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# Loricate Choanoflagellates from the South Atlantic coastal zone (~32 °S) including the description of *Diplotheca tricyclica* sp. nov.

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***Abstract:*** The biodiversity of marine heterotrophic protists is poorly known in the South Atlantic coastal zone (~32 °S) especially regarding the nanoflagellates. The presence of loricate choanoflagellates was reported for the first time in the Patos Lagoon estuary and the adjacent coastal zone. Seventeen species of eleven genera of loricate choanoflagellates were observed between October 1998 and May 2000 in fixed water samples (lugol's solution + glutaraldehyde) in a JEM 100-SX transmission electron microscope. Most species were recorded in euhaline and mixopolyhaline waters during the spring and summer, none in autumn and a few (four) in winter. The absence of choanoflagellates at the more sheltered inshore stations is due freshwater influence, and at the beach station, probably due the strong wave action. The probably cosmopolitan species *Pleurasiga minima*, *Cosmoeca norvegica*, *C. ventricosa* and *Parvicorbicula circularis* were present in spring or summer in the estuary channel and coastal area while *Stephanoeca diplocostata* which apparently prefers lower temperature, was recorded in winter. *Calotheca alata* and *Campyloacantha spinifera* are mainly temperate species and were present in spring. The new species *Diplotheca tricyclica* was recorded at the estuary channel in the summer 1999, in high salinity water.
***Keywords:*** *nanoflagellates, taxonomy, heterotrophic protists, south Brazil.*

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***Resumo:*** A biodiversidade de protistas heterotróficos é pouco conhecida na zona costeira do Atlântico Sul (~32 °S), especialmente dos nanoflagelados. No presente estudo, foi registrada pela primeira vez a presença de coanoflagelados loricados no estuário da Lagoa dos Patos e na zona costeira adjacente. Dezessete espécies de onze gêneros de coanoflagelados loricados foram observadas em amostras de água fixadas (solução de lugol + glutaraldeido) coletadas entre outubro de 1998 e maio de 2000, e analisadas em microscópio de transmissão JEM 100-SX. A maior parte das espécies foi registrada em águas euhalinas e mixopolihalinas durante a primavera e o verão, nenhuma no outono e poucas (quatro) no inverno. A ausência de coanoflagelados nas estações mais protegidas deve-se a influência da água doce, e na estação da praia deve-se, provavelmente, a forte ação das ondas. As espécies, provavelmente cosmopolitas, *Pleurasiga minima*, *Cosmoeca norvegica*, *C. ventricosa* and *Parvicorbicula circularis* ocorreram na primavera ou no verão no canal principal do estuário e na área costeira, enquanto *Stephanoeca diplocostata*, que aparentemente prefere baixas temperaturas, foi registrada no inverno. *Calotheca alata* e *Campyloacantha spinifera* são principalmente de ambientes temperados e estiveram presentes na primavera. Uma espécie nova, *Diplotheca tricyclica*, foi registrada na área do canal principal do estuário, no verão de 1999, em águas de alta salinidade.
***Palavras-chave:*** *nanoflagelados, taxonomia, protistas heterotróficos, sul do Brasil.*

## Introduction

The choanoflagellates are important nanoplankters in the cycling of matter through the microbial loop, as grazers of heterotrophic bacteria and autotrophic picoplankton (Thomsen et al. 1990, Arndt et al. 2000). As consumers, they ingest marine particulate organic carbon and thus make it available to higher levels of the food web. The loricate choanoflagellates (Family Acanthoecidae) are a relatively well circumscribed group of heterotrophic, filter-feeding protists found exclusively in marine and brackish habitats, comprising planktonic or attached unicellular and colony-forming species (Thomsen et al. 1991, Thomsen & Buck 1991). The species all possess a single flagellum whose movement is responsible for creating a water current that brings food particles towards the front end of the cell. The flagellaum is surrounded by a collar of tentacles which serves as a filter for food particles. The taxonomy of the Acanthoecidae is to a large extent based on the unique lorica, constructed from silicified rod-like units. The numerical and dimensional aspects of external coverings, the type of connections between costal strips, costal strip morphology, and the position of the protoplast in the lorica, are the main characteristics for species identification (Leadbeater 1981). Collar and protoplast morphology are additional important characteristics. The knowledge of choanoflagellate diversity is important to understand their role in the microbial food web, and the group is well circumscribed taxonomically in many parts of the world, more so than any other group of bacteriovores in the marine ecosystems (Thomsen et al. 1991).

Most studies on choanoflagellates have been conducted in the Northern Hemisphere (Thomsen et al. 1991, 1995): the North Atlantic (Leadbeater 1972a, Throndsen 1974), the Indian Ocean (Andaman Sea) and Indo-Pacific (Thomsen & Boonruag 1983a, Thomsen & Boonruag 1983b, Thomsen & Moestrup 1983, Thomsen & Boonruag 1984). In the Southern Hemisphere, a striking similarity with the choanoflagellates of the North Atlantic was reported for New Zealand waters (Moestrup 1979, Thomsen & Moestrup 1983). In Antarctic waters the group comprised 10-40% of the total number of living marine nanoplankton (for a review, see Thomsen et al. 1997). In contrast, there is lack of information on choanoflagellate presence, abundance and biodiversity in the Southwest Atlantic. Their small size, slow sinking rate and delicate lorica structure render these forms easy to overlook during routine observations on preserved material (Throndsen 1997).

In southern Brazilian waters, the size structure and composition of phytoplankton have been documented by several authors (Odebrecht & Abreu 1997, Odebrecht & Garcia 1997, Bergesch & Odebrecht 2001, Bergesch et al. 2008) but characterization of the heterotrophic community is still lacking. Here we present detailed information on loricate choanoflagellate species composition and distribution in the South Brazilian coastal waters, and the description of one new species of the genus *Diplotheca* (Thomsen & Buck 1991). Selected electron microscope micrographs are included.

## Materials and Methods

### 1. Study area

The Patos Lagoon in southern Brazil (Figure 1) is the world's largest choked lagoon with a surface of 10,227 $km^2$ (Asmus 1997). Choked lagoons are characterized by their high surface area and narrow mouth communicating with coastal waters. The estuary proper in the south comprises 10% of the lagoon area and presents highly variable hydrographic characteristics, due to short-term, seasonal and long-term fluctuations. Under *El Niño* events in the Pacific Ocean, freshwater discharge exceeds mean values in southern Brazil, and the opposite is observed during *La Niña* phenomena (Berlato & Fontana 2003). The second key factor is wind forcing with SE/SW wind pushing seawater into the estuary during low fluvial discharge, while NE wind favors fluvial discharge and causes salinity decrease in the lower lagoon. Periods of strong freshwater discharge with seawater intrusion induced by southerly winds cause vertical stratification and the formation of a saline wedge (Garcia 1997). Thermohaline conditions of the adjacent continental shelf are influenced by the freshwater outflow from the Patos Lagoon and La Plata River, and by tropical and subantarctic waters during summer and winter, respectively. Tropical waters transported by the Brazilian Current are the main outer shelf and slope waters, while on shelf diluted waters of subtropical and subantarctic origin prevail in winter and summer, respectively (Ciotti et al. 1995, Piola et al. 2000, Soares & Möller Jr. 2001).

