resolved.

This report also establishes the presence of *W. antarctica* in the southern Ross Sea. This species, first described as *Philine antarctica* from the Ross Sea by Smith (1902), was termed "elusive" by Moles *et al.* (2019); prior to their study, it was definitively known only from the shell of a single specimen collected during the British Expedition of the *Southern Cross* (1898–1900) at Cape Adare, close to the northwestern-most point of the Ross Sea (*c.* 71.3362°S, 170.1439°E), in "20 fathoms" (36.5 m). This shell was described by Smith (1902) and later figured by Dell (1990: fig. 441) and Engl (2012: 203). More than 100 years after its collection, Moles *et al.* (2019) identified a single, second specimen that shared its unique shell traits; this specimen was collected in 1998 from 65 m depth by ANTXV/3 on the German R/V *Polarstern* in a cruise along the eastern coast of the Weddell Sea, on Norsel Bank, Kapp Norvegica (71°7.30′S, 11°28.40′W). This specimen from the Weddell Sea was described and sequenced for four molecular markers, including COI, and was renamed and moved into its own new monospecific genus as *Waegelea antarctica* (Moles *et al.*, 2019). Because the Smith (1902) and Moles *et al.* (2019) specimens were collected on opposite sides of the Antarctic continent, separated by >8,000 km of shoreline, Moles *et al.* (2019) considered *W. antarctica* to have a circum-Antarctic distribution. Given that our sequences from McMurdo Sound, which is in the Ross Sea, are only <0.002% divergent from the single Moles *et al.* (2019) sequence from the Weddell Sea, we can confirm that these widely separated populations are conspecific.

In addition to the type specimen described by Smith (1902), there have been numerous, more recent reports of "*Philine antarctica*" and other philinoids from shallow depths in the Ross Sea. Close to McMurdo Station, "*P. antarctica*" was found in benthic cores and in the gut contents of fish (*Trematomus* sp.) at MIJ and three other nearby sites (Keist, 1993). "*Philine falklandica*" was collected directly off McMurdo Station ("Hot Water Outlet", close to MIJ) and at Cinder Cones (*c.* 5 km from MIJ) (Rudman, 1972). Rudman (1972) also reported that "*Philine gibba*" was common at the "Hot Water Outlet" site. Definitive identifications of these collections are largely lacking, so further morphological and genetic work on species from this area is likely necessary to determine whether in fact multiple species are present in this small geographic area. The true distribution of antarctophilinid species in McMurdo Sound and elsewhere

