

Shell structure of two polar pelagic molluscs, Arctic *Limacina helicina* and Antarctic *Limacina helicina antarctica* forma *antarctica*

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Abstract The purpose of this study was to investigate shell growth performance in two thin-shelled pelagic gastropods from cold seawater habitats. The shells of Arctic *Limacina helicina* and Antarctic *Limacina helicina antarctica* forma *antarctica* are very thin, approximately 2–9 µm for shells of 0.5–6 mm in diameter. Many axial ribbed growth lines were observed on the surface of the shell of both *Limacina* species. Distinct axial ribs were observed on the outermost whorl, while weak or no rib-like structures were observed on the inner whorls in the larger shell of *L. helicina antarctica* forma *antarctica*. For *L. helicina*, no ribs were observed on small individuals with three whorls, while larger individuals had distinct ribs on the outer whorls. Shell microstructure was examined in both species. There is an inner crossed-lamellar and extremely thin outer prismatic layer in small individuals of both species, and a distinct thick inner prismatic layer was observed beneath the crossed-lamellar layer in large Antarctic individuals. Various orientations of the crossed-lamellar structure were observed in one individual. Shell structure appeared to be different between the Antarctic and Arctic species and among shells of different size.

Keywords Shell structure · Axial ribs · Pteropoda · *Limacina helicina antarctica* forma *antarctica* · *Limacina helicina* · Crossed-lamellar structure · Prismatic structure

Introduction

After the 4th IPCC (Intergovernmental Panel on Climate Change) report (Solomon et al. 2007), the impact of greenhouse gas emissions on marine ecosystems, including its influence on the life of calcareous organisms (e.g. corals and molluscs), was recognized. Increasing atmospheric CO₂ concentration is known to induce ocean acidification, thereby reducing carbonate ion concentration and directly affecting calcium carbonate metabolism in calcifying organisms (Orr et al. 2005). These negative effects are considered to be especially relevant to polar oceans. Aragonitic shells are specifically susceptible to undersaturated waters. For Arctic surface waters, undersaturation with respect to aragonite is projected to occur as early as 2016 (Steinacher et al. 2008). The IPCC report states that the Southern Ocean may become undersaturated on a seasonal basis by 2030, when atmospheric CO₂ is projected to reach 450 ppm.

There are many forms within the species *Limacina helicina*, and their taxonomy remains unclear. For convenience, during discussion of the two taxa (Arctic *Limacina helicina* and Antarctic *Limacina helicina antarctica* forma *antarctica*), they are here referred to as ‘species.’ In the Arctic and Southern Oceans, the thecosomatous pteropods *Limacina helicina* and *L. helicina antarctica* forma *antarctica* are key mesozooplankton species (Hunt et al. 2008). Pteropod species show large patch biomass with high grazing rates, while also playing a significant role in

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contributing to carbonate and organic carbon flux (Fortier et al. 1994; Pakhomov et al. 2002). Relatively few reports contain information on shell structure in pteropods (e.g. Bé and Gilmer 1977; Lalli and Gilmer 1989; Hodgkinson et al. 1992). Bé and Gilmer (1977) reported that the chemical form of CaCO_3 in the family Limacinidae is aragonite and that shells in *Limacina* species are composed of two microstructures: an outer crossed-lamellar and inner prismatic structure. However, most of this earlier literature on shell structure is derived from work with fossils, and there is little information on shell structure in living polar *Limacina* species.

Shell structure is here examined in *Limacina helicina* and *L. helicina antarctica* forma *antarctica*, comparing the shell characteristics of these two polar *Limacina* species in terms of shell growth performance in calcifiers with the extremely thin shells of cold seawater inhabitants. Also discussed is the advantage of possessing a thin shell in cold seawater. Fundamental studies on shell structure are necessary as a prelude to applied research directed at tackling the anticipated effects of increased greenhouse gas emissions and ocean acidification.

Materials and methods

Sampling of specimens

The pteropod *Limacina helicina* was collected using time-series sediment traps (Nichiyu SMD 26S and Technicup PPS5/5), which were deployed at 100 m depth at CA8 (70°58'N, 126°07'E) in the southeast Beaufort Sea from July 2004 to November 2006. The traps were deployed for approximately 1 year at the longest. All sample bottles were filled with neutral buffered formalin in filtered seawater (final conc. 5%). Samples of *Limacina helicina antarctica* forma *antarctica* were collected using a bucket-type plankton net (100 cm mouth diameter and 180 cm long, 500- μm -mesh size) at St. II-7 in the Indian sector of the Antarctic Ocean (67°10'S, 68°51'E) in January 2009. The net was hauled vertically from 175 m depth to the surface. After recovery, samples with *L. helicina antarctica* were kept in a deep freezer at -80°C .

