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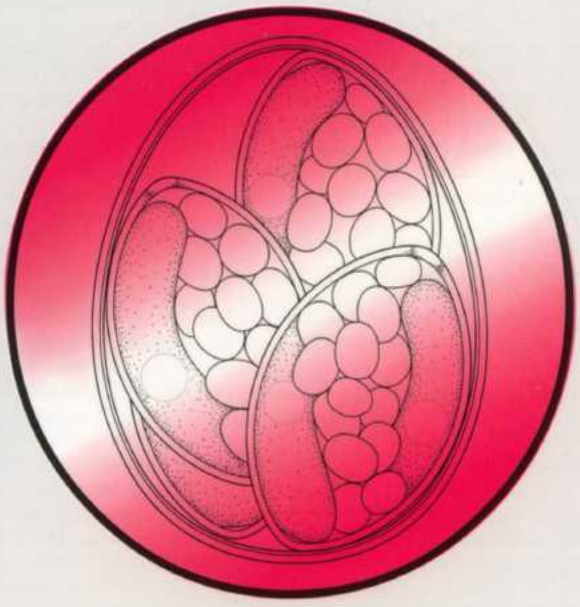

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A Unified Organization of the Stichotrichine Oral Apparatus, Including a Description of the Buccal Seal (Ciliophora: Spirotrichea)

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Summary. We investigated the oral apparatus of several stichotrichine spirotrichs, such as *Stylonychia*, *Saudithrix*, and *Holosticha*. Scanning electron microscopy reveals the oral opening into the buccal cavity covered by a membranous sheet, the "buccal seal", which is very fragile and thus probably restored after each feeding process. Depending on the depth of the buccal cavity, there is an upper or an upper and a lower seal, for example, in *Cyrtohymena* and *Saudithrix*, where the buccal cavity extends near to the dorsal side of the cell. Scanning electron microscopy further reveals special cilia at the right end of the ventral membranelles. These "lateral membranellar cilia" originate mainly from the short fourth row at the anterior side of each membranelle. The lateral membranellar cilia, which are usually covered by the (upper) buccal seal, may be numerous and long (*Cyrtohymena*) or sparse and short (*Holosticha*). Live observations reveal that they are involved in feeding, while the long paroral and endoral cilia remain almost motionless. Based on these and other new observations, especially on the buccal lip and the membranellar bolsters, we propose an improved model for the organization of the stichotrichine oral apparatus. The distribution of buccal seal-like structures throughout the ciliate phylum, the nature and possible functions of the buccal seal and the lateral membranellar cilia, and the alpha-taxonomic significance of the new features are discussed.

Key words: buccal cavity, buccal lip, feeding, hypotrichs, lateral membranellar cilia, membranellar bolsters, SEM.

INTRODUCTION

Stichotrichine spirotrichs are characterized by two features: the cilia are bundled to form cirri and the oral apparatus, which occupies the left anterior quadrant of the cell, possesses an "adoral zone of membranelles" composed of up to two hundred ciliary plates, the adoral membranelles. Right of the membranellar zone is the buccal cavity, at or near to the right margin of which extend the undulating membranes, that is, the paroral and endoral formation (Corliss 1979, Berger 1999).

The first detailed study, still of use today, on the stichotrichine oral apparatus was performed by Sterki (1878). Later, Kahl (1932) and Foissner (1989) used details of the buccal cavity and the arrangement and structure of the undulating membranes to distinguish genera and species. Although this is acknowledged today (Berger 1999), it hardly contributed to a deeper understanding of the structure and function of the stichotrichine oral apparatus. This changed with the fundamental study of Machemer and Deitmer (1987),

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who used *Stylonychia mytilus*, a typical stichotrich, as a model organism for studies on feeding and ciliary motor functions. They analyzed and interpreted the data in terms of the incomplete morphological knowledge available at that time.

During the past 15 years, we have recognized a considerable diversity of the stichotrichine oral apparatus *in vivo* and in the scanning electron microscope (Foissner *et al*. 1991, 1999, 2002). However, most fine structural data remained unpublished because they were only marginally related to the identification and description of species. With the present paper, this gap will be closed, using outstanding scanning electron micrographs, most not published before. However, we cannot show all of our materials, based on over 40 species, because this would surpass the space available in an international journal. Thus, we selected a few representative genera and refer to our previous publications for many others (Foissner *et al.* 1991, 1999, 2002).

MATERIALS, METHODS AND TERMINOLOGY

Material

The species listed below were isolated from limnetic, marine, and soil samples. With the exception of *Saudithrix terricola*, all were cultivated in Eau de Volvic (French table water) or artificial sea water enriched with some squashed wheat grains to stimulate growth of food organisms, viz., bacteria, heterotrophic flagellates, and small ciliates for rapacious species like *Australocirrus* and *Cyrtohymena*. *Saudithrix terricola* did not grow in pure cultures, but became rather numerous in the non-flooded Petri dish raw culture (NFP), where it was discovered. See Foissner *et al*. (2002) for a detailed description of the NFP method, and Berger (1999) for literature and descriptions of most species mentioned below and in the text. All identifications were checked in protargol preparations (Foissner 1991).

Australocirrus oscitans Blatterer and Foissner was found in a NFP culture of mud and soil from a granitic rock-pool on the top of the Table Mountain, Republic of South Africa.

Cyrtohymena candens (Kahl) was found in a NFP culture of bark from a large tree in the fog rainforest of the Henry Pittier National Park on the north coast of Venezuela, South America.

Pleurotricha lanceolata (Ehrenberg) and *Stylonychia mytilus* (Müller) are from the USA, Colorado. Cultures were sent by Prof. Prescott for identification. Site details are not known.

Steinia platystoma (Ehrenberg) was collected from a small pond in the surroundings of Salzburg City, Austria. Note that the endoral membrane of this species is not fragmented. Thus, *S. sphagnicola* Foissner belongs to another, new genus.

Saudithrix terricola, a new genus and species submitted for publication, was discovered in soil of a vegetable field about 20 km north of Riyadh, Saudi Arabia.

Hemiamphisiella wilberti (Foissner) became numerous in a NFP culture of grassland soil from the surroundings of the town of Kefermarkt in Upper Austria.

Holosticha sp., a new, not yet described species was discovered in a brackish pond on the coast of the Saudi Arabian Gulf.

Pseudokeronopsis rubra (Ehrenberg) is a gift from Prof. Dr. A. Schmid (Salzburg University), who collected it on the coast of the Red Sea.

Methods

Live observations were performed with bright field and interference contrast, using a high power oil immersion objective. Protargol impregnation and scanning electron microscopy (SEM) were performed as described in Foissner (1991), with some variation, that is, SEM-stubs covered with graphite-tabs (Gröpl Company, Frauenhofnerstrasse 40, A-3430 Tulln, Austria; order no. G 3347 or G 3348, i.e., tabs with a diameter of 12 or 25 mm) were used to attach the specimens and to obtain a homogenous background. Preservation of stichotrichs is very difficult. Often the cirri spread in their component cilia, and then they look like minute brushes and/or the cortex is strongly wrinkled. Thus, various fixatives were tried. Good fixation is usually obtained when cells are fixed for 30 min in a mixture of 4 ml saturated, aqueous mercuric chloride ($HgCl_2$) and 1 ml aqueous 2 % osmium tetroxide (OsO_4). Some excellent preparations were obtained with the fixative used by Wicklow (1981), that is, a 1:1 mixture of 2% OsO_4 and 3% glutaraldehyde for 30 min.

Terminology

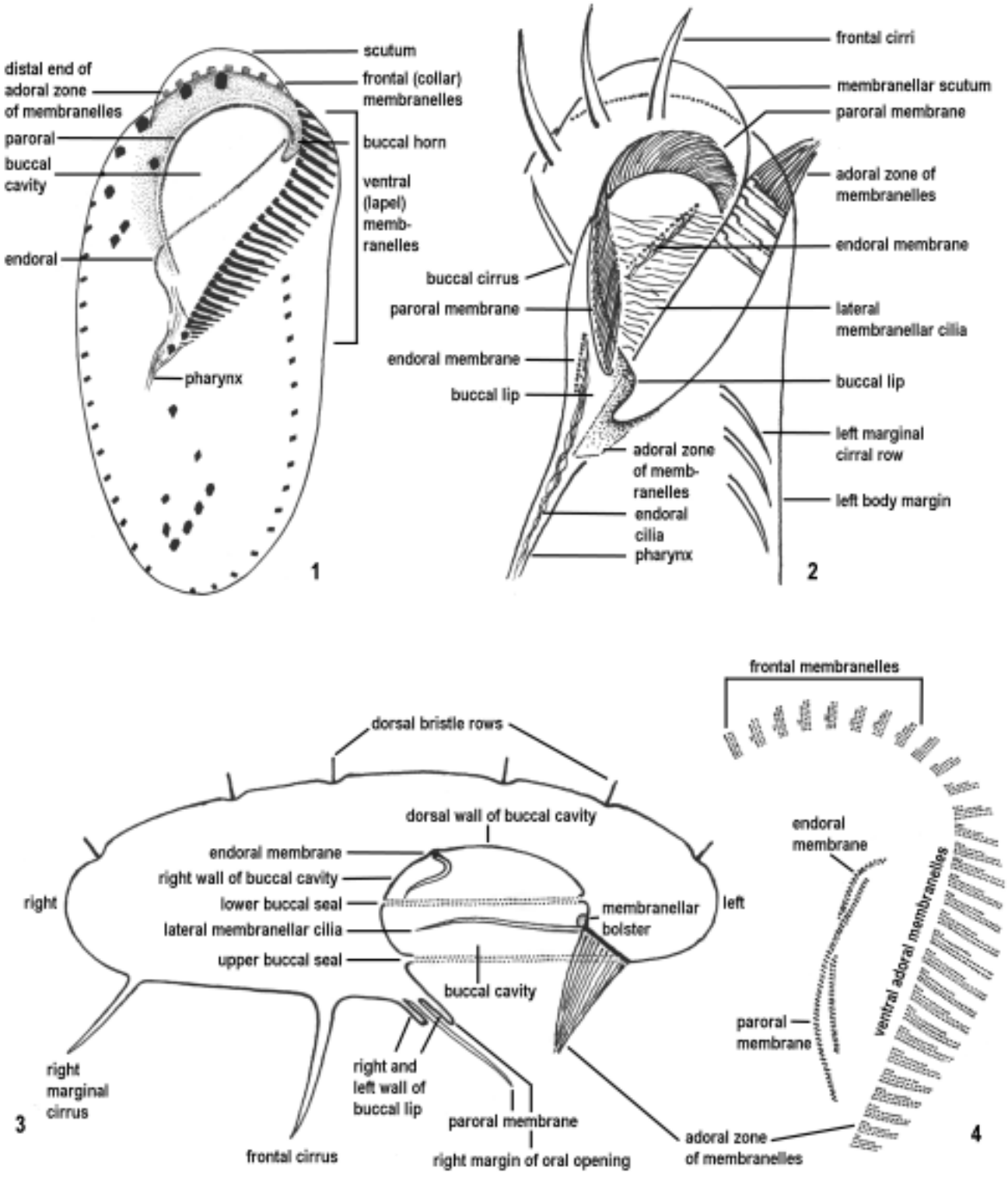
See Figures 1-8 for the terms used. They are based on Corliss (1979) and Berger (1999). Some new terms will be explained in the appropriate sections.

RESULTS

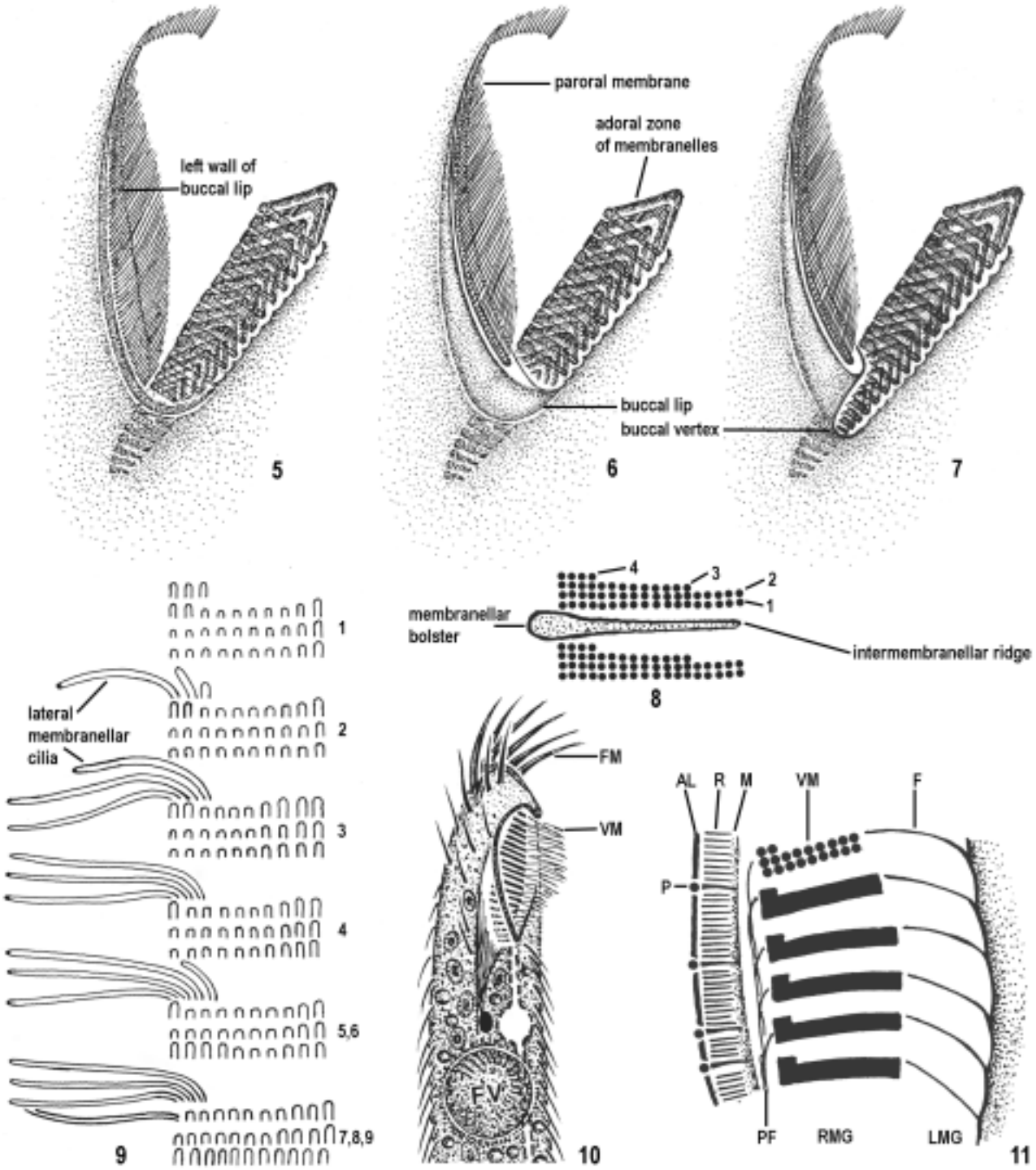
A revised general organization and terminology of the stichotrichine oral apparatus

Terminology of the stichotrichine oral apparatus has been reviewed and clarified by Berger (1999) who, however, did not provide comprehensive schemes and did not know of the new structures described below.

Thus, we provide updated and synoptical schemes of the stichotrichine oral apparatus (Figs 1-8). These schemes, which are based on the fundamental, but almost forgotten paper by Sterki (1878), show the main components of the oral apparatus, that is, the "adoral membranelles" which are composed of the "membranellar" and "lateral membranellar cilia", both distinguished already by Sterki (1878); the "adoral zone of membranelles" consisting of "frontal" and "ventral membranelles", differing in the arrangement and length of the basal body rows; the "membranellar bolsters" at



Figs 1- 4. A unified morphology and terminology of the stichotrichine oral apparatus. The schemes are based on Sterki (1878) and are updated with Berger (1999) and the new observations reported in the present study. For general organization and designation of cirri, see Berger (1999). **1** - ventral view of *Cyrtohymena candens*, a large-mouthed, about 120 µm long stichotrich with a huge paroral membrane very conspicuous in the scanning electron microscope (Figs 12, 13); **2, 3** - schemes of the oral apparatus in ventral and transverse view; **4** - details of the adoral zone of membranelles of *Sterkiella histriomuscorum* (from Augustin and Foissner 1992). Depending on the structure of the membranelles, the zone is divided in a frontal and ventral portion.



Figs 5-11. Details from the oral apparatus of stichotrichine spirotrichs. **5-7** - three main types (flat, curved, angular) of buccal lip occur; **8** - scheme of a typical adoral membranelle composed of four rows of ciliated basal bodies and the intermembranellar ridge; **9** - this scheme shows the very different length of the cilia at the right margin of ventral adoral membranelles 1-9 in *Cyrtohymena candens* (redrawn from SEM micrographs). Note that cilia of membranellar rows 1-3 have double length when compared with the lateral membranellar cilia, that is, they were shortened for the sake of clarity; **10, 11** - the oral apparatus of *Etoschothrix terricola* deviates considerably from the general pattern: frontal and ventral adoral membranelles are widely separated, the adoral membranelles consist of only three (vs. four, Fig. 8) rows of basal bodies, the paroral membrane consists of few, very widely spaced cilia, and the endoral membrane is likely lacking (from Foissner *et al*. 2002). AL - argyrophilic line (endoral?), F - fibres, FM - frontal membranelles, FV - food vacuole, LMG - left half of membranellar groove, M - right margin of buccal opening, P - paroral membrane, PF - pharyngeal fibres or lateral membranellar cilia, R - rods, RMG - right half of membranellar groove, VM - ventral membranelles.

the proximal end of the "intermembranellar ridges"; the "paroral membrane" which usually inserts in a cleft of the "buccal lip"; and the "endoral membrane" on the dorsal wall (bottom) of the "buccal cavity" which is usually covered by a membranous structure, the "buccal seal".

The buccal cavity and the buccal seal

The buccal cavity is right to the adoral zone of membranelles and has, if it is deep and wide as in *Cyrtohymena* (Figs 12, 13), the shape of an elliptical bowl or of a groove, if it is shallow and narrow as in *Holosticha* (Figs 32, 35). Basically, the buccal cavity can be wide and shallow (*Stylonychia*, Figs 17, 18) or wide and deep extending near to the dorsal surface of the cell (*Cyrtohymena*, Figs 12, 13); narrow and shallow (*Holosticha*, Figs 32, 35) or rather narrow and deep (= ordinary; *Pleurotricha lanceolata*, Figs 19-21); and short (*Eschaneustyla lugeri*, Foissner *et al*. 2002) or long relative to the length of the oral apparatus (*Hypotrichidium conicum*, Foissner *et al*. 1999; *Cyrtohymena candens*, Figs 1, 12).

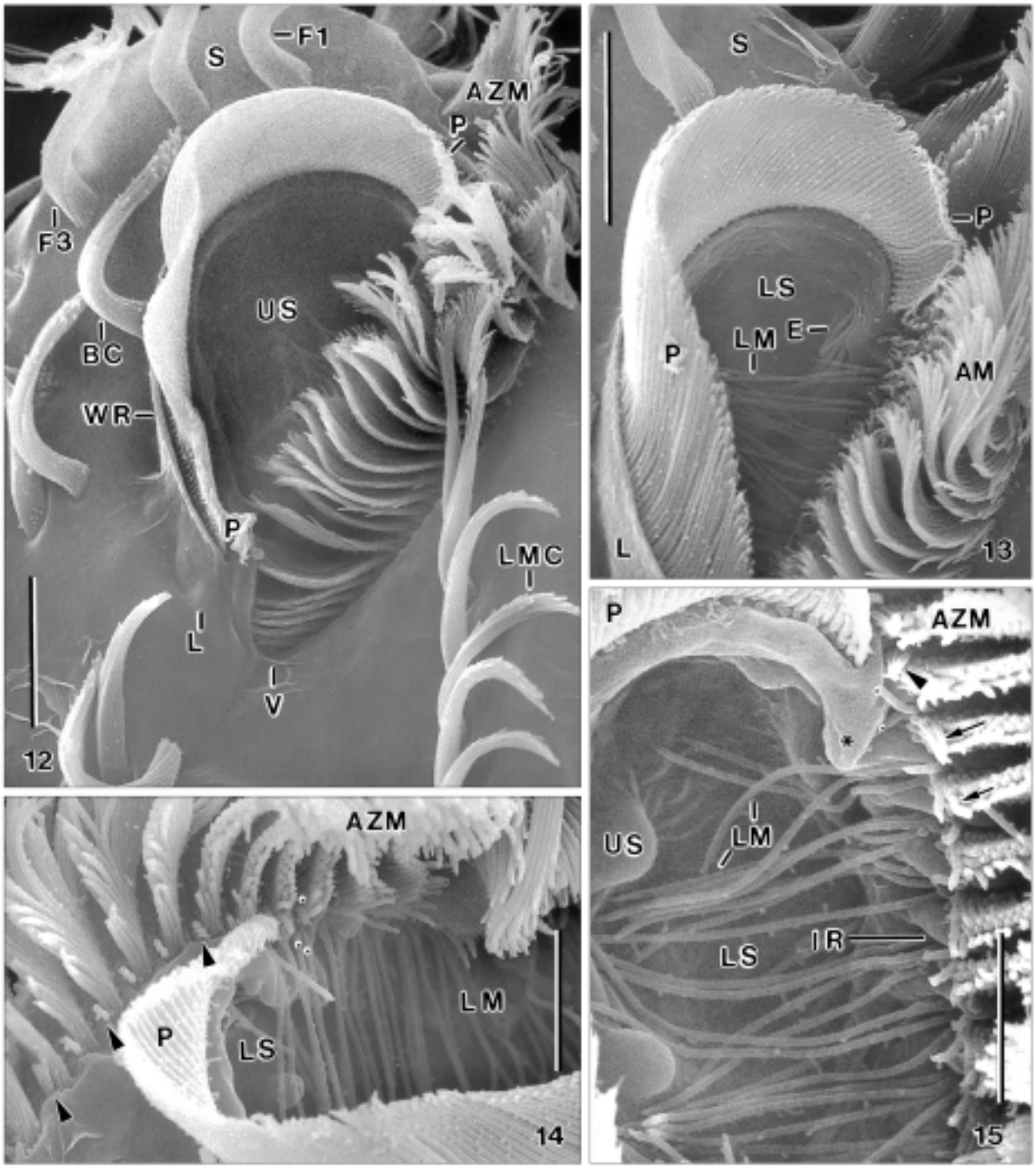
It was a great surprise when we recognized that the buccal cavity and thus the oral opening was covered by a membranous sheet in excellently prepared specimens (Figs 12, 13, 28, 29). Further investigations and a re-evaluation of the literature showed this structure, which we call "buccal seal" (denotes both, viz., that it covers the oral opening and is difficult to recognize), throughout the great diversity of stichotrichine spirotrichs. Here, we demonstrate it in *Cyrtohymena candens* (Figs 12, 13, 15), *Steinia platystoma* (Fig. 24), *Stylonychia mytilus* (Figs 17, 18), *Pleurotricha lanceolata* (Figs 19-21), *Australothrix oscitans* (Figs 25, 26), *Saudithrix terricola* (Figs 28-30), *Hemiamphisiella wilberti* (Figs 38-40), *Holosticha* sp. (Figs 32, 35), and *Pseudokeronopsis rubra* (Fig. 34); in the literature, the buccal seal and/or its remnants can be seen in *Gastrostyla steinii* (Foissner *et al*. 2002), *Steinia sphagnicola* (Voss and Foissner 1996), *Hypotrichidium conicum* (Foissner *et al*. 1999), *Laurentiella strenua* (Berger 1999), *Engelmanniella mobilis* (Wimsberger-Aescht *et al*. 1989), *Urostyla grandis* and *Holosticha multistilata* (Foissner *et al*. 1991); and a seal is present in our unpublished material from *Kahliella bacillifera*, *Pattersoniella vitiphila*, *Oxytricha gigantea*, and *Pseudourostyla cristata*.

The buccal seal is not recognizable *in vivo* and protargol preparations, and usually it is lost or only partially preserved in specimens prepared for scanning electron microscopy; indeed, the micrographs shown in this paper are a selection from over hundred preparations made during the past 15 years. Partially destroyed and well preserved cells show the buccal seal as a sheet-like structure covering the entire oral opening and, in most species, also the lateral membranellar cilia and the proximal quarter of the adoral membranelles. The best preparations reveal that the buccal seal is contiguous with the cell membrane of the frontal cortex and the left wall of the buccal lip (Figs 12, 13, 17, 19, 26, 29, 35, 39).

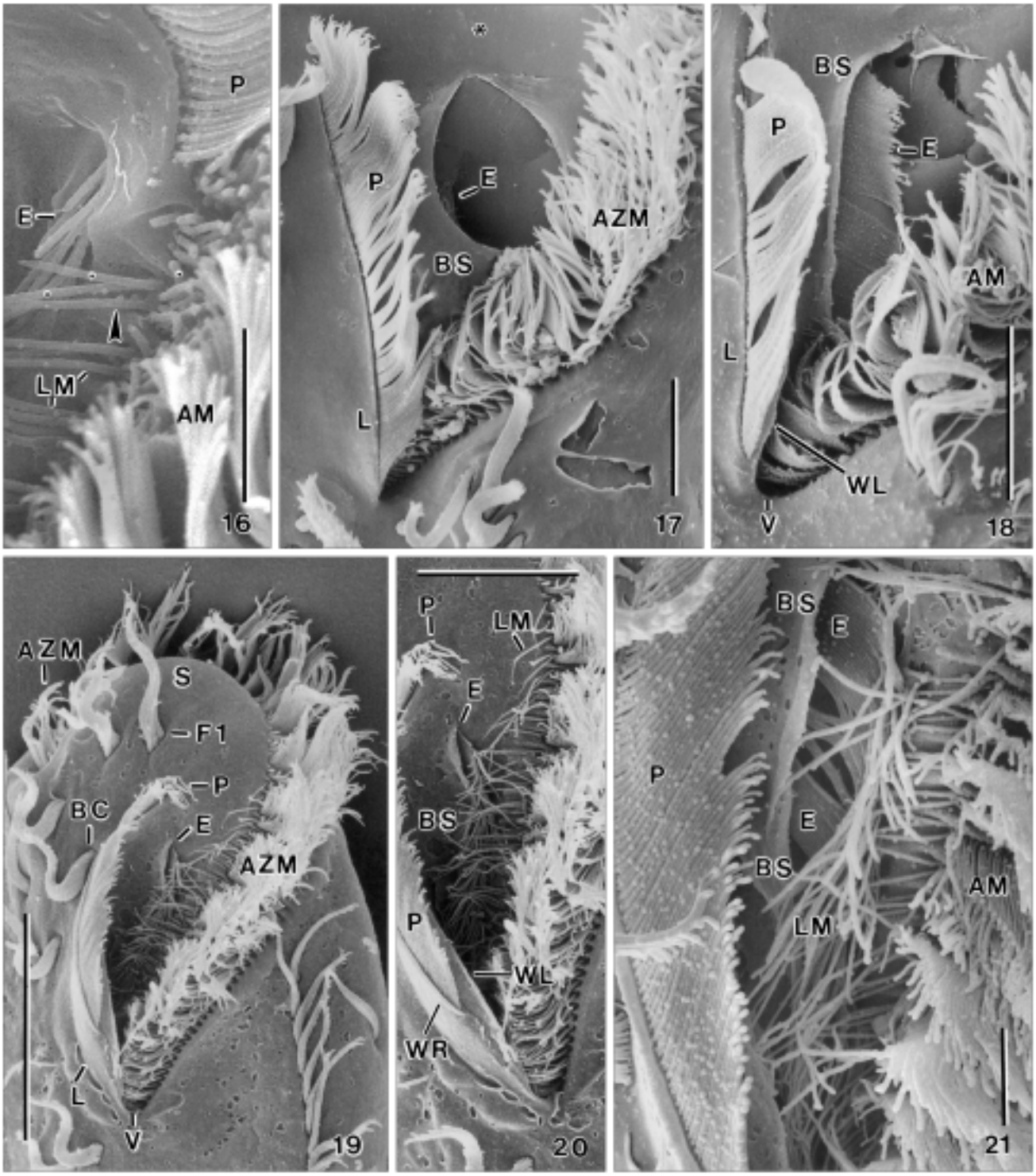
The detailed analysis of the species mentioned above revealed three modes of buccal seal position. In the first mode, the seal covers not only the oral opening but also the lateral membranellar cilia and the proximal portion of the adoral membranelles. Thus, the buccal area is very smooth and devoid of cilia. This pattern is most common and found, for example, in *Stylonychia mytilus* (Figs 17, 18), *Laurentiella strenua* (Berger 1999), *Australocirrus oscitans* (Figs 25, 26), *Pseudokeronopsis rubra* (Fig. 34), and *Holosticha* sp. (Fig. 35). The second mode, we observed as yet only in *Pleurotricha lanceolata* (Figs 19-21). It is similar to the first mode, but the seal extends at a slightly deeper level, exposing the lateral membranellar cilia and the bases of the adoral membranelles.

The third and most complex mode is found in species with a deep buccal cavity, e.g., *Cyrtohymena candens* (Figs 12-16) and *Saudithrix terricola* (Figs 28-30). In these species, there is an upper seal covering the oral opening and the lateral cilia, while the endoral membrane is covered by a second, lower seal, which is not visible unless the upper seal is destroyed. The lower seal is apparently more stable than the upper one because it is usually well preserved, exposing only part of the endoral and only the tip of its cilia (Figs 13, 16, 28-30). Further, the lower seal causes the buccal cavity to appear comparatively flat, although it extends near to the dorsal surface of the cell.

