

LORICATE CHOANOFLLAGELLATES FROM ELLIS FJORD,  
ANTARCTICA INCLUDING THE DESCRIPTION OF  
*ACANTHOCORBIS TINTINNABULUM* SP. NOV.

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**Abstract:** The ice-free Vestfold Hills is deeply dissected by fjords and has a number of lakes and lagoons with seasonal connection to the sea. These diverse aquatic habitats vary greatly in salinity, ice cover, temperature, water depth and water column mixing. As part of ongoing studies on these water bodies, we have sampled the plankton from a basin at the head of Ellis Fjord, some 10 km from the open sea, where the temperature was 2.6°C and the salinity was between 37.6 and 38.4‰. In enriched cultures from a 25 l sample taken from this basin we have found the loricate choanoflagellates, *Acanthoeca brevipoda*, *Acanthocorbis unguiculata*, *Diaphanoeca grandis*, *Stephanoeca complexa*, *S. norrisii* and *S. diplocostata*. From this site we describe the new species *Acanthocorbis tintinnabulum*. The 25 l sample was integrated over 0.8 m which encompassed the boundary layer between oxic and anoxic waters. The sample was stored at ca. 4°C for 3 weeks and was anaerobic for that period. The species enriched from the sample were therefore able to withstand prolonged anoxia.

## 1. Introduction

Whilst considerable attention has been given to the large and robust species of the microplankton from Antarctic waters, especially the diatoms, it is only recently that the smaller and fragile components have come under increasing attention. Loricate choanoflagellates (Acanthoecidae), are a conspicuous and abundant component of the Antarctic marine nanoplankton. Their numbers have been reported as being in the order of  $10^5$  cells  $l^{-1}$  in the Weddell Sea (SILVER *et al.*, 1980; BUCK and GARRISON, 1983) and Prydz Bay (MARCHANT, 1985). The few reports of Antarctic coastal choanoflagellates indicate that while most of the species found also occur in the open sea, some appear restricted to the inshore regions.

The Vestfold Hills, an ice-free area of some 400 km<sup>2</sup> on the coast of Princess Elizabeth Land is dotted with numerous lakes of widely differing salinities and has a number of lagoons with seasonal connection to the sea as well as encompassing four fjords. The plankton of these lakes, lagoons and the inshore region has received recent attention (BAYLY, 1978, 1982; BURTON and HAMOND, 1981; CROOME *et al.*, 1987; EVERITT and THOMAS, 1986; MARCHANT, 1985; MARCHANT and PERRIN, 1986; PERRIN *et al.*, 1987). Some new species have been reported as well as some conspicuous marine species that have not been found in the pelagic region of the Antarctic seas. Some saline lakes and fjords in the Vestfold Hills are permanently stratified (mero-

mictic) due to their seasonal development of surface ice which protects the higher salinity bottom waters from being mixed into the upper waters by wind induced turbulence. A consequence of this stratification is the development of a boundary zone between the upper aerobic waters and lower anoxic waters. This zone allows the development of specialized biota such as photosynthetic bacteria (BURKE and BURTON, 1988a). During the course of our investigations of such biota we cultured seven species of loricate choanoflagellates. We describe here the loricate choanoflagellates that inhabit the upper anoxic basin in Ellis Fjord together with a description of the environmental conditions of the site and compare the assemblage of or-