Stereomicroscopy and scanning electron microscopy observations

Neutralized formalin-fixed specimens of *Limacina helicina* were used for stereomicroscopic observations (MZ16-A, Leica). After careful removal of the flesh, the shells were rinsed with pure water several times and then dried at room temperature (22°C). Small pieces of shell were coated with Au or Pt as per the standard procedure for imaging using

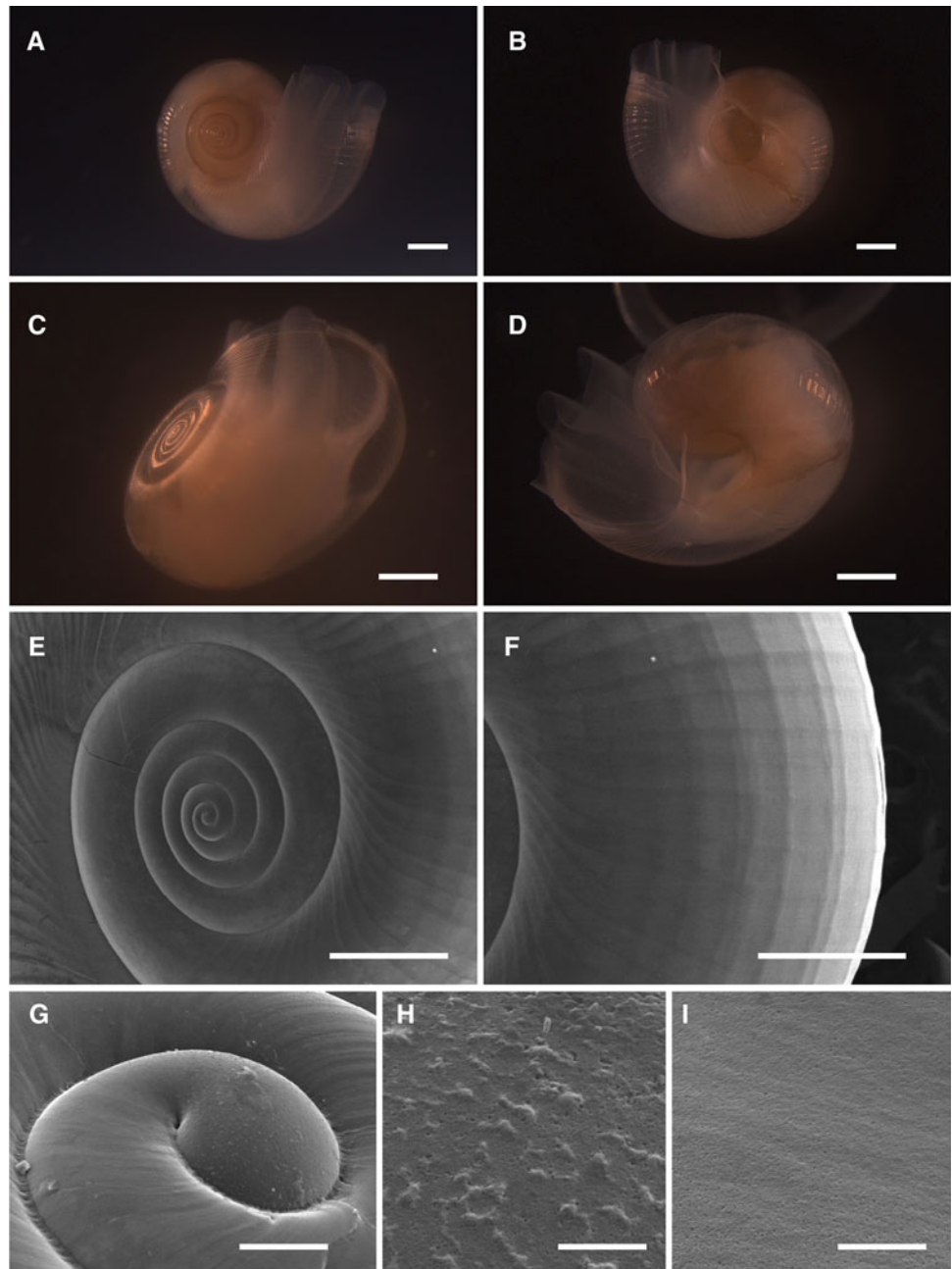
scanning electron microscopy (SEM). The inner shell surface and fractured cross-section of the shell was imaged using one of two SEM's (JSM-6380LV, JEOL or S-4200, Hitachi). Deep-frozen specimens of *L. helicina antarctica* forma *antarctica* were also used after fixation in 5% neutralized seawater formalin for analysis of inner, outer and fractured surface structure in the same manner. We observed 20 individuals for stereomicroscopic analysis (outer morphology) and five to eight individuals for each species for SEM analysis (inner structure).