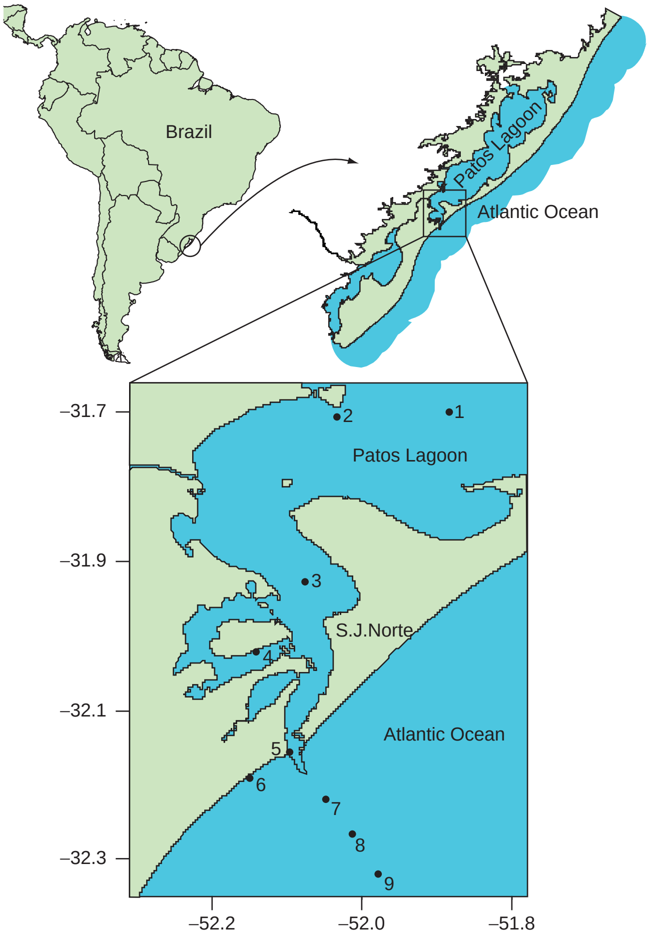


**Figure 1.** Map of Brazilian coastline showing the sampling stations.

**Figura 1.** Mapa da área de estudo com os locais de coleta.

### 2. Sampling

Surface water samples were taken monthly between October 1998 and May 2000 at six stations (Figure 1, Table 1), (a) four stations (1-4) in the Patos Lagoon estuary (Lagoon, Laranjal, Mendanha, Museum), (b) one station (5) near its mouth in the main channel, (c) one station (6) at Cassino Beach, and (d) twice (February and October 2000) at standard depths (Niskin bottle, 5 L) at three stations (7-9) along a

**Table 1.** Geographic location of sampling stations.

**Tabela 1.** Localização geográfica das estações de amostragem.

| Station | | Temperature | Salinity | Latitude | Longitude |
|---|---|---|---|---|---|
| (1) Lagoon | Mean | 20.2 | 3.0 | 31º 41’ 00’’ S | 51º 53’ 30’’ W |
| | Max | 27.8 | 7.0 | | |
| | Mín. | 13.9 | 0.0 | | |
| (2) Laranjal | Mean | 20.3 | 6.0 | 31º 43’ 00’’ S | 52º 03’ 00’’ W |
| | Max | 27.9 | 21.3 | | |
| | Mín. | 14.0 | 0.0 | | |
| (3) Mendanha | Mean | 19.7 | 12.6 | 31º 56’ 30’’ S | 52º 05’ 00’’ W |
| | Max | 27.6 | 31.5 | | |
| | Mín. | 13.1 | 0.0 | | |
| (4) Museum | Mean | 19.8 | 18.8 | 32º 01’ 521’’S | 52º 06’ 358’’ W |
| | Max | 28.0 | 34.0 | | |
| | Mín. | 11.0 | 0.0 | | |
| (5) Main Channel | Mean | 20.2 | 22.5 | 32º 15’ 00’’ S | 52º 06’ 358’’ W |
| | Max | 28.0 | 35.0 | | |
| | Mín. | 12.0 | 1.0 | | |
| (6) Cassino Beach | Mean | 20.2 | 31.5 | 32º 12’ 00’’ S | 52º 10’ 00’’ W |
| | Max | 26.0 | 35.7 | | |
| | Mín. | 12.0 | 26.0 | | |
| (7) Coastal Shelf St.1 | Mean | 23.0 | 35.6 | 33º 28’ 69’’ S | 51º 39’ 86’’ W |
| | Max | 23.3 | 35.6 | | |
| | Mín. | 22.2 | 35.5 | | |
| (8) Coastal Shelf St.2 | Mean | 22.7 | 35.8 | 33º 07’ 940’’ S | 51º 04’ 024’’ W |
| | Max | 23.7 | 35.9 | | |
| | Mín. | 18.0 | 35.7 | | |
| (9) Coastal Shelf St.3 | Mean | 23.0 | 35.6 | 32º 15’ 00’’ S | 52º 03’ 00’’ W |
| | Max | 23.3 | 35.6 | | |
| | Mín. | 22.2 | 35.5 | | |

transect on the adjacent coastal shelf (Figure 1). Water temperature (using a thermometer in shallow waters and a CTD Sea Bird on the continental shelf) and salinity (Yellow Spring 33 SCT or CTD Sea Bird) were measured at each station.

In the laboratory, one liter of water was prefiltered (20 µm) to remove larger particles, and the samples were fixed in brown glass flasks by addition of 10 mL of acidic Lugol’s solution and 10 mL of 25% glutaraldehyde (Mikroskopie MERCK, 25%) (final concentration 1% Lugol; 0.25% glutaraldehyde) following Jensen (1998). The fixed samples were left undisturbed to settle in cool and dark conditions for at least two days before removal of the supernatant. The concentrated sample was transferred to 60 mL brown flasks and stored in the dark, under refrigeration (4 °C). It was gently mixed, one drop transferred to the grids and left to dry, following the usual whole mount preparation of grids for TEM (Moestrup & Thomsen 1980). The grids were shadowcast with chromium at an angle of 20-30º using an Edwards E12E vacuum evaporator. One to three grids per sample were examined in a JEM 100-SX transmission electron microscope (TEM). Loricate choanoflagellates were identified in 21 samples, but broken or incomplete loricas were present in several others of the 198 analyzed samples (Bergesch et al. 2008).