Series of SEM micrographs show various stages of seal destruction due to insufficient preservation in *Cyrtohymena candens* and *Saudithrix terricola*. Both seals commence to break along the border of the adoral zone of membranelles and the buccal cavity. Thus, the lateral membranellar cilia and the anterior portion of the endoral membrane become first exposed (Figs 13, 15, 16, 19, 20, 25, 26). Further destruction causes the seals to become withdrawn to or above the midline of the buccal cavity, where the broken lower seal often forms a minute wall on the endoral membrane (Figs 17-21, 29, 30). If the upper seal is completely destroyed,



Figs 12-15. *Cyrtohymena candens*, oral structures in the scanning electron microscope (see also figures 16, 31). **12, 13** - these overviews show conspicuousness and complexity of the oral apparatus, especially of the paroral membrane (P) and the buccal seals (US, LS). The specimen shown in (12) has preserved the upper seal, and thus the huge buccal cavity and the long lateral membranellar cilia are not recognizable. The specimen shown in (13) and, at higher magnification, in (16) lost the upper buccal seal, exposing the lower seal (LS), the lateral membranellar cilia (LM), and the tips of some endoral cilia (E); **14, 15** - these specimens lost the upper buccal seal due to the preparation procedures, exposing the lateral membranellar cilia, which originate mainly from row 4 of the adoral membranelles: triangles mark ordinary rows 4 distal to the buccal cavity, while the row 4 cilia gradually lengthen (dots) and thus become "lateral membranellar cilia" when the adoral zone enters the buccal area (dots). The lateral membranellar cilia usually extend across the buccal cavity. Note also the highly different length of the cilia of the adoral membranelles (15, arrows) and the buccal horn (asterisk), which is hardly recognizable when the upper buccal seal is preserved (12, 13). AM - adoral membranelles, AZM - adoral zone of membranelles, BC - buccal cirrus, E - endoral (membrane), F1, 3 - frontal cirri, IR - intermembranellar ridge, L - buccal lip, LM - lateral membranellar cilia, LMC - left row of marginal cirri, LS - lower buccal seal, P - paroral (membrane), S - frontal scutum, US - upper seal, V - buccal vertex, WR - right wall of buccal lip. Scale bars: 5 µm (14, 15) and 10 µm (12, 13).



Figs 16-21. *Cyrtohymena candens* (16), *Stylonychia mytilus* (17, 18), and *Pleurotricha lanceolata* (19-21) in the SEM. **16** (overview, see figure 13) - three lateral membranellar cilia (dots), which gradually lengthen, originate from row 4 of the adoral membranelles, while one cilium originates from membranellar row 3 (arrowhead); **17, 18** - only when the buccal seal, which is contiguous with the frontal pellicle (asterisk), is destroyed, the endoral membrane becomes visible; **19-21** - in *Pleurotricha*, the lateral membranellar cilia, which occur only along the buccal cavity, are not covered by the buccal seal (19, 20). The endoral membrane becomes distinct only when the buccal seal is destroyed (21). AM - adoral membranelles, AMZ - adoral zone of membranelles, BC - buccal cirrus, BS - buccal seal, E - endoral, F1 - frontal cirrus, L - buccal lip, LM - lateral membranellar cilia, P - paroral, S - frontal scutum, V - buccal vertex, WL, WR - left and right wall of buccal lip. Scale bars: 5 µm (16, 21), 20 µm (17, 18, 20), and 40 µm (19).

remnants may adhere to the right and upper edge of the buccal cavity (Figs 14, 15). We did not find a specimen in which the lower seal was completely destroyed, emphasizing its stability (see above).

The membranellar bolsters and the lateral membranellar cilia

The individual membranelles of the adoral zone are usually composed of four ciliary rows of different length (Figs 4, 8, 22). These rows have cilia of different length, producing a dome-shaped membranelle or a rectangular plate if the cilia are of similar length, as is usually the case with those of the frontal membranelles (see also below). The membranelles are separated by cortical ridges often increasing in height towards the buccal cavity. The buccal end of the ridges projects slightly to distinctly over the end of the membranelles and is more or less inflated, for instance, in *Cyrtohymena candens* (Fig. 15) and various amphisiellids (Figs 38-40). These inflations, which we term "membranellar bolsters", can form conspicuous arrays (Figs 38-40) and contain protargol-affine granules, possibly some sort of mucocysts (Fig. 41).

At the right margin of each ventral membranelle, there are one or several cilia which beat independently and, in *Cyrtohymena candens*, extend across the buccal cavity when not beating. We term these units "lateral membranellar cilia" or, briefly, "lateral cilia". The lateral cilia, which were named "paroral cilia" by Sterki (1878), can be seen *in vivo* and in appropriate protargol and SEM preparations (Figs 2, 3, 9, 13-15, 19-21, 33-36, 39-41). The lateral membranellar cilia are of very different length and number in various species. Further, lateral cilia occur mainly in that region of the adoral zone which faces the margin of the buccal cavity (for details, see *Cyrtohymena* below). In *Holosticha* sp., where there is only one lateral cilium per membranelle and the length of the cilia gradually increases from distal to proximal, the area with lateral cilia is slightly longer than the buccal cavity, that is, commences near the mid of the adoral zone (Figs 32, 33, 35, 36).

In *Cyrtohymena candens*, we could analyze in detail the origin and distribution of the lateral membranellar cilia, except of those obscured in the proximal fifth of the adoral zone (Figs 9, 13-16). The first membranelle with lateral cilia is opposite to the distal end of the buccal cavity; often, this membranelle has only one or two lateral (= elongated) cilia. Then follow about three membranelles with three lateral cilia each, which usually originate from the three basal bodies comprising membranellar row 4. After that follow several membranelles with four lateral cilia, three of which originate from membranellar row 4 and one from row 3. The membranellar cilia of rows 1-3 have also many length specializations (Figs 9, 14-16). However, there is considerable variability in this respect, and thus the pattern shown in figure 9 can serve only as an example.

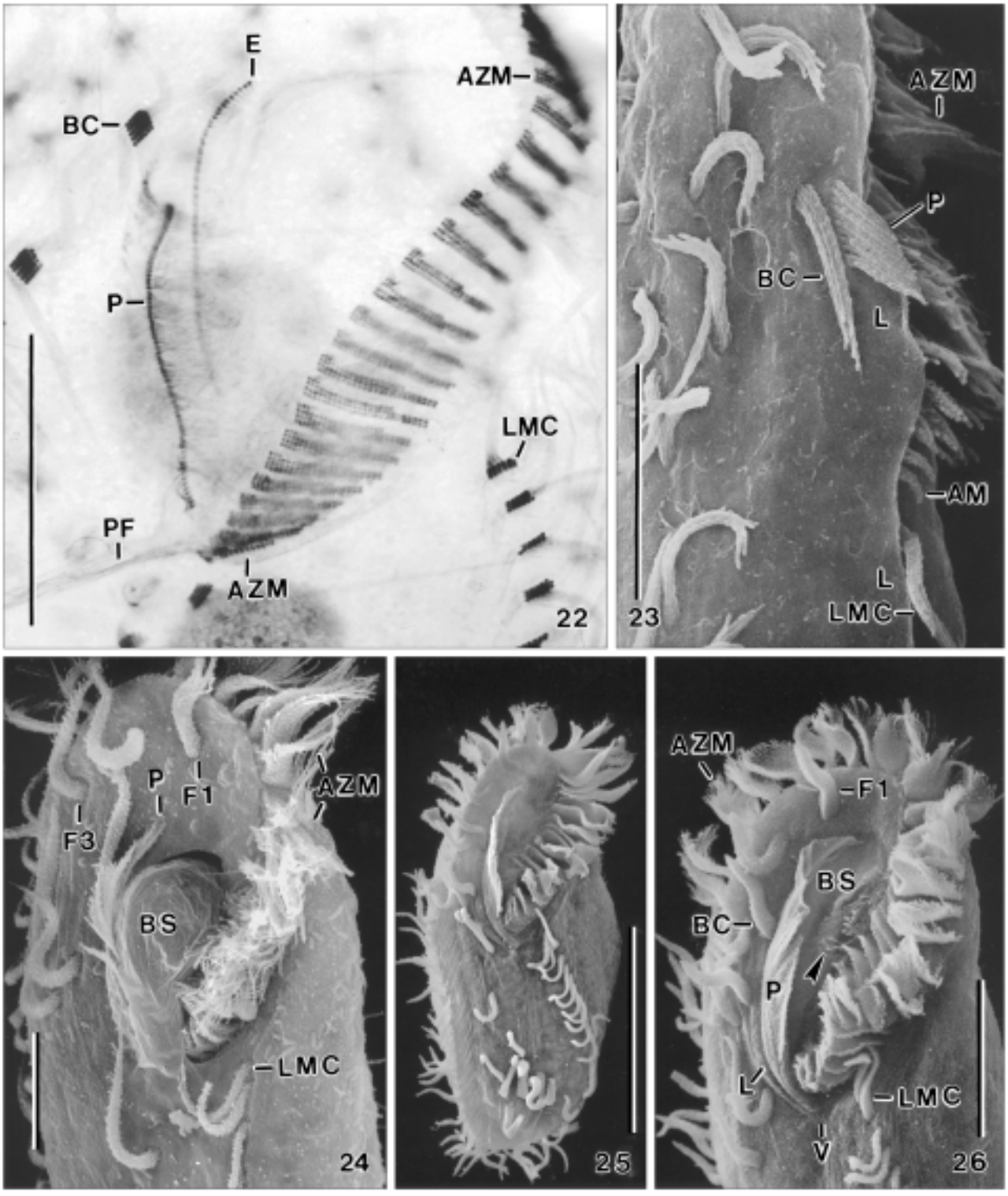
In vivo, in protargol preparations (Fig. 22), in the scanning electron microscope (Figs 14, 15, 33), and during light microscopical ontogenesis (Foissner *et al*. 2002), the lateral membranellar cilia and their basal bodies look like those of the rest of the membranelle. However, transmission electron microscopy shows that the basal bodies of the lateral cilia, i.e., of membranellar row 4 and some basal bodies of row 3 are specialized in that they maintain the transverse microtubule ribbon reduced in all other membranellar basal bodies during ontogenesis (Puytorac *et al*. 1976, Bakowska and Jerka-Dziadosz 1978, Jerka-Dziadosz 1981).

The buccal lip and the buccal horn

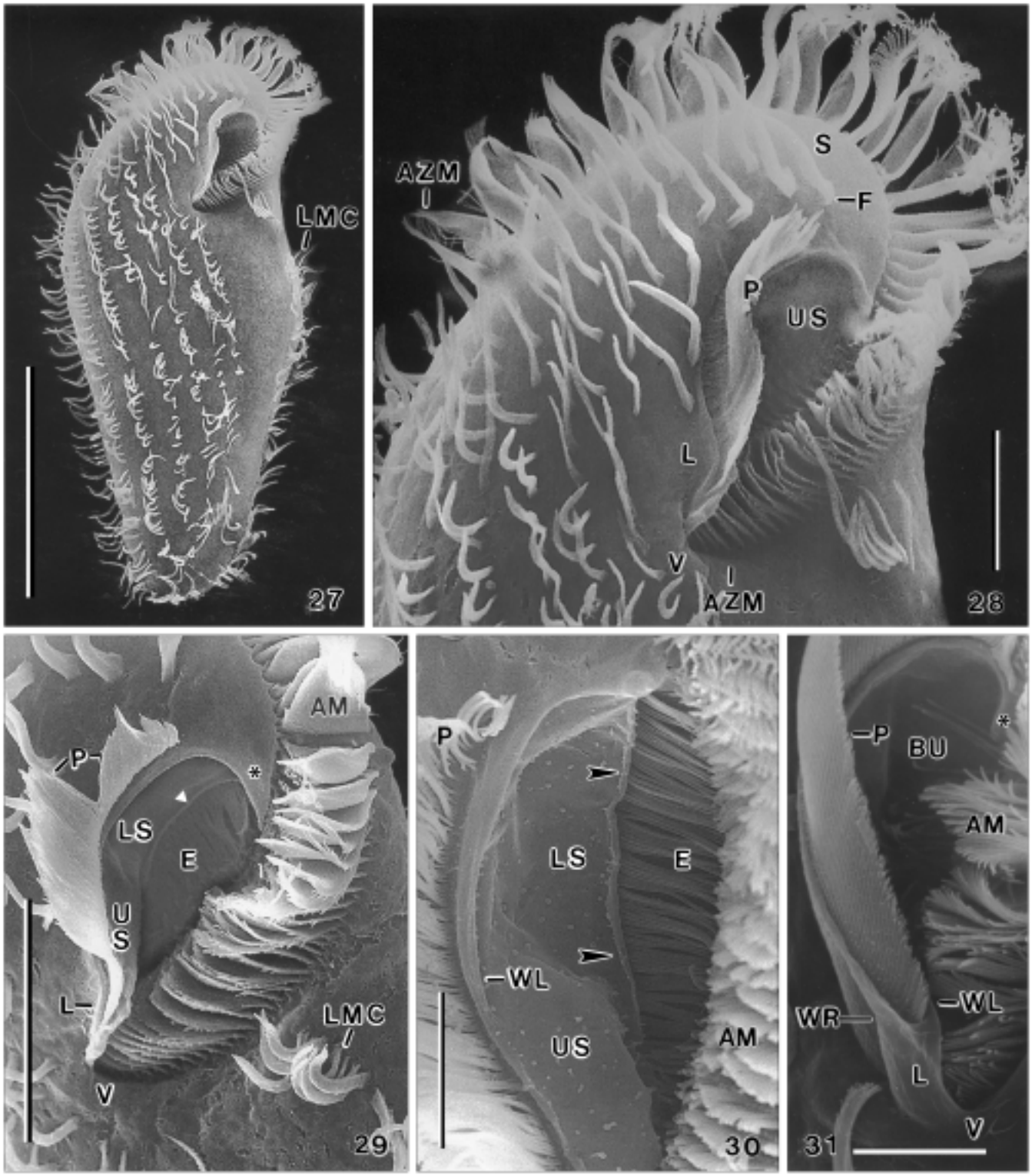
The left margin of the stichotrichine oral (buccal) opening is delimited by the ventral portion of the adoral zone of membranelles, while the right is defined by the edge of the buccal lip, a hyaline structure well recognizable in the scanning electron microscope (Figs 2, 3, 12, 20, 31, 34, 35, 39), but difficult to see *in vivo* and protargol preparations; probably, this has had the result that the right wall of the buccal cavity, which is more distinct, has been and still is frequently considered as the right margin of the oral opening. The buccal lip and/or its projecting lower half may cover the proximal portion of the adoral zone partially or entirely (Figs 23, 28). However, this does not obstruct the function of the membranelles because the lip is elevated relative to the adoral zone of membranelles.

Often, the edge of the buccal lip contains a shallow longitudinal cleft, where the paroral membrane inserts. This causes the lip to be divided into a right (outer) and a left (inner) wall. Three basic types of clefts occur: the right and left lip wall have similar height, for instance, in *Cyrtohymena candens* (Fig. 12); the left wall is considerably higher than the right one, for example, in *Stylonychia mytilus* (Fig. 18) and *Hemiamphisiella wilberti* where it is, additionally, conspicuously thickened (Figs 38-40); the right wall is considerably higher than the left one, a rare pattern found in *Uronychia* (Morelli *et al*. 1996).

The buccal lip does not contain endoplasm and is thus a differentiation of the cortex. Three types and several



Figs 22-26. *Steinia platystoma* (22, 24), *Hemiurosoma terricola* (23, from Foissner *et al.* 2002), and *Australocirrus oscitans* (25, 26), oral structures after protargol impregnation (22) and in the scanning electron microscope (23-26). **22, 24** - figure (22) shows the classical oxytrichid oral apparatus, where the endoral membrane (E) is covered by the buccal seal (24, BS) and the adoral membranelles are structured as shown in figure (8); **23** (overview, see figure 37) - the paroral membrane (P) is slightly above and right of the buccal lip; **25, 26** - the buccal seal (BS) is intact (25), respectively, slightly destroyed (26), exposing part of the lateral membranellar cilia (arrowhead). AM - adoral membranelles, AZM - adoral zone of membranelles, BC - buccal cirrus, BS - buccal seal, E - endoral, F1, 3 - frontal cirri, L - buccal lip, LMC - left row of marginal cirri, P - paroral, PF - pharyngeal fibres, V - buccal vertex. Scale bars: 10 µm (23, 24), 25 µm (22), 40 µm (26), and 75 µm (25).



Figs 27- 31. *Saudithrix terricola* (27-30) and *Cyrtohymena candens* (31) in the SEM. **27, 28 -** overview and oral detail of a specimen with intact buccal seal covering the deep buccal cavity and the proximal portion of the adoral membranelles; **29, 30 -** these figures show convincingly the presence of an upper buccal seal (US) and a lower seal (LS). The upper seal disappeared almost completely, while the lower seal was destroyed only right of the adoral zone, exposing the narrowly spaced and long endoral cilia (E). Note the buccal horn (asterisk), the curled margin (arrowheads) of the lower seal, and the inconspicuous lateral membranellar cilia not recognizable in these micrographs; **31 -** detail of the angular buccal lip. Asterisk marks buccal horn. AM - adoral membranelles, AMZ - adoral zone of membranelles, BU - buccal cavity, E - endoral, F - frontal cirri, L - buccal lip, LMC - left row of marginal cirri, LS - lower buccal seal, P - paroral, S - frontal scutum, US - upper buccal seal, V - buccal vertex, WL, WR - left and right wall of buccal lip. Scale bars: 10 µm (30, 31), 20 µm (28, 29), and 100 µm (27).

variations occur (Figs 5-7). Most frequent is the angular type (Fig. 7), which is generated by a more or less curved lip gradually increasing in height from less than 1 µm anteriorly to up to 10 µm posteriorly, where it makes a rather sharp angle producing a more or less conspicuous process before it merges with the cortex of the buccal vertex. This type is common in oxytrichids (Figs 12, 19, 28), amphisiellids (Figs 38, 39), and kahliellids. A remarkable variation is found in *Stichotricha*, where the paroral cilia are very near to the edge of the distinctly curved lip, which lacks the right wall of the cleft (Foissner *et al*. 1991). The curved lip type is as the angular type, but lacks the angle and thus the process (Fig. 6). It is found in several oxytrichids (Figs 17, 18, 25, 26) and in urostylids, for instance, *Holosticha* sp. (Fig. 35) and *Urostyla grandis* (Foissner *et al*. 1991, p.226, Fig. 15). In the flat type (Fig. 5), the lip is near to the level of the cell surface. Thus, the right lip wall is very flat, while the left may be conspicuously high, for instance, in *Hypotrichidium conicum* and *Amphisiella multinucleata* (Foissner *et al*. 1999, 2002).

When the buccal cavity is deep and its anterior margin is semicircularly curved, the left end of the cavity margin may form a conspicuous, acute process, which we term "buccal horn". The buccal horn is well recognizable in *Cyrtohymena* (Fig. 15), *Steinia* (Fig. 24), *Saudithrix* (Figs 29, 31), several amphisiellids (Foissner *et al*. 2002 and unpubl.), and *Hypotrichidium* (Foissner *et al*. 1999). The function and systematic significance of the buccal horn are not known.

The paroral and endoral membrane

As concerns the paroral and endoral membrane, we shall not deal with their basic features and variations, which have been reviewed by Sterki (1878), Fernandez-Leborans (1985) and Berger (1999). However, it has been overlooked that there are two basic patterns in the location of the paroral membrane. Although these patterns are not related to current stichotrichine classifications, they might be more important than presently recognized, especially at genus level.

In most species, the paroral inserts on the (environmental) edge of the buccal lip, where a longitudinal cleft extends, as described in the previous section. This type occurs, for instance, in the oxytrichid *Cyrtohymena candens* (Figs 12, 31) and the urostylid *Holosticha* sp. (Fig. 35). The second type, which is much rarer, has the paroral membrane near or close to the base of the buccal lip, for instance, the oxytrichid *Hemiurosoma terricola* (Figs 23, 37) and the supposed amphisiellid *Orthoamphisiella breviseries* (Foissner *et al*. 2002).

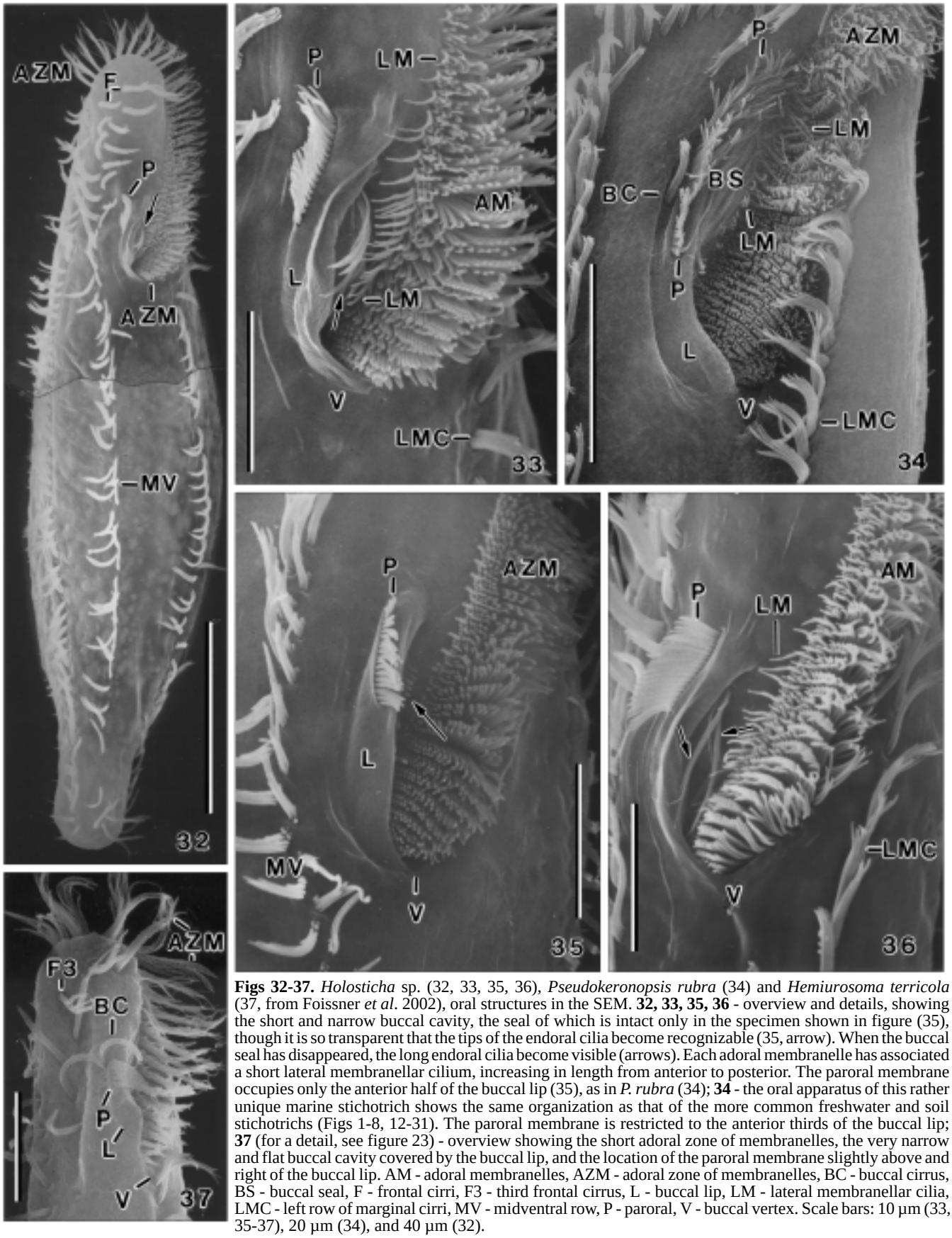
The endoral and paroral membrane can be distinguished by their location, viz., on the dorsal wall of the buccal cavity vs. outside the cavity on or near to the buccal lip. In some species, the cilia of the endoral membrane gradually lengthen from anterior to posterior, for instance, in *Bakuella granulifera* and *Amphisiella binucleata multicirrata*, where they increase from about 20 µm anteriorly to 40 µm posteriorly and become recognizable by their wavy movements deep in the cytopharynx (Foissner *et al*. 2002); in protargol preparations, the endoral cilia are often indistinguishable from the lateral membranellar cilia and the fibres supporting the cytopharyngeal wall.

Live observations on feeding

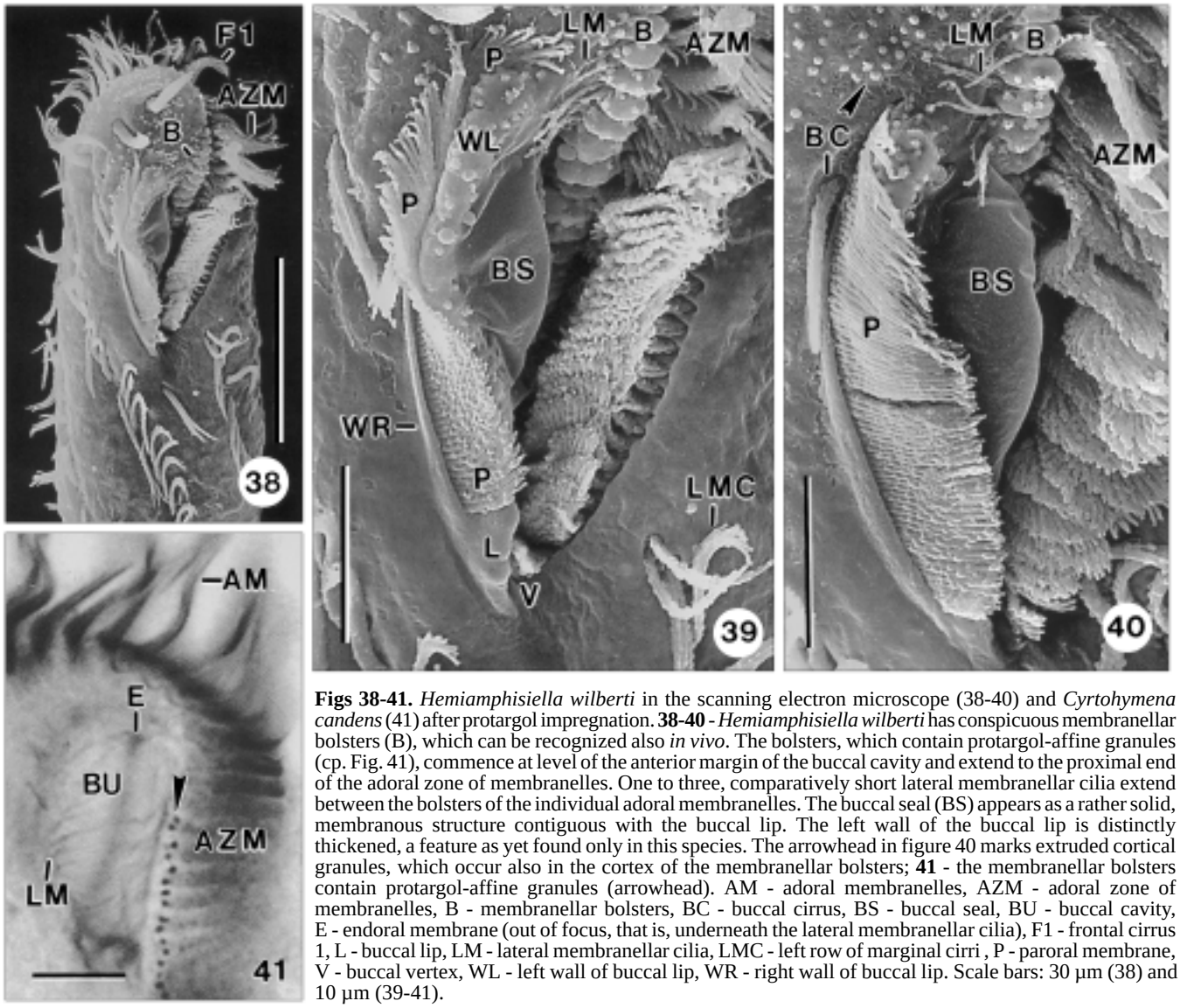
The food vacuoles of wild stichotrich populations show that they can feed on large prey, such as other ciliates, testate amoebae, resting cysts, and so on (Foissner *et al*. 1991, Foissner 1998). This applies also to the species used in this study, except *Holosticha* sp., which feeds mainly on bacteria.

Unfortunately, detailed investigations on feeding in stichotrichine spirotrichs are rare (see Discussion). Thus, we report our anecdotal observations, which show that the predator attaches to ciliate prey with its frontal and oral area, whereby the buccal cavity becomes wider and the victim is engulfed within a few seconds or minutes. First, the prey-predator contact is weak and the victim may escape. However, as soon as part of the prey is in the buccal cavity, the contact is firm and the prey rarely lost. Sometimes, the prey dissolves and/or dies during engulfment, but often it survives for minutes in the food vacuole, showing slow or fast rotation for up to 10 min (Foissner and Schiffmann 1974).

In *Cyrtohymena candens*, we studied feeding *in vivo* using the heterotrophic flagellate *Polytomella* sp. (~ 20 x 15 µm) as a food. *Cyrtohymena candens* has a huge oral apparatus, occupying almost half of body length and about 65% of body width. The very wide buccal cavity is semicircularly curved anteriorly and very deep, extending near to the dorsal side of the cell (Figs 1, 12-15, 41). The ciliary structures involved in feeding have the following lengths: longest bases of ventral adoral membranelles 10 µm; longest cilia of ventral adoral membranelles 12 µm; longest cilia of frontal adoral membranelles 20 µm; lateral membranellar



Figs 32-37. *Holosticha* sp. (32, 33, 35, 36), *Pseudokeronopsis rubra* (34) and *Hemiurosoma terricola* (37, from Foissner *et al*. 2002), oral structures in the SEM. **32, 33, 35, 36** - overview and details, showing the short and narrow buccal cavity, the seal of which is intact only in the specimen shown in figure (35), though it is so transparent that the tips of the endoral cilia become recognizable (35, arrow). When the buccal seal has disappeared, the long endoral cilia become visible (arrows). Each adoral membranelle has associated a short lateral membranellar cilium, increasing in length from anterior to posterior. The paroral membrane occupies only the anterior half of the buccal lip (35), as in *P. rubra* (34); **34** - the oral apparatus of this rather unique marine stichotrich shows the same organization as that of the more common freshwater and soil stichotrichs (Figs 1-8, 12-31). The paroral membrane is restricted to the anterior thirds of the buccal lip; **37** (for a detail, see figure 23) - overview showing the short adoral zone of membranelles, the very narrow and flat buccal cavity covered by the buccal lip, and the location of the paroral membrane slightly above and right of the buccal lip. AM - adoral membranelles, AZM - adoral zone of membranelles, BC - buccal cirrus, BS - buccal seal, F - frontal cirri, F3 - third frontal cirrus, L - buccal lip, LM - lateral membranellar cilia, LMC - left row of marginal cirri, MV - midventral row, P - paroral, V - buccal vertex. Scale bars: 10 µm (33, 35-37), 20 µm (34), and 40 µm (32).