Results

The shells of Antarctic *L. helicina antarctica* forma *antarctica* (Figs. 1, 3) and Arctic *Limacina helicina* (Figs. 2, 3) were both minute and helicoid. All specimens had a low spire, including small specimens with three to four whorls (Fig. 2c–e, h) and a large specimen of 6 mm in diameter with six to seven whorls (Fig. 1a–g). The shell was also very thin and fragile. The thickness of shells of 0.5–6 mm in diameter was 2–9 μm by SEM observation. A thin periostracal layer covered the calcareous surface. Stereomicroscopic (Figs. 1a–d, 2a, b) and SEM (Figs. 1e, f, 3a–d) observations revealed that the surface of the shell was smooth, with many regularly spaced axial or longitudinal ribs on the shell surface of both species. However, although visible axial ribs were observed on the shell surface of the most outer whorl, the surface of the innermost embryonic shell (Fig. 1g) and the inner several whorls (Fig. 1i) showed no distinct axial ribs and very weak ribs or no rib-like structure on the shell of 6 mm in diameter with seven whorls in Antarctic *Limacina* (Fig. 1e, f). Similar shell architecture with visible axial ribs on outer whorls was also observed in Arctic *Limacina helicina* (Fig. 2a, b). Smaller shells of *L. helicina* with three to four whorls (shell diameter ca. 0.5–0.8 mm) showed no rib-like structure on the entire surface (Fig. 2c–f). The surface was smooth and simple without any other morphological characteristics. The surface of the embryonic shell of both species was sometimes irregular (Figs. 1h, 2g). Although there was no visible sculpture on the whorls, many growth line-like structures were observed under strong transmitted light (Fig. 2i) in both species, which indicates that there are some differences in inner structure.

Although both species had axial ribs, the structure was different between the Arctic and Antarctic species. In *Limacina helicina*, the distinct ribs were straight and spaced at regular intervals (Fig. 3a, c), while in the Antarctic species, multiple and irregular axial ribs were observed (Fig. 3b, d). The distance between ribs tended to increase gradually from the apex to the aperture in both *Limacina* species. The distance between ribs near the outer

Fig. 1 *Limacina helicina antarctica* forma *antarctica*. **a–d** Note the low spires with six to seven whorls. *Bar* 1 mm. **e** Shell surface of a larger individual with no visible sculpture except axial ribs on the most outer whorl. *Bar* 700 μm (SEM). **f** Outer surface of the outermost whorl showing spiral cords and axial ribs in a regular alignment. *Bar* 700 μm (SEM). **g** Apex with protoconch and a part of suture. *Bar* 30 μm (SEM). **h** Higher magnification of the protoconch showing a rough surface. *Bar* 3 μm (SEM). **i** Higher magnification of the surface of first to second whorl. Note the smooth surface with no sculpture. *Bar* 3 μm (SEM)