For the new species of *Diplotheca,* light microscope whole mounts were prepared on coverslips using the upside-down dry-mounting technique, which enables the use of immersion oil lenses (Thomsen & Moestrup 1983). The silicified costae of loricate choanoflagellates are readily visible in this type of preparation which usually allows for species identification.

## Results

Sixteen species of choanoflagellates were identified, and one new species was described. The local distribution of species is summarized in Table 2. In addition, the material comprised some incomplete loricae, which could not be identified. The organisms size could usually but not always be measured because some lorica were incomplete or partially broken.

*Diplotheca tricyclica*. Bergesch et Moestrup, sp. nov. (Figures 2-11).

### *1. Diagnosis*

Cell solitary (Figure 2). The lorica is barrel-shaped, ca. 26 µm long and 13 µm in its widest part, composed of two chambers, one anterior and the other posterior, with a pedicel (Figures 2-4). The protoplast is located in the posterior chamber. The flagellum and collar tentacles protrude into the anterior chamber, which is about twice as long as the posterior chamber. The anterior chamber consists of nineteen longitudinal and two transverse costae. One series of costal

**Table 2.** Choanoflagellate species observed in southern Brazilian coastal waters and respective station and season (W = Winter, Sp = Spring, Su = Summer, Au = Autumn).

**Tabela 2.** Espécies de coanoflagelados observadas nas águas costeiras do sul do Brasil com a respectiva estação de coleta e estação do ano (Inverno, Primavera, Verão, Outono).

| **Species** | **Station** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Lagoon** | **Laranjal** | **Mendanha** | **Museu** | **Channel** | **Beach** | **Cont. Shelf** |
| *Acanthocorbis* sp. | - | - | ● (Wi, Sp) | - | - | - | - |
| *Calotheca alata* | - | - | - | - | ● (Sp) | - | ● (Sp) |
| *Campyloacantha spinifera* | - | - | - | - | - | - | ● (Sp) |
| *Cosmoeca norvegica* | - | - | - | - | - | - | ● (Sp) |
| *C. subulata* | - | - | - | - | - | - | ● (Sp) |
| *C.* aff. *ventricosa* | - | - | - | - | ● (Sp) | - | - |
| *Diplotheca tricyclica* | - | - | - | - | ● (Su) | - | - |
| *Parvicorbicula circularis* | - | - | - | - | - | - | ● (Su) |
| *Pleurasiga minima* | - | - | - | - | - | - | ● (Su- Sp) |
| *Polyfibula elatensis* | - | - | - | - | ● (Su) | - | - |
| *Stephanacantha dichotoma* | - | - | - | - | - | - | ● (Su) |
| *Stephanoeca apheles* | - | - | ● (Su) | - | ● (Wi) | - | - |
| *S. cupula* | - | - | ● (Sp) | - | - | - | - |
| *S. diplocostata* | - | - | - | - | ● (Wi) | - | - |
| *S. urnula* | ● (Wi) | - | ● (Sp) | - | - | - | - |
| *Stephanoeca* sp. | - | - | ● (Sp) | - | - | - | - |
| *Syndetophyllum pulchellum* | - | - | - | - | - | - | ● (Su) |

strips forms the anterior ring, and another is located in the midregion of the anterior chamber. The posterior chamber is separated from the anterior by transverse costae converging at the posterior end. The structure of the posterior chamber is difficult to see because of the tightly appressed costae and the presence of the protoplast. The longitudinal costal strips are of three types (Figures 5a-c). The anterior sets of longitudinal costal strips are also enlarged and an inflated proximal tip bears a small spine (Figure 5a). The second longitudinal costal strips are heteropolar and spatulate (7.3 x 1.3 µm), one extremity rod- shaped with enlarged ends, and the other broad and flattened with a thickened midrib and an elaborate pattern of perforations on both sides (Figure 5b). The posterior lorica chamber probably has a third type of costal strip, a broad and flattened heteropolar strip with a thick midrib approximately 7, 5 µm long (Figure 5c). The apical transverse costal strips (the anterior ring) have slightly enlarged ends (7.2 x 0.5 µm, Figure 5d). The pedicel is composed of four costal strips (5.8 x 0.5 µm) that comprise the fourth type of strip (Figure 5e). The complete cell (Figure 2) shows at the second transverse costae (anterior chamber) an accumulation of costal strips which are morphologically similar to those of the posterior chamber (third type), produced in preparation for the next division of the cell. The waist has also an accumulation of thicker strips with a convex side, as seen also in the posterior chamber of *Diplotheca costata* (Jackson & Leadbeater 1991).

The sample containing *D. tricyclica* was collected in the main channel of the mouth of Patos Lagoon, in March 1999, when water temperature and salinity were 24 °C and 33.5, respectively. We found seven cells in LM dry mounts (Figure 6-11), but these did not contribute towards understanding the lorica details. The electron microscope preparations included an almost complete cell and part of the posterior chamber. This genus shows a high number of costal strip types in contrast to most choanoflagellates where the costal strips are morphologically uniform (Jackson & Leadbeater 1991). Number of organisms observed in the TEM: two.

### *2. Holotype*

Figure 2. The sample has been deposited in the Federal University of Rio Grande Herbarium (HURG) under number HURG-11032.

### *3. Etymology*

Tricyclic: three rings, referring to the presence of three transverse costal rings.