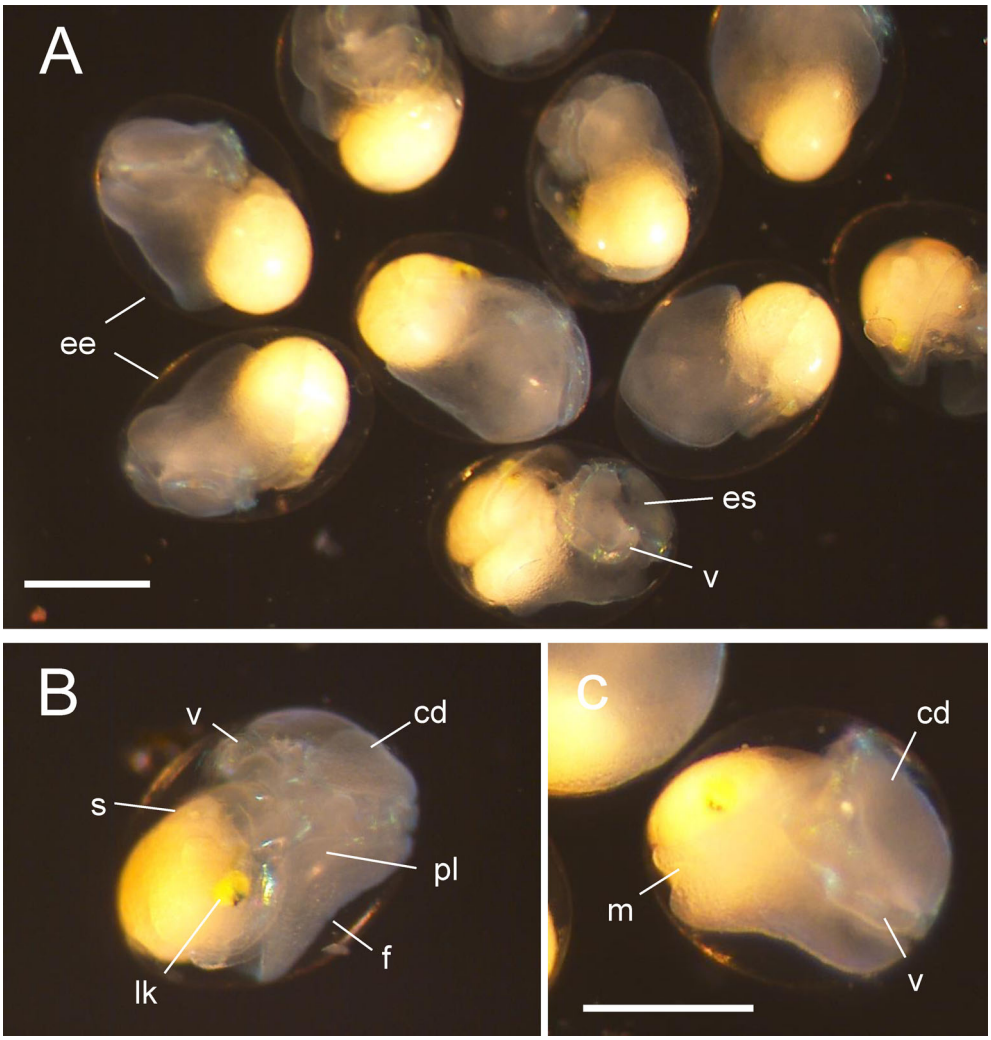

Figure 5. Developing veligers of *Waegelea antarctica* removed from a single egg mass. **A.** Multiple individuals removed from a single mass. **B, C.** Individuals shown at higher magnification to show morphological features. Abbreviations: cd, cephalic disc; ee, egg envelope; es, eyespot; f, foot; pl, parapodial lobe; s, shell; v, velum (mostly retracted behind cd in **B** and **C**). Scale bars = 500 μm.

in Antarctica is likely obscured by taxonomic uncertainty (Chaban, 2016) and by limited external morphological divergence among species (Valdés *et al.*, 2016; Moles *et al.*, 2019).

Hatchlings of *W. antarctica* retained their velum and swam, but they also had well-developed juvenile features that suggest a short pelagic period. This is consistent with the overall (though by no means universal) trend towards Antarctic species having short or no pelagic periods (reviewed in Peck, 2018). We did not specifically test for metamorphic competence, but unlike Hain & Arnaud (1992), who saw metamorphosed juveniles 2 d after hatching in *A. alata*, we did not observe any metamorphosed juveniles of *W. antarctica* over several weeks of maintaining masses in the laboratory, suggesting long-distance dispersal potential. Overall, Antarctic taxa show only weak predictive relationships between larval developmental mode and genetic differentiation (Halanych & Mahon, 2018). A long dispersal stage would be consistent with the close sequence similarity at COI (<0.002% divergent) between all six of our specimens and the single specimen from the Weddell Sea (Moles *et al.*, 2019), and with the distributional data reported in Moles *et al.* (2021). However, low levels of genetic differentiation over large geographic distances may also reflect a recent range expansion (Marko, 2004; Maggs *et al.*, 2008; Provan & Bennett, 2008; Halanych & Mahon, 2018; Lau *et al.*, 2020). In either case, even if the pelagic duration is short, demersal larvae could be transported considerable distances away from the hatching site. Current speeds just above the bottom in the region of McMurdo Station averaged 3.6 cm/s over 10 months in 2015–2016 (Moran *et al.*, 2018), suggesting that in directional currents demersal larvae could move >3 km per day. Even a very short dispersal period of a few days or less would facilitate recolonization and recovery of populations of *W. antarctica* in nearshore Antarctic habitats that are heavily impacted by iceberg scour.

For most Antarctic ectotherms, development is extended by the extreme cold temperatures; a combination of cold temperature and 'late' hatching stages in the Antarctic has led to some of the longest developmental periods known for molluscs or any other animal (Moles *et al.*, 2017; Moran *et al.*, 2019). All of the masses of *W. antarctica* that we examined in November and December 2019 were close to hatching save one, and that single one was recently laid; this suggests an annual reproductive pattern with spawning and hatching both occurring in the austral spring, and development in the egg mass taking ≥1 year. This time frame was supported by three egg masses that were collected from the same site in January 2020 at an early (pre-veliger) stage, and were outplanted in mesh cages at the site. When these masses were collected and photographed in November 2020, larvae were at the late veliger stage (Supplementary Material Fig. S1).