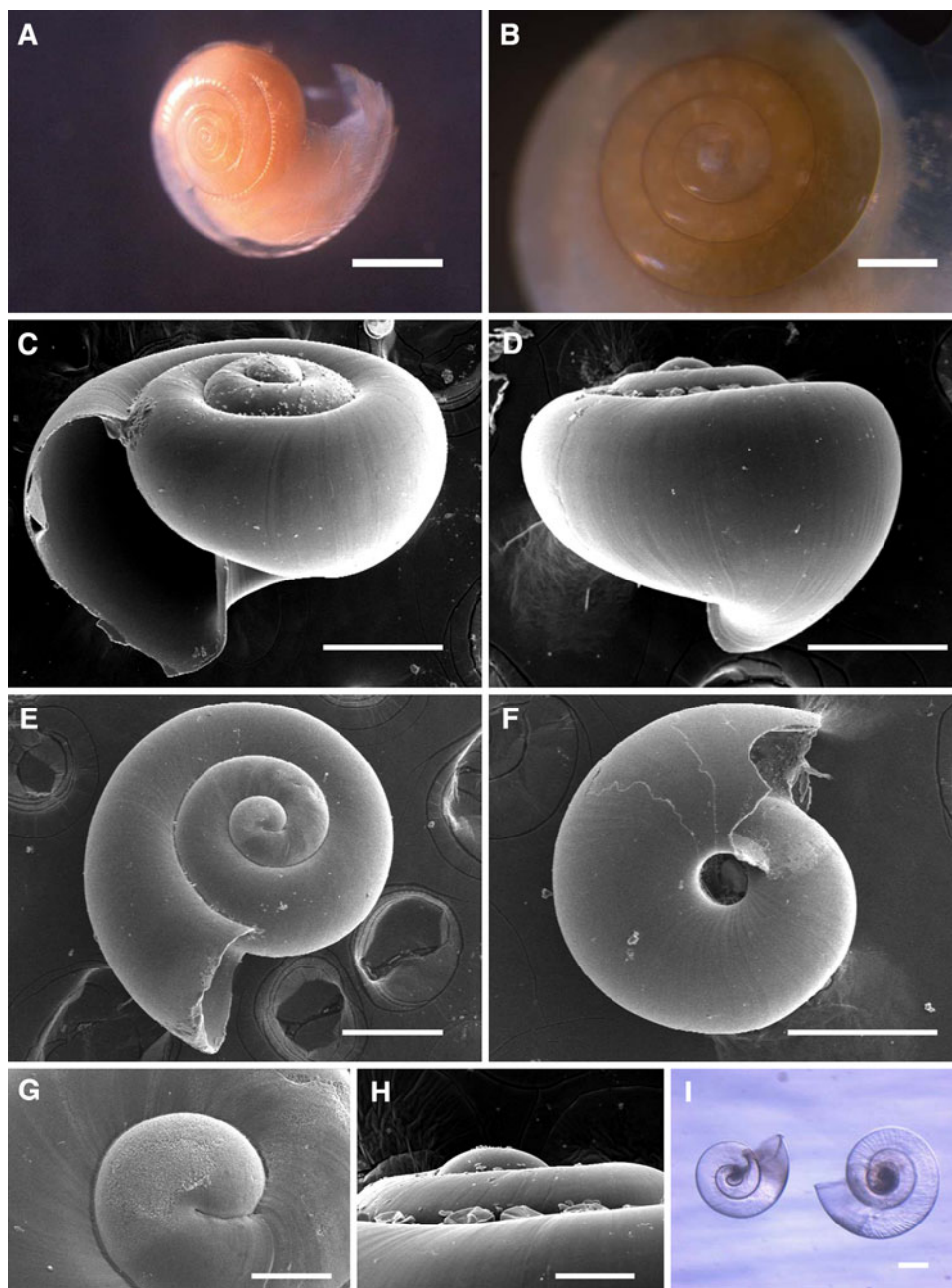


margin of the aperture was ca. 70 μm in a shell of 2.5 mm in diameter in *L. helicina* (Fig. 3a) and ca. 100 μm in a shell of 6 mm in diameter in *L. helicina antarctica* forma *antarctica* (Fig. 3b). Another shell feature characteristic of only *L. helicina antarctica* forma *antarctica* was a series of lines (spiral ribs or spiral cords) which run parallel to the circumference of the shell, perpendicular to the axial rib structure such that the surface appeared to be constructed of rectangular plates (Fig. 1f).

SEM micrographs of the shells of both species indicated that the greater part of the calcareous shell structure is composed of a crossed-lamellar layer in small

individuals (Fig. 3e, f). In the Arctic species (shell diameter 1.5 mm), the inner crossed-lamellar layer was extremely thin (ca. 3–5 μm) (Fig. 3e). In *L. helicina antarctica* forma *antarctica* (shell diameter 1.4 mm), the thickness of the inner crossed-lamellar layer at the central part of the spire was ca. 5–7 μm (Fig. 3f). Arrangement of the crossed-lamellar structure appeared to be flexible in both species, with some being oriented parallel to the shell surface (Fig. 3f) and others vertical (Fig. 3e). Various gradient arrangements were observed in various parts of the shell within one individual shell in both species. However, when analysing the shell microstructure of the