### *4. Additional observations and comments*

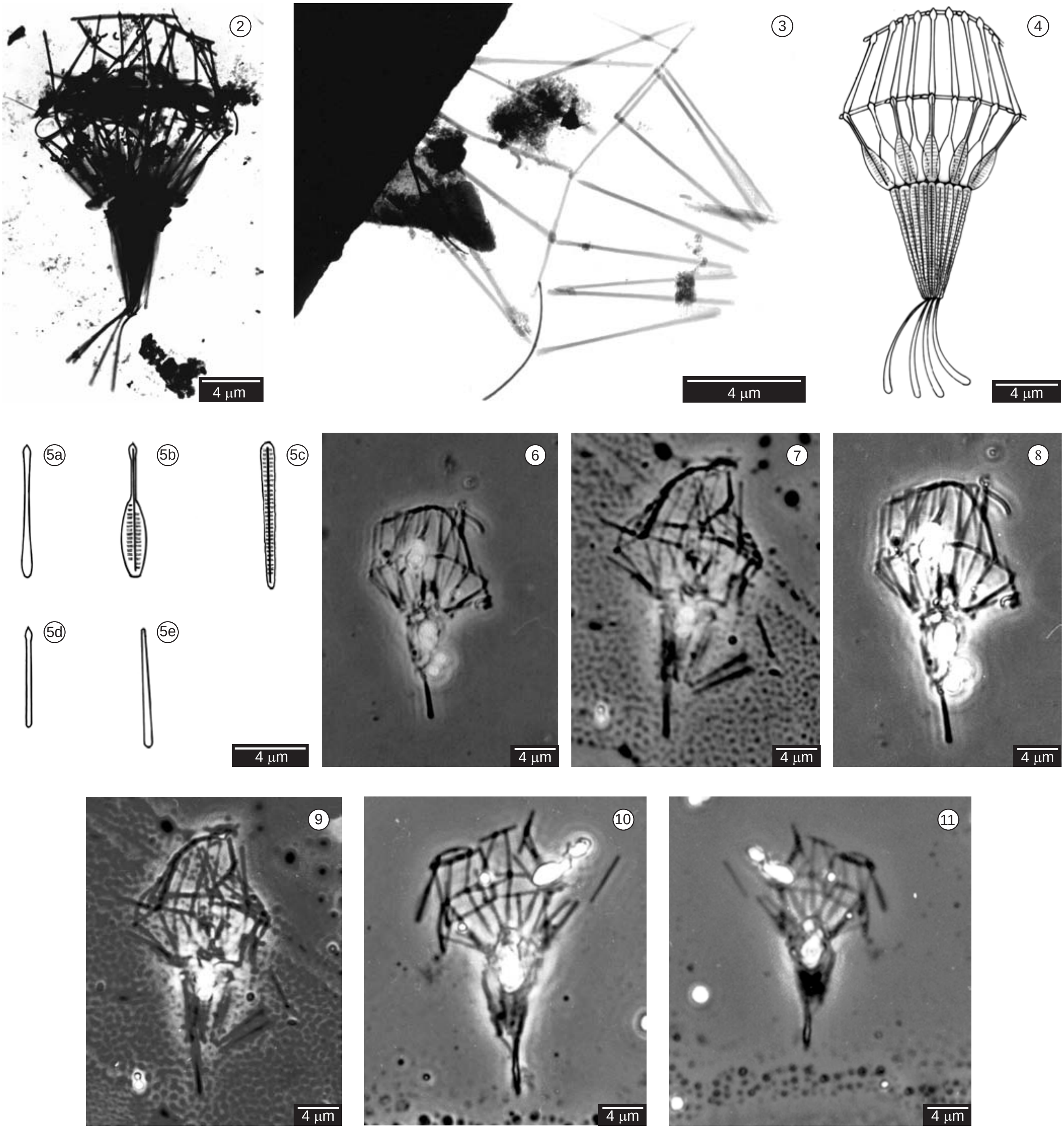
The cell observed in Figure 2 shows at the second transverse costae (in the middle of the anterior chamber) an accumulation of costal strips morphologically similar to those of the posterior chamber (third type). The presence of these strips is related to the following cell division process. The waist has also an accumulation of thicker strips with a convex side. They differ from those present in a similar position in the posterior chamber of *Diplotheca costata* (Jackson & Leadbeater 1991).

### *5. Comparison with D. costata*

*Diplotheca tricyclica* differs from *D. costata* in (1) the presence of three rather than two transverse costal rings, (2) the presence of a pedicel, (3) the morphology of the costal strips in the third transverse ring (crescentic, rod-shaped in *D. costata*, wider and flattened in *D. tricyclica)*. We include the new species in the genus *Diplotheca* because of the similarity in the longitudinal costal strips of the anterior chamber. The costal strips of the posterior chamber are also rather similar. In both species of *Diplotheca*, 2-3 longitudinal costal strips insert on each strip of the anterior costal ring, the anterior tips of the strips being slightly spatulate.

1. *Acanthocorbis* sp. (Hara et Takahashi 1984) (Figure 12)

The lorica is 13,9 to 15,2 µm long with the posterior conical end composed of a high number of longitudinal costal strips (fourteen?). The anterior part is basket shaped and composed by



**Figures 2-11.** *Diplotheca tricyclica*. 2-5) TEM. 6-11) LM (x1000). 2) Complete lorica. 3) Anterior chamber detail. 4) Schematic drawing of the complete lorica. 5 a-e) The costal strips: a) first longitudinal set of the anterior chamber; b) second longitudinal set of the anterior chamber; c) longitudinal set of the posterior chamber; d) transversal set of the anterior chamber; and e) pedicel. 6-11) cells in dry mounts.

**Figuras 2-11.** *Diplotheca tricyclica*. 2-5) MET. 6-11. MO (x1000). 2) Lorica completa. 3) Detalhe da câmara anterior. 4) Desenho esquemático da lorica completa. 5 a-e) Peças costais: a) primeiro conjunto longitudinal da câmara anterior; b) segundo conjunto longitudinal da câmara anterior; c) conjunto longitudinal da câmara posterior; d) conjunto transversal da câmara anterior; e e) pedicelo. 6-11) células em "dry mounts".

sixteen (?) longitudinal costal strips. Between the anterior and posterior parts there is a transverse set of loosely costal strips. Incomplete organisms were observed in August (13 °C; salinity 28) and December 1999 (23 °C; salinity 14) at the station Mendanha. Number of organisms observed in the TEM: three.

2. *Calotheca alata* Thomsen et Moestrup 1983 (Figure 13)

The lorica is ca. 12 µm long. Although it was not possible to count the number of coastal strips in the posterior chamber, cellular characteristics of the anterior lorica containing six longitudinal costae, individual costal strips 6 µm long and 1.5 µm wide, and costal strips of the anterior transverse ring 4.4 µm long and 1.3 µm wide, are all characteristic of *C. alata*. Found in December 1999 and October 2000, respectively, in the estuary main channel (22 °C; salinity 19) and on the continental shelf (13 °C; salinity 22). Number of organisms observed in the TEM: two.

3. *Campylocantha spinifera* (Leadbeater 1973) Hara et Takahashi 1987 (Figure 14) (Syn. *Parvicorbicula spinifera* Leadbeater 1973)

In this species the anterior projections fuse into a transverse costal ring, forming L-shaped structures; in our material only part of this structure was left. Found in October 2000 at the continental shelf (13 °C; salinity 32). Number of organisms observed in the TEM: one.

4. *Cosmoeca norvegica* Thomsen 1984 (Figure 15)

The cup-shaped lorica is composed by ten longitudinal and three transverse costae. The two anterior transverse costae have a spacing of approximately one costal strip (4 µm). Interconnections between longitudinal costal strips as well as those in the anterior and middle transverse costae are end-to-end joints. Found in October 2000, at the continental shelf (13 °C; salinity 32). Number of organisms observed in the TEM: one.