Figs 38-41. *Hemiamphisiella wilberti* in the scanning electron microscope (38-40) and *Cyrtohymena candens* (41) after protargol impregnation. **38-40** - *Hemiamphisiella wilberti* has conspicuous membranellar bolsters (B), which can be recognized also *in vivo*. The bolsters, which contain protargol-affine granules (cp. Fig. 41), commence at level of the anterior margin of the buccal cavity and extend to the proximal end of the adoral zone of membranelles. One to three, comparatively short lateral membranellar cilia extend between the bolsters of the individual adoral membranelles. The buccal seal (BS) appears as a rather solid, membranous structure contiguous with the buccal lip. The left wall of the buccal lip is distinctly thickened, a feature as yet found only in this species. The arrowhead in figure 40 marks extruded cortical granules, which occur also in the cortex of the membranellar bolsters; **41** - the membranellar bolsters contain protargol-affine granules (arrowhead). AM - adoral membranelles, AZM - adoral zone of membranelles, B - membranellar bolsters, BC - buccal cirrus, BS - buccal seal, BU - buccal cavity, E - endoral membrane (out of focus, that is, underneath the lateral membranellar cilia), F1 - frontal cirrus 1, L - buccal lip, LM - lateral membranellar cilia, LMC - left row of marginal cirri , P - paroral membrane, V - buccal vertex, WL - left wall of buccal lip, WR - right wall of buccal lip. Scale bars: 30 µm (38) and 10 µm (39-41).

cilia 15 µm; cilia of paroral membrane 15 µm; cilia in anterior third of endoral membrane 20 µm.

When flagellates are swirled to the buccal cavity by the adoral zone of membranelles, they remain at or near to the cavity's surface and commence a fast rotation due to the action of the lateral membranellar cilia which beat along the cavity's margin. In contrast, the paroral membrane remains motionless; rarely a single, slow undulation passes along it, showing that the cilia are glued together (Fig. 12). After some seconds of rotation, the prey is either repelled and released or glides into the pharynx without touching the bottom of the cavity, giving support to the observation of a lower seal. The endoral cilia, which form a dense bundle left of the endoral row, remain motionless during these processes, but those within the pharynx show wavy movements, described already by Sterki (1878), when the prey arrives; probably, these movements transport the prey into the forming food vacuole.

DISCUSSION

The nature of the buccal seal

We could not find any mention of a covered buccal cavity in stichotrichine spirotrichs, neither in the light microscopical literature (Berger 1999) nor in detailed

transmission electron microscopical investigations (Grim 1972, Puytorac *et al*. 1976, Bakowska and Jerka-Dziadosz 1978). Obviously, the dorsal wall of the buccal cavity has been considered as the border to the environment. Probably, the seal escaped the transmission electron microscopists due to its fragility and the paucity of detailed investigations. None the less, transmission electron microscopy is necessary to clarify the nature of the buccal seal, that is, whether it is a membrane, as indicated by scanning electron microscopy (Figs 12, 17, 30, 39), or, for instance, mucous material secreted by the membranellar bolsters (Figs 39-41). However, the buccal seal of *Cyrtohymena* does not stain with alcian blue, suggesting that it does not consist of acid mucopolysaccharides. On the other hand, the buccal field of *Meseres corlissi*, an oligotrichine spirotrich, is covered by several slimy layers staining with alcian blue (Foissner *et al*. 2005).

Buccal seals and lateral membranellar cilia in other ciliates?

A literature search showed buccal seal-like sheets in a variety of ciliate groups. However, they were never recognized as a definite structure, and it is not known whether they are morphologically and functionally homologous. Thus, only a few examples will be mentioned. For instance, Eisler (1988) noted that the distal end of the oral basket of the nassulid *Furgasonia* is covered by a membranous, lid-like structure with a central slit. Similar differentiations have been observed, for instance, in the cyrtophorids *Trithigmostoma* (Foissner *et al*. 1991) and *Phascolodon* (Foissner *et al*. 2002) as well as in various haptorids, e.g., *Belonophrya*, *Cyclotrichium* and *Balantidion* (Foissner *et al*. 2002). In contrast, buccal seal-like structures are lacking, for instance, in hymenostomes, except for the big-mouthed *Lembadion* (Foissner *et al*. 1994). However, hymenostomes often have a rather large buccal lip, for instance, *Glaucoma scintillans*, where the lip covers part of the buccal cavity, just as does the buccal lip in stichotrichs (Foissner *et al*. 1994).

Is there a buccal seal in other spirotrichs, especially in the oligotrichs and euplotids? Unfortunately, the matter is difficult in the latter because the cortex is made of plates looking similar to a buccal seal in the scanning electron microscope. However, *Euplotes* probably lacks both, lateral membranellar cilia and a buccal seal (Foissner *et al*. 1991 and unpubl. data). The oligotrich spirotrichs, for instance, *Halteria grandinella*, *Pelagostrombidium mirabile*, and *Rimostrombidium lacustris*, lack lateral membranellar cilia, while the undulating (endoral) membrane is covered by a membranous sheet similar to the buccal seal of the stichotrichs (Foissner *et al*. 1999). The Heterotrichida, e.g., *Blepharisma* and *Linostomella*, which were formerly also included into the spirotrichs (Corliss 1979) but are now in a different subclass (Lynn 2003), lack a buccal seal, while lateral membranellar cilia are present in *Linostomella* (Foissner *et al*. 1999 and unpubl. data).

As all living things are covered by at least a cell membrane, our buccal seal might be considered as trivial. But it isn't. Actually, it is more comprehensible to assume the dorsal wall of the buccal cavity or the inner basket surface as the environmental border than the buccal seal or seal-like structures on the organism's surface which requires restoration after each feeding process. Thus, we assume that the buccal seal has important functions.

Functional aspects

Our investigations show that the stichotrichine oral apparatus is more complex than previously recognized (Machemer and Deitmer 1987, Berger 1999, Verni and Gualtieri 1997), both in terms of the buccal seal as well as the length and movement of the membranellar cilia (Figs 1-4, 9). Likely, all these specializations are involved in the feeding process, but accurate data are rare (for reviews, see Machemer and Deitmer 1987, Ricci and Erra 2001, Wilks and Sleigh 2004). Our preliminary observations show that the lateral membranellar cilia are involved in feeding, though covered by the buccal seal in most species, while the function of the motionless endoral cilia remains obscure. In *Stylonychia mytilus*, the endoral membrane possibly directs the water flow towards the cytostome (Machemer and Deitmer 1987, Wilks and Sleigh 2004). As concerns the buccal seal, we will now discuss three hypotheses.

Food recognition and selection: The mechanisms of food recognition and selection are poorly understood. However, it is known that size and surface properties of the food and the adoral membranelles are important for phagocytosis, suggesting the glycocalyx of the plasma membrane as a main receptor (Laybourn-Parry 1984, Fenchel 1987, Hausmann *et al*. 2003, Wilks and Sleigh 2004). Assuming that the buccal seal is a surface membrane, then it is one of the first structures contacting potential food, suggesting that it could play a major role in food recognition and selection.

Hydrodynamic forces: Although the buccal cavity is usually small as compared to the total cell surface, it may

be very deep, for instance, in *Cyrtohymena*, *Saudithrix*, and *Lembadion* (Foissner *et al*. 1991, Berger 1999). Probably, such a deep hole or groove, if not sealed, would disturb the hydrodynamical properties of the cell (Machemer and Deitmer 1987).

Protective function: When not feeding, the buccal seal may protect the buccal cavity and the organelles contained from involuntary stimuli. In contrast, protection from mechanical forces seems unlikely due to the fragility of the seal.

The lateral membranellar cilia were first described by Sterki (1878) under the term "paroral cilia", but then fell into oblivion. Berger (1999) mentioned only Sterki's term, and they were not recognized in detailed studies of the adoral membranelles (Grim 1972, Grimes 1972, Jerka-Dziadosz 1981, Machemer and Deitmer 1987, Ricci and Erra 2001). Our observations show that the lateral membranellar cilia are involved in the feeding process. They hold close contact with the prey, suggesting important functions in food selection and uptake. This is emphasized by the considerable diversity of the lateral membranellar cilia within (Figs 9, 13, 15, 33) and between (Figs 13, 15, 33, 36, 39, 40) species.

We studied only the morphology of the buccal seal and lateral membranellar cilia. However, their physiology and behaviour during the life cycle are very likely even more interesting. Is the seal physiologically different in different species? Is the seal destroyed in species feeding on bacteria? How fast and when is the seal restored after feeding? What is the specific function of the lower seal? Is the seal membrane different from that of the neighbouring cortex? Do the seal and the lateral membranellar cilia interact? These and other questions will be difficult to answer considering the fragility and, perhaps, complexity of the buccal seal.

How general and complete is our knowledge of the stichotrichine oral apparatus?

We suppose that our scheme of the stichotrichine oral apparatus is now fairly complete in terms of gross morphology (Figs 1-8), while highly incomplete functionally. However, a more detailed analysis of the adoral cilia/membranelles and the interkinetal ridges might bring some surprises (Ricci and Erra 2001, Wilks and Sleigh 2004); and freeze-fracture and deep-etch rotary-shadow replicas might show additional specializations of the buccal seal and/or the buccal cavity. Further, there are exceptions and distinct variations, e.g., some amphisiellids which have the left wall of the buccal lip so strongly developed that it covers the ventral part of the adoral zone (Foissner *et al*. 2002). One can consider this as a variation only, but when compared to, e.g., *Stylonychia*, it seems to be a type of its own. Likewise, such curious genera as *Etoschothrix* (Figs 10, 11) and *Erniella* (Foissner 1987) considerably deviate from the scheme in the structure of the adoral membranelles (composed of only three ciliary rows), the adoral zone (distinctly bipartite), and the undulating membranes (paroral made of few, widely spaced cilia, endoral perhaps lacking).

Significance for alpha-taxonomy

Our data are not only of interest for cell biologists and physiologists, but also for taxonomists of stichotrichine spirotrichs. Most of the structures described can be recognized *in vivo* and with the light microscope. Thus, they should be included in future species descriptions: membranellar bolsters (recognizable or not), lateral membranellar cilia (length, many or few), type of buccal lip, buccal horn (present or absent), location of paroral membrane. In future, when occurrence and variation of these features are better known, they might be of significance for distinguishing genera and species.

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Hapantotypification and Morphological Redescription of the Marine Planktonic Ciliate, *Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et* Wilbert, 1995 (Ciliophora: Oligotrichida)

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Summary. The living morphology and infraciliature of a poorly-known marine planktonic ciliate, *Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et* Wilbert, 1995, collected from coastal mollusc farming ponds near Qingdao, north China, are investigated based on observations of live and protargol impregnated specimens. Since modern studies on *S. cinctum* are unavailable, an improved diagnosis based on the Qingdao population is given: medium-sized marine *Spirostrombidium*, *in vivo* about 55 × 40 μm; dorsoventrally *ca* 2:3 flattened; cell asymmetric barrel-shaped with inconspicuous apical protrusion; on average 25 anterior and 14 ventral membranelles; 3-4 posteriorly-directed thigmotactic membranelles; one macronucleus and one micronucleus; extrusomes prominent, about 6-10 μm long, arranged along somatic kineties; extra, girdle and ventral kinety consisting of about 17, 62 and 23 dikinetids, respectively.

Key words: infraciliature, marine planktonic ciliate, Oligotrichida, *Spirostrombidium cinctum*.

INTRODUCTION

Oligotrich ciliates are ubiquitous in the ocean surface and usually dominate planktonic ciliate communities (Lynn and Montagnes 1988, Lynn *et al.* 1988). Their ecology, especially of the vegetative stage, has been frequently studied since they serve as an important link between smaller unicellular organisms and those of higher trophic levels in the marine microbial food webs (*e.g.* Pierce and Turner 1992). Being small and fragile, many of these organisms have been only rather superficially investigated, which were usually based on living observations (Fauré-Fremiet 1924, Kahl 1932, Maeda and Carey 1985). Because of this, numerous ambiguities concerning the identification of taxa have accumulated in last century and hence, most nominal species need to be re-investigated using modern methods. This is especially true for the marine forms, which were relatively poorly considered as revealed in some recent studies (Lynn *et al.* 1988, Montagnes *et al.* 1990, Martin and Montagnes 1993, Petz *et al.* 1995, Song *et al.* 2000).

In the present paper, one oligotrich ciliate, *Spirostrombidium cinctum*, isolated from coastal waters off Qingdao, is redescribed from life and after

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protargol impregnation. The results are documented here.

MATERIALS AND METHODS

Ciliates were collected with 20 µm mesh plankton nets from some mollusc-culturing ponds in the Jiaozhou Bay near Qingdao, China (36°08´N; 120°43´E). The water temperature was ~15°C and the salinity ~33‰.

Specimens were observed *in vivo* with phase contrast and differential interference contrast microscopy. The infraciliature was revealed by protargol impregnation (Wilbert 1975). Counts, drawings (with help of a Camera Lucida) and measurements were performed at a magnification of × 1250. Terminology is mainly according to Agatha *et al.* (2005) and Corliss (1979).

RESULTS

Order Oligotrichida Bütschli, 1887
Family Strombidiidae Fauré-Fremiet, 1969
Genus *Spirostrombidium* Jankowski, 1978

Spirostrombidium cinctum (Kahl, 1932) Petz, Song *et* Wilbert, 1995 (Figs 1-3, Table 1)

To the authors' knowledge, this species has never been investigated using modern methods. Hence we supply an improved diagnosis based on an examination of the Qingdao population.

Improved diagnosis: Medium-sized marine *Spirostrombidium, in vivo* about 55 × 40 µm; dorsoventrally *ca* 2:3 flattened; cell asymmetric barrel-shaped with inconspicuous apical protrusion; on average 25 anterior and 14 ventral membranelles; 3-4 posteriorly-directed thigmotactic membranelles; one macronucleus and one micronucleus; extrusomes prominent, about 6-10 µm long, arranged along somatic kineties; extra, girdle and ventral kinety consisting of about 17, 62 and 23 dikinetids, respectively.

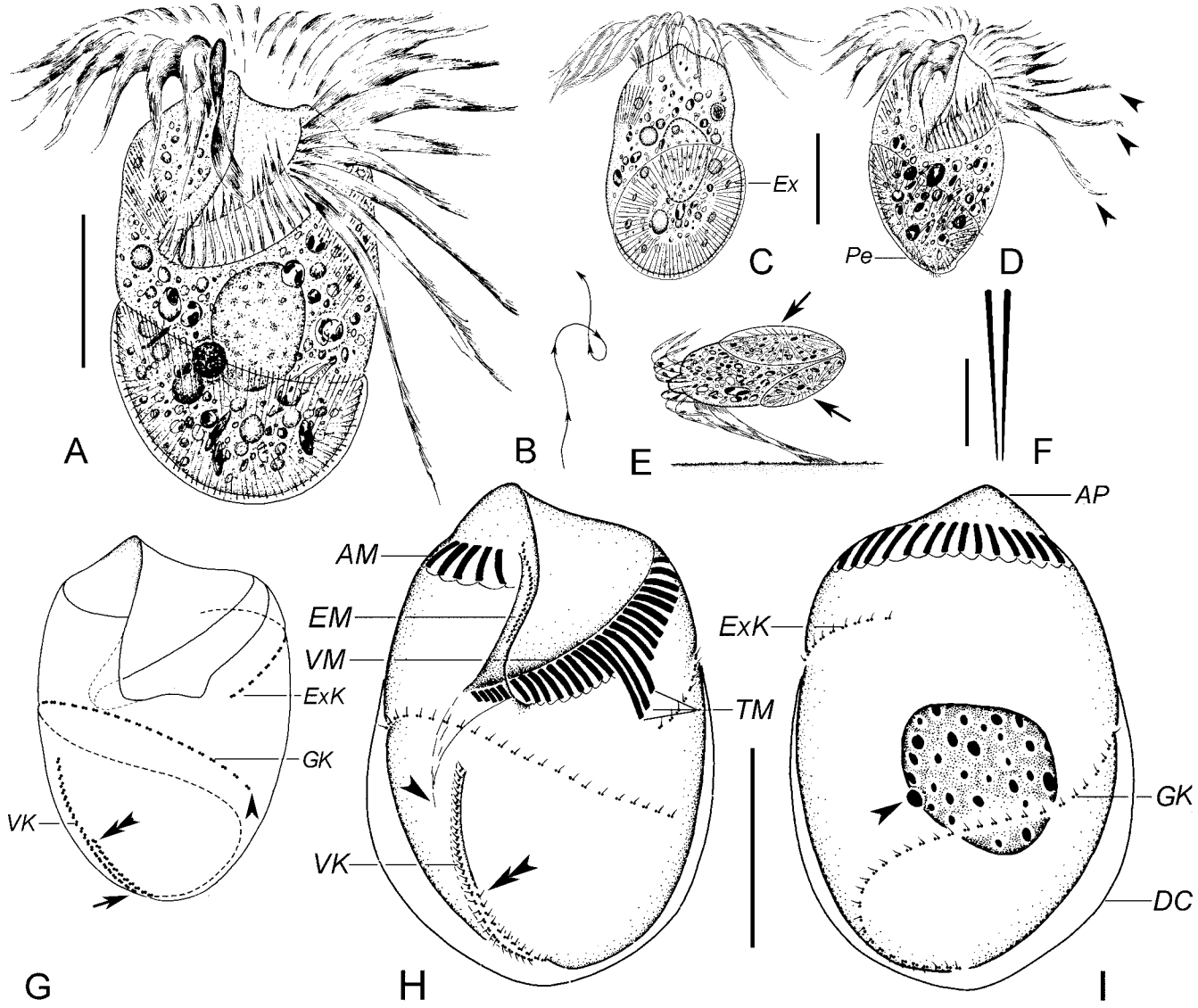
Deposition of slides: Since no silver impregnated specimens have previously been preserved, one neotype (registration number 2005:24:17) is deposited in the Natural History Museum, London and a second slide (registration number 03102901) is deposited in the Laboratory of Protozoology, Ocean University of China.

Description of the Qingdao population: Cells *in vivo* mostly about 45-65 × 30-45 µm. Body shape generally invariable, slightly asymmetric and barrel-shaped with posterior end widely rounded; when viewed from ventral side, broadest mostly at the equatorial area (Figs 1A, 2A). Dorsoventrally flattened and thickness:width about 2:3 (Figs 1D, 2D). Apical protrusion (collar) inconspicuous *in vivo*, which may disappear or be undetectable after fixation (Figs 1A; 2A, D, arrowhead). Buccal cavity relatively deep and prominent, extending obliquely towards right body side and terminating at about 2/5 of cell length (Figs 1A, 2A). Locomotion

Table 1. Morphometric characterization of *Spirostrombidium cinctum*. Data based on protargol-impregnated specimens. All measurements in µm. Abbreviations: Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of specimens, SD - standard deviation.

Character	Min	Max	Mean	SD	n
Body length	40	58	47.3	3.71	23
Body width	27	44	32.4	4.07	23
Distance from apex to cytostome*	13	28	20.4	3.13	22
No. of anterior membranelles**	23	28	25.5	1.55	15
No. of ventral membranelles	12	15	13.7	1.11	13
No. of thigmotactic membranelles	3	3	3.0	0.00	15
No. of dikinetids in girdle kinety	51	77	62.4	6.67	15
No. of dikinetids in ventral kinety	18	29	22.8	3.26	16
No. of dikinetids in extra kinety	13	19	16.8	1.80	12
No. of macronucleus	1	1	1.0	0.00	23
No. of micronucleus	1	1	1.0	0.00	4
Macronucleus length	12	22	16.8	2.84	22
Macronucleus width	8	18	13.2	2.28	22

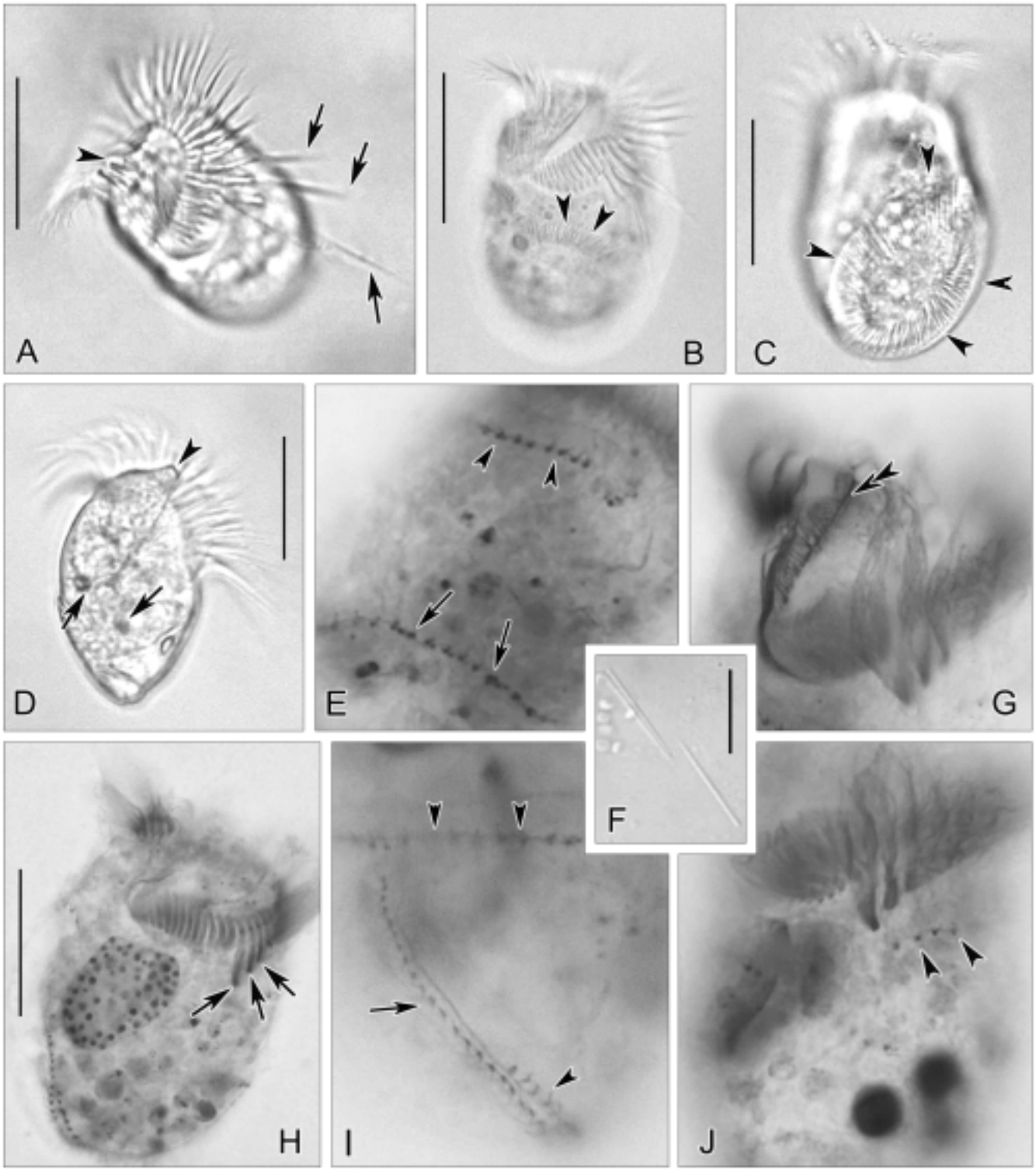
*Measured from anteriormost to the posterior end of buccal zone of membranelles; **Including the thigmotactic membranelles.



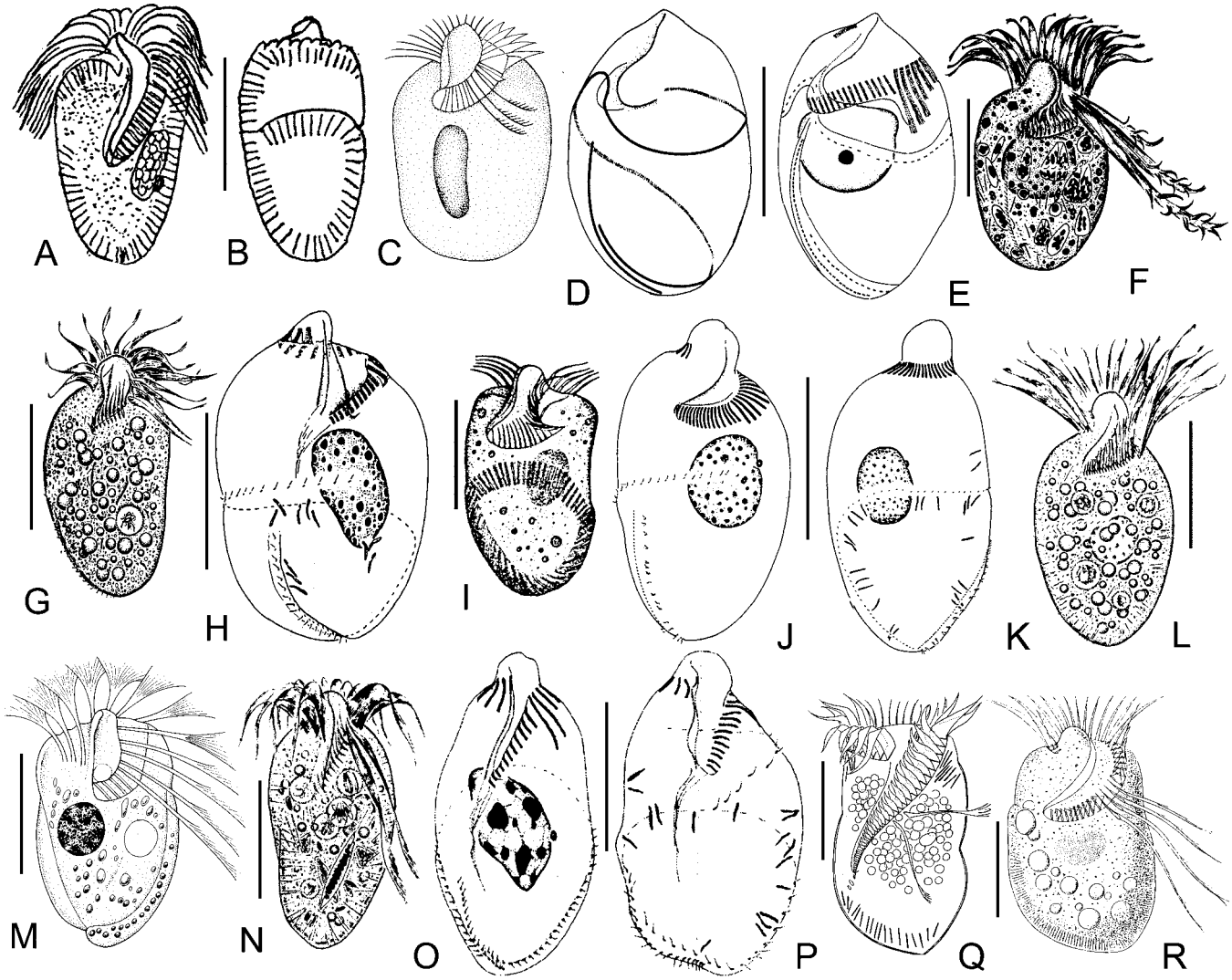
Figs 1A-I. *Spirostrombidium cinctum* from life (A-F) and after protargol impregnation (G-I). **A** - a representative specimen; **B** - swimming trace; **C** - dorsal view; **D** - lateral view, arrowheads to show the three thigmotactic membranelles; **E** - to show the creeping state, note the cell attached to the substrate with its three thigmotactic membranelles, arrows mark the bipartited subpellicular platelet layer; **F** - extrusomes; **G** - pattern of somatic ciliature, to show the beginning (arrowhead) and the end of the girdle kinety (double arrowheads), arrow to indicate the end of the ventral kinety; **H, I** - ventral and dorsal view of ciliary pattern, arrowhead in (H) indicates pharyngeal fibres, in (I) marks the micronucleus and double arrowheads in (H) show the end of the girdle kinety. AM - anterior membranelles; AP - apical protrusion; DC - distended cell surface; EM - endoral membrane; Ex - extrusomes; ExK - extra kinety; GK - girdle kinety; Mi - micronucleus; TM - thigmotactic membranelles; VK - ventral kinety; VM - ventral membranelles. Scale bars: 20 µm (A, C, D, H, I); 5 µm (F).

with two patterns: moderately fast and irregular when swimming (Fig. 1B), or very fast when crawling on the debris, using its 3 thigmotactic membranelles for attachment with ventral side down (Fig. 1E).

Cilia of most anterior membranelles about 20 µm long, stretching anteriorly as shown in Figs 1A and 2A. The three thigmotactic membranelles about 28 (range 25-30), 35 (range 33-37), 42 (range 40-45) µm in length, respectively, which are shortened from proximal to distal ones and always directed posteriorly like three tails (Figs 1D, arrowheads; 2A, arrows). Bases of three thigmotactic membranelles about 7-11 µm in length, connecting



Figs 2A-J. *Spirostrombidium cinctum* from life (A-D, F) and after protargol impregnation (E, G-J). **A** - a typical specimen, the apical protrusion (arrowhead) and the three thigmotactic membranelles (arrows); **B, C** - ventral and dorsal view, the arrangement of extrusomes (arrowheads); **D** - lateral view, the apical protrusion (arrowhead) and the ingested algae (arrows); **E** - dorsal view, the extra kinety (arrowheads) and girdle kinety (arrows); **F** - extrusomes after cell bursts; **G** - apical view, the endoral membrane (double arrowheads); **H** - arrows indicate the three thigmotactic membranelles; **I** - right side view, to demonstrate the girdle (arrowheads) and ventral kinety (arrow); **J** - the extra kinety. Scale bars: 40 µm (A-D); 8 µm (F); 20 µm (H).