Fig. 2 *Limacina helicina*. **a–b** Note the low spires with four to six whorls. Bars 1 and 0.5 mm, respectively. **c–f** Shells of young individuals with no visible surface sculpture. Bars 200 μm (SEM). **g** Apex with protoconch showing a rough surface. Bar 50 μm (SEM). **h** Lateral view of the spire with low whorls. Bar 50 μm (SEM). **i** Young shells. Note the many growth line-like structures viewed with transmitted light. Bar 200 μm

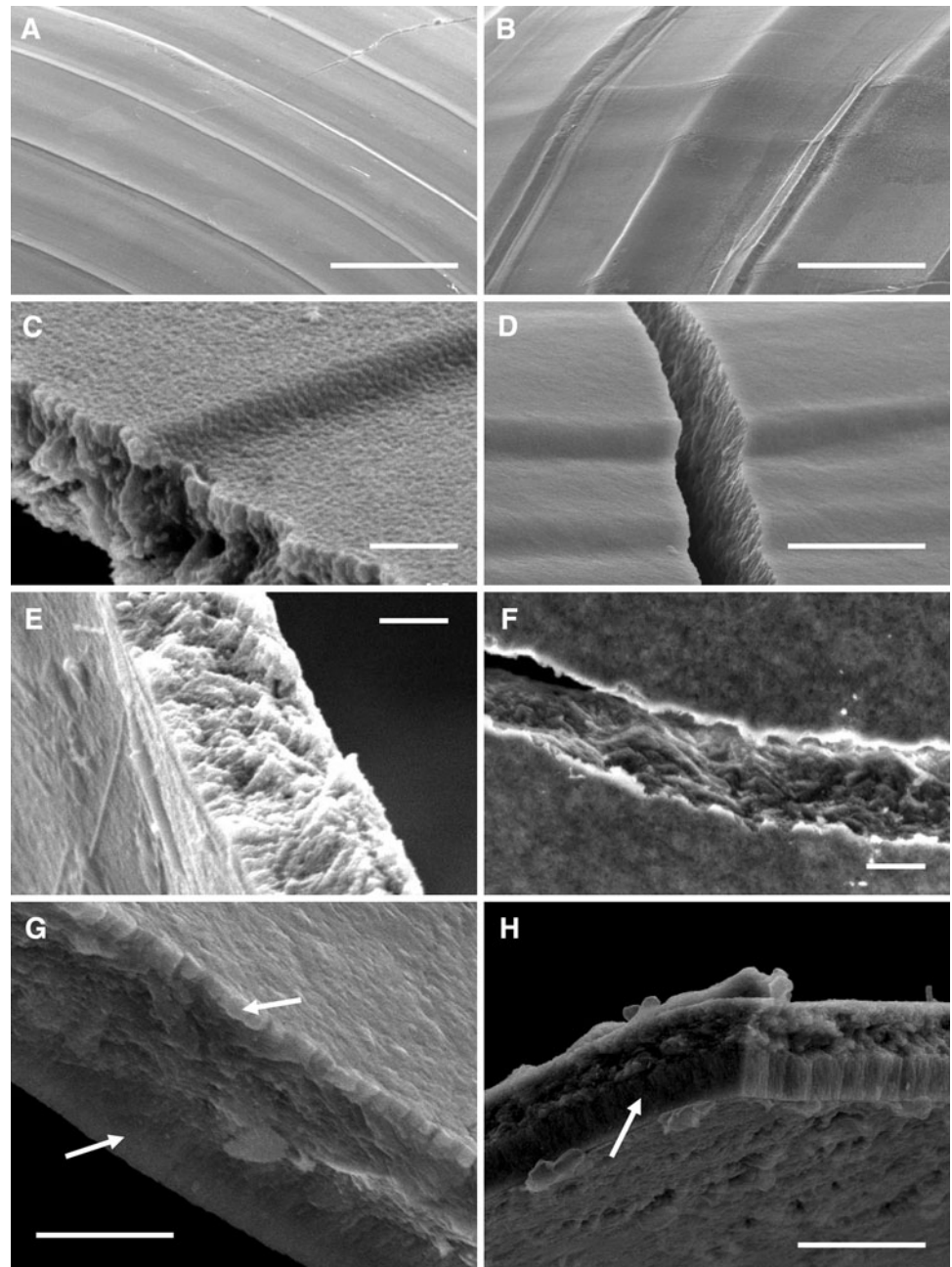


fracture surface carefully, an extremely thin outer prismatic layer was observed in both species (Fig. 3e, g). Of particular interest was the crossed-lamellar structure observed within the shell fracture and beneath it a distinctive thick prismatic structure in larger Antarctic *Limacina* species (Fig. 3g, h). In adult-sized shells, three layers were clearly present, with a layer of crossed-lamellar structure situated between the outer and inner prismatic layers. As the thickness of the outermost prismatic-like structure was less than 1 μm in the small specimens, it was sometimes difficult to observe and identify.