5. *Cosmoeca subulata* Thomsen in Thomsen and Boonruang 1984 (Figure 16)

Lorica funnel-shaped ca. 12,9 µm long, composed of two transverse costal rings, overlaid externally by ten longitudinal costae with a reduction of costae number below the posterior transverse costae. The incomplete lorica makes it difficult to count the number of longitudinal costae. The anterior longitudinal costae, project shortly (less than one half) above the anterior transverse costal ring, which attaches at the joints between neighboring strips. *Cosmoeca subulata* closely resembles *C. ceratophora*, the main differences being the position and size of the posterior transverse costal rings: in *C. subulata* the rings are composed of six–seven costal strips, located at the level of the joints between the second and the third longitudinal costal strip, whereas in *C. ceratophora* the posterior transverse costal rings comprises only four costal strips and crosses at the joints between the third and the fourth longitudinal costal strips. Found in October 2000 (12° C; salinity 34). Number of organisms observed in the TEM: one.

6. *Cosmoeca* aff. *ventricosa* Thomsen and Boonruang 1984 (Figure 17).

Lorica barrel-shaped, composed of twelve (?) longitudinal costae and three transverse rings. The two anterior costal rings are located in the anterior part of the lorica chamber, at the level of the joints between the second and the third longitudinal costal strip. The third transverse costal strip was not visible. The costal strips are shaped as narrow rods, and strips from the transverse and longitudinal costae are of the same length. Transverse costae are more heavily silicified and furnished with a slightly enlarged tip, which attaches the longitudinal costae. The lorica of this organism is similar to *Cosmoeca ventricosa*, but smaller (ca. 12,8 µm long), compared to 23-32 µm; and the comparison with *C. ventricosa* forms A, B, C and D was impaired as only one organism with an incomplete lorica was found in December 1999 at the estuary main channel (22 °C; salinity 19) (Thomsen & Boonruang 1984, Thomsen et al. 1990). Number of organisms observed in the TEM: one.

7. *Pleurasiga minima* Throndsen 1970 (Figure 18)

The cell is ca. 7 µm long, has a long flagellum and amphora-shaped lorica, eight (?) longitudinal costae and two equally wide, circular transverse costal rings. The longitudinal costae join the transverse costae in their middle part. This species was observed in continental shelf samples in February 2000 at the surface (24 °C; salinity 36) and 75 m depth (18 °C; salinity 36), and in October 2000 at the surface (14 °C; salinity 32), and at 20 and 30 m depth (13 °C; salinity 32). Number of organisms observed in the TEM: seven.

8. *Parvicorbicula circularis* Thomsen 1976 (Figure 19)

The cell is ca. 6 µm long and approximately 2 µm wide. The flagellum is ca. 15 µm long, and surrounded by a collar of tentacles measuring ca. 2.5 µm in length. Four longitudinal costae extend from the anterior costal ring to the antapex of the lorica. Only two of three costal strips could be observed in each longitudinal costa, probably due the cell observed being seen from the antapical side. Found in February 2000 at the continental shelf, 20 m depth (24 °C; salinity 35.7). Number of organisms observed in the TEM: one.

9. *Polyfibula elatensis* Manton (Manton et Bremer, 1981) (Figure 20) (Syn. *Pleurasiga sphyrelata* subsp. *elatensis* Thomsen 1978)

Lorica ca. 19,3 µm long, has nine (?) longitudinal and three transverse costae. Longitudinal costae form a fringe projecting at the anterior end, each strip projecting for approximately half its length. The stipitate posterior end which characterizes this species was not observed. The number of longitudinal costal strips is higher than the seven- eight described by Manton and Bremer (1981). Found at the estuary main channel (23 °C; salinity 33). Number of organisms observed in the TEM: one.

10. *Stephanacantha dichotoma* Thomsen et Boonruang, 1983 (Figure 21).

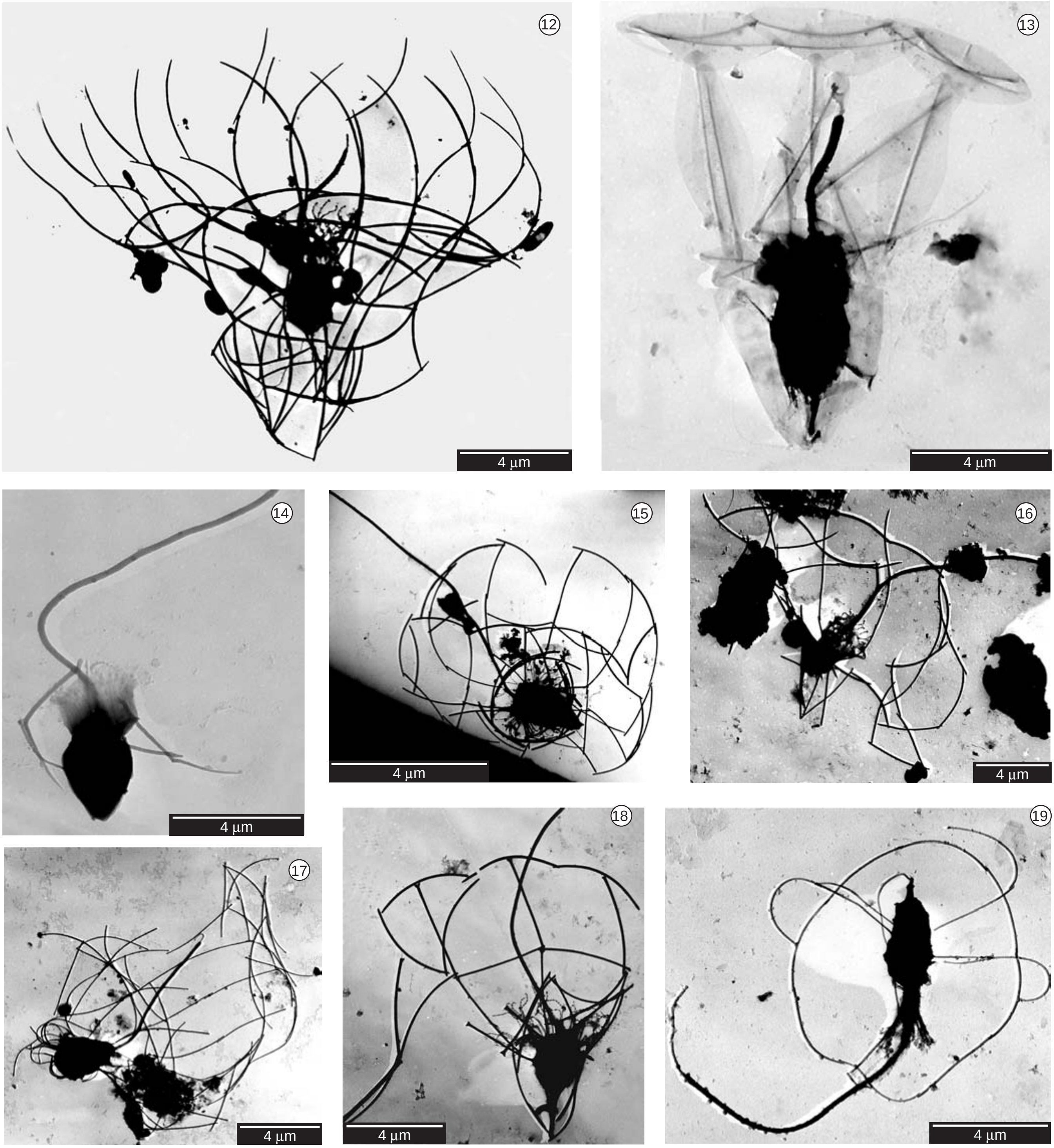
Lorica is ca. 13 µm long, composed of three different costal strips. The anterior transverse ring is composed of strips each measuring ca. 3.8 µm in length, with an upright spine at one end. Three longitudinal costal strips attach to the centre of each transverse costal strip. The longitudinal costal strips converge at the posterior end, joining with a flattened, ca. 3 µm long pedicel. Some parts of the lorica were not clear enough to allow for counting and measuring of the different types of costal strips. Found in February 2000 at the continental shelf, 50 m depth (24 °C; salinity 36). Number of organisms observed in the TEM: one.