Figs 3A-R. Comparison of some closely related species. **A, B** - *Spirostrombidium cinctum* (from Kahl 1932); **C** - *Spirostrombidium urceolare* (from Maeda and Carey 1985); **D-F** - *Spirostrombidium urceolare* (from Lei *et al.* 1999); **G, H** - *Spirostrombidium platum* (from Song and Packroff 1997); **I** - *Spirostrombidium pseudocinctum* (from Wang 1934); **J-L** - *Spirostrombidium echini* (from Song *et al.* 1999); **M** - *Strombidium elegans* (from Maeda and Carey 1985); **N-P** - *Strombidium elegans* (from Song *et al.* 2000); **Q** - *Strombidium latum* (from Fauré-Fremiet 1950); **R** - *Strombidium clavellinae* (from von Buddenbrock 1922). Scale bars: 30 µm (A, B, D-F, H, J-N, R); 50 µm (G, Q); 20 µm (I, O, P).

ventral membranelles (length of bases not the same, range 3-6 µm) and other anterior ones (bases about 6 µm).

Cell fragile, highly sensitive to pressure of cover-slip and easy to burst; pellicle rather delicate with thin and distinct subpellicular platelet layer covering the posterior 1/2 of body, but no polygonal cortical platelets detected (Figs 1A, C; 2C). This structure is obliquely positioned and seems in two parts: ventral one is smaller (Fig. 1A) while the one on dorsal side is dominant (Figs 1C, E, arrows; 2C). Cytoplasm colourless to grayish, sometimes yellow-green due to ingested algae (Figs 1A; 2D, arrows). Extrusomses prominent and acicular-shaped (*ca* 10 µm long), evenly arranged along somatic kineties,

Table 2. Morphometric comparison between *Spirostrombidium cinctum* and morphologically similar species. 1 - *Spirostrombidium cinctum;* 2 - *Spirostrombidium echini;* 3 - *Spirostrombidium urceolare;* 4 - *Spirostrombidium platum;* 5 - *Spirostrombidium pseudocinctum;* 6 - *Strombidium latum;* 7 - *Strombidium clavellinae;* 8 - *Strombidium elegans.* Measurements in μm.

Characters	1	2	3	4	5	6	7	8
Cell length (*in vivo*)	45-65	50-70	70-80	70-100	-	110-170	70-80	30-50
Body shape	barrel-shaped	conical to ovoid	widely oval	conical	elongate elliptical	widely oval	widely oval	slim-conic
Length of buccal field: body length	2/5	<1/4	*ca* 1/3	*ca* 1/3	*ca* 1/3	*ca* 2/3	1/4-1/3	1/3-1/2
No. of ventral membranelles	12-15	15-18	13-16	8-11	14-17	*ca* 15*	*ca* 10*	8-10
No. of anterior membranelles	23-28	19-24	20-22	15-18	26-29	>20*	*ca* 25*	12-15
No. of thigmotactic membranelles	3-4	absent	3	absent	absent	2	4	2
No. of dikinetids in girdle kinety	51-77	>70	87-121	-	-	-	-	37-44
No. of dikinetids in ventral kinety kinety	18-29	*ca* 15*	38-49	absent	-	-	-	9-21
Habitats	marine	marine (endo-commensal)	marine	marine (meso-saprobic)	marine	marine	marine	marine (meso-saprobic)
Data resource	Present work	Song *et al.* (1999)	Lei *et al*. (1999)	Song and Packroff (1997)	Petz *et al.* (1995)	Fauré-Fremiet (1950)	von Buddenbrock (1922)	Song *et al.* (2000)

*Assumed from the illustrations; - Data unavailable or structure difficult to judge (or doubtful if present).

but not in bundles (Figs 1A, C, D; 2B, C, arrowheads). Contractile vacuole not observed.

Macronucleus ovoid to ellipsoidal, centrally located, contains numerous small globular nucleoli (about 2 μm across) (Figs 1I, 2H). Single spherical micronucleus about 3 μm in diameter, closely attached to macronucleus (Fig. 1I, arrowhead).

All somatic kineties composed of dikinetids, one basal body of each with a short cilium (about 2-3 μm long) (Figs 1H, I). Girdle kinety consisting of about 62 (51-77) widely spaced dikinetids, extending from left/mid ventral side transversely across ventral and dorsal side, curving posteriad along left margin, across posterior pole and terminates subcaudally on right ventral-lateral area (Figs 1G, H, double arrowheads; 2E, arrows; 2I, arrowheads). Ventral kinety, which is composed of about 23 (18-29) densely arranged dikinetids, extends anteriad from posterior pole, parallel to the distal end of girdle kinety and terminates at equatorial level (Figs 1G, H; 2I, arrow). Thus, girdle kinety and ventral kinety both with inverse orientation. Ventral kinety positioned mainly in the right ventral-lateral area (Figs 1H; 2I, arrow), but in some specimens, ventral kinety could shifted almost to the right side of the cell (Fig. 2H). One fragment-like "extra" kinety positioned around "shoulder" area of left side of the cell and composed of about 17 (13-19) sparsely arranged dikinetids (Figs 1H, I; 2E, J, arrowheads).

Oral apparatus consisting of a single endoral membrane on inner wall of buccal lip and a membranellar zone (Figs 1H, 2H). Membranellar zone distinctly opens ventrally, bipartited into an anterior portion of about 25 (23-28) membranelles and a ventral portion of about 14 (12-15) membranelles, which are all composed of 3 kinety rows. Endoral membrane extending to center of protrusion (Figs 1H; 2G, double-arrowheads). Pharyngeal fibres about 10 μm long (Fig. 1H, arrowhead).

DISCUSSION

Comparison with related species

Our population corresponds well with Kahl's original description regarding the basic morphology (viz. the size, dorsoventrally flattened body shape, the distribution of extrusomes, behavior etc.) (Kahl 1932). Kahl also mentioned that 3 or 4 pointed membranelles were found in his population rather than "thigmotactic membranelles" (Figs 3A, B). We suppose that this could be due to a misinterpretation since these 3 thigmotactic membranelles might be sometimes less prominent like some individuals in our observations (Fig. 1D, arrowheads), hence it is reasonable to identify our population to be conspecific with Kahl's.

In 1995, Petz *et al.* (Petz *et al.* 1995) - without knowledge of its infraciliature - transferred *Strombidium cinctum* Kahl, 1932 into the genus *Spirostrombidium*. The pattern of somatic ciliature revealed by the present study corresponds well with the new diagnosis for *Spirostrombidium* (Petz *et al.* 1995, Agatha 2004), thus the new combination made by Petz *et al.* is confirmed.

In 1973, Hartwig found *S. cinctum* in the sand of North Sea around the Island of Sylt (Hartwig 1973). His organism corresponded well with Qingdao population considering the cell shape (oval), size (40-50 μm) and habitat (marine). The cell shape, habitat, distribution of extrusome of the Mexican population reported by Aladro-Lubel resembled Qingdao population except that the former is a little smaller (31.5-35 × 21 *vs.* 45-65 × 30-45 μm) (Aladro-Lubel 1984).

To date, four species in *Spirostrombidium* with flattened body shape inhabiting marine habitat have been reported, all of which have been studied using protargol impregnation method: *Spirostrombidium pseudocinctum* (Wang, 1934) Petz, Song *et* Wilbert, 1995; *Spirostrombidium urceolare* (Stein, 1867) Lei, Xu *et* Song, 1999; *Spirostrombidium platum* (Song *et* Packroff, 1997) Song, Wilbrt *et* Warren, 1999; *Spirostrombidium echini* Song, Wilbrt *et* Warren, 1999 (Wang 1934, Song and Packroff 1997, Lei *et al.* 1999, Song *et al.* 1999). The key morphological features of these taxa are given in Table 2. *Spirostrombidium pseudocinctum* (Wang, 1934) Petz, Song *et* Wilbert, 1995 is similar to *S. cinctum* in terms of cell size, habitat, number of anterior and ventral membranelles, but the former can be distinguished by: (1) the arrangement of extrusomes which are distributed only in ventral side (*vs*. present also in dorsal side), (2) absence of thigmotactic membranelles (*vs*. present), and (3) lacking of the extra kinety (*vs*. present) (Fig. 3I) (Wang 1934, Petz *et al.* 1995).

Spirostrombidium urceolare (Stein, 1867) Lei, Xu *et* Song, 1999 resembles *S. cinctum* in the presence of 3 thigmotactic membranelles, similar number of anterior and ventral membranelles. However, the former can be recognized by the following combined features: (1) larger size (70-80 *vs*. 45-65 μm), (2) extrusomes widely

arranged (*vs*. densely arranged along somatic kineties), (3) abscence of an extra kinety (*vs*. present), and (4) evidently higher number of dikinetids in girdle (87-121 *vs*. 51-77) and ventral kinety (38-49 *vs*. 18-29) (Figs 3C-F) (Lei *et al*. 1999).

Spirostrombidium platum (Song *et* Packroff, 1997) Song, Wilbert *et* Warren, 1999 differs from *S. cinctum* in: (1) larger size (70-100 *vs*. 45-65 μm), (2) absence of thigmotactic membranelles (*vs*. present), (3) fewer anterior (15-18 *vs*. 23-28) and ventral membranelles (8-11 *vs*. 12-15), and (4) abscence of a ventral kinety (*vs*. present) (Figs 3G, H) (Song and Packroff 1997).

Spirostrombidium echini Song, Wilbert *et* Warren, 1999 is an endocommensal form which can be clearly separated from *S. cinctum* by its biotope within the digestive tract of the urchins (*vs*. free-living) and the opened girdle kinety, in which the posterior end is divided into two parts giving the impression that this species has two ventral kineties (Figs 3J-L) (Song *et al*. 1999).

Considering the general morphology, comparisons should also be made with three *Strombidium*-species: *Strombidium elegans* Florentin, 1901; *S. clavellinae* von Buddenbrock, 1922 and *S. latum* Kahl, 1932, in which the infraciliature of the latter two remain unknown (Table 2). *Spirostrombidium cinctum* differs from *Strombidium clavellinae* mainly in terms of presence of 3-4 thigmotactic membranelles (*vs*. 4) and different arrangement of extrusomes (covering completely the body portion *vs*. limited in the posterior half when viewed ventrally) (Fig. 3R) (von Buddenbrock 1922).

Strombidium latum Kahl, 1932 is characterized by its giant body size (110-170 *vs*. 45-65 μm), deeply extended buccal field (*ca* 2/3 *vs*. 2/5 of body length), presence of 2 thigmotactic membranelles (*vs*. 3-4) and possibly less dominant extrusomes (Fig. 3Q) (Fauré-Fremiet 1950), thus it can be easily distinguished from *Spirostrombidium cinctum* (Table 2).

Strombidium elegans Florentin, 1901 was redefined very recently (Song *et al*. 2000). It differs from *Spirostrombidium cinctum* in several aspects, i.e. (1) the number of thigmotactic membranelles (2 *vs*. 3-4), (2) the somatic ciliary pattern (girdle kinety closed and inserted by ventral kinety *vs*. girdle kinety dextrally spiraled and its posterior portion parallel to ventral kinety), and (3) less dominant buccal apparatus with fewer ventral membranelles (8-10 *vs*. 12-15), much less number of anterior membranelles (12-15 *vs*. 23-28), dikinetids in girdle kinety (37-34 *vs*. 51-57) and in ventral kinety (9-21 *vs*. 18-29) (Figs 3M-P).

Occurrence and ecology

Kahl (1932) discovered *Spirostrombidium cinctum* in the sand of Kieler Bay at a salinity of 9‰. Raikov (1963) observed this species in sands of the Ussuri Gulf (Japan Sea). Furthermore, it occurred in the sand of North Sea around the Island of Sylt (Hartwig 1973). The Mexican population reported by Aladro-Lubel (1984) was from tropical and calcareous sediments near Enmedio Island, Mexico.

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Redescriptions of Three Marine Peritrichous Ciliates, *Zoothamnium alternans* Claparède *et* Lachmann, 1859, *Z. sinense* Song, 1991 and *Z. commune* Kahl, 1933 (Ciliophora, Peritrichia), from North China

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Summary. Three marine peritrichous ciliates, *Zoothamnium alternans* Claparède *et* Lachmann, 1859, *Z. sinense* Song, 1991 and *Z. commune* Kahl, 1933, were isolated from coastal waters near Qingdao, China. The living morphology, infraciliature and silverline system were studied in both living and silver-impregnated specimens. Based on the Qingdao populations, improved diagnoses and redescriptions are provided for each species: *Z. alternans* is distinguished from its congeners by the alternately branched stalk, the macrozooids on the main stalk trunk, the J-shaped macronucleus and in having 40-55 silverlines between the oral area and the trochal band and 20-30 between the trochal band and the scopula; *Z. sinense* can be recognized by the small zooid size, the alternately branched stalk, and in having 55-70 silverlines between the oral area and the trochal band and 20-35 between the trochal band and the scopula; *Z. commune* can be distinguished from other species of similar zooid shape and size by the form of peniculus 3 and the number of silverlines between the oral area and the trochal band (59-70) and between the trochal band and the scopula (38-43).

Key words: marine ciliate, morphology, Peritrichida, *Zoothamnium*.

INTRODUCTION

Zoothamnium Bory de St. Vincent, 1826 is a well-known genus with more than 70 species, many of which are abundant in marine biotopes (Kent 1880-1882, Kahl 1935, Stiller 1971, Küsters 1974, Corliss, 1979, Jankowski 1985). In common with most other genera of sessile peritrichs, however, few *Zoothamnium* species have been studied using modern methods (e.g. silver impregnation) as recommended by Foissner and Schiffmann (1974). Furthermore, there has been no revision of this genus since that of Kahl (1935). Consequently species separation and identification among *Zoothamnium* spp. is often very difficult (Kahl 1933; Precht 1935; Sommer 1951; Stiller 1953a, b; Stiller and Stevčić 1967).

In the present study we report on three marine *Zoothamnium* species collected from the littoral zone of coastal waters near Qingdao, China, following observations on both living and silver- impregnated specimens.

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All three species are known forms for which detailed information concerning the infraciliature and silverline system are lacking. Therefore, based on the Qingdao populations, an improved diagnosis and redescription of each species is presented here.

MATERIALS AND METHODS

Sample collection. Ciliates were collected from coastal waters at three sites in the Qingdao region, P.R. China, (36°08'N; 120°43'E) from both natural (*Zoothamnium alterans*) and artificial (*Zoothamnium sinense, Z. commune*) substrates. The natural substrate was the shell of the scallop *Chlamys* sp. The artificial substrates were glass slides fixed to a frame that was immersed in water and left for 10 days to allow colonization to occur. After this time the slides were retrieved and transported to the laboratory for examination.

Zoothamnium alternans was collected on 19 April 2004 from a scallop (*Chlamys* sp.) farming pond, water temperature 12°C, salinity about 30‰.

Zoothamnium sinense was collected on 10 July 2002 from the littoral zone of coastal waters off Qingdao, water temperature 26°C, salinity 30‰.

Zoothamnium commune was collected on 22 May 2002 from a shrimp-farming pond, water temperature 20°C, salinity 28‰.

Observations: Ciliates were observed *in vivo* using bright field and differential interference contrast microscopy. The infraciliature was revealed with protargol impregnation according to Wilbert (1975). The "dry" silver nitrate method (Foissner 1976) was used to demonstrate the silverline system.

Drawings and terminology: Drawings of impregnated specimens were made with the help of a camera lucida at ×1250 magnification. Terminology is mainly according to Foissner and Schiffmann (1974) and Warren (1986).

Deposition of slides: Two slides of *Zoothamnium alternans* (No. 0404190101, 0404190102) with protargol-impregnated specimens are deposited at the Laboratory of Protozoology, OUC, China. Another slide (No. 2005:3:24:7) is deposited in the collections of the Natural History Museum, London, U.K.

One slide of *Zoothamnium sinense* (No. 0207100101) with protargol-impregnated specimens is deposited in the collection of the Laboratory of Protozoology, OUC, China.

One slide of *Zoothamnium commune* (No. 0205220401) with protargol-impregnated specimens is deposited at the Laboratory of Protozoology, OUC, China. Another slide (No. 2005:3:24:8) is deposited in the collections of the Natural History Museum, London, U.K.

RESULTS

Class: Oligohymenophora de Puytorac *et al.*, 1974
Subclass: Peritrichia Stein, 1859
Order: Sessilida Kahl, 1933
Family: Vorticellidae Ehrenberg, 1838
Genus: *Zoothamnium* Bory de St. Vincent, 1826

Redescription of *Zoothamnium alternans* Claparède *et* Lachmann, 1859 (Figs 1, 2; Table 1)

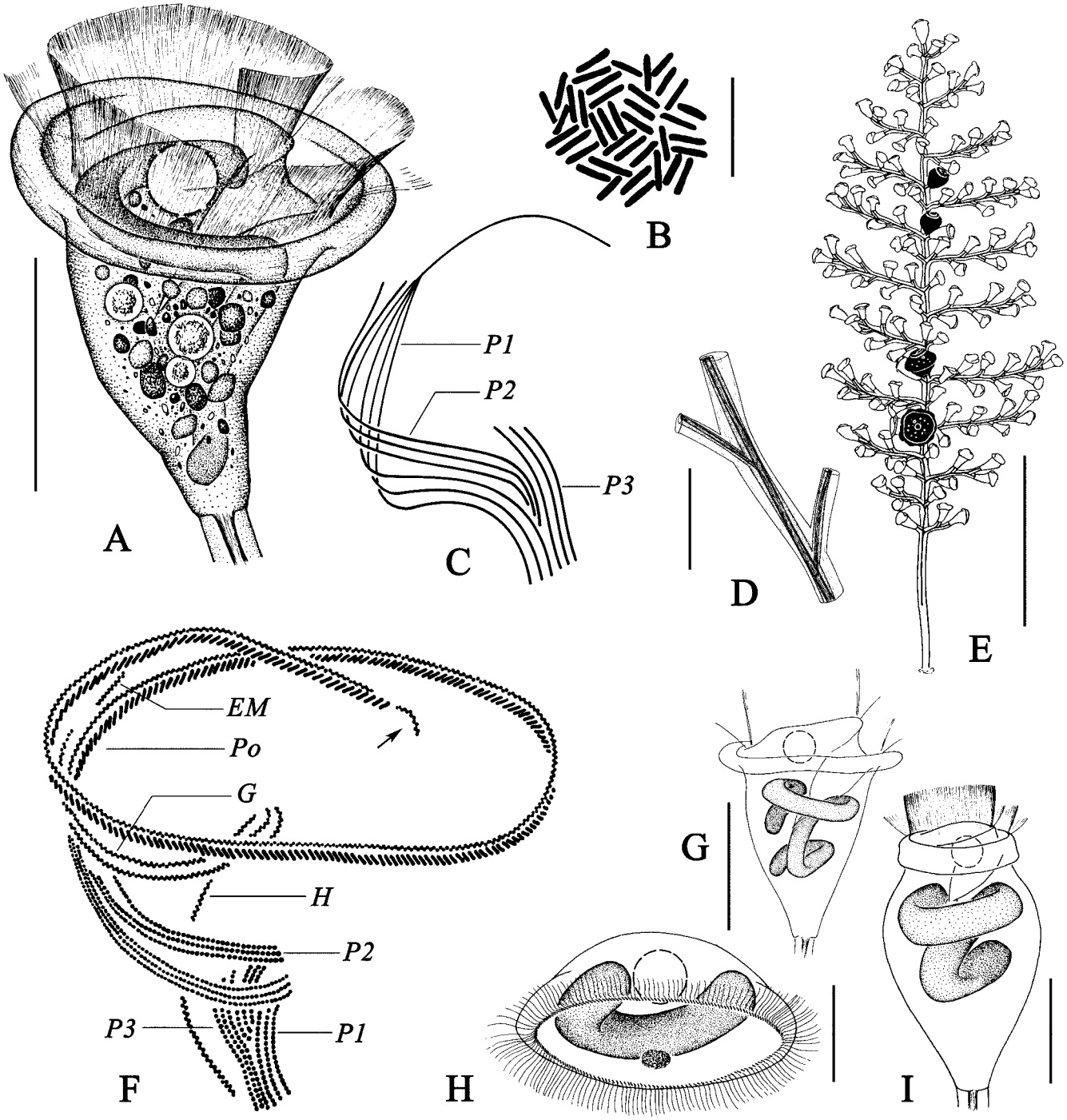
Synonym: *Zoothamnium chlamydis* Hu *et* Song, 2001 (p216)

Although *Zoothamnium alternans* has been known for a long time (Claparède and Lachmann 1859; Kent 1880-1882; Kahl 1933, 1935), it has never been investigated using silver impregnation methods. Therefore its infraciliature and silverline system remain unknown. Based on the population found in Qingdao, a detailed redescription and an improved diagnosis are presented here.

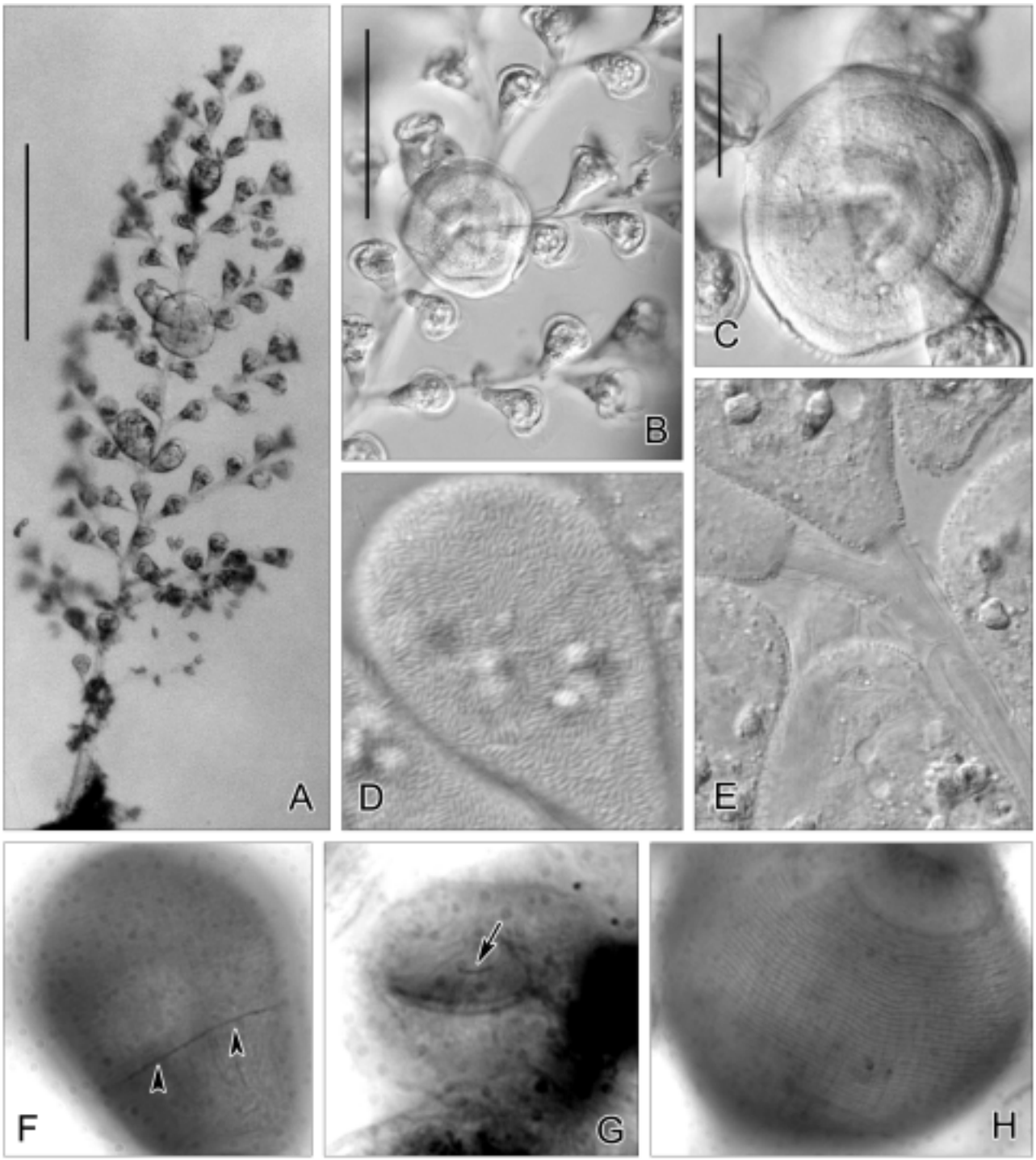
Amended diagnosis: Marine *Zoothamnium* with alternately branched stalk. Cells differentiated into micro- and macrozooids. Macrozooids ovoid, *ca* 70-90 × 45-55 μm *in vivo*, located only on main stalk trunk; microzooids inverted bell-shaped, 40-56 × 26-32 μm in size. Both micro- and macrozooids have a J-shaped macronucleus and an apically located contractile vacuole. Number of silverlines from oral area to aboral trochal band *ca* 40-55, from aboral trochal band to scopula *ca* 20-30.

Amended description: Colonies with micro- and macrozooids. Microzooid inverted bell-shaped, 40-56 × 26-32 μm *in vivo*, with thin, distinctly everted peristomial lip and moderately elevated peristomial disc (Figs 1A, G). Cytoplasm colourless to slightly grey, usually containing granules and several vacuoles (Fig. 1A). Macronucleus J-shaped in lateral view. Oral section of macronucleus elongate, cylindroid in cross-section, semicircular, transversely oriented. End of oral section sometimes recurved aborally (Fig. 1G). Aboral continuation of macronucleus cylindroid, straight, oriented diagonally to longitudinal axis. Aboral extremity of macronucleus usually recurved orally. Single contractile vacuole located slightly above peristomial lip and pulses slowly (3-5 min intervals). Pellicle appears smooth, usually covered with a layer of rod-shaped bacteria (Figs 1B, 2D). Fine pellicular striations can be observed occasionally under ×1000 magnification (Fig. 2H). Number of silverlines from aboral trochal band to scopula *ca* 20-30, from peristome to aboral trochal band *ca* 40-55.

Macrozooid oval-shaped, 70-90 × 45-55 μm *in vivo*, widest at mid-body, with thick peristomial lip, J-shaped macronucleus and single, apically located contractile vacuole (Figs 1I, 2A). Macrozooids located only on main trunk of stalk, one to several per colony, but absent at newly formed colonies. Telotroch acetabuliform (sau-



Figs 1A-I. *Zoothamnium alternans* from life (A, B, D, E, G-I) and after protargol impregnation (C, F). **A** - a micozooid; **B** - bacteria on pellicle; **C** - detail of infundibular polykineties; **D** - stalk structure; **E** - colony form, from Grell (1968); **F** - oral infraciliature, arrow marks the distal kinety fragment; **G** - lateral view of a microzooid; **H**- a telotroch; **I** - a macrozooid. EM - epistomial membrane; G - germinal kinety, H - haplokinety, P1-3 - infundibular polykinety 1-3, Po - polykinety. Scale bars: 30 µm (1A, G-I); 10 µm (1B); 20 µm (1D); 300µm (1E).



Figs 2A-H. Photomicrographs of *Zoothamnium alternans* from life (A-E) and after protargol impregnation (F-H). **A-** colony form; **B -** showing a telotroch, a macrozooid and several microzooids; **C** - a telotroch; **D** - to show bacteria covering the pellicle; **E** - to show structure of stalk; **F** - lateral view, arrowheads mark the aboral trochal band; **G** - to show the epistomial membrane (arrow); **H** - silverline system. Scale bars: 300 µm (2A); 200 µm (2B); 50 µm (2C).

Table 1. Morphometric characters of *Zoothamnium alternans* (first line, data for microzooids), *Z. sinense* (second line) and *Z. commune* (third line). Min - minimum, Max - maximum, Mean - arithmetic mean, SD - standard deviation, n - sample number.

	Min	Max	Mean	SD	n
Body length *in vivo* in μm	40	56	48.4	4.91	16
	36	48	40.7	3.46	9
	60	104	84.4	12.9	10
Body width *in vivo* in μm	26	32	29.3	1.65	16
	30	38	33.3	2.65	9
	48	56	50.0	2.83	9
No. of silverlines from oral area to aboral trochal band	40	55	-	-	4
	55*	70*	-	-	4
	59	70	65.0	3.80	10
No. of silverlines from aboral trochal band to scopula	20	30	-	-	3
	20*	35*	-	-	4
	38	43	40.6	1.67	9

* Detected from living cells.

cer-shaped), 100-120 μm in diameter and 40-50 μm in height, with large C-shaped macronucleus (Figs 1H; 2B, C). Telotrochs only formed by macrozooids.

Colonies up to 1.2 mm high, alternately branched, and containing up to 120 zooids (Figs 1E, 2A). Stalk with smooth surface, main stalk trunk 18 μm in diameter, 8 μm at distal end. Stalk spasmoneme 3-6 μm in width, comprising a dark fibrous bundle within a transparent thecoplasmic sheath, sometimes with a few mitochondria (Figs 1D, 2E).

Buccal apparatus of similar structure to most congeners. Haplokinety and polykinety describing one and one-third turns around peristomial disc before entering infundibulum, where they make another turn (Fig. 1F). One short kinety fragment is always visible at the peristomial end of the haplokinety (Fig. 1F, arrow).

In infundibulum, polykinety (P1) is accompanied by two other polykineties, P2 and P3, each of which consists of three ciliary rows (Fig. 1C). P1 and P2 much longer than P3, and originate from mid-infundibulum. P1 extends posteriorly to end of infundibulum, where it converges with P3 and the two terminate at the same level. Three rows of P2 terminate at different levels and significantly above the posterior ends of P1 and P3 (Fig. 1C). Germinal kinety parallel to haplokinety within abstomal half of infundibulum (Fig. 1F). Epistomial membrane short, located at opening of oral cavity (Figs 1F; 2G, arrow).

Aboral trochal band composed of a row of dikinetids which encircle the cell in aboral third of zooid (Fig. 2F).

Remarks: Given its highly distinctive morphology, i.e. alternately branched stalk, zooid size and appearance, marine habitat and the presence of macrozooids, the identity of the Qingdao population as *Z. alterans* is beyond reasonable doubt (Claparède and Lachmann 1859, Grell 1968).

Zoothamnium alternans is similar to *Z. niveum* Ehrenberg, 1838, which also has an alternately branched stalk, macrozooids on the main trunk of the stalk and a marine habitat, but the latter forms considerably larger colonies (> 1 cm high *vs.* 1.2 mm high in *Z. alternans*) and has a relatively shorter P2 (about equal to P3 in length *vs*. more than twice as long as P3 in *Z. alternans*), thus the two can be clearly separated (Bauer-Nebelsick *et al.* 1996).