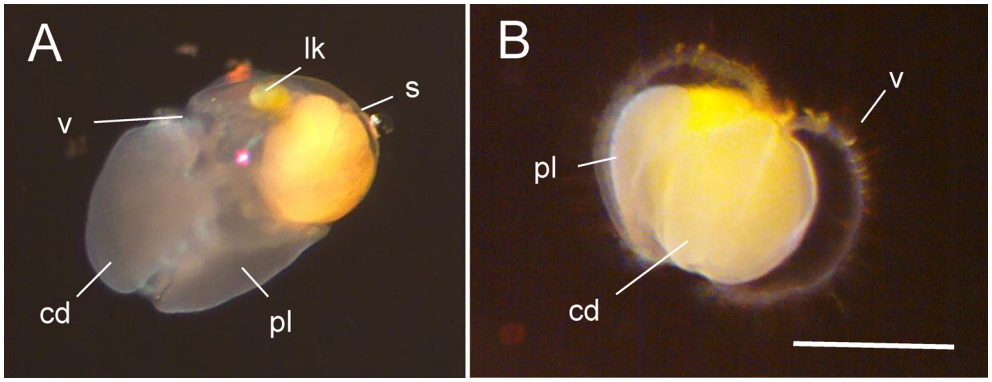

Figure 6. Hatchlings of *Waegelea antarctica* released from a single egg mass. **A.** Dorsolateral view of crawling hatchling with foot on substrate and velum retracted. **B.** Anterior view of hatchling swimming with cephalic disc and velum upwards. Abbreviations: cd, cephalic disc; pl, parapodial lobe; s, shell; v, velum (mostly retracted behind cd in **B**). Scale bars = 500 μm.

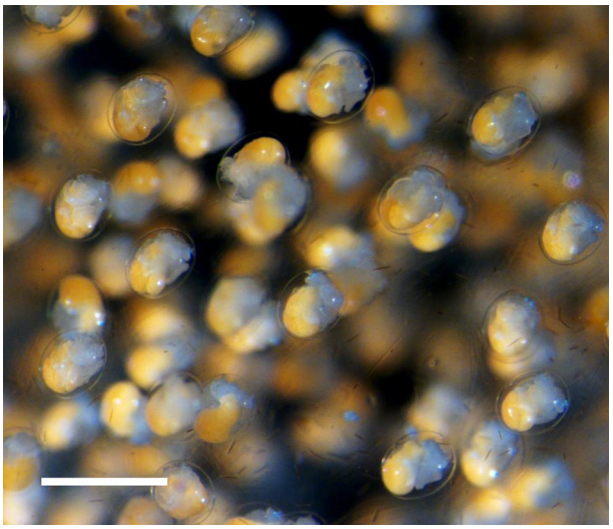

Figure 7. Veligers of *Antarctophiline alata* inside egg mass. Scale bar = 1 mm.

One effect of slow development is that embryos are exposed to conditions in the egg mass for prolonged periods. Oxygen availability in egg masses is crucial to development (Chaffee & Strathmann, 1984; Cohen & Strathmann, 1996), and work with masses of other heterobranch gastropods (the nudibranchs *Tritoniella belli* and *Tritonia challengeriana*) from McMurdo Sound has shown that oxygen levels in masses drop with increased temperature, larger mass size and decreased water flow (Woods & Moran, 2008). In the field, Moran & Woods (2010) found that oxygen levels in nudibranch masses ranged from close to ambient to >25% saturation and that oxygen levels decreased as development progressed and embryos became more metabolically active. Oxygen in masses of *W. antarctica* was comparably low but quite variable between masses. All masses we measured were similar in size and developmental stage, so mass size and embryo age likely did not drive the greater-than-three-fold difference between the lowest and highest mass averages (13.8% *vs* 49.6% saturation). Instead, the differences we saw were more likely attributable to high variation in embryo density between masses. Because *W. antarctica* lays large, easily manipulated egg masses within SCUBA depths, this species is an excellent candidate for further experiments testing how changes in ocean temperature, oxygenation and flow will affect Antarctic organisms with benthic development. The close genetic similarly between very distant populations also makes this species potentially valuable for understanding biogeography, dispersal and gene flow in shallow-water, nearshore Antarctic ecosystems.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

ACKNOWLEDGEMENTS

We thank the United States Antarctic Program ASC staff for invaluable help with field- and laboratory work in 2019 and 2020. We also thank P. Dayton, G. Robillard, B. Brueggeman and C. Collins for insights on past benthic observations near MIJ and Jeff Goddard and an anonymous reviewer for helpful comments on the manuscript. This project was supported by NSF-OPP-1745130 to A.L. Moran. This is publication no. 132 from the School of Life Sciences, University of Hawai'i at Mānoa.