11. *Stephanoeca* sp. Ellis 1930 (Figure 22).

The cell observed is ca. 3 µm long, 2 µm wide and the flagellum 8 µm long. The lorica is approximately 13 µm long, and composed by eight (?) longitudinal costae, each formed of four (?) costal ribs. The anterior transverse costae encircle the lorica aperture, and the connection between transverse and longitudinal costae are of the T type. The second transverse costal ring is located at the widest part of the lorica, crossing the middle part of the second longitudinal strip (counting from the lorica opening). The costal ribs of the first and second transverse costa ring overlap about one third of their length. Found in December 1999 at station Mendanha (23 °C; salinity 14). Number of organisms observed in the TEM: one.

12. *Stephanoeca apheles* Thomsen *et al*. 1991 (Figure 23)

Lorica ca. 14,1 µm long with anterior and posterior chamber separated by a waist-like constriction; the latter is smaller and contains the cell. The anterior chamber is expanded and contains the flagellum and collar. The lorica is composed of sixteen longitudinal costae, each costa composed of three costal strips, and



**Figures 12-19.** TEM 12) *Acanthocorbis* sp x2860. 13) *Calotheca alata* x3800. 14) *Campyloacantha spinifera* see L-shaped structures on the left side x4000. 15) *Cosmoeca norvegica* x2400. 16) *C. subulata* x2500. 17) *C.* aff. *ventricosa* x3000. 18) *Pleurasiga minima* x2500. 19) *Parvicorbicula circularis* x3600.

**Figuras 12-19.** MET 12) *Acanthocorbis* sp x2860. 13) *Calotheca alata* x3800. 14) *Campyloacantha spinifera*, observe as estruturas em forma de "L" no lado esquerdo x4000. 15) *Cosmoeca norvegica* x2400. 16) *C. subulata* x2500. 17) *C.* aff. *ventricosa* x3000. 18) *Pleurasiga minima* x2500. 19) *Parvicorbicula circularis* x3600.

two transverse costal rings. Found in July and December 1999 at the estuary main channel (14 °C; salinity 8) and station Mendanha (26 °C; salinity 32), respectively. Number of organisms observed in the TEM: three.

13. *Stephanoeca cupula* (Leadbeater 1972) Thomsen 1988 (Figure 24) (*Pleurasiga cupula* Ledbeater 1972)

The lorica consists of two chambers separated by a waist. The posterior chamber contains the protoplast, while the flagellum and the collar are located in the anterior chamber. The anterior chamber is lined by eight longitudinal costae (each composed of three costal strips), which cross at approximately right angles to the (at least) three transverse costal rings. The apical transverse costa is located at the anterior opening and the others at the junctions between longitudinal costae. The space between the third transverse costa and the waist may be partially filled with additional transverse strips. The two anterior transverse costal rings are located externally to the longitudinal costae, and the third is overlaid externally by longitudinal costae. The interconnections between anterior longitudinal and transverse costal strips are three-point joints. The tip of each longitudinal costal strip attaches to a transverse costal strip approximately midway along its length, and there is considerable overlapping between transverse costal strips. The posterior chamber is formed by converging longitudinal costal strips and several transverse costal strips, some of which are spiraled. Found in December 1999 at station Mendanha (23 °C; salinity 23). Number of organisms observed in the TEM: one.

14. *Stephanoeca diplocostata* Elis 1930 Ledbeater 1972 (Figure 25).

The lorica ca. 13,8 µm long and ca. 9 µm wide the anterior chamber. This species has a high number of longitudinal (fifteen) and transverse costae (seven). The posterior chamber is encircled by four transverse and the anterior chamber by three costae. The high number of costal strips gives the lorica a complex architecture, making counting of the costae difficult. Leadbeater (1972a) states that although the species appears somewhat disorganized in whole mounts, its general appearance, the distinction between anterior and posterior lorica chambers, the presence of thirteen to fifteen longitudinal costae inwardly curved at their anterior end, and the possession of a high number of transverse costae, are diagnostic features. Found in July 1999 at the estuary main channel (13.5 °C; salinity 32). Number of organisms observed in the TEM: one.

15. *Stephanoeca urnula* Thomsen 1973 (Figure 26)

The lorica ca. 10,5 µm long, consists of one small posterior and one large anterior chamber. The anterior chamber is of a highly complicated construction, with sixteen longitudinal costae, attached to the costal curved strips forming the proximal part of the lorica. Four transverse costal rings encircle the lorica at its maximum diameter. The transition region between the anterior and posterior chambers seems to have few transverse costae, making it difficult to count the number of oblique oriented costal strips (more than twenty). Found at stations Lagoa (July; 14 °C; salinity 14) and Mendanha (December 1999; 23 °C; salinity 14). Number of organisms observed in the TEM: two.

16. *Syndetophyllum pulchellum* Thomsen et Moestrup 1983 (Figure 27).

Cell ca. 3,6 µm long and 1,8 µm wide. The description of costal organization was impossible, as only one type of costae with broad strips, and heavily silicified midribs characteristic for this species, were observed. Found in February 2000 at surface water of the continental shelf (23 °C; salinity 36). Number of organisms observed in the TEM: one.