Another marine species, *Zoothamnium plumula* Kahl, 1933, also has enlarged zooids and an alternately branching stalk, and so is somewhat similar as the current organism. However, these two species can be separated by the number of silverlines between the aboral trochal band and the peristomial border (*ca* 70 in *Z. plumula vs.* 40-55 in *Z. alternans*) and the location of the macrozooids which are located at the distal ends of the accessory branches in *Z. plumula vs*. on the main trunk in *Z. alternans* (Song *et al.* 2002).

Additionally, two other forms of *Z. alternans* were reported by Greeff (1870) and Kent (1880-1882) respectively, both of which are considered to be misidentifications. The population described by Kent has many enlarged zooids on the accessory branches but

Table 2. Morphological comparison between three *Zoothamnium* spp. and morphologically similar congeners. Measurements in μm.

Species	Body length *in vivo*	Body width *in vivo*	Number of silverlines from peristome to aboral trochal band	Number of silverlines from aboral trochal band to scopula	Shape of macronucleus	Habitat	Data source
Z. alternans	40-56	26-32	40-55	20-30	J-shaped	marine	present study
Z. chlamydis	50-60	-	27-47	19-29	vertically C-shaped	marine	Hu and Song (2001)
Z. commune	60-104	48-56	59-70	38-43	C-shaped	marine	present study
Z. hentscheli	63-84	35-42	-	-	C-shaped	freshwater	Kahl (1935)
Z. niveum	54-66	16-22	-	-	C-shaped	marine	Bauer-Nebelsick *et al.* (1996)
Z. plumula	50-100	30-50	70	24-28	C-shaped	marine	Song *et al.* (2002)
Z. sinense	36-48	30-38	55-70	20-35	C-shaped	marine	present study
*Z. sinense**	33-61	29-53	-	-	C-shaped	marine	Song (1991)
Z. thiophilum	90-95	-	-	-	C-shaped	freshwater	Stiller (1946)

*Called *Z. commune* sensu Song, 1991

lacks typical macrozooids on the main stalk trunk while Greeff's population possesses long cylindrical microzooids of a type that are not found in *Z. alternans*.

Zoothamnium chlamydis Hu *et* Song, 2001 has a strong similarity to *Z. alternans* in all key characters (Table 2). Therefore *Z. chlamydis* is considered to be a junior synonym of *Z. alternans* (Hu and Song 2001).

Redescription of *Zoothamnium sinense* Song, 1991 (Figs 3, 4; Table 1)

Synonyms: *Zoothamnium commune* sensu Song, 1991 (p47); *Z. truncatum* Song, 1986 (primary homonym) (p229)

Amended diagnosis: Marine *Zoothamnium* with alternately branched stalk; zooids inverted bell-shaped, measuring about 36-48 × 30-38 μm *in vivo*; peristomial disc flat, occasionally very slightly elevated above distinctly everted peristomial lip; macronucleus C-shaped and transversely oriented; single contractile vacuole apically located; pellicle finely striated, with *ca* 20-35 striations from scopula to aboral trochal band and *ca* 55-70 striations from aboral trochal band to oral area.

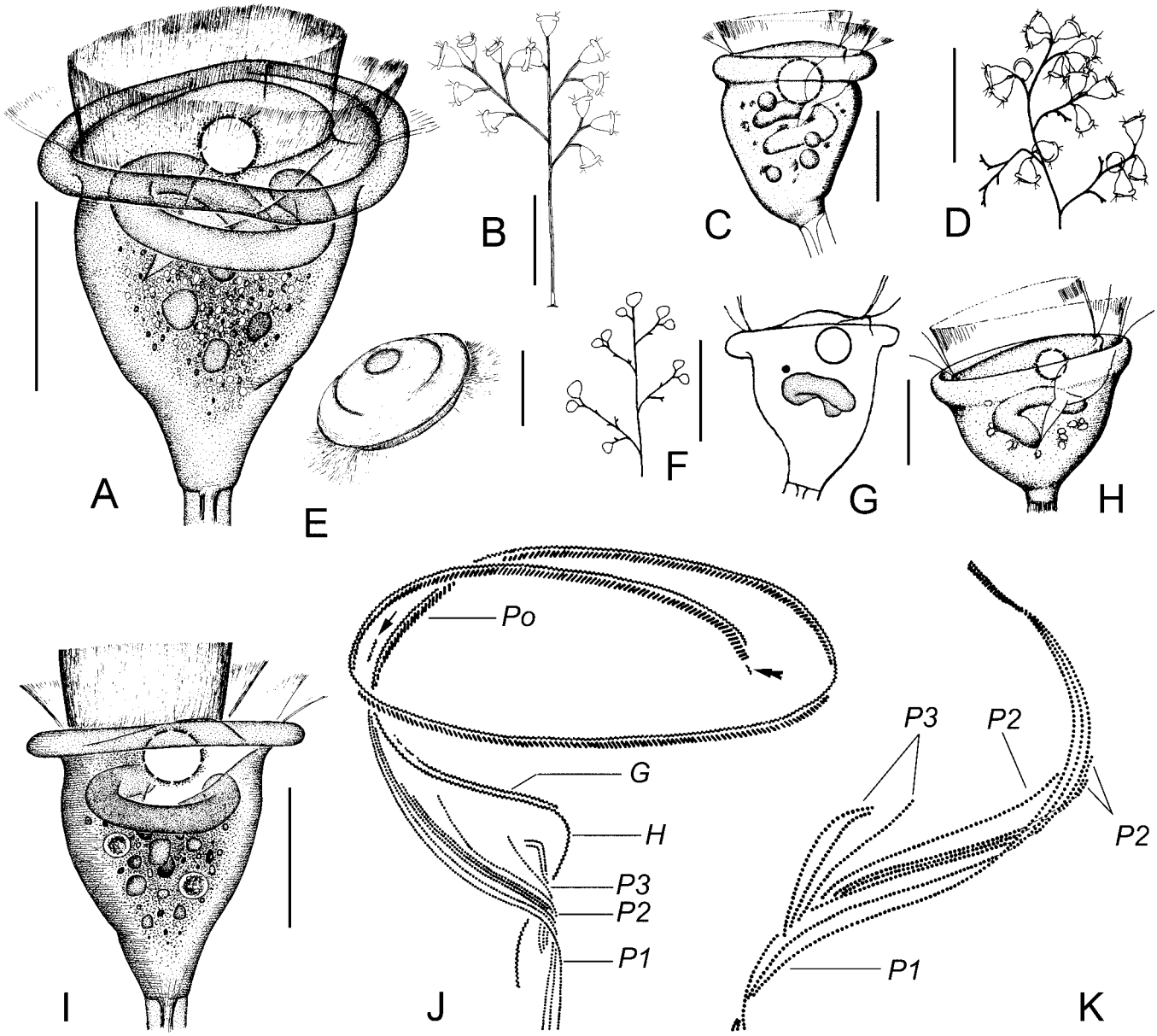
Amended description: Zooid inverted bell-shaped, usually measuring 36-48 × 30-38 μm *in vivo*, with maximum width just below everted, single-layered peristomial lip (Figs 3A, I; 4B). Peristomial disc flat, occasionally slightly elevated above peristomial lip (Figs 3I, 4B). Pellicle appears smooth at low magnification, fine pellicle striations only visible at ×1000 magnification or higher.

Cytoplasm colourless, usually containing granules and a few food vacuoles (Figs 3A, I). Single contractile vacuole apically positioned (Figs 3A, I; 4C, arrow). Macronucleus generally C-shaped, transversely oriented, located in oral 2/3 of body and surrounds the aboral portion of infundibulum (Figs 3A, I).

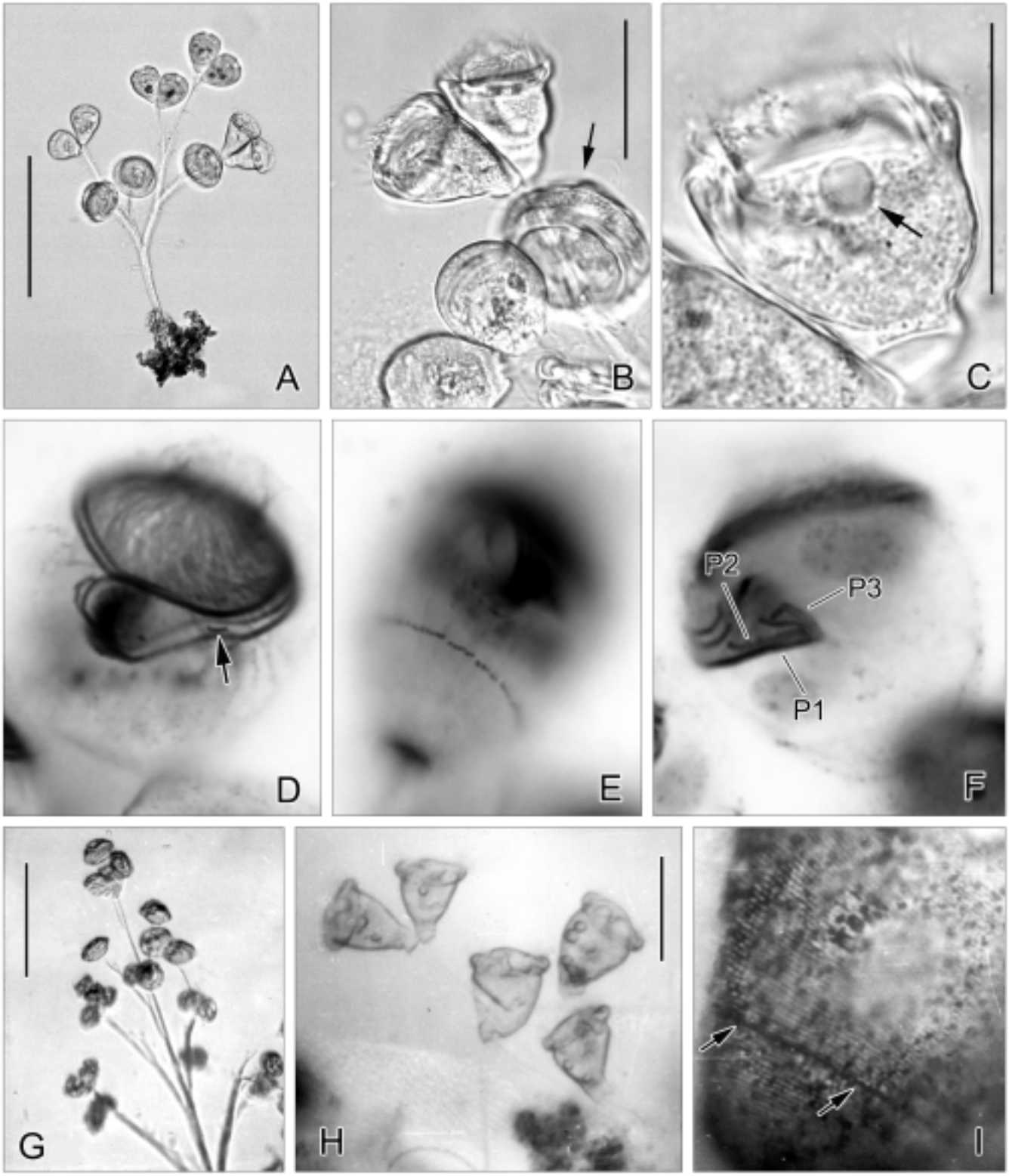
Cells highly sensitive to stimuli and contract readily. Also zooids readily convert to telotrochs, which are acetabuliform, *ca* 25 μm high and 45 μm in diameter (Figs 3E; 4B, arrow).

Colony *ca* 400 μm high, containing up to 40 zooids (Figs 3B, 4A); macrozooid formation was not observed. Stalk smooth, typically alternately branched, 6-9 μm in diameter. Spasmoneme 1.5-3 μm in diameter, mitochondria not observed.

Oral infraciliature as shown in Figs 3J, K and 4D, F. Haplo- and polykinety both make one and one-third turns of peristome before entering infundibulum, where they make a further turn. At adoral end of polykinety, a short kinety fragment is always recognizable (Fig. 3J, double arrowhead). Each of three infundibular polykineties consisting of three ciliary rows. Rows of P1 about equal in length, terminating at end of infundibulum. P2 about half length of P1. Inner two rows of P2 lie close to P1 while outer one is separated from the other two; all three



Figs 3A-K. *Zoothamnium sinense* Song, 1991 from life (A-I) and after protargol impregnation (J, K). **A** - a typical zooid; **B** - colony form; **C, D** - zooid (C) and colony (D), after Song (1991) (called *Z. commune*); **E** - telotroch; **F**-**H** - colony (F) and zooid (H), after Song (1986) (called *Z. truncatum*); **I** - zooid, lateral view; **J** - general oral infraciliature, arrow depicts the epistomial membrane, double arrowhead marks the distal kinety fragment; **K** - detail of infundibular polykineties. G - germinal kinety, H - haplokinety, P1-3 - infundibular polykinety 1-3, Po - polykinety. Scale bars: 20 µm (3A, C, E, G-I); 150 µm (3B, D, F).



Figs 4A-I. Photomicrographs of *Zoothamnium sinense* from life (A-C, G, H) and after protargol (D-F) and silver nitrate impregnation (I). **A** - a small colony; **B** - to show zooid shape and a developing telotroch (arrow); **C** - zooid at high magnification, to show the contractile vacuole (arrow); **D** - oral infraciliature, arrow marks the epistomial membrane; **E** - to show aboral trochal band; **F** - to show infundibular polykineties. **G** - photomicrograph of holotype of *Z. sinense* as described in Song (1991); **H** - photomicrograph of specimen from population described by Song (1991) (called *Z. commune*); **I** - silverline system of specimen from population described by Song (1991), arrows mark the aboral trochal band. P1-3 - infundibular polykinety 1-3. Scale bars: 150 µm (4A, G); 50 µm (4B, C, H).

rows of P2 end conspicuously above the posterior end of P1(Figs 3J, K). P3 about 2/3 length of P2, anterior end considerably below those of P1 and P2 and terminates posteriorly above P1 but below P2. Abstomal half of inner row of P3 separated from the other two ciliary rows but converges with them adstomally (Figs 3J, K;4F). Haplokinety passes around infundibulum on wall opposite to peniculi; germinal kinety runs parallel to haplokinety in abstomal half of infundibulum before terminating in mid-region of infundibulum (Fig. 3J). Epistomial membrane located near opening of infundibulum as commonly seen in other peritrichs (Figs 3J arrow; 4D, arrow).

Aboral trochal band appears as a row of loosely arranged dikinetids that encircle the body about ¼ of the body length from the scopula (Fig. 4E).

Silverline system typical of genus, consisting of many parallel, transverse rows about 0.5 μm apart, *ca* 55-70 between peristomial area and aboral trochal band, *ca* 20-35 between aboral trochal band and scopula, and with sparsely distributed pelliclular pores (Fig. 4I). Aboral trochal band stains heavily with silver, probably composed of two rows of closely arranged silverlines (Fig. 4I, arrows).

Remarks: *Zoothamnium sinense* was originally reported by Song (1986) under the name *Z. truncatum* (Figs 3F-H, 4G), and later renamed *Z. sinense* (Song 1991). According to the original description, *Z. sinense* has small body (33-59 × 35-64 μm), an alternately branched stalk, a single-layered peristomial lip, a C-shaped macronucleus and a marine habitat, all of which correspond well with the present population (Song 1986).

Considering general living appearance, the following congeners should be compared with *Z. sinense*: *Z. alternans* Claparède *et* Lachmann, 1859, *Z. commune* Kahl, 1933 and *Z. plumula* Kahl, 1935 (Table 2). According to our present study, *Zoothamnium alternans* is similar to *Z. sinense* in zooid size, stalk branching style and habitat, but can be easily recognized from the latter by the presence of several large macrozooids (*vs.* absent in *Z. sinense*), its J-shaped macronucleus (vs. C-shaped in *Z. sinense*) and in having 40-55 silverlines between the peristome and the aboral trochal band (vs. 55-70 in *Z. sinense*).

Zoothamnium commune and *Z. plumula*, like *Z. sinense*, also possess alternately branching stalks, inverted bell-shaped zooids and marine habitats. However, both can be separated from *Z. sinense* by their distinctly larger body size (>50 μm *vs.* 36-48 μm long *in vivo* in *Z. sinense*) and differences in the arrangement of the kineties of P3 (all three parallel in *Z. commune* and *Z. plumula vs*. innermost kinety separated and with a different orientation in *Z. sinense*) (Song *et al.* 2002). Furthermore, *Z. commune* has 38-43 silverlines between the aboral trochal band and the scopula (*vs.* 20-35 in *Z. sinense*).

Another marine *Zoothamnium*, which also has a small body size (33-61 μm long) and an alternately branched stalk, was reported by Song (1991) under the name *Z. commune* (Figs 3C, D; 4H). Considering the apparent difference from the original description of *Z. commune* by Kahl (1933) and the similarity with the current form, we consider that the population described by Song (1991) was misidentified and should be *Z. sinense*.

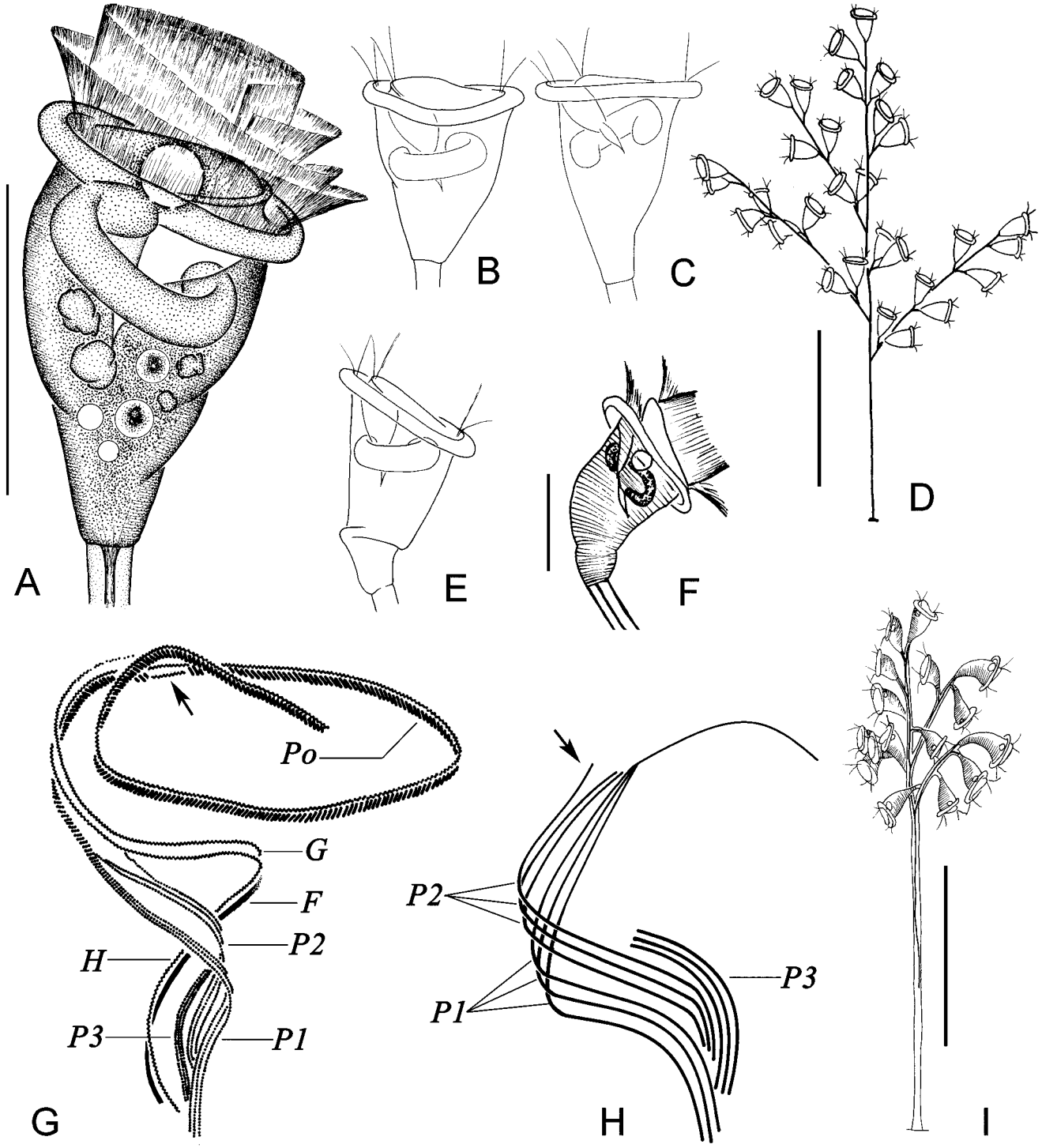
Redescription of *Zoothamnium commune* Kahl, 1933 (Figs 5, 6; Table 1)

Amended diagnosis: Marine *Zoothamnium* with alternately branched stalk. Zooids elongate, measuring about 60-104 × 48-56 μm *in vivo*. Single contractile vacuole apically located; macronucleus C-shaped and transversely to obliquely oriented. About 59-70 silverlines between aboral trochal band and peristomial lip, and 38-43 between aboral trochal band and scopula. Three ciliary rows in the third infundibular polykinety (P3) lie parallel to each other; P3 about half-length of P2 and terminates posteriorly conspicuously above P1.

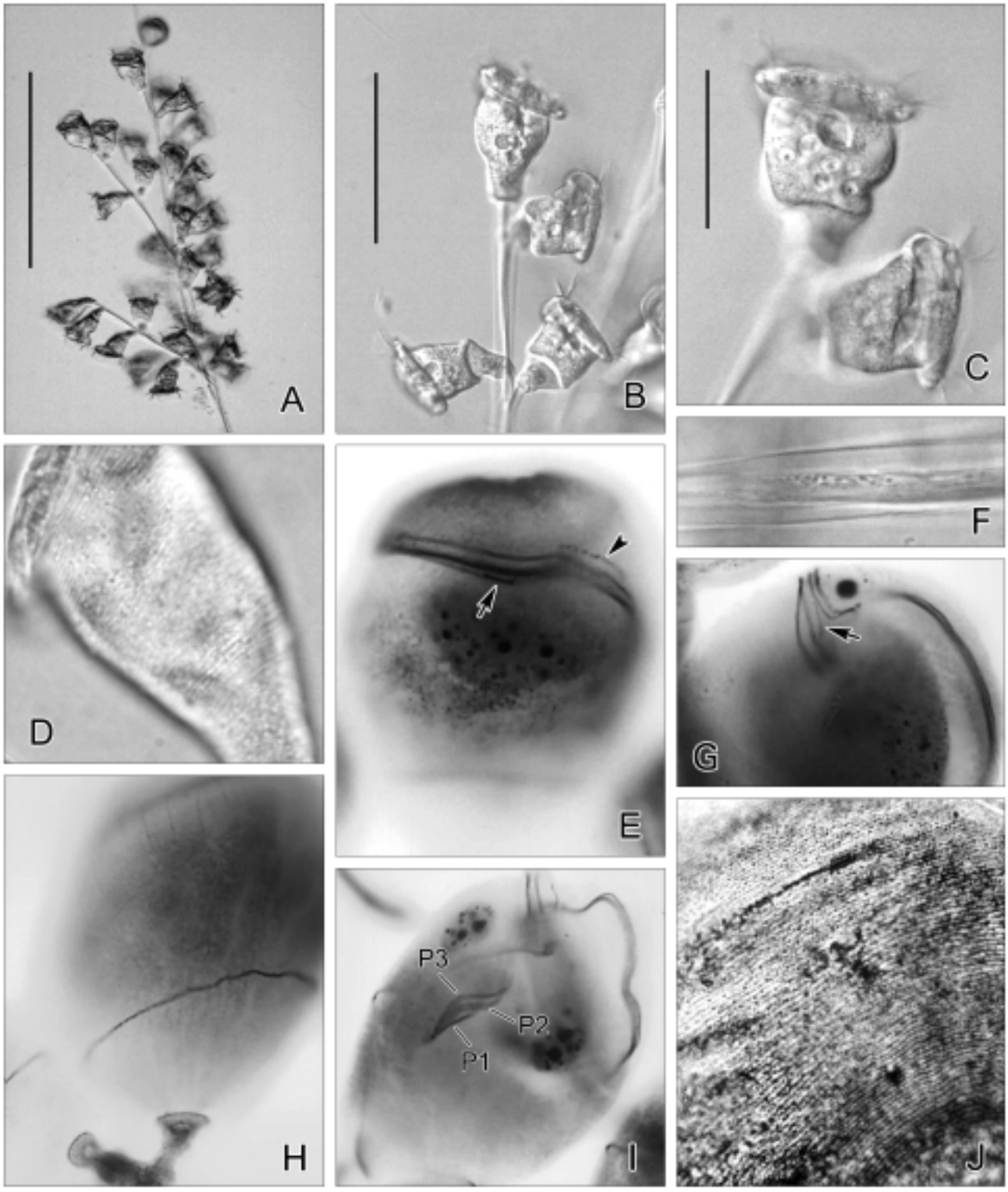
Amended description: Zooids elongate, inverted bell-shaped to conical; 60-104 × 48-56 μm *in vivo* (Figs 5A-C, E, F; 6A-C). Body widest just below peristomial lip. Peristomial disc slightly elevated above peristomial lip and contains the single contractile vacuole. Macronucleus C-shaped, more or less transversely oriented in third of body length to oral area and surrounding adstomal portion of infundibulum. Cytoplasm colourless and transparent, usually with granules and several food vacuoles (Figs 5A; 6B, C).

Pellicle appears smooth at low magnification; fine pellicular striations observed only above ×400 magnification (Fig. 6D). After staining with silver nitrate, many tiny pellicular pores visible along pellicular striations (Fig. 6J). Aboral trochal band not detectable in living zooids and only faintly stained with silver nitrate. About 59-70 pellicular striations between aboral trochal band and peristomial lip, and 38-43 between aboral trochal band and scopula.

Colony alternately branched, up to 1mm or more in height and contains up to 50 zooids (Figs 5D, I; 6A).



Figs 5A-I. *Zoothamnium commune* from life (A-F, I) and after protargol impregnation (G, H). **A** - a typical zooid at high magnification; **B, C, E** - shape variability; **D** - colony; **F** - zooid (after Kahl, 1933); **G** - oral infraciliature, arrow marks the epistomial membrane; **H** - detail of infundibular polykineties, arrow marks the separated anterior end of outer ciliary row in P2. **I** - colony (after Kahl, 1933). F - filamentous reticulum; G - germinal kinety, H - haplokinety, P1-3 - infundibular polykinety 1-3, Po - polykinety. Scale bars: 50 µm (5A); 300 µm (5D, I); 30 µm (5F).



Figs 6A-J. Photomicrographs of *Zoothamnium commune* from life (A-D, F), after protargol (E, G-I) and silver nitrate impregnation (J). **A**- colony form; **B, C** - zooids at low magnification; **D** - zooid at high magnification, to show pellicular striations; **E** - to show epistomial membrane (arrow) and germinal kinety (arrowhead); **F** - detail of stalk, to show mitochondria in spasmoneme; **G** - to show the separated anterior end of outer kinety in peniculus 2 (arrow); **H** - lateral view, to show the aboral trochal band; **I** - to show infundibular polykineties; **J** - silverline system. P1-3 - infundibular polykinety 1-3. Scale bars: 300 µm (A); 100 µm (B); 50 µm (C).

Stalk with smooth surface, 16 μm in diameter in main trunk and 10 μm in diameter at distal ends of branches. Spasmoneme 7 μm in diameter in main trunk and 4 μm in diameter at distal ends of branches, and with numerous dark mitochondria (Fig. 6F).

Oral infraciliature of *Z. commune* formed of peristomial and infundibular parts separated by the epistomial membrane (Figs 5G, H; 6E arrow, G., I). Peristomial part consisting of haplo- and polykinety, which are parallel to one another throughout their length and make one and one-quarter turns of the peristome before entering the infundibulum. Within infundibulum haplokinety and polykinety spiral on opposite walls and end at border of cytostome. Infundibular part of haplokinety accompanied by germinal kinety in adoral half (Figs 5G; 6E, arrowhead) and a filamentous reticulum in aboral half (Fig. 5G). Infundibular polykinety transforms into three polykineties at adstomal half, designated P1-3 (Figs 5G, H; 6I). P1 consists of three parallel rows of kinetosomes that extend to the edge of the cytostome. The three rows of P2 originate in mid-infundibulum, adjacent to point where the polykinety differentiates into P1, the outermost row being slightly separated from the other two at this point (Figs 5H arrow; 6G, arrow); P2 terminates between and above adstomal ends of P1 and P3 (Fig. 5H). P3 is about half the length of P2. It originates in aboral half of infundibulum and comprises three parallel ciliary rows that terminate slightly above those of P1 (Fig. 5H).

Aboral trochal band composed of a row of loosely arranged double kinetosomes that encircle the aboral quarter of the cell (Fig. 6H).

Remarks: The current population is identified as *Zoothamnium commune* based on the following: (1) the stalk is alternately branched; (2) accessory branches are unequal in length giving the colony an asymmetrical shape; (3) zooids are similar in shape; (4) marine habitat (Kahl 1933).

In terms of the stalk branching pattern, body shape and size, *Zoothamnium hentscheli* Kahl, 1935 and *Z. thiophilum* Stiller, 1946 are similar to *Z. commune* (Table 2), although their infraciliature and silverline system remain unknown. However, both of these taxa are freshwater forms unlike the Qingdao population which is marine (Kahl 1935, Stiller 1946).

Zoothamnium sinense also has an alternately branched stalk and marine habitat, but differs from *Z. commune* in having distinctly smaller size zooids (*ca* 36-48 × 30-38 μm *vs.* 60-104 × 48-56 μm).

Zoothamnium plumula Kahl, 1933 also possesses an alternately branched stalk and is marine, but differs from the Qingdao population in having enlarged zooids at the distal ends of the stalk branches (*vs*. absent) in *Z. commune* and fewer silverlines between scopula and aboral trochal band (24-28 *vs*. 38-43 in *Z. commune*) (Song *et al.* 2002).

In general, the present study contains three alternatively branched species, which have similar living appearance and can be easily confused with each other as well as related congeners. Their previous misidentifications indicate that the criterion for identification on *Zoothamnium* species should be adequately improved.