Discussion

In general, the shell thickness of adult molluscs ranges from several millimetres to tens of centimetres in the giant clam *Tridacna gigas*. The shell thickness of the two *Limacina* species examined here was among the thinnest observed for shelled gastropods. While the function of calcareous shells is normally considered to be that of a defence mechanism, especially against benthic predators, its effectiveness for this purpose in some pelagic groups is doubtful. It is more reasonable to speculate that these light-weight shells may be advantageous to be pelagic.

Fig. 3 SEM micrographs of *Limacina helicina* (a, c, e) and *Limacina helicina antarctica* forma *antarctica* (b, d, f, g, h). **a–b** Shell surface of larger individuals showing axial ribs. Bars 100 μm . **c–d** Fracture surface of the axial ribs. Note the step-like structures; the distance from step to step was wider and more regular in *L. helicina*. Multiple and irregular steps were observed in *L. helicina antarctica* forma *antarctica*. Bar 2 μm in c and 10 μm in d. **e–f** Fracture surface of the shells showing crossed-lamellar structure. Different directions of arrangement of thin lamellae are shown: predominantly vertical in e, parallel in f. Bar 2 μm in e and 5 μm in f. **g** Fracture surface of the shell showing outer prismatic (top arrow), middle crossed-lamellar, and inner prismatic (bottom arrow) structures in a large individual. Bar 5 μm . **h** Fracture surface of a different part of the shell in g showing the thick inner prismatic layer (arrow). Bar 10 μm



Almost all Limacinidae from the Eocene (Hodkinson et al. 1992) and Quaternary (Gerhardt et al. 2000) sediments have a shell, which consists of crossed-lamellar structure beneath the periostracum. The prismatic layer is also observed in some species of *Limacina* collected from Quaternary sediments. The helical structure is only observed in *Limacina inflata* (Glaçon et al. 1994). In the case of present day *Limacina*, here we confirm the presence and thickness of crossed-lamellar structure and prismatic structure in both polar species, *L. helicina* and *L. helicina antarctica* forma *antarctica*.

Observations of the inner shell layer at the growth edge of the shell, in *Limacina* fossils from late Quaternary deposits, have revealed the presence of a thin prismatic layer (Glaçon et al. 1994). Although we could not obtain similar longitudinal orientations at the growth edge, we could observe the prismatic structure in the outer layer for small individuals of both species and on both the inner and outer layers in larger individuals of the Antarctic *Limacina* species. Inspection of fully developed adult specimens should confirm not only the presence of the prismatic layer but also the arrangement

of constituent parts of the shell to clarify its microstructure in the genus *Limacina*.

Shell structure is related both to the mode of life and to environmental factors. Most molluscs have a multilayered shell. Taylor and Layman (1972) and Carter (1980) determined various mechanical properties of the bivalve shell and attempted to relate these to function. The crossed-lamellar structure was found to be the hardest but least elastic shell microstructure. The prismatic structure, which is constructed from calcium carbonate and interprismatic organic material, was found to have elastic properties. As *Limacina* shell is constructed from layers of both prismatic and crossed-lamellar material, it is likely to have mechanical properties that combine elasticity, flexibility and strength, despite the shell being incredibly thin. The thin elastic and hard multilayered *Limacina* shell may exhibit resistance to chemical dissolution and mechanical degradation. Toughness and lightness of the shell would be an advantage in a thin-shelled pelagic pteropod.