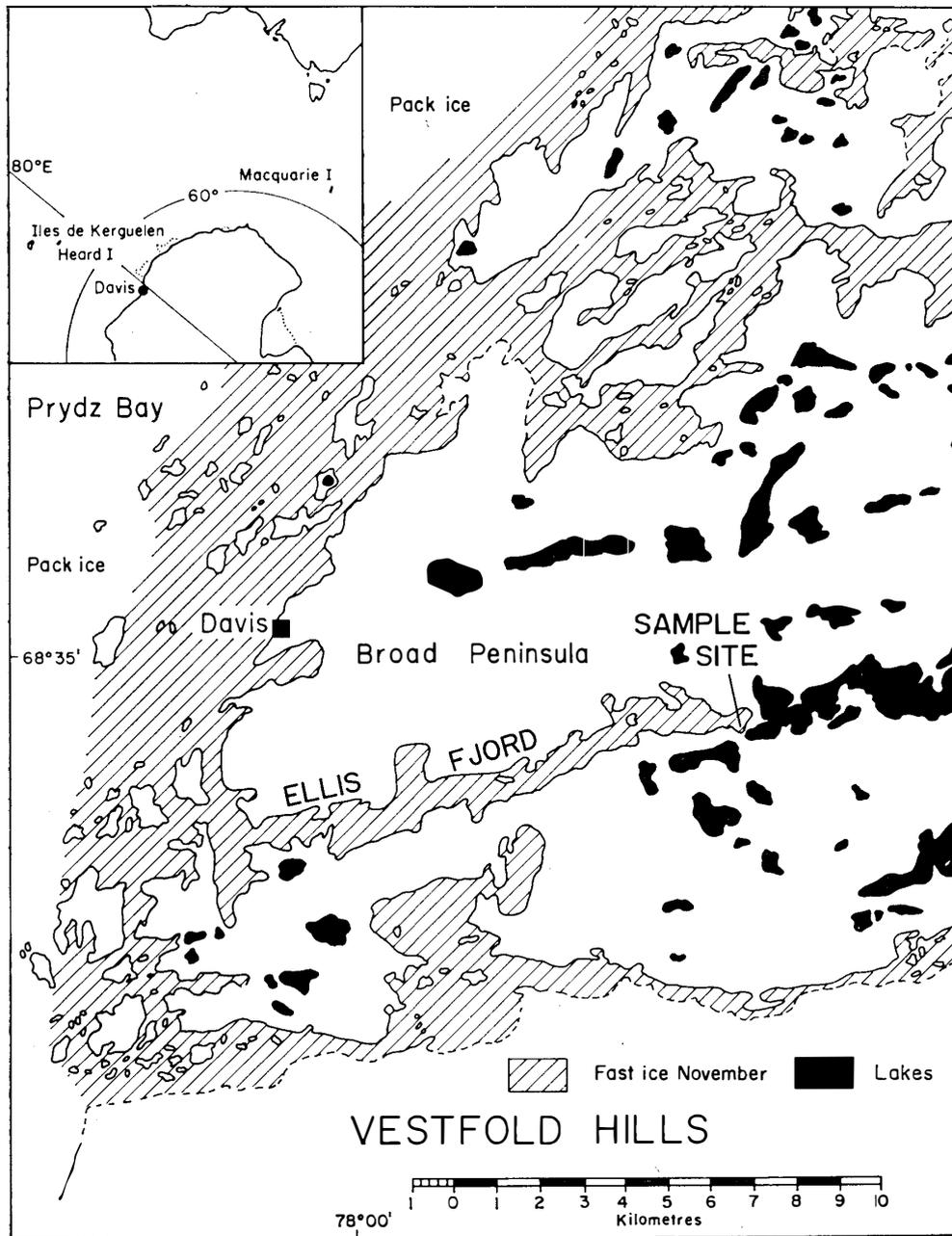


Fig. 1. Map of the Vestfold Hills showing the location of the collecting site at the head of Ellis Fjord. The inset indicates the location of the Vestfold Hills on the coast of Antarctica.

ganisms at this site with those from other habitats.

## 2. Materials and Methods

An integrated 25 l water sample was taken from 5.7 to 6.5 m depth (the boundary zone) on the 20th January 1986 from the eastern most basin of Ellis Fjord (68°36'S, 78°13'E) Antarctica (Fig. 1), with an electrical peristaltic pump through a hole drilled in the ice. This sample included both oxygenated and anoxic water. The anoxic water contained hydrogen sulfide (H<sub>2</sub>S) which led to the whole 25 l sample becoming anoxic when mixed. Protists were later (3 weeks) cultured in GP medium (LOEBLICH and SMITH, 1968), maintained at 4°C in subdued light and examined after 5 weeks for any organisms present. The direct culture method used was to add 1 drop of the integrated sample to 250 ml of GP medium in fully filled and sealed centrifuge bottles. These sub-cultures contained populations of unicellular algae and bacteria as well as the choanoflagellates. Sub-culture samples were fixed for scanning electron microscopy (SEM) in 1% glutaraldehyde, settled onto a poly-l-lysine coated glass cover slips (MARCHANT and THOMAS, 1983), dehydrated in an acetone series, critical point dried, sputter coated with gold, and examined with a JEOL JSM-840 SEM. Samples for transmission electron microscopy (TEM) were pipetted onto poly-l-lysine treated, formvar coated copper grids and fixed with 2% OsO<sub>4</sub> for 30 s, allowed to settle for 1 h, washed with distilled water, air dried, shadow cast with platinum/palladium at an angle of 30°, and viewed using a JEOL JEM-1200EX TEM.

*In situ* water temperatures were measured with a CSIRO YEO-KAL submersible data logger (PAPIJ, 1986), light intensity was measured with a Biospherical Instruments QSP-160 profiling quantum scaler irradiance system fitted with a QSP-200D underwater scaler irradiance sensor. Salinity was calculated after WHITFIELD and JAGNER (1981) using density values determined at 20°C ( $\pm 0.02^\circ\text{C}$ ) with an Anton Paar DMA 55 density meter.

## 3. Results

The sampling site was in the most inland basin at the head of the 10 km long Ellis Fjord. The fjord is open to the sea and consists of 6 basins separated by shallow sills varying in depth from 1 to 30 m. The second most inland basin is 107 m deep, the most inland only 13 m. Both are meromictic with anoxic bottom water. The 13 m deep basin at the head of the Fjord is fed by a freshwater melt-stream through Ellis Rapids in most summers (TIERNEY, 1975). However the Rapids remained frozen in the 1985/86 austral summer. The profiles of water temperature, salinity and irradiance at this site are shown in Fig. 2. At the depth from which organisms were collected the temperature was 2.6°C and the salinity *ca.* 38‰. The integrated water sample was obtained 0.4 m above and below the depth at which H<sub>2</sub>S was first smelt. The H<sub>2</sub>S concentration increased (as estimated by nose) below the depth of first detection. This depth does vary seasonally (BURKE and BURTON, 1988a). The water colour, at the depth where H<sub>2</sub>S was first detected, was very green, whereas the water above and below this depth was uncoloured.