## Discussion

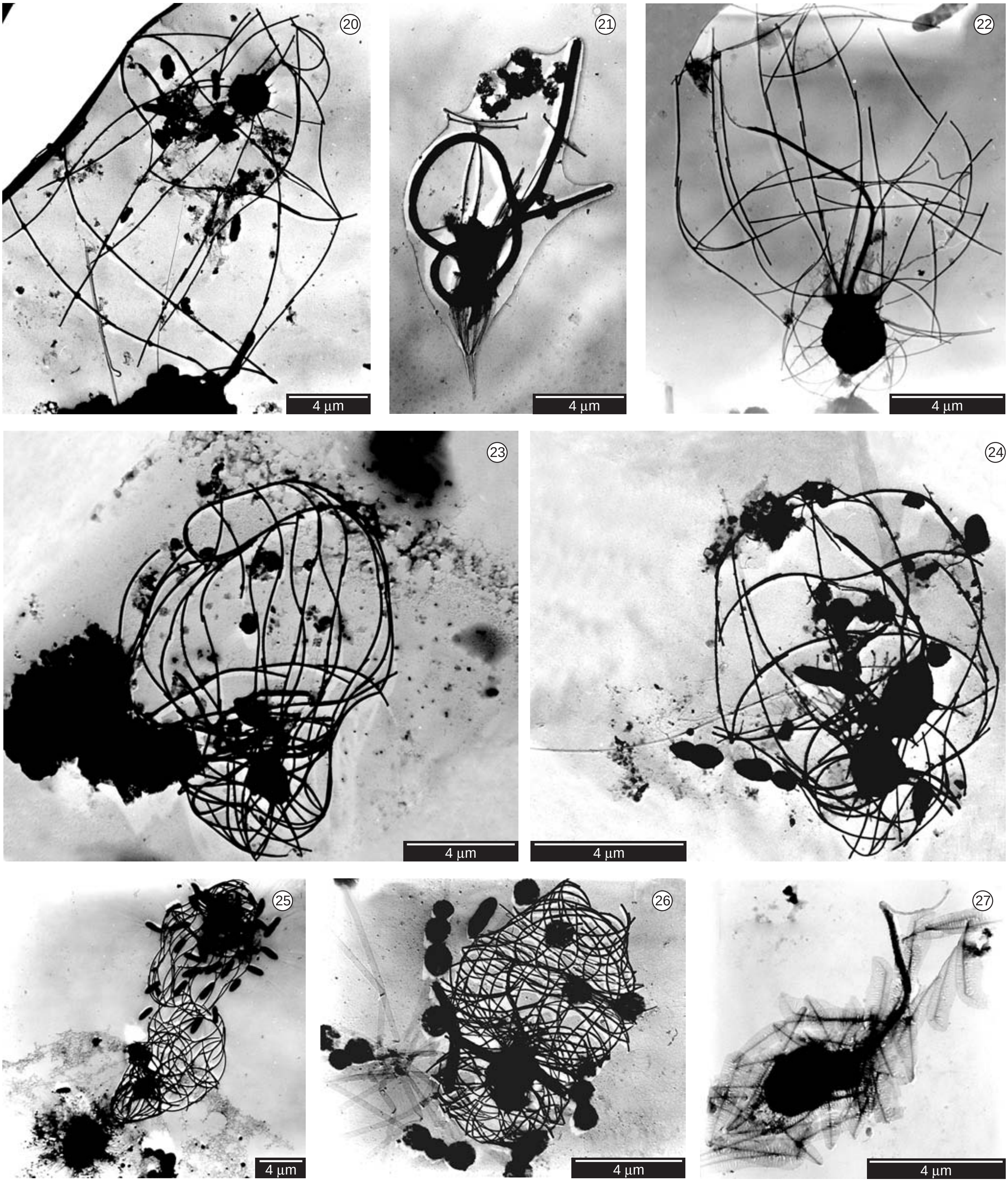
The nanoflagellates are abundant in the estuarine and coastal areas of southern Brazil (Odebrecht & Abreu 1997, Odebrecht & Garcia 1997, Bergesch & Odebrecht 2001), and comprise several taxonomic groups including haptophytes, prasinophytes, chrysophytes and pedinellids (Bergesch et al. 2008). The present article is the first report of loricate choanoflagellates in the area.

Choanoflagellates were recorded in mixopolyhaline and euhaline waters during the spring and summer both at the continental shelf, in the Patos Lagoon estuary main channel and at the Mendanha embayment; a few were recorded in the winter. During summer, mixopolyhaline and euhaline waters are frequent in the estuary as a result of reduced continental freshwater discharge and the action of southerly winds forcing the coastal water through the main channel to reach the inner lagoon (Möller Jr. et al. 1996, Odebrecht et al. 2005). During summer, the coastal shelf is influenced by tropical water from the Brazil Current, with warm temperature (>20 °C), reduced nutrients and suspended matter, while in spring it is influenced by nutrient-rich cold water flowing northwards (Ciotii et al. 1995), and phytoplankton diatom blooms are then commonly observed in the Patos Lagoon estuary (Odebrecht & Abreu 1997). These conditions also seem to be favourable for the growth of choanoflagellates.

In contrast, we did not observe choanoflagellates at the inshore stations Lagoon, Laranjal and Museum, and at Cassino Beach. Lagoon and Laranjal are mostly oligohaline to limnic (Bergesch 2003, Odebrecht et al. 2005). As members of the Acanthoecidae are exclusively marine and brackish organisms (Thomsen & Buck 1991), the absence in these stations can be explained by unfavorable low salinity water. The station Museum, although closer to the main channel, is located in the western area of the estuary where the marine influence is generally less than in the eastern side. This is reflected in zooplankton species distribution, which is significantly different in the eastern and western sides of the estuary depending on the marine influence (Montu et al. 1997). Also, the freshwater non-loricate colonial choanoflagellate *Kentrosiga* sp. was previously recorded in the Museum station, as an important picoplankton consumer (Hickenbick 2002). The absence of choanoflagellates at the beach station can be related to the high energy and turbulence from the action of waves in the surf-zone, probably destroying or dispersing the delicate cells.

Most choanoflagellates found in our study present geographic distribution (Table 3). *Pleurasiga minima* is the most frequently reported species, occurring from the extreme environments in the Arctic and Antarctic to tropical waters. In our study it was found in spring and summer, in surface and deeper waters (30-75 m) of the continental shelf. *Cosmoeca norvegica*, *C. ventricosa*, *Parvicorbicula circularis* have been reported in many parts of the world and can also be expected to have a cosmopolitan distribution; they were present in spring or summer in the southern Brazilian coastal areas. *Polyfibula elatensis* observed by us in summer (23 °C) has been reported from climatic extremes, the North Alaska and Galapagos Islands. Manton & Bremer (1981) suggested that it temporarily enters the Arctic Sea via Bering Strait, but it generally prefers warm water, as the presence in the Red Sea initially suggested. In contrast, *Stephanoeca diplocostata* apparently prefers lower temperature as it is mainly reported at high latitudes like Greenland, Norway and the Antarctic (Thomsen 1982, Leadbeater 1972a, Marchant 2005); at lower latitude (36 °N, California; Thomsen et al. 1991) the temperature was low when it occurred (13 °C). In this study it was observed in the estuary main channel in winter (13.5 °C). Other species like *Calotheca alata* and *Campyloacantha spinifera* are largely temperate (Thomsen et al. 1991) and were observed in spring (13-22 °C).