Based on our study, we suggest that the following characters should be considered for species identification/separation: The branching pattern, the length of the main trunk below the first ramification, zooid differentiation (micro-/macrozooid), zooid size, appearance of oral border (single/double-layered), shape of macronucleus, location of contractile vacuole, arrangement of the third oral polykinety, number of silverline and habitat.

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Observation on a Japanese Population of *Pseudoamphisiella alveolata* (Kahl, 1932) Song *et* Warren, 2000 (Ciliophora: Hypotrichida): Morphology and Morphogenesis

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Summary. A population of *Pseudoamphisiella alveolata* was isolated from the coastal waters off Nagasaki, Japan. Its morphology and morphogenesis were studied by observations on organisms from life and following protargol impregnation. Morphometrical data of this form correspond well with those of the Chinese population, thus the validity of this species is confirmed. Its morphogenetic processes show a few that differ from those of *P. lacazei*, the type species of this genus, and main events are documented here: (1) The oral primordium of the opisthe and primary primordium of the fronto-ventral transverse cirri are possibly derived from an anarchic field of kinetosomes originating *de novo* on cell surface, and most ventral cirri do not contribute to the formation of these primordia; (2) The posterior portion of the parental adoral zone of membranelles is renewed *in situ* while the anterior part is retained by the proter; (3) Oblique streaks of FVT-anlagen in both dividers are generated from the division of primary primordium. Two buccal cirri are derived from the anterior two streaks of the FVT-anlagen; (4) The left marginal rows and dorsal kineties equally develop by "within proliferation"; the caudal cirri develop from the posterior end of each dorsal kinety anlage; (5) The anlagen for the right marginal rows originate parallel to FVT-anlagen; (6) So-called caudal cirri near the rightmost transverse cirri are actually migratory right marginal cirri. The present study demonstrates that morphogenetic data can be useful in differentiating species and genera with similar morphologies.

Key words: Hypotrichida, marine ciliate, morphology, morphogenesis, *Pseudoamphisiella alveolata.*

INTRODUCTION

Ontogenetic data have been widely used in the reconstruction of ciliate phylogenies (Corliss 1968). These data might be employed to separate morphologically similar taxa at genus or species levels (Foissner 1996). Recently studies showed that species and genera are often separated by minor - but none the less important - ontogenetic details (Wirnsberger *et al.* 1985; Hu and Song 2001a, b; Hu *et al.* 2000, 2003, 2004a, b; Blatterer and Foissner 2003).

The genus *Pseudoamphisiella* has been included in the recently erected family Pseudoamphisiellidae due to its unique cortical pattern in non-dividers and dividers (Song 1996, Song *et al.* 1997, Song and Warren 2000). Up to date, it comprises two forms: *P. lacazei*, the type species and *P. alveolata*. Hitherto, morphogenetic events have only been known for *P. lacazei*.

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In the present paper, new data on the morphology and morphogenesis of a Japanese population of *Pseudoamphisiella alveolata* are provided. These observations are compared with those from its congener described by Song *et al.* (1997). This study shows how morphogenetic data might be useful in separating genera or species with very similar morphologies.

MATERIALS AND METHODS

Samples were collected from the coastal waters in the New Fishing Port of Nagasaki (32° 48'N; 129° 46'), Japan. Water salinity was about 34‰, water temperature 21-23°C and pH 8.1-8.2. After isolation, specimens were cultured in boiled seawater to which squeezed rice grains were added to enrich bacteria. Cells were observed in life using differential interference contrast microscope. Mainly saturated mercury bichloride solution was used to fix organisms. To reveal the infraciliature, the protargol silver staining method according to Wilbert (1975) was applied. Body shape of live cells was drawn from slides without coverslips. Drawings of stained specimens were carried out with the help of a camera lucida. To make plain the changes during morphogenetic processes, old cirri are depicted only by contour whereas the new ones are blocked. Measurements were performed at magnifications of 100-1250×.

Terminology and systematic arrangements are according to Corliss (1979), Foissner (1982) and Borror and Wicklow (1983).

RESULTS

Morphology of the non-divider

Prior to this investigation, *Pseudoamphisiella alveolata* has not been reported from East China Sea, Japan, although it has previously been isolated at least three times (Kahl 1932, Borror 1963, Song and Warren 2000).

Description (Figs 1-3, 19-33, 38; Table 1): Cell *in vivo* 100-200 × 40-80 µm, usually 150 × 40 µm, length to width ratio 2.5-4.5:1. Body generally elongated, rather fragile and contractile, thus variable in shape; when extended during motion, both ends rounded, and left and right margins distinctly sigmoidal (Figs 19, 25), when stationary cell outline oval. Dorsoventrally flattened about 2:1. Adoral zone of membranelles conspicuous, about 1/3 of cell length, with distal end bending posteriorly far onto right side and towards posterior of cell (Figs 3, arrows; 21, double-arrowheads; 24, arrowheads).

Cell surface covered by conspicuous, hyaline alveolar layer, about 3 µm thick and visible even under low magnification (Fig. 25, arrows). When viewed dorsally, this alveolar layer is seen to have irregular polygonal structure (Fig. 27). Within alveolar layer are sparsely arranged extrusomes which are bar-like, about 1.5-2 µm long (Fig. 20, arrows).

Cytoplasm usually grayish to dark grey when examined under dissecting microscope, due to presence of numerous granular inclusions 5 to 10 µm in size. Neither contractile vacuoles nor food vacuoles observed.

Cilia of adoral membranelles *ca* 13 µm long. Frontal and transverse cirri strong, about 15-20 µm in length. Other cirri *ca* 12-14 µm long. Marginal cirri bending towards ventral side or lying along the ventral grooves, not protruding from outline and not discernible when observed from dorsal aspect, except in the posterior region. Dorsal cilia about 3 µm long, derived from the base of the alveolar layer (Fig. 26, arrows). Caudal cirri (CC) close-set, normally lying along posterior margin (Figs 1, 3; 31, arrowheads).

Locomotion typified by continuous crawling on substrate or on bottom of Petri dish, reacting quickly when disturbed by contracting and remaining motionless for a short while.

Usually two (rarely 3) ellipsoidal macronuclear nodules positioned in center of body (Figs 2, 21; 30, arrows). One to six micronuclei, about 1-2 µm long, often within indentations of macronuclear nodules (Figs 2, arrows; 30, arrowheads).

Infraciliature Figs 1-3, 21, 28, 29, 31-33: Adoral zone of membranelles (AZM) composed of about 40 membranelles. Paroral membrane (PM, Figs 3; 28, arrowhead; 29, arrow; 33) conspicuously short, almost parallel to endoral membrane (EM, Figs 3; 28, double-arrowhead; 29, arrowhead). Constantly three large frontal cirri (FC; Figs 1; 21, arrowheads; 33, 38, arrows), of which the rightmost one is located posterior to the distal end of the AZM, and two buccal cirri (BC) separated from each other near mid-body of endoral membrane (Figs 3; 28, arrows; 33, arrowheads). Ventral row 1 (VR1) relatively short, terminating anteriorly at the rightmost frontal cirrus; anterior end of ventral row 2 (VR2) extending to buccal cirri. Highly developed, close-set transverse cirri (TC) arranged in a J-shaped row, terminating near the posterior end of body (Figs 1, 3, 21; 22, arrow; 29, 32). Posterior end of left marginal row (LMR) continues with row of caudal cirri (CC), which renders it difficult to determine where LMR terminates during interphase (Figs 1; 31, arrowheads). Right marginal row toward the center of the cell and parallel to ventral row 1, thus not convergent with row of

Table 1. Morphometrical characteristics of *Pseudoamphisiella alveolata* (Kahl, 1932) Song *et* Warren, 2000 from Nagasaki New Fishing Port. Data are based on protargol-impregnated specimens. Measurements in µm. CV - coefficient of variation in %, Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of specimens examined, SD - standard deviation, SE - standard error of the mean.

Character	Min	Max	Mean	SD	SE	CV	n
Body length	93	150	113.7	12.95	2.59	11.4	25
Body width	45	96	68.2	15.01	4.75	22.0	10
Length of adoral zone of membranelles	32	43	37.3	3.44	0.69	9.2	25
Number of adoral membranelles	37	43	40.2	1.83	0.37	4.6	25
Number of frontal cirri,	3	3	3	0	0	0	25
Number of buccal cirri	2	2	2	0	0	0	25
Number of cirri in ventral row 1	11	15	12.7	0.79	0.16	6.2	25
Number of cirri in ventral row 2	10	15	12.0	1.10	0.22	9.2	25
Number of cirri in left marginal row	15	30	20.5	3.14	0.50	15.3	39
Number of cirri in right marginal row	12	16	13.0	0.89	0.18	6.8	25
Number of extra right marginal cirri	2	4	3.4	0.90	0.14	2.3	40
Number of caudal cirri	8	12	10.1	0.98	0.16	2.5	40
Number of transverse cirri	13	17	14.3	0.95	0.19	6.6	25
Number of dorsal kineties	9	12	10.8	1.09	0.36	10.1	9
Number of macronuclear nodules	2	3	2.1	0.27	0.04	0.7	40
Length of macronuclear nodule	14	30	20.0	4.13	0.83	20.7	25
Width of macronuclear nodule	7	25	13.6	3.95	0.79	29.0	25
Number of micronuclei	1	6	3.1	1.18	0.19	3.0	40

CC posteriorly. Nevertheless, a few extra, sparsely distributed right marginal cirri located nearby the rightmost caudal cirrus (Figs 1; 3, arrowheads; 21, arrows; 38, arrowheads). Fibrils from TC are highly developed and associated with those of ventral cirri (Fig. 29). Mostly dorsal kineties extending over entire length of body, with 2 or 3 kineties on lateral sides (Figs 1-3; 31, 32 arrows).

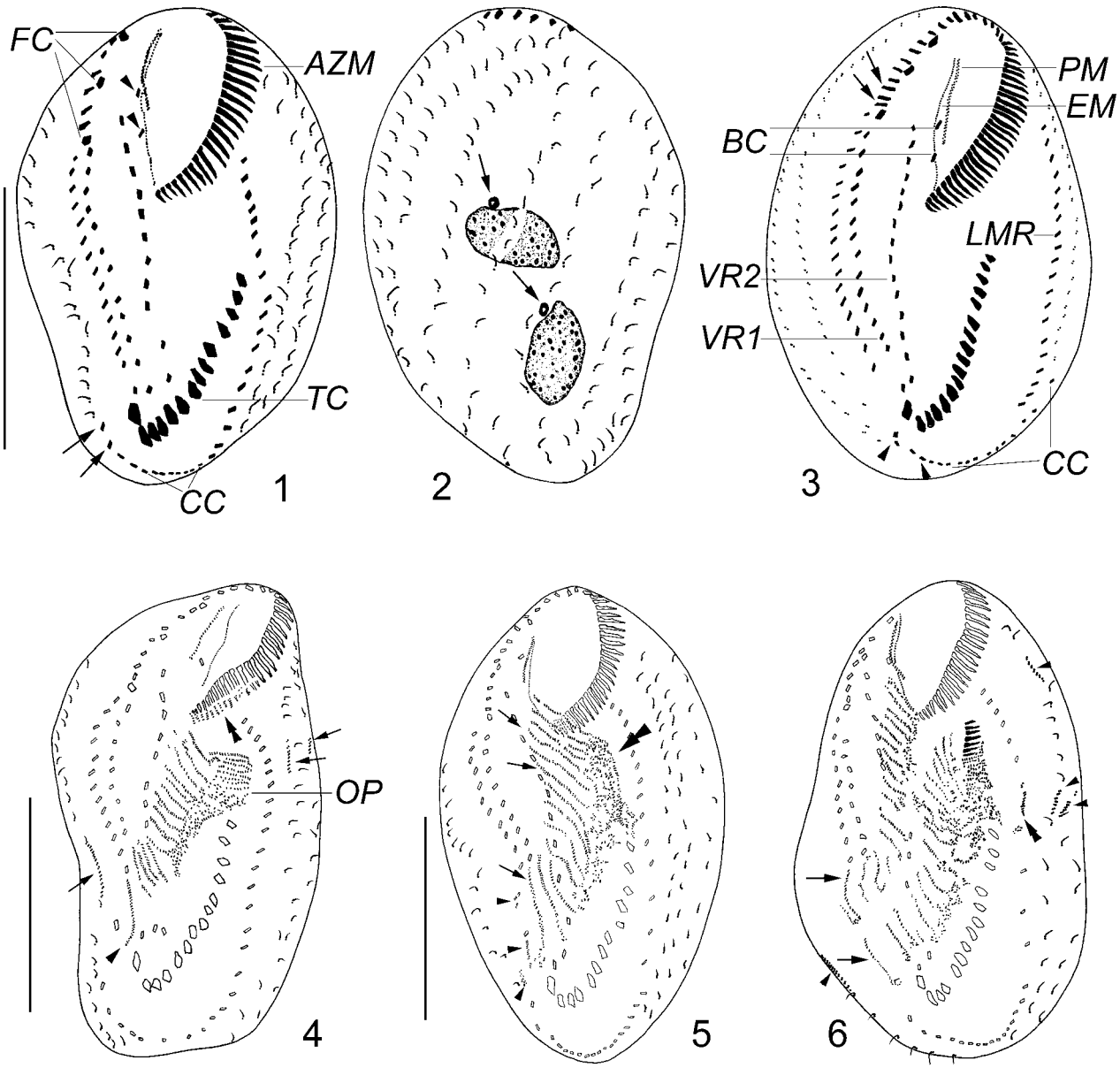
Divisional morphogenesis (Figs 4-18, 34-37, 39-49)

The first stage of morphogenesis was not observed. However, observations on an early stage of recognization (Figs 4, 35) allows us to deduce that morphogenesis seems to commence apokinetally with the appearance of loosely arranged basal bodies behind buccal cavity and between rows of left marginal cirri and ventral cirri. Furthermore, with the proliferation of these basal bodies this anarchic field is enlarged, later gradually splits into two parts, one is oral primordiun (OP) of the opisthe and the other is the cirral anlagen (FVT-anlagen, Figs 4; 35, arrows). During this process, most old cirri appear to remain intact and thus do not contribute to the formation of these anlagen.

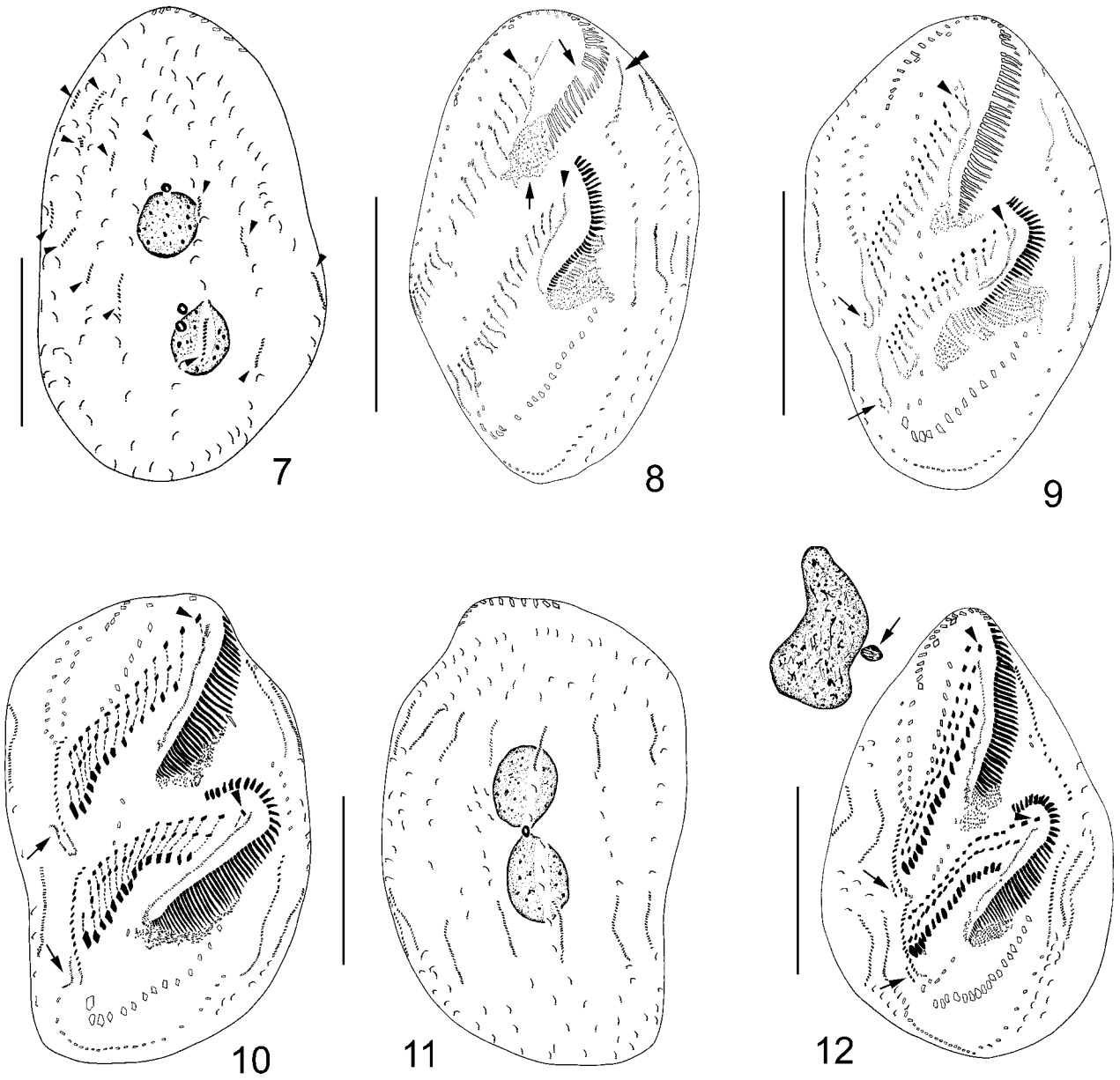
Then, the FVT-anlagen become larger with further joining of more basal bodies, that is, the number of the cirral steaks increases, and each steak is lengthened. Some steaks begin to divide at their mid-point (Figs 5, 36, arrows). Posterior to and right of them, a small anlage begin to develop intrakinetally in the posterior portion of the right marginal row (Fig. 5, arrowheads).

In the next stage, each streak of FVT-anlagen divide into two parts, thus two groups of FVT-anlagen, each of which has about 13-19 oblique streaks, are formed for the proter and the opisthe respectively (Fig. 39, arrows). The anlage for right marginal row is formed parallel to FVT anlagen and then splits into two (Figs 6, arrows; 39, arrowheads), each part accompanied by several loosely arranged kinetosomes. Meanwhile, new membranelles differentiate at the left anterior end of the OP. At the same time, the anlage for the left marginal row of the opisthe begins to appear in the middle port of the parental row (Fig. 6, double-arrowhead), and two separate anlagen occur in each old dorsal kinety (Figs 6, 7, arrowheads; 37, arrows), which develop to replace the old structures (Figs 8-18; 34, 42, arrows). One caudal cirrus will be derived from the posterior end of each dorsal kinety (Figs 13, 14, arrowheads; 45, 47, arrows).

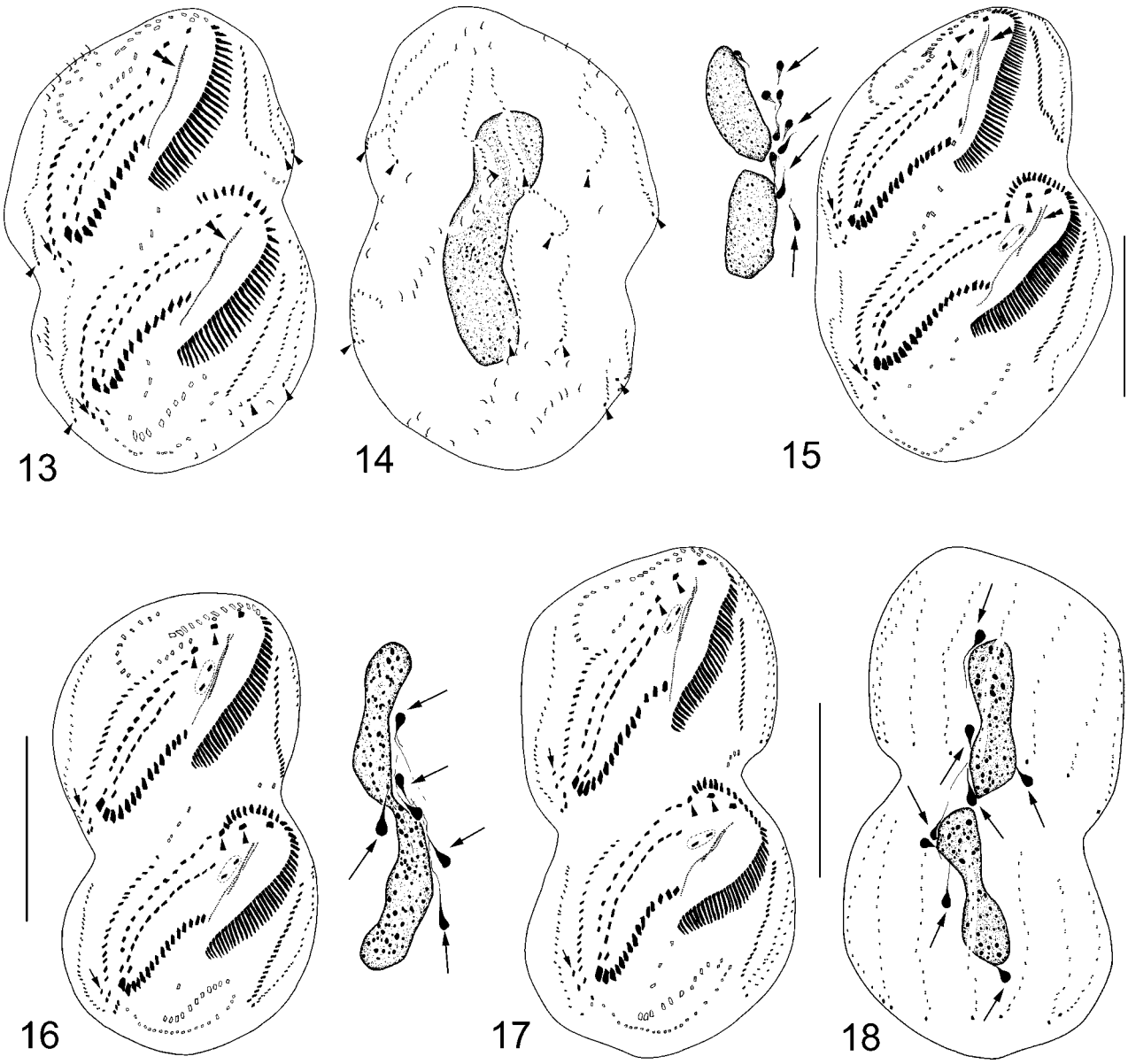
Slightly later, all cirral anlagen start to divide into segments (Figs 8; 41, arrows). In the proter, the parental paroral and endoral membranes begin to dissolve and participate in the formation of the undulating membrane



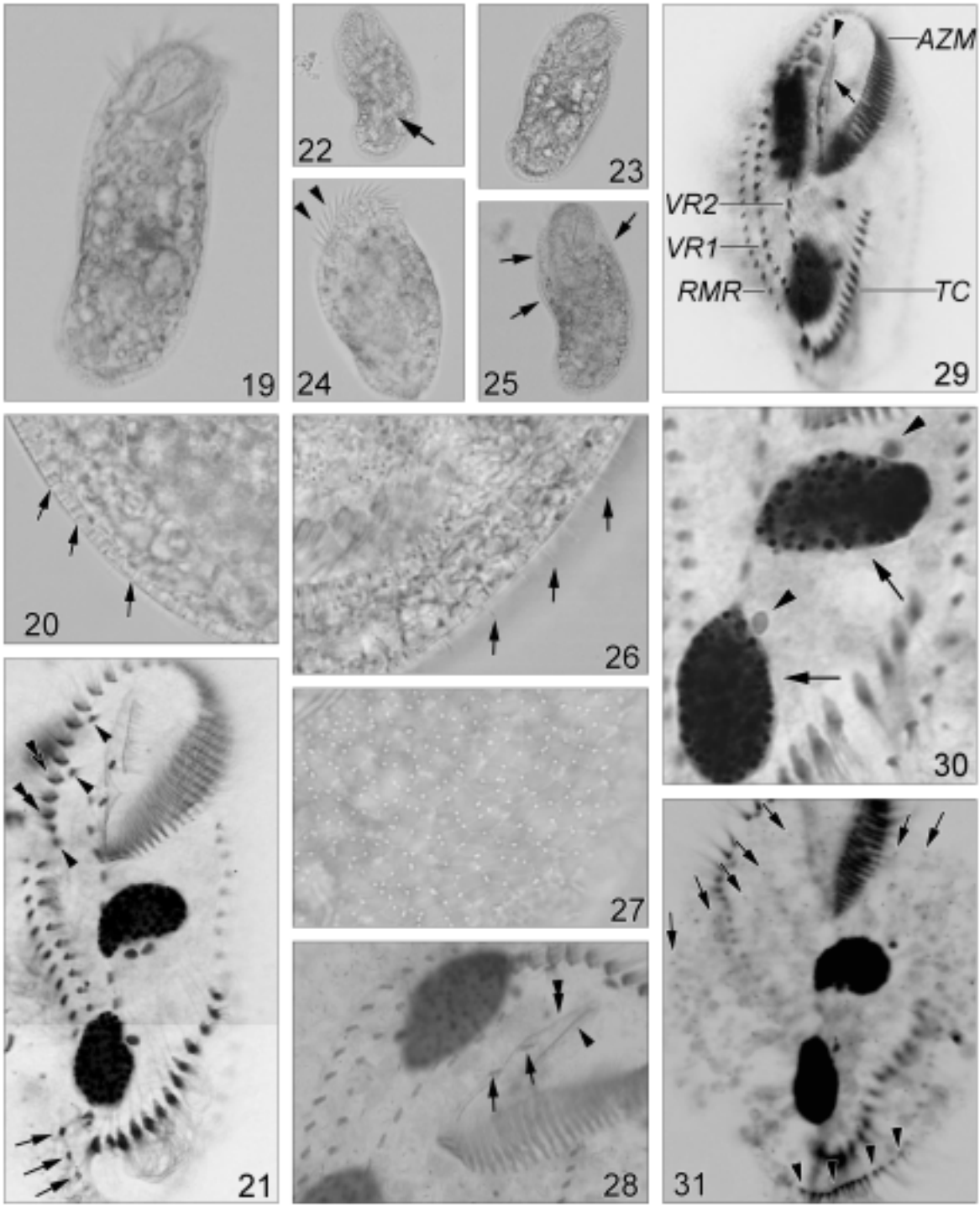
Figs 1-6. *Pseudoamphisiella alveolata* after protargol impregnation. **1, 2** - infraciliature of the ventral and dorsal side and nuclear apparatus in the same specimen. Arrows in Fig. 1 mark the extra right marginal cirri, while in Fig. 2 denote micronuclei. Arrowheads indicate buccal cirri. **3** - infraciliature of ventral side in the cell just after division. Arrows mark the bases of the membranelles at the distal end of the adoral zone. Arrowheads denote extra right marginal cirri. **4-6** - infraciliature of the ventral side of an early recognizer or divider. Arrows in Figs 4-6 mark the anlagen for the dorsal kineties (4), fronto-ventral transverse cirri (5) and the right marginal row (6). Arrowheads in Figs 4, 5 mark the anlagen for right marginal row, while in Fig. 6 denote dorsal kineties anlagen. Double-arrowheads in Figs 4-6 marks the disaggregated the adoral zone of membranelles at its posterior part (4), the oral primordium of the opisthe (5) and the anlage for the left marginal row of the opisthe (6). AZM - adoral zone of membranelles; BC - buccal cirri; CC - caudal cirri; EM - endoral membrane; FC - frontal cirri; LMR - left marginal row; OP - oral primordium; PM - paroral membrane; TC - transverse cirri; VR1, 2 - ventral row 1, 2. Scale bars: 50 μm.