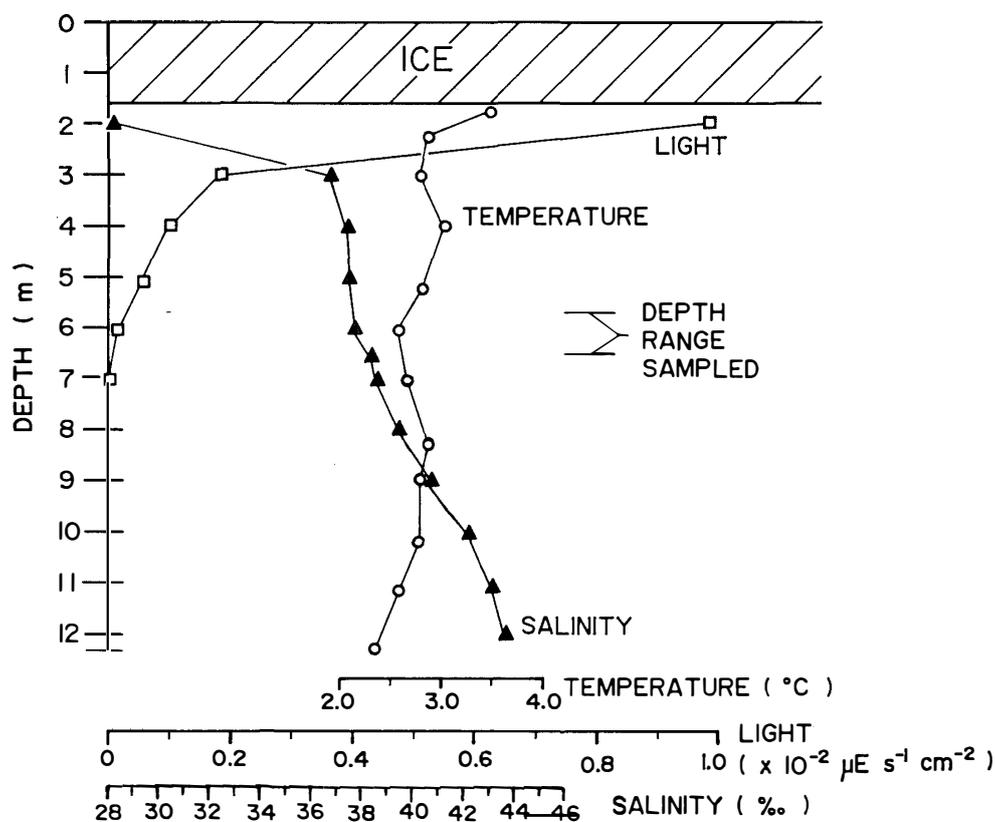


Fig. 2. Profile of temperature, salinity and irradiance at the collecting site. The fjord is covered with ice to a thickness of 1.6 m.

*Acanthocorbis unguiculata*, *Acanthocorbis tintinnabulum* sp. nov., *Acanthoea brevipoda*, *Diaphanoeca grandis*, *Stephanoeca complexa*, *S. diplocostata* var. *paucicostata* and *S. norrisii* were all cultured from this sample.

*Acanthocorbis unguiculata* (Thomsen) HARA and TAKAHASHI, 1984 (Fig. 3)

The longitudinal costae of the lorica of this species vary in number between 13 and 15. Transverse costae are confined to the posterior half of the lorica. The protoplast is located posteriorly in the lorica and is enclosed by a sheath. The tips of the longitudinal costae are in-curving and bear small claws. The length of the lorica chamber is 7–8  $\mu\text{m}$  and its diameter is 6  $\mu\text{m}$ . The spines extend 5.4  $\mu\text{m}$  beyond the lorica chamber.

This species has also been found at inshore stations in Prydz Bay (MERCHANT, 1985) and in hypersaline Organic Lake in the Vestfold Hills (VAN DEN HOFF and FRANZMANN, 1986). It is also known from Arctic Greenland (THOMSEN, 1982). Prior to the reinvestigation of *Polyoeca dichotoma* and *Acanthoea spectabilis* by HARA and TAKAHASHI (1984), this organism was named *Acanthoecopsis unguiculata* (THOMSEN, 1973).

*Acanthoea brevipoda* ELLIS, 1930 (Figs. 4 and 5)

This is a small choanoflagellate with a characteristic spiral arrangement of the costal strips and the anterior longitudinal costae protruding as spines. Interestingly,