The axial ribs on the shell of Antarctic *L. helicina antarctica* forma *antarctica* and Arctic *L. helicina* are similar to those observed on the shell of Antarctic *Limacina helicina* (probably *L. helicina antarctica*) by Boltovskoy (1974). Those shells also exhibited a roughly ornamented embryonic shell (protoconch), while the remainder of the shell surface was smooth, with small granules and rib-like structures. However, adding to the previous observation, this study showed that prominent rib structures are only present on the surface of the outermost whorl in larger Antarctic individuals (shell diameter 6 mm) or outer whorls in Arctic individuals (shell diameter 2.5 mm). Smaller individuals (shell diameter 0.5–0.8 mm) exhibited no, or only weak, rib structures on the entire surface and on inner whorls, including the central embryonic shell region, of the medium-sized individual in *L. helicina* (shell diameter 1.5 mm). Similarly, on inner whorls of the surface of the larger individual of *L. helicina antarctica* forma *antarctica* (shell diameter 6 mm; Fig. 1), no visible ribs were observed (Fig. 1e). Thus, it appears that shell structure is different between the Antarctic and Arctic species and among shells of different size (developmental stage).

The intervals between the prominent ribs gradually increased from the inner whorl to the outer whorl in both species. The visible axial rib structure may be of use in understanding growth performance.

The rib structures on the shell of *L. helicina* may provide a means for calculating the daily age and growth rate of the linear shell length and for making semi-quantitative observations of shell growth under experimental conditions. Comeau et al. (2009) studied shell growth in *L. helicina* under different conditions of pH using fluorescent calcein and ^{45}Ca and demonstrated decreased growth at a pH of 7.8 compared to that at a pH of 8.1. Notably, although the

authors made no mention of rib features on the shell of *L. helicina*, their results clearly show that the number of ribs constructed on the newly grown shell was six or seven at a pH of 8.1 in 5 days. This rib number may indicate that one rib is constructed each day (the interval between two ribs reveals the growth in 1 day; six ribs represent 5 days' growth). Moreover, although less shell formed at low pH compared to high pH, the rib structure was still constructed under both conditions. This may indicate that even with a fall in environmental pH, the mechanism of shell formation and characteristics of the basic structure are retained.

Detailed analyses are needed in order to compare the characteristics of shell structure in temperate and cold water pteropods, not only from a structural perspective but also in terms of the functional role of shell reduction in these species and its effect on mechanical properties. Although shell architecture is said to be the same throughout the genus, previous results with the Antarctic bivalve *Laternula elliptica* showed different microstructural characteristics in terms of both quality and quantity compared with a temperate bivalve of the same genus, *Laternula marilina* (Sato-Okoshi and Okoshi 2008). Variability in shell thickness between individuals at different stages of development should also be investigated, since it may be related to the ecology of *Limacina*, including such factors as distribution, vertical migration ecology, and life history.

It is interesting to note that the benthic gastropod *Euspira fortunei* (Naticidae), which undergoes floating migration from its early adult stage, has the same shell architectural characteristics as the two *Limacina* species, with respect to the inner crossed-lamellar structure and the thinly distributed outer prismatic layer. In comparison with the shell characteristics of other Naticidae, such as *Glossaulax didyma* (Okoshi, unpublished data), *E. fortunei* has a thinner shell, which may have evolved as an adaptation to reduce weight in order to facilitate vertical migration.

The molluscs *Limacina helicina* and *L. helicina antarctica* forma *antarctica* are among the most dominant and widely distributed mesozooplanktonic organisms in Arctic and Antarctic waters, respectively. This success may be, in part, due to their characteristic feeding behaviour of spreading mucus webs and apparently low-cost means of shell formation. The shell is constructed from calcium carbonate and interprismatic organic materials. The former is considered to be absorbed mainly from surrounding seawater and the latter from the soft tissues. It is also notable that one-third or one-fourth of the total energy directed towards growth is used for shell formation (Dame 1976; Jørgensen 1976; Griffiths and King 1979; Wilbur and Saleuddin 1983). Thin-shelled molluscs need less materials and energy to construct their shell. Shell reduction in these two species would free more energy for the development of

the soft tissues, as mentioned for *Laternula elliptica*, a dominant burrowing bivalve inhabiting the same cold Antarctic seawaters (Ahn 1994; Sato-Okoshi and Okoshi 2002, 2008; Ahn et al. 2003). It is suggested that both *Limacina* species described here are well adapted for inhabiting the polar sea environment, where food is limited and water temperature is extremely low and thus slowing many biological processes including development and growth.

The present report was an initial fundamental study of shell growth in the genus *Limacina* in terms of shell structure. Further experiments are planned to use these shelled animals as models for investigating shell construction. Studies with other species will compare the effects of increasing CO₂ emission and ocean acidification. Investigations are also required to clarify ecological effects on growth rate, migration behaviour and life history.

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