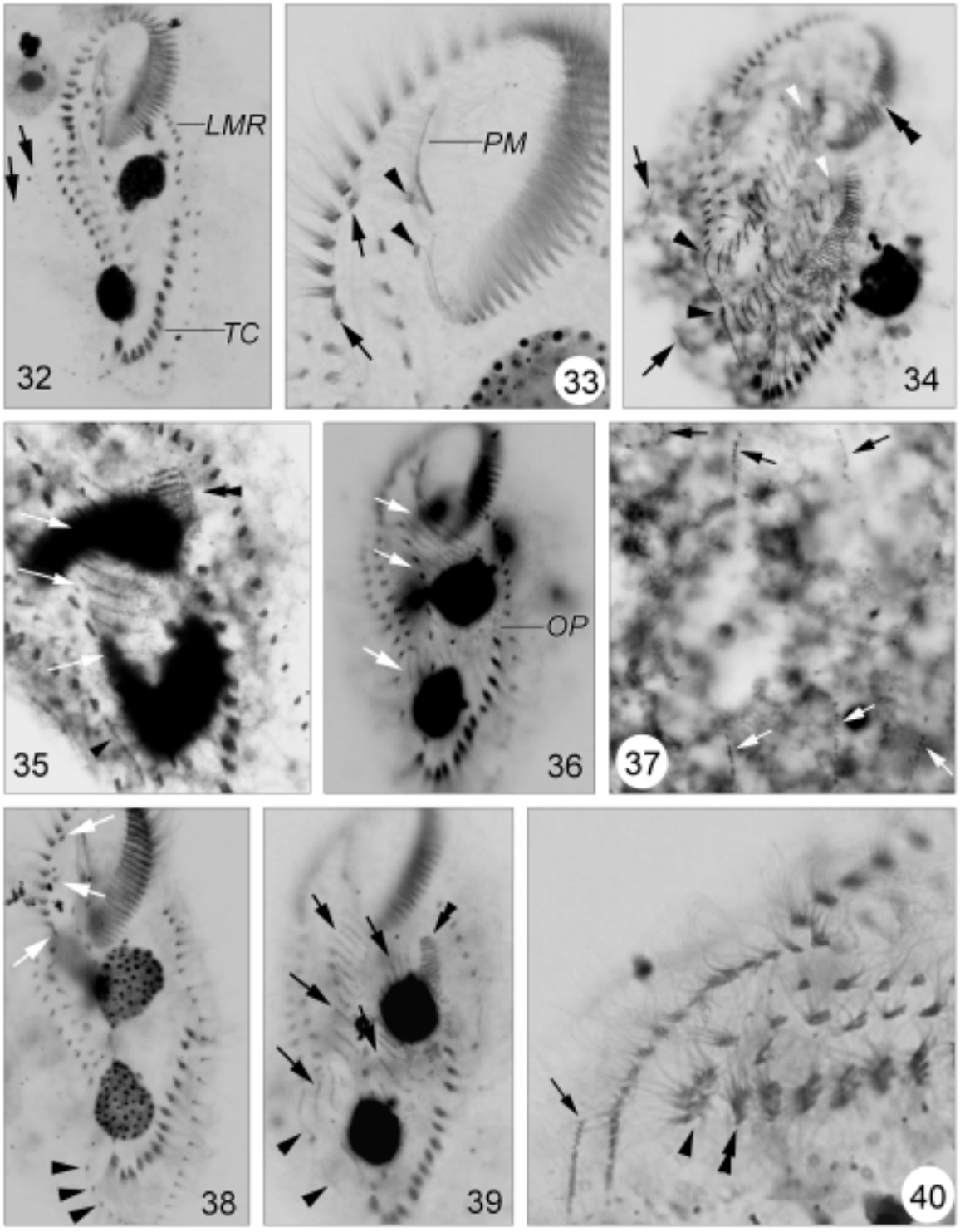
Figs 7-12. *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (8-10, 12) and dorsal (7, 11) sides. Parental structures in white, new structures in black. **7** - the same cell as that in Fig. 6. Arrowheads mark the dorsal kineties anlagen. **8** - early divider, which has two sets of anlagen for the cirri and the dorsal kineties. Arrows mark dedifferentiation of the parental proximal portion. Arrowheads mark the anlagen for the undulating membranes, and show the deorganization of the posterior buccal cirrus. Double-arrowhead denotes the anlage for the left marginal row of the proter. **9** - early to middle divider. Note that fronto-ventral transverse cirral anlagen begins to generate new cirri. Arrows mark extra small anlagen beside the posterior end of the anlagen for the right marginal rows. Arrowheads denote the leftmost frontal cirri derived from the anterior end of the undulating membranes anlage. **10, 11** - middle divider. Note that almost all fronto-ventral transverse cirral anlagen have produce three cirri each except for the posterior two anlagen, which form 4 and 2 cirri respectively. The cirri originating from the same anlage are connected by a broken line. Arrows mark lengthened extra anlagen. Arrowheads show the anteriormost frontal cirri. Two separate anlagen occur within each parental dorsal kinety, and two macronuclear nodules move together. **12** - middle divider. Note that cirri are formed from the extra anlagen (arrow) and that the new cirri are beginning to move. Arrowheads mark the anteriormost frontal cirri. Inset: fused macronuclear mass and micronucleus (arrow). Scale bars: 40 μm (7), 80 μm (8, 9) and 50 μm (10-12).



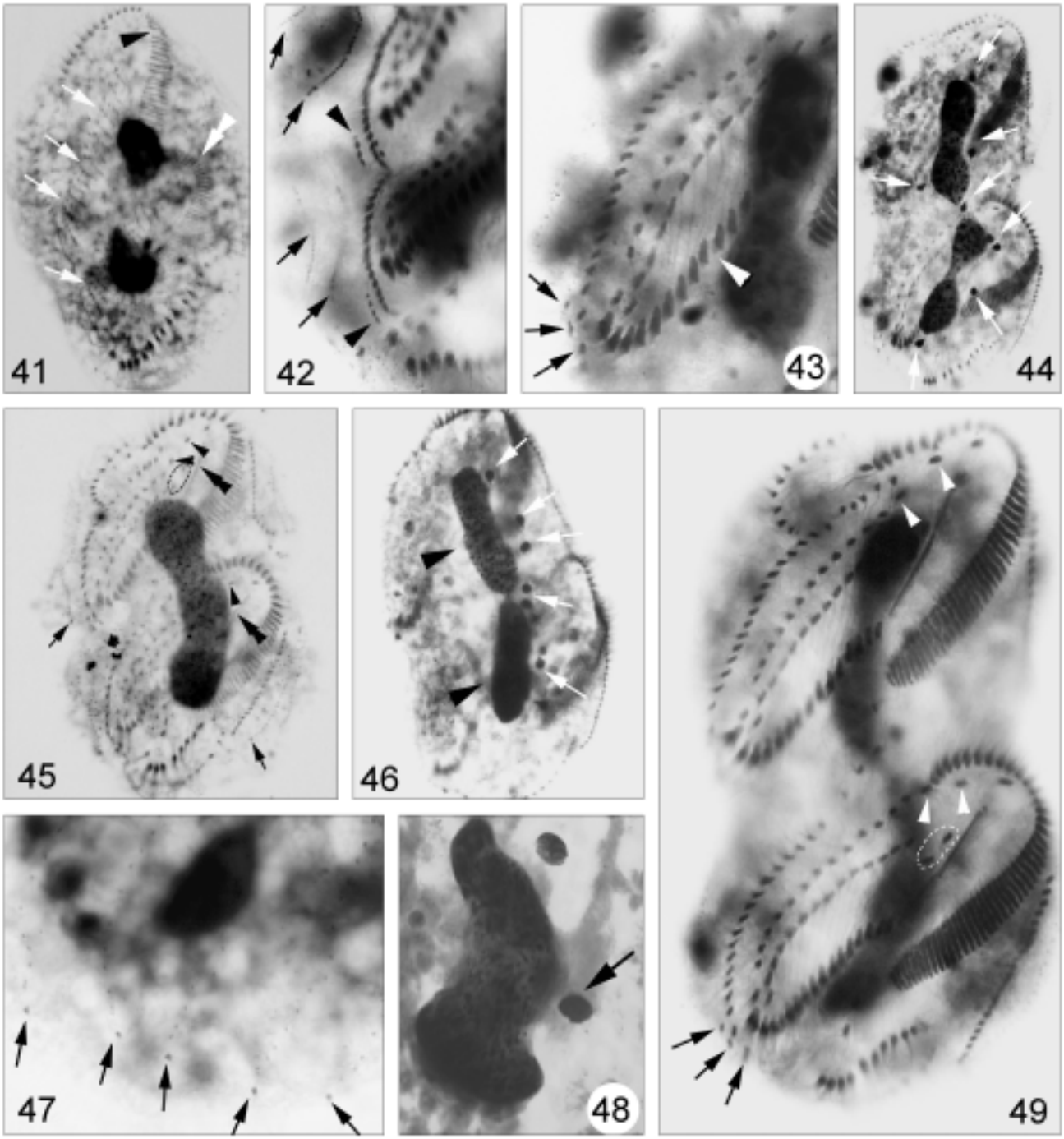
Figs 13-18. *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (13, 15-17) and dorsal sides (14, 18). Parental structures in white, new structures in black. **13, 14** - middle to late divider. Note the formation of the new oral and somatic ciliature. Small arrows in Figs 13, 15-17 mark the extra right marginal cirri which start to move posteriorly. Arrowheads denote caudal cirri originated from the posterior end of the dorsal kineties anlagen. Double-arrowheads mark the streak-like undulating membranes anlagen composed of disorganized kinetosomes. **15-18** - very late divider to note the migration of all new structures and the formation of paroral and endoral membranes (double-arrowheads). The two buccal cirri encircled by a broken line. Arrowheads mark the other two frontal cirri. Insets: division of macronucleus and micronuclei (long arrows). Scale bars: 50 µm



Figs 19-31. Photomicrographs of *Pseudoamphisiella alveolata* from life (19, 20, 22-27) and after protargol impregnation (21, 28-31). Ventral (19-30) and dorsal (31) views. **19** - extended body with anterior part cephalized. **20, 26, 27** - portion of cortex in side (20, 26) and top (27) views to show extrusomes (arrows in 20), dorsal bristles (arrows in 26) and the polygonal-shaped alveolar layer. **21, 29** - infraciliature. Arrows, arrowheads and double-arrowheads in Fig. 21 mark the extra marginal cirri, frontal cirri and the bases of adoral membranelles at distal end respectively. Arrow and arrowhead in Fig. 29 indicate endoral and paroral membranes respectively. **22-25** - different cells in contracted state. Arrows mark highly developed transverse cirri (22) and hyaline alveolar layer covering cell surface (25). Arrowheads denote the adoral membranelles at distal end. **28** - portion of infraciliature, note paroral (arrowhead) and endoral (double-arrowahead) membranes and buccal cirri (arrows). **30** - note macronuclear nodules (arrows) and micronuclei (arrowheads). **31** - note dorsal kineties (arrows) and caudal cirri (arrowheads). AZM - adoral zone of membranelles; RMR - right marginal row; TC - transverse cirri; VR1, 2 - ventral row 1, 2.



Figs 32-40. Photomicrographs of *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (32-36, 38 - 40) and dorsal (37) sides. **32** - arrows mark dorsal kineties. **33, 38** - note frontal cirri (arrows), buccal cirri (arrowheads in 33) and the extra marginal cirri (arrowheads in 38). **34, 37** - note the anlagen for the right marginal rows (black arrowheads), dorsal kineties anlagen (arrows), anlagen for undulating membranes (white arrowheads) and dedifferentiation of the parental oral apparatus (double-arrowhead). **35, 36, 39** - note oral primordium (double-arrowheads), fronto-ventral transverse cirral anlagen (FVT-anlagen, arrows) and the anlagen for right marginal rows (arrowheads). **40** - note the extra anlage beside the posterior end of the anlage for the right marginal row (arrow), and the posterior two FVT-anlagen which have produced two and four cirri respectively (arrowhead and double-arrowhead). LMR - left marginal row; OP - oral primordium; PM - paroral membrane; TC - transverse cirri.



Figs 41-49. Photomicrographs of *Pseudoamphisiella alveolata* after protargol impregnation. Infraciliature of ventral (41-46, 48, 49) and dorsal (47) sides. **41** - note FVT-anlagen which start to develop into new cirri (arrows), disaggregation of the parental adoral membranelles (arrowhead) and the new membranelles curving to the right (double-arrowhead). **42, 48 -** the same cell. Note the dorsal kineties anlagen (arrows in 42), the newly formed extra marginal cirri (arrowheads), fused macronuclear mass and micronucleus (arrow in 48). **43** - note the newly-formed extra marginal cirri (arrows) and transverse cirri (arrowhead). **44, 47** - the same cell. Note the dividing of macronuclear nodules and micronuclei (arrows in 44) and caudal cirri derived from the posterior end of the dorsal kineties (arrows in 47). **45** - middle divider. Note that the development of the oral and somatic ciliature is nearly completed. Arrows mark the new caudal cirri at the posterior end of the dorsal kineties. Arrowheads mark the frontal cirri from the anterior two streaks of fronto-ventral transverse cirral anlagen. Double-arrowheads denote the streak-like undulating membranes anlagen. Two buccal cirri are encircled by a broken line in the proter. **46** - late divider. Note division of macronuclear nodules (arrowheads) and micronuclei (arrows). **49** - very late divider. Note that all new cirri continue to migrate. Arrows mark the extra marginal cirri. Arrowheads mark new frontal cirri from the anterior two streaks of the fronto-ventral transverse cirral anlagen. Two buccal cirri are encircled by a broken line in the opisthe.

Table 2. Morphological and morphometrical comparison of *Pseudoamphisiella alveolata* and *P. lacazei*. Measurements in µm.

Character	*Pseudoamphisiella alveolata*	*P. alveolata*	*P. lacazei*	*P. lacazei*
Body size *in vivo*	120-200 × 50-70	120-240 × 50-80	120-300 × 40-80	200-300 × 70-100
Number of adoral membranelles	37-43 (n=25)	47-59 (n=9)	39-49 (n=25)	50-56
Number of buccal cirri	2 (n=25)	2 (n=18)	2 (n=25)	2
Number of frontal cirri	3 (n=25)	3 (n=18)	3 (n=25)	3
Number of cirri in ventral row 1	11-15 (n=25)	10-14 (n=11)	11-15 (n=26)	14-16
Number of cirri in ventral row 2	10-15 (n=25)	11-15 (n=11)	16-23 (n=26)	16-21
Number of cirri in left marginal row	15-30 (n=39)	14-20 (n=11)	21-31 (n=16)	66-73*
Number of cirri in right marginal row	12-16 (n=25)	12-14 (n=11)	20-29 (n=16)	-
Number of transverse cirri	13-17 (n=25)	12-16 (n=11)	16-23 (n=26)	*ca* 20
Number of caudal cirri **	11-16 (n=40)	11-16 (n=9)	9-11 (n=5)	-
Number of dorsal kineties	9-12 (n=9)	10-12 (n=16)	8-11 (n=25)	9-11
Number of macronuclei	2-3 (n=40)	2 (n=16)	24-57 (n=20)	50-60
Number of micronuclei	1-6 (n=40)	2-5 (n=11)	7-10 (n=6)	-
Alveolar covering	present	present	absent	absent
Data source	This study	Song and Warren (2000)	Song *et al*. (1997)	Song (1996)

* including caudal, left and right marginal cirri; ** including the extra right marginal cirri

anlage (UM-anlage; Fig. 8, anterior arrowhead); the posterior part of the old adoral zone of membranelles becomes dedifferentiated and the middle membranelles get resorbed (Figs 8, arrows; 41, arrowhead). While in the opisthe, the undulating membranes anlage (Fig. 8, posterior arrowhead) detaches from the main body of the oral primordum, which extends anteriorly and curves to right with the increase in the number of membranelles (Fig. 41, double-arrowhead). Meanwhile another anlage occurs at the anterior of the parental left marginal row (Fig. 8, double-arrowhead). Subsequently, new cirri are gradually formed in the FVT-anlagen (Fig. 9). A small anlage is derived from the anterior end of the undulating membranes anlage in both dividers (Fig. 9, arrowheads), which eventually forms the anteriormost frontal cirrus (Figs 10, 12, arrowheads). Other anlagen develop further. Near the posterior part of the right marginal row anlagen, two small extra anlagen appear (Figs 9, arrows; 40, arrow).

Thereafter, three new cirri are formed in each streak of the FVT anlagen except the posteriormost two, which produce four and two cirri respectively (Figs 10, 12; 40, double-arrowhead and arrowhead). The extra anlagen lengthen and fragment into 2-4 cirri (Figs 10, 12, 13, 15-17, 40, arrow; 42, arrowheads; 43, 49, arrows), which will move posteriorly to the newly formed caudal cirri in the interphase cell (Fig. 3, arrowheads). As the cell elongates in the next stages, all these new cirri migrate and are accurately positioned to form the mature cortical pattern: the first cirrus from the two anterior streaks develop into two frontal cirri (Figs 15-17, 45, arrowheads), while the second cirrus develop into two buccal cirri (encircled in Figs 15-17, 45, 49); each streak of FVT-anlagen contributes the last cirrus to form the row of transverse cirri (Fig. 43, arrowhead) the remainder will constitute the two ventral rows (Figs 15-17, 45, 49). At this time, new cirri begin to organize proteriad in the anlagen of the marginal rows (Figs 10, 12, 13). In oral primordium, new adoral membranelles continue to be arched in the opisthe, while in the old oral appratus the posterior part is gradually renewed *in situ* (Figs 10, 12), and the re-built membranelles combine with the anterior old ones to generate the new adoral zone (Fig. 13). Then the undulating membranes anlagen split longitudinally to form paroral and endoral membranes (Fig. 15, double-arrowheads). Next, the daughter dividers start to separate (Figs 16, 17, 45, 49) and with the completion of the cytostome become trophic cells once again.

Nuclear apparatus evolves in the usual way: the fused macronuclear mass (Figs 12, inset; 48) can be observed with an enlarged micronucleus (Figs 12, inset; 48, arrow)

in the middle stage. Both divide and be assigned to two daughters at late stages (Figs 14, 15, 16, insets; 18; 44, 46, arrows and arrowheads).

DISCUSSION

Within the genus *Pseudoamphisiella*, only two species have been described using silver staining method. Based on the data from this study and previous descriptions, the most readily observed differences (Table 2) between the two are: the number of macronuclei (2-3 in *P. alveolata vs.* 24-60 in *P. lacazei*) and the conspicuous alveolar layer (present in *P. alveolata vs.* absent *P. lacazei*).

The Japanese population of *Pseudoamphisiella alveolata* has relatively stable morphological characters as indicated by the low coefficients of variation for the morphometrical data (Table 1). Our population corresponds well with the descriptions by Kahl (1932), Borror (1963) and Song and Warren (2000) in terms of body shape and size, characteristic cortical structure (e.g. cell surface covered by alveolar layer), nuclear apparatus and the cirral pattern, especially the widely separated ventral rows, all of which indicate that these four forms are conspecific. Nevertheless, minor differences exist in morphometrical data, e.g. number of adoral membranelles (Table 2, Kahl 1932, Borror 1963), which can be population dependent. Compared with the Qingdao population, Japanese population has relatively fewer caudal cirri because, due to a lack of morphogenetic data, Song and Warren (2000) misidentified the extra right marginal cirri at the posterior end of body as caudal cirri.

In terms of morphogenesis, *Pseudoamphsiella* is very similar to the genera of the suborder Urostylina, e.g. *Holosticha*, *Pseudokeronopsis*, *Thigmokeronopsis*, in having only one marginal cirral anlage on each side and numerous oblique cirral anlagen (Hu and Song 2001a, b; Hu *et al.* 2000, 2003, 2004a, b). However, these anlagen do not develop into frontoterminal cirri and a zig-zag structured midventral row but rather into two widely separated ventral rows. Within this genus, *P. alveolata* resembles *P. lacazei* in the combination of the following features: the oral primordium of the opisthe and FVT-anlagen originate *de novo*, and most ventral cirri do not contribute to the formation of these primordia; the two buccal cirri are derived from the anterior two streaks of the FVT-anlagen; the left marginal row and dorsal kineties develop by "within-proliferation"; one caudal cirrus is derived from the posterior end of each dorsal kinety anlage; the anlage for the right marginal row develops simulatenously and parallel to the posterior streaks of the FVT-anlagen; the macronuclear nodules evolve in the usual way as in other hypotrichs (Song and Wilbert 1994, Eigner 1995). However, difference between *P. alveolata* and *P. lacazei* include: (1) the parental adoral zone of membranelles is partly renewed in *P. alveolata*, that is, its posterior portion is rebuilt *in situ* whilst the anterior part is retained by the proter (vs. completely replaced in *P. lacazei*); (2) the presence of extra migratory marginal cirri in *P. alveolata* (vs. absence in *P. lacazei*); (3) joining of the old undulating membranes and (possibly) a few posterior right marginal cirri in the formation of new anlagen in *P. alveolata* (vs. not involvement of these structures in *P. lacazei*). Additionally, the oral primordium of the opisthe and the primary primordium of the fronto-ventral transverse cirri are possibly derived from an anarchic field of kinetosomes on the cell surface, and the FVT-anlagen of both dividers are formed by the separation of the primary primordium, which was not confirmed by the work of Song *et al.* (1997). Therefore, the present study further demonstrates the value of morphogenetic data in discriminating between species and genera with similar morphologies.

According to Borror and Wicklow (1983) the suborder Urostylina includes genera where "frontal ciliature differentiate from the longitudinal field of more than 5 oblique ciliary streaks". In a sense, *Pseudoamphisiella* undoubtedly has the same developmental pattern as the urostyline genera, e.g. *Holosticha* (Hu and Song 2001a, Hu *et al.* 2003) despite it lacks typical midventral rows; therefore it is reasonable to assign *Pseudoamphisiella* to the suborder Urostylina (Shi 1999). Furthermore, absence of frontoterminal (migratory) cirri in *Pseudoamphisiella* also exists in several urostyline genera such as *Urostyla* and *Australothrix* (Song *et al.* 1997).

Considering the similarity of the general developmental process of ciliary primordia between *Pseudoamphisiella alveolata* and *Psammocephalus faurei* (Wicklow 1982), it is possible that the family Pseudoamphisiellidae should belong to the suborder Discocephalina Wicklow, 1982, despite the fact that the infraciliature of *Pseudoamphiella* is very similar to that of *Pseudouroleptus* Hemberger, 1985 which Lynn and Small (2000) assigned to the order Stichotrichida (formerly Stichotrichina).

As mentioned above, the systematic assignment of *Pseudoamphisiella*/Pseudoamphisiellidae depends on which one is adopted among criteria such as similarity of infraciliature and/or morphogenetic pattern. However, the question about the significance of these criteria at the suborder level has not yet solved to date and thus we cannot assign the family Pseudoamphisiellidae to any suborder at present (Song *et al.* 1997).

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ACTA
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Morphology and morphogenesis of *Rubrioxytricha indica* n. sp. (Ciliophora: Hypotrichida)

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Summary. *Rubrioxytricha indica* n. sp. 80 × 30 µm *in vivo* was isolated from a pond in New Delhi, India. It possesses a single caudal cirrus and numerous brown coloured crystalloids in the cytoplasm and three contractile vacuoles. Uniformly scattered dark granules are present either as single entities or in clusters of 2-5. The ventral ciliature consists of 18 frontal-ventral-transverse cirri, one row each of right and left marginal cirri, three long dorsal kineties, two dorsomarginal rows, two undulating membranes (UMs) in *Cyrtohymena* pattern (Berger and Foissner 1997) and a question mark shaped adoral zone of membranelles. The cirrus V/3 participates in morphogenesis at very late stages of division; its kinetosomes merging with the posterior part of the developing UMs of the opisthe. There is no splitting of the third dorsal primordium. Consequently three dorsal kineties (DK_{1-3}) are formed and only one caudal cirrus develops at the end of DK_3.

Key words: diagnosis, morphogenesis, morphometry, *Rubrioxytricha indica* n. sp.

Abbreviations: AZM - adoral zone of membranelles, BL - body length, BW - body width, CC - caudal cirrus, CV - coefficient of variance, DK - dorsal kinety, DM - dorsomarginal, FVT - frontal-ventral-transverse, LMC - left marginal cirri, n - number of cells, OP - oral primordium, RMC - right marginal cirri, SD - standard deviation, UMs - undulating membranes.

INTRODUCTION

The genus *Rubrioxytricha* was erected by Berger (1999) by separating it from the genus *Oxytricha* on the basis of the presence of one or two caudal cirri and distinctly or slightly homogeneously coloured cytoplasm. Other features of the genus include, highly flexible body, question mark shaped adoral zone of membranelles, 18 frontal-ventral-transverse (FVT) cirri wherein posterior frontal cirri are arranged in a 'V' shaped pattern, 5 ventrals and transverse each and one row each of left marginal (LMC) and right marginal cirri (RMC). Two undulating membranes are arranged in the *Oxytricha* pattern (Berger and Foissner 1997). Cells also possess cortical granules. Berger (1999) has listed two species of this genus; *R. haematoplasma* comb. n. and *R. ferruginea* comb. n., originally reported as

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O. haematoplasma (Blatterer and Foissner 1990) and *O. ferruginea* (Stein 1859) respectively.

In the present study, we report the morphometric and morphogenetic data of *Rubrioxytricha indica* n. sp. and its comparison with the two described species of *Rubrioxytricha*, similar sized species of *Cyrtohymena* and similar sized species of *Oxytricha* possessing cortical granules.

MATERIALS AND METHODS

Rubrioxytricha indica n. sp. was isolated from a small pond in New Delhi (28°34'N, 76°07'E). Cells were acclimatized to the laboratory conditions and then grown at 23 ± 1°C with axenically cultured *Chlorogonium elongatum* as the food organism (Ammermann *et al.* 1974).

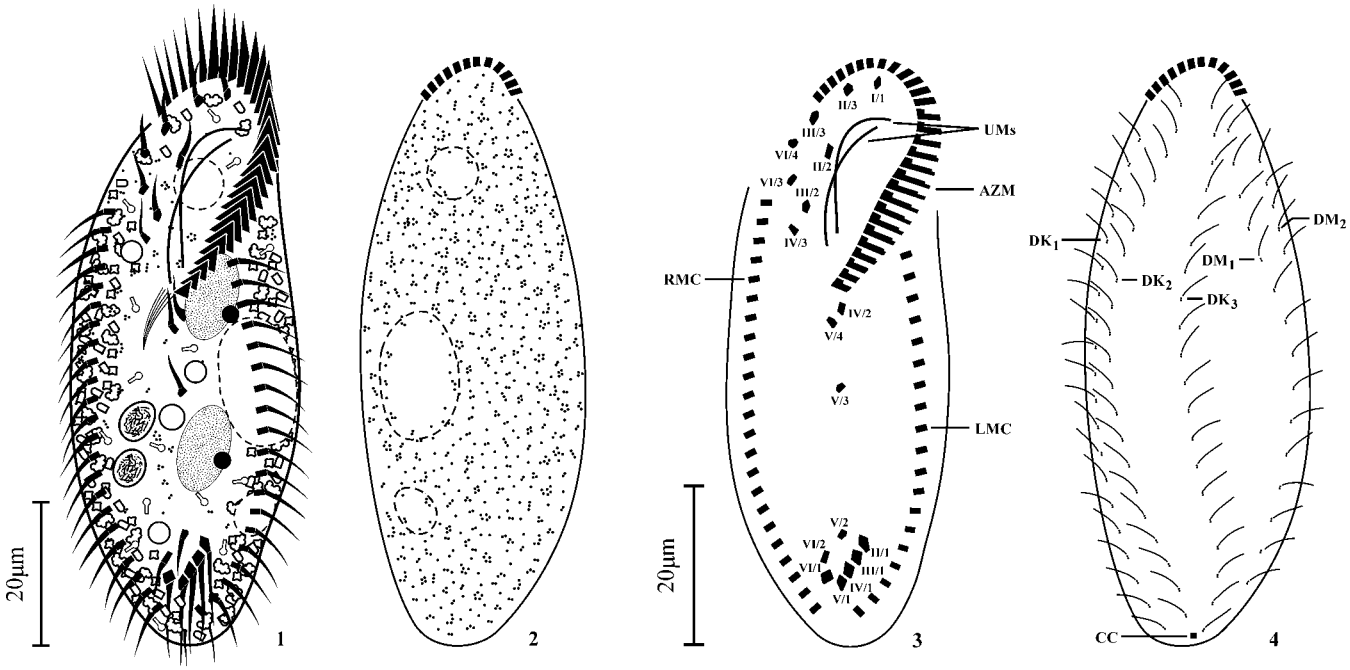
Morphometric characterization of non-dividing cells was performed after staining with the modified protargol method (Kamra and Sapra 1990). The enrobed cells were bleached for 4 minutes in a 5% dilution of commercial sodium hypochlorite solution before staining in 0.5% protargol (Roque). All measurements were performed using an ocular micrometer (Jena) with one unit = 32.5 µm. Measurements and counts were done at the magnification of 400× and 1000× respectively. Live cells were observed under differential interference contrast microscope at the magnification of 1000×. Observations on cortical granules and cytoplasmic crystalline inclusions were made under bright field microscope for confirming their colour etc. Magnification was calculated using a micrometer slide (E. Leitz). The general terminology followed was according to Wallengren (1900), Borror (1972) and Berger (1999).

For making schematic illustrations, protargol impregnated cells were projected on a table using a slide projector. The diagrams were further reproduced by scanning and using the computer program coral draw (version 8.0).

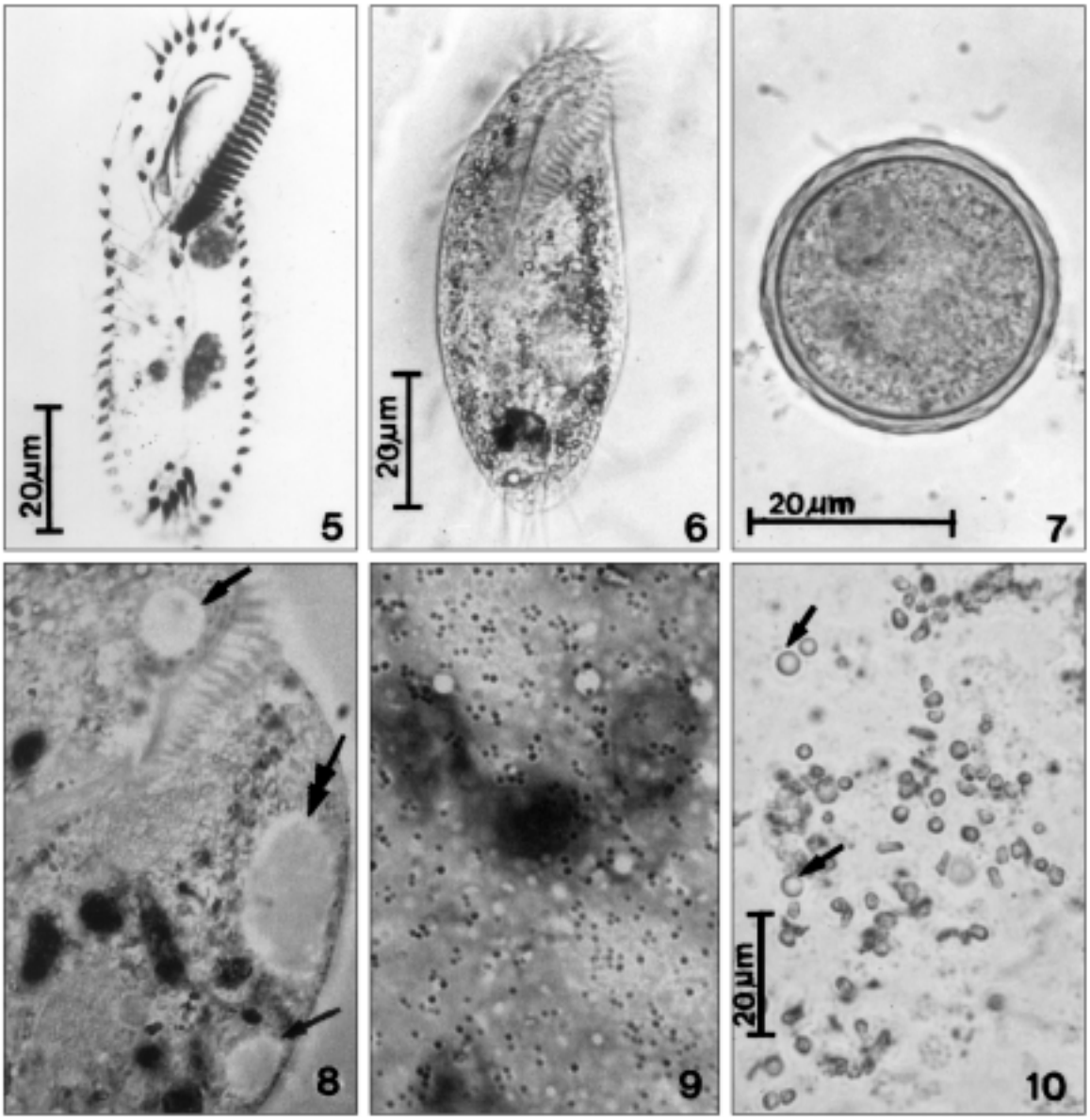
RESULTS

Rubrioxytricha indica n. sp.