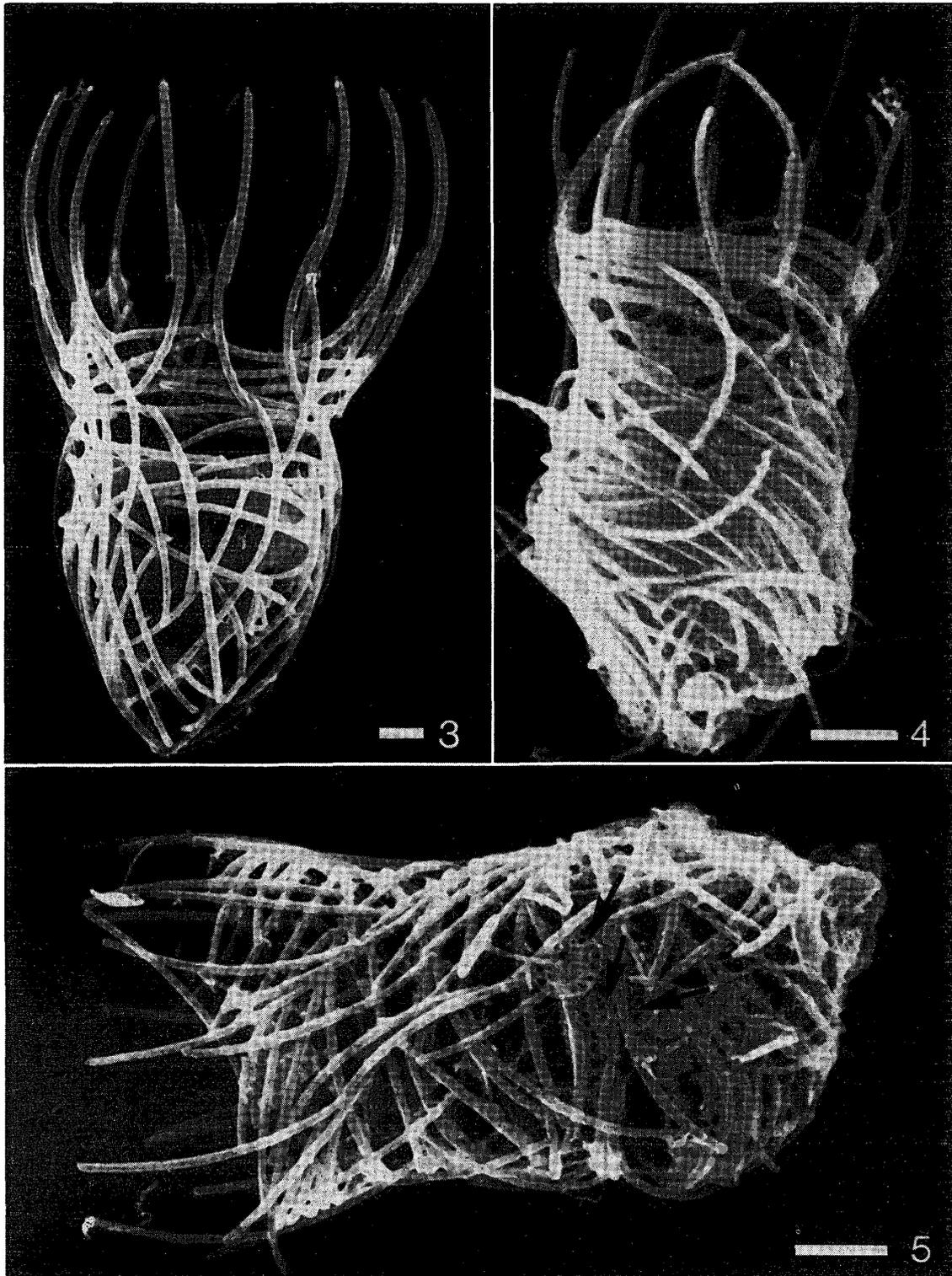


Fig. 3. *Acanthocorbis unguiculata*, note the claws at the tips of the spines. SEM. Scale marker = 1  $\mu$ m.

Fig. 4. *Acanthoeca brevipoda*. SEM. Scale marker = 1  $\mu$ m.

Fig. 5. A lorica of *Acanthoeca brevipoda* with a more open arrangement of costae to that shown in Fig. 4. The lorica also contains scales of *Paraphysomonas butcheri* (arrows). SEM. Scale marker = 1  $\mu$ m.

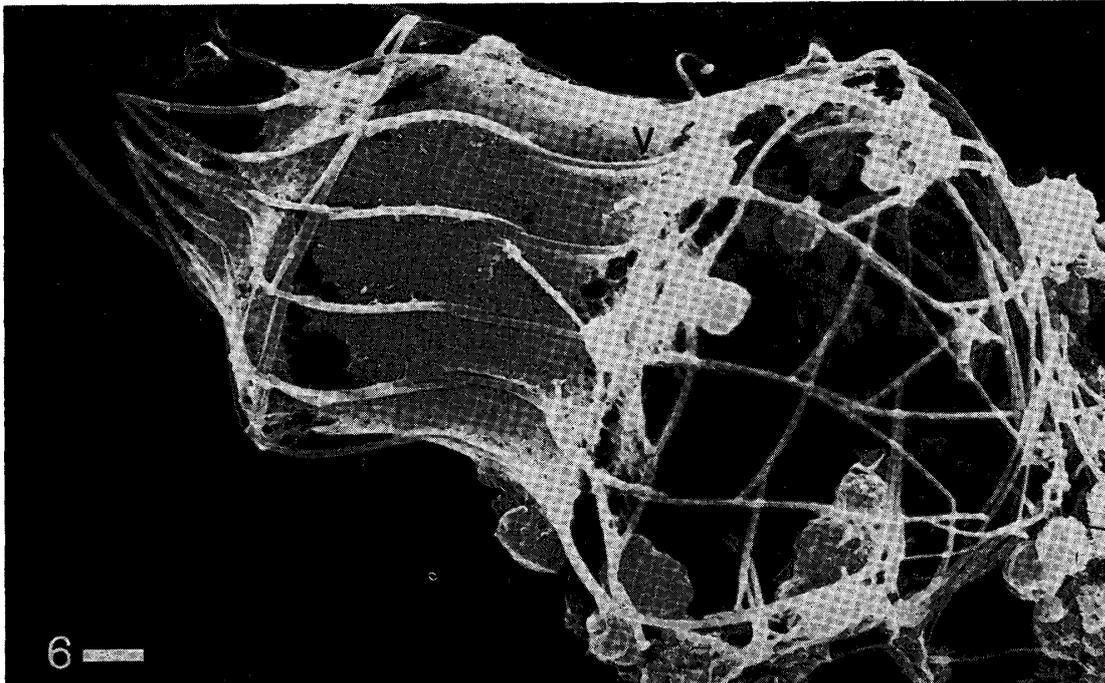


Fig. 6. An empty lorica of *Diaphanoeca grandis* showing the characteristic double row of costal strips in the anterior transverse costa (arrow). The anterior part of the lorica is distorted apparently by the shrinkage of the veil (v) that lines this part of the lorica. SEM. Scale marker = 2  $\mu\text{m}$ .

we have found scales of *Paraphysomonas butcheri* attached to the lorica of this organism. This species has been reported from the Weddell Sea (BUCK and GARRISON, 1983) as well as the coast of Europe (ELLIS, 1930; LEADBEATER, 1972b; THOMSEN, 1973; LEADBEATER and MORTON, 1974) and the North Pacific (NORRIS, 1965).

#### *Diaphanoeca grandis* ELLIS, 1930 (Fig. 6)

This distinctive species has 12 longitudinal and 4 transverse costae; the anterior transverse costae is composed of a double row of costal strips. The longitudinal costae extend beyond the anterior transverse costae. The total length of the lorica is around 30  $\mu\text{m}$ , its greatest diameter is 20  $\mu\text{m}$ . The protoplast is suspended in the middle of the lorica chamber, its tentacles extend almost to the anterior transverse costae. Some preparations, especially those for scanning electron microscopy, revealed a veil-like mesh on the inside of the lorica. As discussed in MARCHANT (1985) the role of this structure has yet to be elucidated. This veil appears to shrink during preparation for SEM, thus distorting the lorica. This species has also been found in the inshore area of the Vestfold Hills (MARCHANT, 1985), in Burton and Clear Lakes located in the Vestfold Hills (MARCHANT and PERRIN, 1986) but we have not seen it in collections from oceanic stations in Prydz Bay. It is also known from arctic Greenland (THOMSEN, 1982) and has grown up in enrichment cultures from the ice-edge region of the Weddell Sea (BUCK and GARRISON, personal communication).