REFERENCES

ARNAUD, P.M. 1977. Adaptations within the Antarctic marine benthic ecosystem. In: *Proceedings of the Third SCAR Symposium on Antarctic Biology: adaptations within Antarctic ecosystems* (G.A. Llano, ed.), pp. 135–137. Smithsonian Institution, Washington, DC.

BARNES, D.K.A. 2017. Iceberg killing fields limit huge potential for benthic blue carbon in Antarctic shallows. *Global Change Biology*, **23**: 2649–2659.

BARNES, D.K.A. & CONLAN, K.E. 2007. Disturbance, colonization and development of Antarctic benthic communities. *Philosophical Transactions of the Royal Society B*, **362**: 11–38.

CHABAN, E.M. 2016. New genus of opisthobranch molluscs *Antarctophiline* gen. nov. (Cephalaspidea: Philinoidea) from the Cooperation Sea, Antarctica. *Ruthenica*, **26**: 49–56.

CHAFFEE, C. & STRATHMANN, R.R. 1984. Constraints on egg masses. I. Retarded development within thick egg masses. *Journal of Experimental Marine Biology and Ecology*, **84**: 73–83.

COHEN, C.S. & STRATHMANN, R.R. 1996. Embryos at the edge of tolerance: effects of environment and structure of egg masses on supply of oxygen to embryos. *Biological Bulletin*, **190**: 8–15.

DELL, R.K. 1990. Antarctic Mollusca with special reference to the fauna of the Ross Sea. *Bulletin of the Royal Society of New Zealand, Wellington*, **27**: 1–311.

ENGL, W. 2012. *Shells of Antarctica*. ConchBooks, Hackenheim, Germany.

GELLER, J., MEYER, C., PARKER, M. & HAWK, H. 2013. Redesign of PCR primers for mitochondrial cytochrome *c* oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, **13**: 851–861.

GODDARD, J.H.R. 2004. Developmental mode in benthic opisthobranch molluscs from the northeast Pacific Ocean: feeding in a sea of plenty. *Canadian Journal of Zoology*, **82**: 1954–1968.

GODDARD, J.H.R. & HERMOSILLO, A. 2008. Developmental mode in opisthobranch molluscs from the tropical eastern Pacific Ocean. *Veliger*, **50**: 83–96.

HAIN, S. 1992. Maintenance and culture of living benthic molluscs from high Antarctic shelf areas. *Aquaculture and Fisheries Management*, **23**: 1–11.

HAIN, S. & ARNAUD, P.M. 1992. Notes on the reproduction of high-Antarctic mollusks from the Weddell Sea. *Polar Biology*, **12**: 303–312.

HALANYCH, K.M. & MAHON, A.R. 2018. Challenging dogma concerning biogeographic patterns of Antarctica and the Southern Ocean. *Annual Review of Ecology, Evolution, and Systematics*, **49**: 355–378.

HUELENBECK, J.P. & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**: 754–755.

HURST, A. 1967. The egg masses and veligers of thirty northeast Pacific opisthobranchs. *Veliger*, **9**: 255–288.

KEARSE, M., MOIR, R., WILSON, A., SONE-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THIERER, T., ASHTON, B., MEINTJES, P. & DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **15**: 1647–1649.

KEIST, K.A. 1993. A relationship of diet to prey abundance and the foraging behavior of *Trematomus bernacchii*. *Polar Biology*, **13**: 291–296.

LAU, S.C.Y., WILSON, N.G., SILVA, C.N.S. & STRUGNELL, J.M. 2020. Detecting glacial refugia in the Southern Ocean. *Ecography*, **43**: 1639–1656.

MAGGS, C.A., CASTILHO, R., FOLTZ, D., HENZLER, C., JOLLY, M.T., KELLY, J., OLSEN, J., PEREZ, K.E., STAM, W., VAINOLA, R., VIARD, F. & WARES, J. 2008. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*, **89**: S108–S122.

MARKO, P.B. 2004. 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. *Molecular Ecology*, **13**: 597–611.

MOLES, J., AVILA, C.B. & MALAQUIAS, A.E. 2019. Unmasking Antarctic mollusc lineages: novel evidence from philinoid snails (Gastropoda: Cephalaspidea). *Cladistics*, **35**: 487–513.

MOLES, J., DERKARABETIAN, S., SCHIAPARELLI, S., SCHRÖDL, M., TRONCOSO, J., WILSON, N. & GIRIBET, G. 2021. An approach using ddRADseq and machine learning for understanding geographic and bathymetric patterns of speciation in Antarctic gas tropods (Mollusca). *Scientific Reports*, **11**: 8473.

MOLES, J., WAEGELE, H., CUTIGNANO, A.M., FONATANA, A., BALLESTEROS, M. & AVILA, C. 2017. Giant embryos and hatchlings of Antarctic nudibranchs. *Marine Biology*, **164**: 114.