The new species *Diplotheca tricyclica* of the previous monotypic genus *Diplotheca* was observed in polyhaline waters at the estuary main channel in the summer 1999 (salinity 33.5; 24 °C). At this time, the phytoplankton was composed mainly of marine diatoms reinforcing the importance of the advection of marine water for the local distribution of choanoflagellates in the lagoon.



**Figures 20- 27.** TEM 20) *Polyfibula elatensis* x2100. 21) *Stephanacanta dichotoma* x4000. 22) *Stephanoeca* sp. x2500. 23) *S. apheles* x4600. 24) *S. cupula* x4600. 25) *S. diplocostata* x1900. 26) *S. urnula* x3900. 27) *Syndetophhyllum pulchellum* x6000.

**Figuras 20- 27.** MET 20) *Polyfibula elatensis* x2100. 21) *Stephanacanta dichotoma* x4000. 22) *Stephanoeca* sp. x2500. 23) *S. apheles* x4600. 24) *S. cupula* x4600. 25) *S. diplocostata* x1900. 26) *S. urnula* x3900. 27) *Syndetophhyllum pulchellum* x6000.

**Table 3.** Geographic distribution of choanoflagellate species reported in the southern Brazilian coastal waters.

**Tabela 3.** Distribuição geográfica das espécies de coanoflagelados registradas nas águas costeiras do sul do Brasil.

| **Species** | **Artic** | **Greenland** | **Finland** | **Norway** | **Denmark** | **Alaska** | **USA-California** | **Galapagos** | **New Zealand** | **Antarctica** | **Thailand** | **Red Sea** | **Mediterraneum** | **Adriátic** | **Equatorial Pacific** | **Sargasso Sea** | **Egipt** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Acanthocorbis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Calotheca alata* | - | - | - | - | - | - | - | - | 14 | - | 14 | 12 | - | - | - | - | - |
| *Campyloacantha spinifera* | - | - | - | - | - | - | 19 | - | - | - | - | - | - | 5 | 25 | - | - |
| *Cosmoeca norvegica* | - | 20 | - | 2 | 16 | - | - | - | 9 | 8, 18 | 16 | - | - | - | 25 | 16 | 16 |
| *C. subulata* | - | - | - | - | - | - | - | - | - | - | 16 | - | - | - | - | - | - |
| *C.* aff. *ventricosa* | - | 20 | 16 | 2 | 16 | - | 19 | - | - | 8, 18 | 16 | 2 | 5 | - | 25 | - | 16*$^{1}$ |
| *Diplotheca tricyclica* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Parvicorbicula circularis* | - | 20 | - | - | 11 | - | 19 | - | - | 1, 8, 18 | - | - | - | - | 25 | - | - |
| *Pleurasiga minima* | 22 | 20, 14 | - | 2, 23, 24 | 10 | - | 19 | - | - | 1, 8, 18 | - | - | - | 5 | 25 | - | - |
| *Polyfibula elatensis* | - | - | - | - | - | 7 | 19 | 7 | - | - | - | 7 | - | - | - | - | - |
| *Stephanacantha dichotoma* | - | - | - | - | - | - | - | - | - | - | 15 | - | - | - | 25 | 15 | - |
| *Stephanoeca apheles* | - | - | - | - | - | - | 19 | - | - | - | - | - | - | - | - | - | - |
| *S. cupula* | - | 20 | - | - | 17 | - | - | - | - | 8 | - | - | - | - | - | - | - |
| *S. diplocostata* | - | 13, 20 | - | 3, 21 | - | - | 19 | - | - | 8, 20 | - | - | - | 5 | - | - | - |
| *S. urnula* | - | - | - | - | 10 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Stephanoeca* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Syndetophyllum pulchellum* | - | - | - | - | - | - | - | - | 9, 14 | - | 14 | 12 | 6 | - | - | - | - |

References: 1) Buck& Garrison (1983); 2) Espeland & Throndsen (1986); 3) Leadbeater (1972a); 4) Leadbeater (1972b); 5) Leadbeater (1973); 6) Leadbeater, (1974); 7) Thomsen, 1978 in Manton & Bremer (1981); 8) Marchant (2005); 9) Moestrup (1979); 10) Thomsen. (1973); 11) Thomsen (1976); 12) Thomsen, 1978 in Espeland & Throndsen (1986); 13) Thomsen (1982); 14) Thomsen & Moestrup (1983); 15) Thomsen & Boonruang (1983b); 16) Thomsen, &. Boonruang, (1984); 17) Thomsen (1988); 18) Thomsen et al. (1990); 19) Thomsen et al. (1991); 20) Thomsen et al. (1995); 21) Thomsen et al. (1997); 22- 23) Throndsen (1970); 24) Throndsen (1974); 25) Vørs et al. (1995). *$^{1}$ Thomsen & Pedersen unpublished in Thomsen & Boonruang (1984).

It is worthwhile noting that loricate choanoflagellates were not observed during phytoplankton counting with the inverted microscope (magnification 400x) in lugol-fixed samples taken as duplicates samples of the present study (Bergesch 2003). The main difficulties are probably related to their delicate lorica structure and low sinking rate, which render these forms easy to overlook during routine observations of preserved material (Throndsen 1997). In contrast, Thomsen & Larsen (1992) mention that collar flagellates were easily observed in settled water samples from the Antarctic examined under the inverted microscope and on filters prepared for epifluorescence microscopy. The relatively high amount of detritus in the estuarine and coastal waters certainly hampered their detection in our samples, but the kind of fixative also plays a role. Better results are obtained using glutaraldehyde solely (Leakey et al. 2002) or glutaraldehyde combined with Lugol's solution (Jensen 1998), the last as the most adequate for preserving the cell appendages.

Thomsen (1982) mentions that the most relevant method to study choanoflagellates is in dry mounts. To roughly estimate the number of choanoflagellates in samples, Thomsen & Larsen (1992) suggest to use phase or Nomarski optics and "air-mounted" whole cell preparations, and to calibrate the technique with epifluorescence microscopy. Thus, the abundance of choanoflagellates should be estimated using the light microscope whole dry mounts technique combined with epifluorescence microscopy in order to assess their contribution to the microbial loop in the southern Brazilian coastal waters.

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