#### *Stephanoeca complexa* (NORRIS) THRONDSSEN, 1974 (Figs. 7 and 8)

The lorica has a "waist" and anterior and posterior chambers can thus be defined

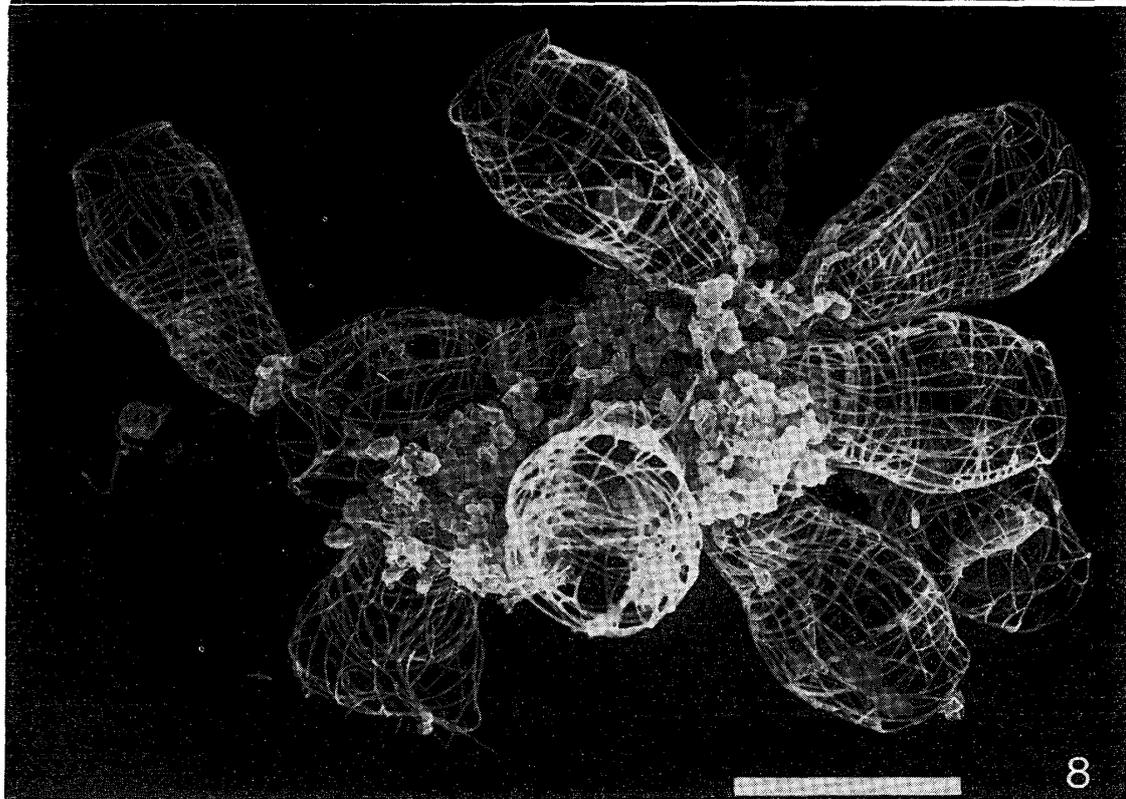
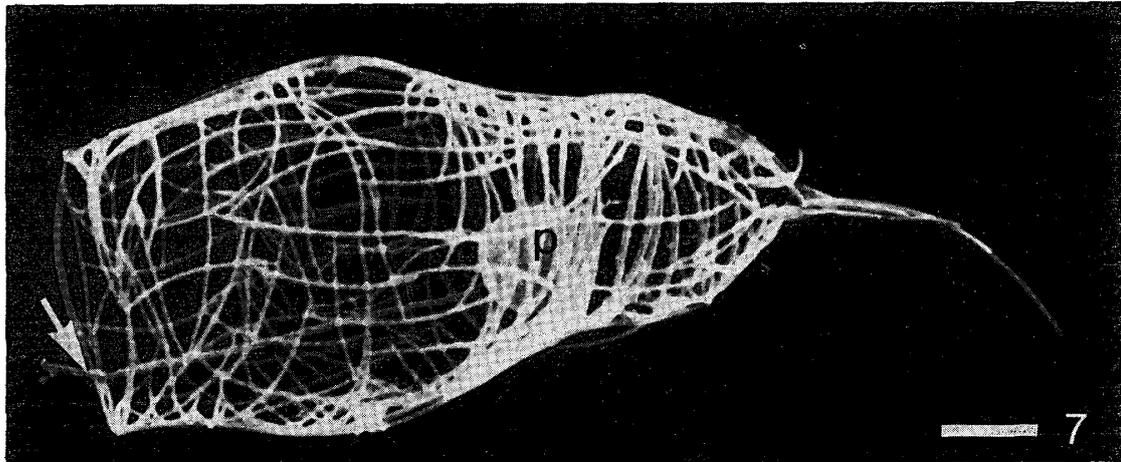


Fig. 7. *Stephanoeca complexa* with protoplast (p) and flagellum (arrow) tipped with a hairpoint. SEM. Scale marker = 2  $\mu\text{m}$ .