MORAN, A.L., HARASEWYCH, M.G., MILLER, B.A., WOODS, H.A., TOBALSKE, B.W. & MARKO, P.B. 2019. Extraordinarily long development of the Antarctic gastropod *Antarctodomus thielei* (Powell 1958) (Neogastropoda: Buccinoidea). *Journal of Molluscan Studies*, **85**: 319–326.

MORAN, A.L. & WOODS, H.A. 2010. Limits to diffusive oxygen transport: flow, form, and function in nudibranch egg masses from temperate and polar regions. *PLoS One*, **5**: 1–8.

MORAN, A.L. & WOODS, H.A. 2012. Why might they be giants: towards understanding polar gigantism. *Journal of Experimental Biology*, **215**: 1995–2002.

MORAN, A.L., WOODS, H.A., SHISHIDO, C.M., LANE, S.J. & TOBALSKE, B.W. 2018. Predatory behavior of giant Antarctic sea spiders (*Colossendeis*) in nearshore environments. *Invertebrate Biology*, **137**: 116–123.

MUÑOZ-RAMÍREZ, C.P., BARNES, D.K.A., CARDENAS, L., MEREDITH, M.P., MORLEY, S.A., ROMAN-GONZALEZ, A., SANDS, C.J., SCOURSE, J. & BRANTE, A. 2020. Gene flow in the Antarctic bivalve *Aequiyoldia eightsii* (Jay, 1839) suggests a role for the Antarctic Peninsula Coastal Current in larval dispersal. *Royal Society Open Science*, **7**: 200603.

PEARSE, J.S., McCLINTOCK, J.B. & BOSCH, I. 1991. Reproduction of Antarctic benthic marine invertebrates: tempos, modes, and timing. *American Zoologist*, **31**: 65–80.

PECK, L.S. 2018. Antarctic marine biodiversity: adaptations, environments, and responses to change. *Oceanography and Marine Biology: An Annual Review*, **56**: 105–236.

PROVAN, J. & BENNETT, K.D. 2008. Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution*, **23**: 564–571.

ROGERS, A.D. 2007. Evolution and biodiversity of Antarctic organisms: a molecular perspective. *Philosophical Transactions of the Royal Society B*, **362**: 2191–2214.

RONQUIST, F. & HUELSENBECK, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

RUDMAN, W.B. 1972. The genus *Philine* (Opisthobranchia, Gastropoda). *Proceedings of the Malacological Society of London*, **40**: 171–187.

SCHAEFER, K. 1996. Review of data on cephalaspid reproduction, with special reference to the genus *Haminaea* (Gastropoda, Opisthobranchia). *Ophelia*, **45**: 17–37.

SCHINDELIN, J., ARGANDA-CARRERAS, I., FRISE, E., KAYNIG, V., LONGAIR, M., PIETZSCH, T., PREIBISCH, S., RUEDEN, C., SAALFELD, S., SCHMID, B., TINEVEZ, J.-Y., WHITE, D.J., HARTENSTEIN, V., ELICEIRI, K., TOMANCAK, P. & CARDONA, A. 2012. Fiji: an open-source platform for biological-image analysis. *Nature Methods*, **9**: 676–682.

SEAGER, J.F. 1979. Reproductive biology of the Antarctic opisthobranch *Philine gibba* Strebel. *Journal of Experimental Marine Biology and Ecology*, **41**: 51–74.

SMITH, E.A. 1902. VII. Mollusca. In: *Report on the collections of natural history made in the Antarctic regions during the voyage of the Southern Cross*, pp. 201–213. Natural History Museum, London.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**: 2688–2690.

THATJE, S. 2012. Effects of capability for dispersal on the evolution of diversity in Antarctic benthos. *Integrative and Comparative Biology*, **52**: 470–482.

VALDÉS, A., CADIEN, D.B. & GOSLINER, T.M. 2016. Philinidae, Laonidae and Philinorbidae (Gastropoda: Cephalaspidea: Philinoidea) from the northeastern Pacific Ocean and the Beaufort Sea (Arctic Ocean). *Zootaxa*, **4147**: 501–537.

WOODS, H.A. & MORAN, A.L. 2008. Temperature–oxygen interactions in Antarctic nudibranch egg masses. *Journal of Experimental Biology*, **211**: 798–804.

WOODS, H.A. & MORAN, A.L. 2020. Reconsidering the oxygen–temperature hypothesis of polar gigantism: successes, failures and nuance. *Integrative and Comparative Biology*, **60**: 1438–1453.