Fig. 8. A cluster of *Stephanoeca complexa*, most with their protoplasts intact, together with detritus and other organisms, mostly flagellates. SEM. Scale marker = 10  $\mu\text{m}$ .

to occur above and below it. Some of the costal strips of the posterior chamber are thickened. The length of the lorica, excluding any posterior appendage, is 13.5–15  $\mu\text{m}$  and the diameter of the protoplast is 2.2  $\mu\text{m}$ . This organism has also been found in Clear Lake in the Vestfold Hills (MARCHANT and PERRIN, 1986).

Clusters of *Stephanoeca complexa*, associated with detritus and other organisms, mostly flagellates were occasionally found (Fig. 8). While this tendency is a feature of *Parvicorbicula socialis* (DEFLANDRE, 1960) it has not been widely reported for other species of choanoflagellates. In Antarctic waters we have observed clusters of *P.*

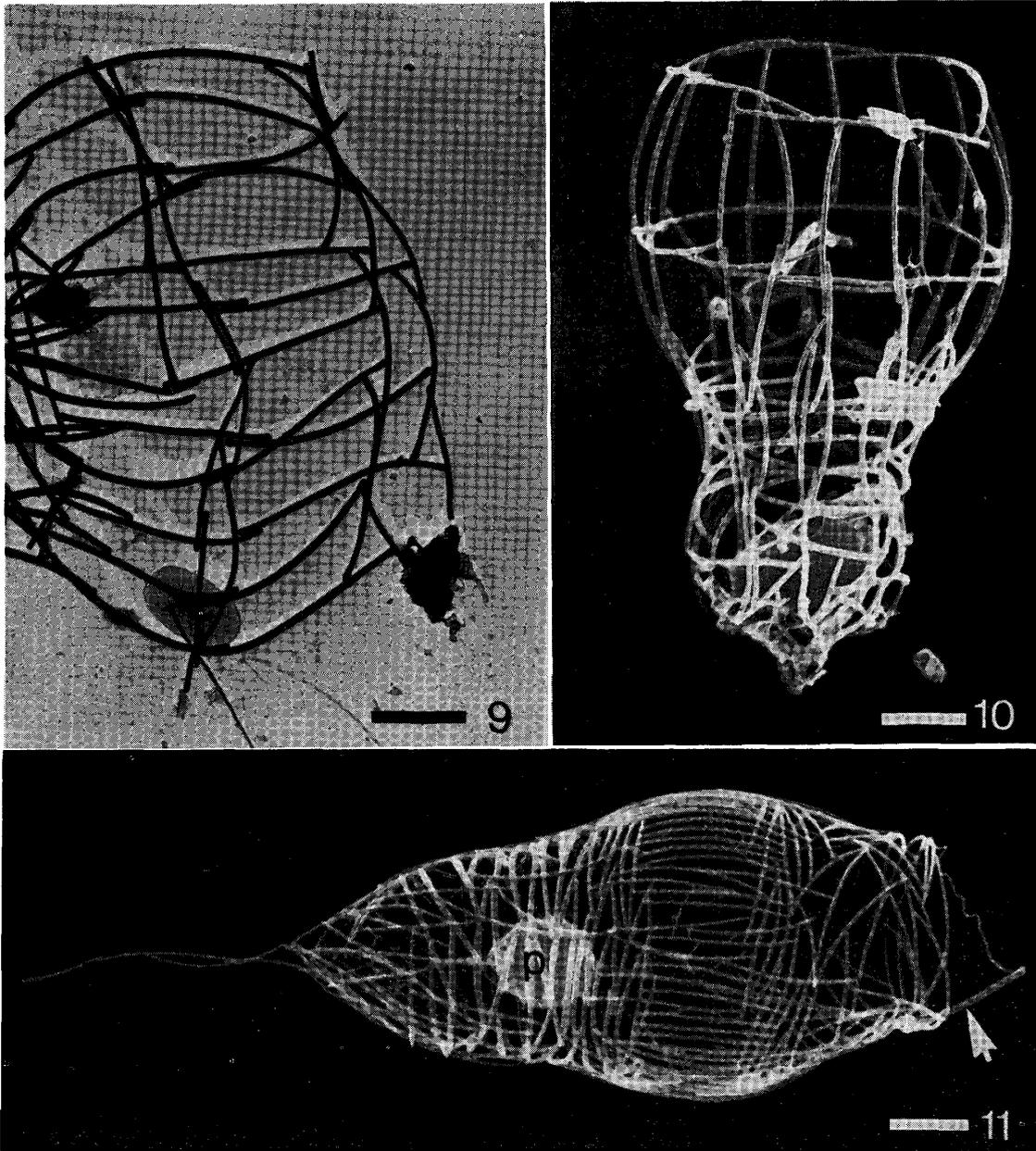


Fig. 9. Detail of the anterior part of the lorica of *Stephanoeca diplocostata* var. *paucicostata* showing the arrangement of, and junctions between, the longitudinal and transverse costae. TEM. Scale marker = 2  $\mu$ m.

Fig. 10. Whole lorica of *S. diplocostata* var. *paucicostata*. SEM. Scale marker = 2  $\mu$ m.

Fig. 11. *Stephanoeca norrisii* with a pedicel and intact protoplast (p) which bears a flagellum (arrow) that extends beyond the lorica. Note the long hairpoint on the flagellum. SEM. Scale marker = 2  $\mu$ m.

*socialis*, *Crinolina aperta* and now *S. complexa*, and HARA *et al.* (1986) reported aggregates of *Pleurasiga cupula* and *Savillea parva* on the surface of detrital particles.

*Stephanoeca diplocostata* var. *paucicostata* THRONDSSEN, 1969 (Figs. 9 and 10)

This organism has 12–14 longitudinal costae and 3 transverse costae, one of which is the anterior rim, in its anterior chamber and an irregular arrangement in the

posterior chamber. Overall length of the lorica is 16–17  $\mu\text{m}$ . The anterior lorica chamber is 10  $\mu\text{m}$  long and 9  $\mu\text{m}$  wide. The length of posterior lorica chamber is 6.5  $\mu\text{m}$ , its width is 6.4  $\mu\text{m}$ . The taxonomy of *Stephanoeca diplocostata* Ellis (ELLIS, 1930) is a little confusing as the original description was based solely on light microscopy. Using electron microscopy, LEADBEATER (1972b) described *Stephanoeca pedicellata*, an organism closely resembling *S. diplocostata* in many ways but lacking the arrangement of double transverse costae in the anterior chamber and bearing a pedicel. His subsequent studies on clonal cultures of *Stephanoeca diplocostata* revealed cells with and without pedicels (LEADBEATER, 1979). The dimensions and number of longitudinal costae in our Antarctic material closely resembles those reported from Denmark (LEADBEATER, 1972b; THOMSEN, 1973), England (ELLIS, 1930), France (BOUCAUD-CAMOU, 1967), Greenland (THOMSEN, 1982), Yugoslavia (LEADBEATER, 1973) and Norway (LEADBEATER, 1972a). *Stephanoeca diplocostata* var. *paucicostata* differs from *Stephanoeca diplocostata* principally in the number and arrangement of the transverse costae, particularly in the anterior lorica chamber. The type specimen of *Stephanoeca diplocostata* var. *paucicostata* has paired transverse costae only where the lorica has its maximum diameter. Our Antarctic material has no double transverse costae.

*Stephanoeca norrisii* THOMSEN, 1973 (Fig. 11)

The lorica of this choanoflagellate is most distinctive with a median band of about 80 longitudinal costal strips. Immediately anterior and posterior to this band the costae are transversely arranged and obliquely oriented at the extremities. It may or may not have a short posterior stalk. The costal strips of the posterior part of the lorica are thicker than the rest. The lorica of *S. norrisii* from Ellis Fjord is 15–18  $\mu\text{m}$  long, excluding any posterior stalk. The protoplast is about 2.6  $\mu\text{m}$  in diameter. The flagellum extends beyond the lorica and has a hairpoint which accounts for about 25% of the total flagellar length. This organism has previously been reported from Danish waters, from where it was described (THOMSEN, 1973) and the North Pacific by NORRIS (1965), who referred to it as *Sportelloeca kentii*. This is the first record of it from Antarctica.

*Acanthocorbis tintinnabulum* sp. nov. (Figs. 12–14)

Diagnosis: Lorica consisting of a single bell-shaped chamber, 6.5  $\mu\text{m}$  long, 4.5  $\mu\text{m}$  in diameter and 12–14 longitudinal costae extending beyond the chamber to form spines, 5–5.5  $\mu\text{m}$  long. The spines are tipped with a small claw (Fig. 13). The overall length of the organism is 11–12.5  $\mu\text{m}$ . The arrangement of costae in the lorica chamber is complex with about 16 longitudinal costae and about 40 costal strips roughly spirally arranged but with a distinct anterior transverse costa. The anterior longitudinal costae are 6–7  $\mu\text{m}$  long, although on some organisms these costal strips are as short as 3.6  $\mu\text{m}$ . They are rounded at their posterior end and taper from about 90 nm to 60 nm in diameter along their length and bear a claw on their anterior end. These costal strips extend about 75% of their length beyond the lorica chamber. The costal strips of the lorica chamber are about 100 nm in diameter, about 4  $\mu\text{m}$  long and have rounded ends.

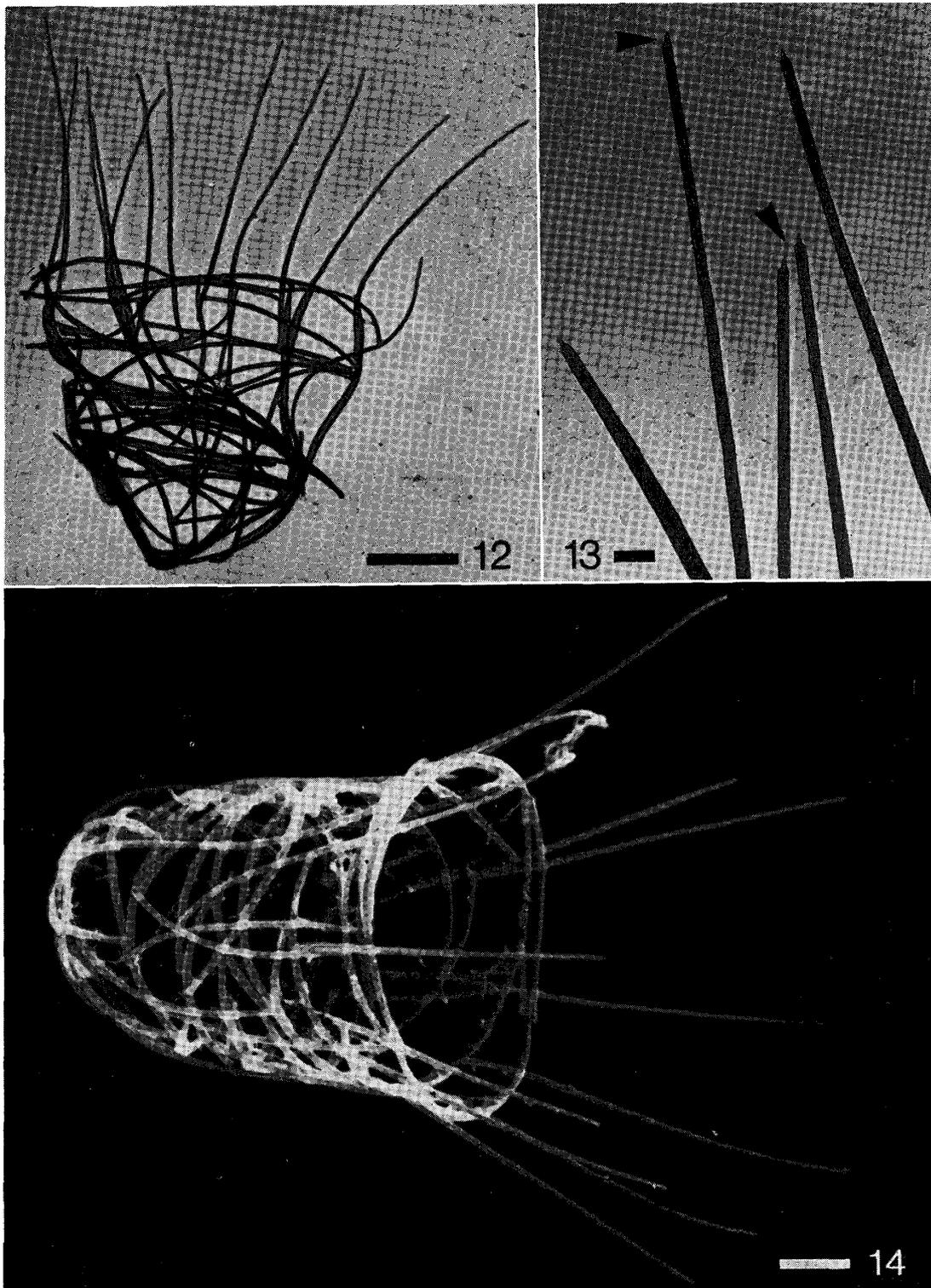


Fig. 12. Shadow-cast lorica of *Acanthocorbis tintinnabulum*. The anterior spines consist of part of a single costal strip. TEM. Scale marker = 2  $\mu$ m.

Fig. 13. Tips of the spines of *Acanthocorbis tintinnabulum* showing the taper of these costal strips and the claws (arrowheads) that closely resemble those of *Acanthocorbis unguiculata*. TEM. Scale marker = 200 nm.

Fig. 14. Intact bell-shaped lorica of *Acanthocorbis tintinnabulum*. SEM. Scale marker = 1  $\mu$ m.

Holotype: Fig. 14

Type location: Ellis Fjord, Vestfold Hills, Antarctica collected on 20th January 1986.

This new species has a close resemblance to *Acanthocorbis unguiculata* with the anterior spines bearing claw-tips and arrangement of the costae of the lorica chamber. However this species differs to the others assigned to *Acanthocorbis* in the structure of the spines. In *A. apoda*, *A. asymmetrica* and *A. unguiculata* the spines are at least one and a half costal strips long (THOMSEN, 1977) whereas in *Acanthocorbis tintinnabulum* the spines are less than a single costal strip in length. Only organisms without a protoplast have been seen. The specific name refers to the bell-shape of the lorica.

#### 4. Discussion

Few lagoons and fjords occur on the coast of Antarctica and little has been published on the plankton of these areas. Reports of near shore or ice edge choanoflagellates in Antarctica are confined to the vicinity of Lützow-Holm Bay (TAKAHASHI, 1981), off the coast of Oates Land (TANOUE and HARA, 1984; HARA and TANOUE, 1984), the Weddell Sea (SILVER *et al.*, 1980; BUCK, 1981; BUCK and GARRISON, 1983) and Prydz Bay and the Vestfold Hills (MARCHANT, 1985; MARCHANT and PERRIN, 1986; PERRIN *et al.*, 1987; VAN DEN HOFF and FRANZMANN, 1986). These studies indicate considerable differences in the species composition of choanoflagellate assemblages between coastal sites and those of the open sea.

Choanoflagellates feed on a variety of small organisms and extracellular products (MARCHANT, 1985). In a stratified environment like the shallow meromictic basin of Ellis Fjord with its abundant photosynthetic, heterotrophic and sulfate reducing bacteria in the boundary layer (BURKE and BURTON, 1988a) at approximately 6 m depth, it is not surprising that heterotrophic choanoflagellates also find it a suitable habitat. This study further exemplifies this. A transition zone between oxygenated and anoxic water is necessarily formed of layered micro-niches.

It is possible that some of the choanoflagellates cultured from the boundary layer in this study in fact originated from the fully aerobic waters above. However the 25 l container did smell of H<sub>2</sub>S at the time of collection and even 3 weeks later. Thus the choanoflagellates were at least capable of withstanding anaerobic conditions for an extended period. Unfortunately no depth profile of oxygen or H<sub>2</sub>S was taken during the sampling.

Anaerobic bacteria, at the bottom of the boundary layer in meromictic environments in the Vestfold Hills, become seasonally incorporated into the upper water by brine driven convection in winter and wind mixing during the short ice-free period in summer (BURKE and BURTON, 1988a, b). These bacteria may be an annual and abundant food source for choanoflagellates at these times.

But as well as bacteria, the nanoplankton in this basin are also a potential food source. They reach high population numbers (WALKER and MARCHANT, unpublished data). This is also shown by the light profile in Fig. 2 where little light penetrates below 5 m.

However, extinction below 6 m is likely to be almost entirely due to bacteria and algae in the boundary layer by analogy with similar layers elsewhere in the Vestfold

Hills (BURCH, 1988; BURKE and BURTON, 1988b). A number of microhabitats based on food particle type and size and environmental characteristics such as salinity, temperature and oxygen concentration can be presumed to exist and thus allow several species of micro- and nanoplankton feeders, such as choanoflagellates (this paper) and heliozoans (CROOME *et al.*, 1987) to become established.

*Acanthocorbis unguiculata* was reported by VAN DEN HOFF and FRANZMANN (1986) from Organic Lake (which has a salinity approximately five times that of seawater) and thus this species was well within its maximum salinity tolerance when cultured from the boundary layer of the top basin of Ellis Fjord. Further investigations may reveal that some of the other choanoflagellates reported here also have high salinity tolerances.

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