Diagnosis: Size about 69 × 27 µm in protargol preparation and 80 × 30 µm *in vivo*; highly flexible and elliptical in shape. 2 macronuclear nodules and 2-3 micronuclei. Cytoplasm shows numerous dark brown crystalline inclusions, present in scattered dense aggregates. Cortical granules spherical and dark green in colour, uniformly distributed, arranged either singly or in clusters of 2-5. Adoral zone of membranelles (AZM) about 40% of body length. Two undulating membranes



Figs 1-4. *Rubrioxytricha indica* n. sp. **1** - ventral view *in vivo*; **2** - dorsal surface showing cortical granules; **3** - infraciliature of ventral surface: adoral zone of membranelles (AZM), undulating membranes (UMs), frontal cirri (II/2, I/1, II/3, III/3, VI/4, VI/3, IV/3 and III/2), ventral cirri (IV/2, V/4, V/3, V/2 and VI/2), transverse cirri (II/1, III/1, IV/1, V/1 and VI/1), right marginal cirral row (RMC), left marginal cirral row (LMC); **4** - dorsal surface: dorsal kineties (DK_{1-3}), dorsomarginal rows ($DM_{1\&2}$) and caudal cirrus (CC).



Figs 5-10. *Rubrioxytricha indica* n. sp. (5 - protargol impregnation; 6-10 *in vivo*). **5 -** infraciliature of ventral surface and nuclear apparatus of the holotype specimen. For detailed labeling of infraciliature see Fig. 3; **6 -** ventral view showing characteristic body outline; **7 -** resting cyst; **8 -** three contractile vacuoles in diastole in slightly squeezed (flattened) specimen; second contractile vacuole (double arrow) largest in size, the other two are smaller (arrows) (Mag. 1000×); **9 -** dorsal surface showing uniformly scattered spherical cortical granules arranged either singly or in clusters of 2-5 (Mag. 1000×); **10 -** crystalline inclusions of various shape and size and spherical lipid droplets (arrows) of squashed cell.

in *Cyrtohymena* pattern (See morphology and morphometry section for further details). On average about 22 cirri in right and 19 in left marginal row, 8 frontal, 5 ventral and 5 transverse cirri. Three dorsal kineties, 2 dorsomarginals and 1 caudal cirrus. Participation of cirrus V/3 in morphogenesis at very late stages and its kinetosomes merge with undulating membranes of opisthe.

Etymology: Species named after the country from where it has been isolated.

Slide deposition: Two slides have been deposited in the Oberösterreichische Landes-museum in Linz (LI), Austria.

Morphology and morphometry (Figs 1-10, 28; Table 1): Size about 69 × 27 µm in protargol preparations and about 80 × 30 µm *in vivo*, average length to width ratio 2.6:1. Shape elliptical, very flexible, dorsoventrally flattened. Generally bottom dwellers showing sluggish creeping movements. Two macronuclear nod-

Table 1. Morphometric characteristics of *Rubrioxytricha indica* n. sp. (Data from protargol impregnated non-dividing cells, n=20).

Characters	Mean	Min	Max	SD	CV
Body length (µm)	69.1	64.7	74.1	3.30	4.77
Body width (µm)	26.6	23.5	31.8	2.80	10.53
Body length/ Body width	2.6	2.2	3.0	0.22	8.46
Macronuclear nodules, number	2.0	2	2	0.00	0.00
Macronuclear nodules, length (µm)	11.8	10.8	12.7	0.63	5.34
Macronuclear nodules, width (µm)	7.8	6.9	8.4	0.46	5.89
Micronuclei, number	2.3	2	3	0.47	0.00
Micronuclei, diameter (µm)	3.2	3.1	3.3	0.10	3.13
Adoral Membranelles, number	29	27	31	1.36	4.69
Adoral zone length (µm)	27.6	23.5	30.6	2.14	7.75
Adoral zone length/ Body length	0.4	0.3	0.4	0.03	7.50
Frontal cirri, number	8.0	8	8	0.00	0.00
Ventral cirri, number*	5.2	5	7	0.64	0.12
Transverse cirri, number*	5.2	5	7	0.52	0.10
Number of cirri in left marginal cirral row**	19.2	17	21	1.25	6.51
Number of cirri in right marginal cirral row	22.3	21	24	1.09	4.89
Number of dorsal kineties (DKs)	3.0	3	3	0.00	0.00
Number of dorsomarginal rows (DMs)	2.0	2	2	0.00	0.00
Dorsal bristles, number in					
DK_1	19.6	18	21	1.07	0.05
DK_2	20.7	20	22	0.67	0.03
DK_3	19.8	18	23	1.47	0.07
DM_1	15.1	12	17	1.38	0.09
DM_2	4.6	3	7	1.56	0.34
Dorsal bristles, total number	79.0	76	88	3.33	0.04
Caudal cirri, number	1.0	1	1	0.00	0.00

*Nearly 14 % cells show one or two supernumerary ventral/ transverse cirri (n=400),** 12% cells show a second LMC with few cirri (n=100)

Table 2. Parental structures associated with the origin of FVT primordia and undulating membranes during division morphogenesis of *Rubrioxytricha indica* n. sp.

Daughter cell	Parental structure	Primordium number/structure
Proter	Parental undulating membranes	I
	II/2 + kinetosomes of streak II of opisthe	II
	III/2	III
	IV/3	IV
	Streak V of opisthe	V
	Streak VI of opisthe	VI
Opisthe	Oral primordium	I
	Oral primordium	II
	Oral primordium	III
	IV/2	IV
	V/4	V
	V/4	VI
	V/3	Posterior part of undulating membranes of opisthe

Table 3. Morphometric comparison of three species of *Rubrioxytricha.* All dimensional measurements are averages in µm.

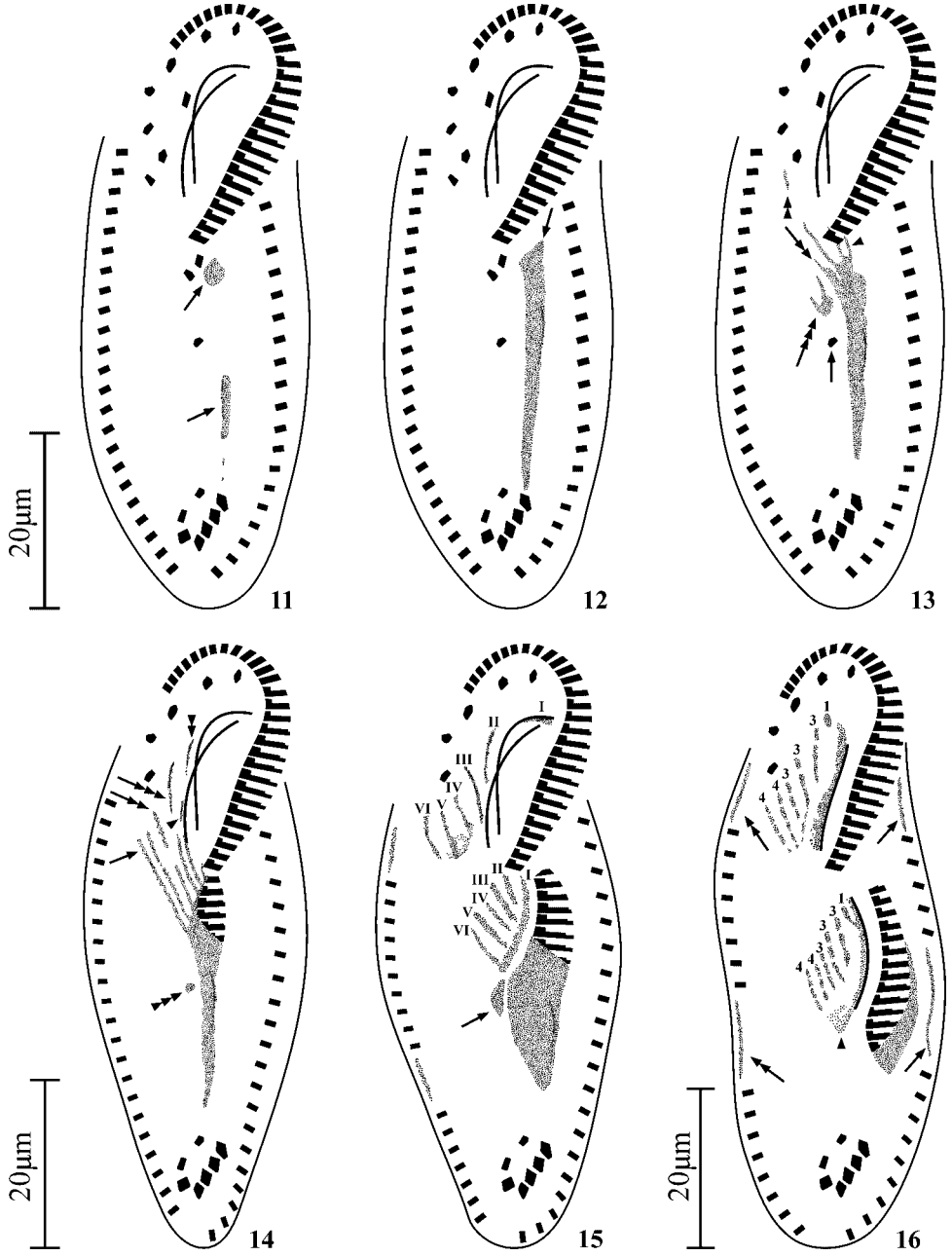
Characters	*R. indica* n. sp. (Present report)	*R. haematoplasma* (Blatterer and Foissner 1990, Berger 1999)	*R. ferruginea* (Stein 1859, Song and Wilbert 1989, Berger 1999)
Body shape	Elliptical, both ends rounded	*	Both ends rounded with concave margins
Body length (BL)	69.1	113.3	143.4
Body width (BW)	26.6	41.4	47.2
Body length/ Body width	2.6:1	2.7:1	3.0:1
Macronuclear nodules, length	11.8	15.9	*
Macronuclear nodules, width	7.8	8.6	*
Micronuclei, number	2-3	2	2
Adoral membranelles, number	29	38.4	38.3
Number of cirri in left marginal cirral row	19.2	35.8	33.0
Number of cirri in right marginal cirral row	22.3	33.4	32.7
Dorsal rows	5.0	4.0	5.0
Caudal cirri	1	1	1-2
OP origin	Dual; in two parts	*	*
Colour of cortical granules	Dark green	Lemon yellow to green	Brownish
Arrangement of cortical granules	Uniformly scattered	In rows	In rows
Colour of the cytoplasm	More or less colourless	Usually distinctly or rarely slightly homogeneously orange to reddish	Homogeneously rusty brown
Cytoplasmic inclusions	Numerous sharp dark crystalline bodies in groups	*	*

* Not mentioned by the authors.

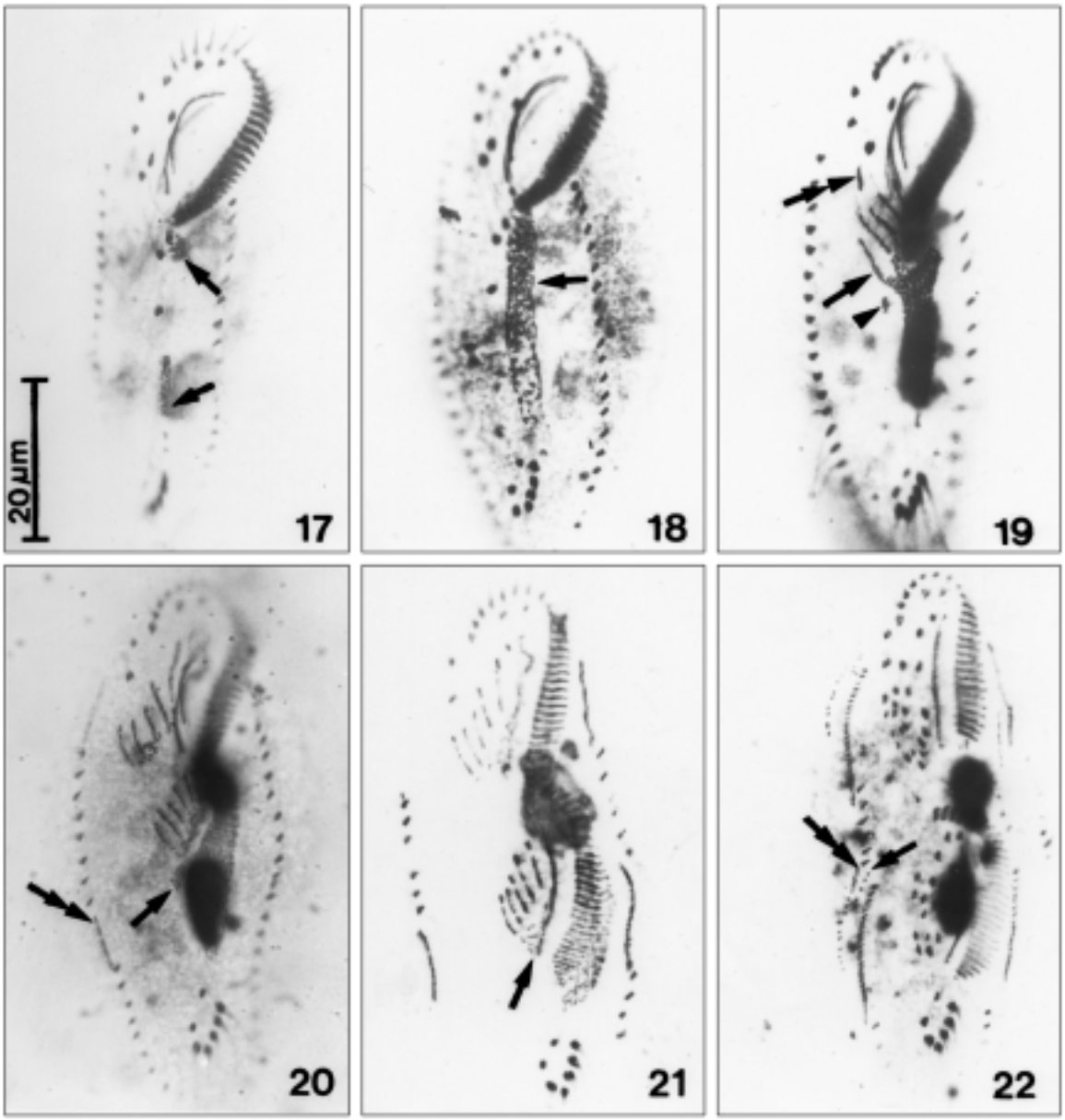
ules situated slightly left of mid line of the cell, ellipsoidal, about 12 × 8 µm. Two to three micronuclei, spherical, about 3.2 µm, attached to the macronuclear nodules at variable positions. Three contractile vacuoles consistently seen in live, swimming specimens (Fig. 8). One (first) contractile vacuole present in the anterior region near the tip of UMs, the second in the mid region below the AZM near the left cell margin and the third one below the second. The second contractile vacuole is the largest whereas the other two are smaller and almost similar in size. All contractile vacuoles show synchronous systolic activity. The diastolic activity of the third contractile vacuole is delayed in relation to the other two. Encystment and excystment frequent. Mature cyst with smooth cyst wall (Fig. 7), 28 µm in protargol stained preparations. Cortical granules (1-1.5 µm), dark green in color, uniformly distributed, and arranged either singly or in clusters of 2-5 (Figs 2, 9), more abundant on the dorsal surface, do not impregnate with protargol.

Cytoplasm with lipid droplets (3-5 µm) and numerous small crystalline inclusions of different shapes roughly spherical (2-2.4 µm) or irregular, rusty brown in color (Fig. 10). Morphometric features of cells are shown in Table 1.

Adoral zone occupies 40% of the body length with about 29 membranelles. Buccal cavity large and deep, undulating membranes (UMs) in *Cyrtohymena* pattern (one may however note that the curvature of the paroral membrane in *R. indica* n. sp. is not as pronounced as that seen in the *Cyrtohymena* pattern). Cirral pattern with 18 FVT cirri (Figs 3, 5). Anterior frontals (II/2, I/1, II/3 and III/3) slightly thicker than the posterior ones. Three postoral ventral cirri (IV/2, V/4 and V/3) near the cytostome, with IV/2 and V/4 appearing as a pair while V/3 placed at a distance from them. About 14% cells (n=400) show one or two supernumerary cirri, generally in the group of ventrals and occasionally as additional transverse cirri. Right marginal row starts at the level of III/2 and terminates below the level of VI/1. Left marginal row 'J' shaped and terminates at the midline. About 12% cells show a second LMC row with few cirri (n=100).



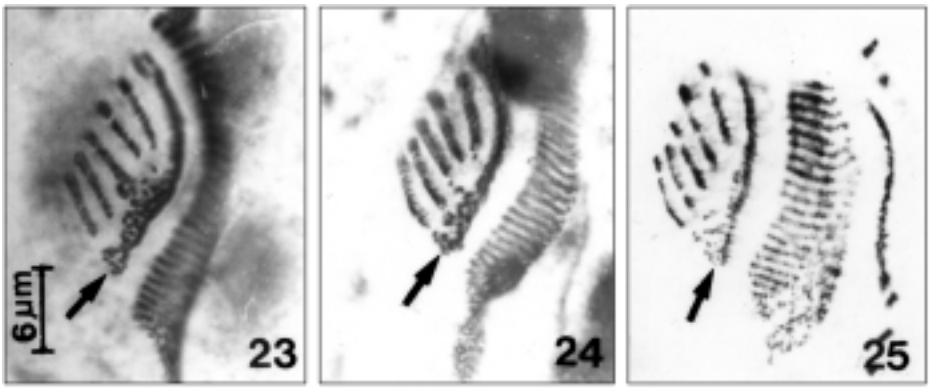
Figs 11-16. Division morphogenesis of *Rubrioxytricha indica* n. sp. on the ventral surface. **11 -** OP in parts (arrows); **12 -** long OP after joining of both the parts (arrow); **13 -** origin of streaks of opisthe: I, II and III from OP (arrow head), streaks IV from IV/2 (double arrow), streaks V and VI for opisthe from V/4 (triple arrow), intact V/3 (arrow), disaggregating IV/3 (double arrow head); **14 -** movement of streaks V and VI (arrow), disaggregated IV/3 (double arrow) and III/2 (triple arrow), migrated kinetosomes of streak II of opisthe (arrow head) to join disaggregated II/2 (double arrow head), disaggregating V/3 (triple arrow head); **15 -** two sets of six FVT ciliary streaks (I-VI), disaggregated V/3 (arrow); **16 -** cirri differentiation of FVT streaks in proter and opisthe in 1,3,3,3,4,4 pattern, LMC primordia (arrows), RMC primordia (double arrows), kinetosomes of V/3 merging with the posterior part of UMs of opisthe (arrow head). FVT - frontal-ventral-transverse, LMC - left marginal cirri, OP - oral primordium, RMC - right marginal cirri, UMs - undulating membranes.



Figs 17-22. Division morphogenesis of *Rubrioxytricha indica* n. sp. after protargol impregnation on ventral surface. **17 -** origin of OP in parts (arrows); **18 -** long OP (arrow); **19 -** anterior movement of streaks V and VI (arrow), disaggregating IV/3 (double arrow) and V/3 (arrow head); **20 -** disaggregated V/3 (arrow), RMC primordium (double arrow); **21 -** kinetosomes of V/3 merging with the posterior part of developing UMs of opisthe (arrow); **22 -** very long DM_1 (double arrow), short DM_2 (arrow). DM - dorsomarginal, OP - oral primordium, RMC - right marginal cirri,UMs - undulating membranes.

Dorsal bristles about 4-5 µm long *in vivo*, arranged in 5 rows (DK_{1-3} and $DM_{1,2}$) (Figs 4, 28). DK_{1-3} complete rows and curved in the middle, a few posterior bristles of DK_3 row arranged in a curvature that runs opposite to that of $DK_{1,2}$. DM_1 long with about 15 bristles while DM_2 short with only 3-7 bristles. Single inconspicuous caudal cirrus present at the end of DK_3.

Divisional morphogenesis (Figs 11-27, 29; Table 2): Stomatogenesis starts with the de novo appearance of kinetosomes between the left marginal cirral row and the post oral ventral cirrus IV/2 forming an oral primordium (OP). The kinetosomes proliferate and extend the field posteriorly. Simultaneously a small field of kinetosomes also appears near the transverse cirrus II/1 (Figs



Figs 23-25. The oral region of opisthe of *Rubrioxytricha indica* n. sp. after protargol impregnation. Kinetosomes of disaggregated V/3 merge with the posterior part of developing UMs (arrows). UMs - undulating membranes.

Table 4. Morphometric comparison of *R. indica* n. sp. with *Cyrtohymena* species. All dimensional measurements are averages in µm.

Characters	*R. indica* n. sp. (Present report)	*C. citrina* (Foissner 1989)	*C. primicirrata* (Foissner 1984)	*C. quadrinucleata* (Foissner 1984)
Body shape	Elliptical, both ends rounded	Slender, slightly 'S' shaped, both ends rounded	Elliptical, both ends rounded	Orthogonal, both ends rounded
Body length (BL)	69.1	99.0	84.7	86.6
Body width (BW)	26.6	33.5	36.0	40.8
Body length/ Body width	2.6:1	2.9	2.3	2.1
Macronuclear nodules, length	11.8	7-10	10-15	9-16
Macronuclear nodules, width	7.8	*	7-9	7-10
Macronuclear nodules, number	2	2	2	4
Micronuclei, number	2-3	1-4	2-3	2-4
Adoral membranelles, number	29	33.6	30.2	40.0
Number of cirri in left marginal cirral row	19.2	21.5	15.6	18.3
Number of cirri in right marginal cirral row	22.3	21.1	20.3	20.3
Dorsal rows	5.0	5.8	6.0	6.0
Caudal cirri	1	3	3	3
OP origin	Dual; in two parts	*	*	*
Colour of cortical granules	Dark green	Orange yellow	Shiny yellow	Absent
Arrangement of cortical granules	Uniformly scattered	Arranged around cirri and dorsal bristles	Arranged in groups of 3-10 along cirri, AZM, and dorsal bristles	Absent
Colour of the cytoplasm	More or less colourless	*	Colourless	Colourless
Cytoplasmic inclusions	Numerous sharp dark crystalline bodies in groups	*	*	*

*Not mentioned by the authors.

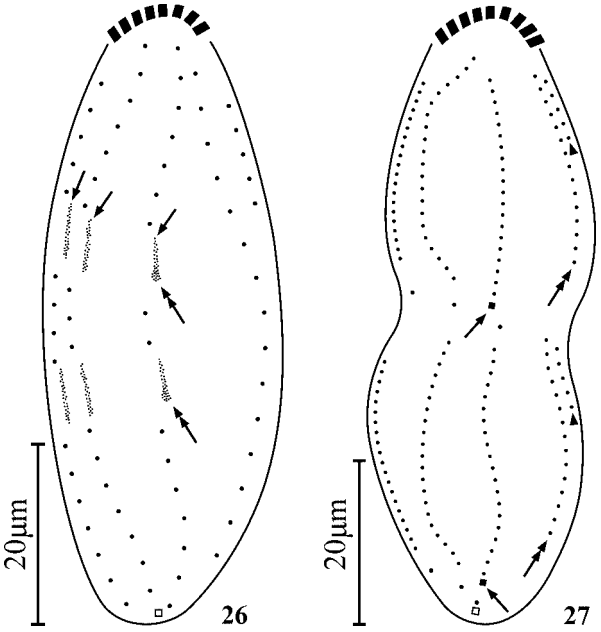
11, 17); it extends anteriorly becoming continuous with the anterior kinetosomal field (Figs 12, 18). As the posterior field of kinetosomes moves anteriorly, its continuity with the transverse cirrus is broken.

Two sets of six FVT ciliary streaks originate from the OP and six parental cirri II/2, IV/3 and III/2 and IV/2, V/4 and V/3. In the proter, the parental UMs function as streak I (Fig. 15). The streak II shows composite origin

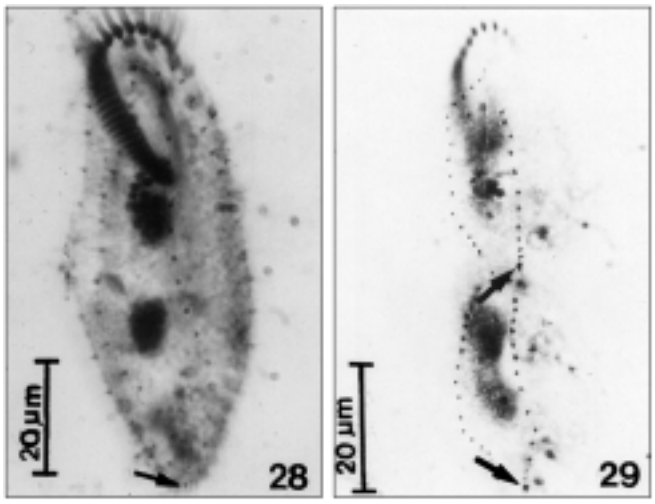
Table 5. Morphometric comparison of *R. indica* n. sp. with *Oxytricha* species possessing cortical granules. All dimensional measurements are averages in µm.

Characters	*R. indica* n. sp. (Present report)	*O. tenella* (Song and Wilbert 1989)	*O. granulifera* (Foissner and Adam 1983)	*O. longigranulosa* (Berger and Foissner 1989)	*O. procera* (Kahl 1932)	*O. durhamiensis* (Kahl 1932, Berger 1999)	*O. oxymarina* (Kahl 1932, Berger 1999)
Body shape	Elliptical, both ends rounded posterior end broadly rounded	Anterior end narrowly and with parallel margins	Broad or slender oval	Elliptical	Slightly spindle shaped	Elliptical	Both ends broadly rounded
Body length (BL)	69.1	50.9	80.6	87.6	*	*	*
Body width (BW)	26.6	26.3	33.6	31.9	*	*	*
Body length/ Body width	2.6:1	1.9	2.4	2.7	*	*	*
Body length (BL) *in vivo*	80	50-70	80-130	80-135	100-120	60-100	80-120
Body width (BW) *in vivo*	30	30-40	35-50	35-55	*	33-45	*
Macronuclear nodules, length	11.8	10.9	14.4	14.8	*	*	14-48
Macronuclear nodules, width	7.8	8.2	7.7	7.6	*	*	*
Micronuclei, number	2-3	2-3	2	1-2	2	2	2-3
Adoral membranelles, number	29	26.0	31.2	26.5	*	25-30	*
Number of cirri in left marginal cirral row	19.2	22.3	34.0	22.9	*	*	31-33
Number of cirri in right marginal cirral row	22.3	25.6	32.1	25.9	*	24	33-39
Dorsal rows	5.0 (3DKs and 2 DMs)	5 (4DKs and 1 DM)	6	6	*	5-6	*
Caudal cirri	1	3	3	3	3	3-4	*
OP origin	Dual; in two parts	*	Between POVCs and LMC	Dual; in parts	*	*	*
Colour of cortical granules	Dark green	*	Colourless to yellowish	Colourless	Dark granules in posterior part	*	Dark granules in posterior part
Arrangement of cortical granules	Uniformly scattered	Irregularly arranged	Absent only along DKs	In groups; arranged in rows	*	In groups of 2-5, further arranged in rows	*
Colour of the cytoplasm	More or less colourless	Slightly yellowish	Colourless	Colourless	*	*	Colourless
Cytoplasmic inclusions	Numerous sharp dark crystalline bodies in groups	Often dark grayish inclusions	Yellowish crystals	Greasy shiny globules	*	*	*

*Not mentioned by the authors.



Figs 26-27. *Rubrioxytricha indica* n. sp. showing division morphogenesis on the dorsal surface. **26 -** within row primordia formation (DP_{1-3}; arrows), Proliferation of kinetosomes at the posterior ends of DP_3 (double arrows); **27 -** presence of one caudal cirrus each at the ends of newly formed DK_3 (arrows), long DM_1 (double arrows), Short DM_2 (arrow heads). DK - dorsal kinety, DM - dorsomarginal, DP - dorsal primordium.



Figs 28-29. *Rubrioxytricha indica* n. sp. showing vegetative and division morphogenesis on the dorsal surface after protargol impregnation. **28 -** dorsal surface showing the presence of one caudal cirrus at the end of DK_3 (arrow); **29 -** newly formed caudal cirrus at the end of each DK_3 (arrows). DK - dorsal kinety.

from II/2 and streak II of opisthe (Fig. 14). The streaks III and IV originate from the disaggregating III/2 and F_7 IV/3 respectively (Figs 13, 14, 19). The streaks V and VI of the proter originate from advancing V and VI streaks of the opisthe (Figs 14, 19). In the proter, outer UM reorganizes completely (Fig. 16). In the opisthe, streaks I, II and III originate from the OP (Fig. 13). The streaks IV, V and VI are formed from the parental IV/2 and V/4 respectively (Fig. 13). The parental cirrus V/3 participates at very late stages of morphogenesis (Figs 14, 15, 19, 20); its participation is noticed subsequent to formation of two sets of six streaks. The kinetosomes of V/3 merge with those of the posterior part of developing UMs of the opisthe (Figs 16, 21, 23-25; Table 2).

The FVT streaks differentiate in the usual pattern of 1, 3, 3, 3, 4, 4 forming 18 FVT cirri, one set each for the two daughter cells (Fig. 16). The development of the marginal cirri occurs by within row primordia formation. Initiation of RMC primordia formation occurs earlier than LMC primordia (Figs 15, 20).

On the dorsal surface, two sets of three primordia arise in the three dorsal kineties by within row proliferation of kinetosomes (Fig. 26). These primordia elongate and differentiate into new dorsal rows (DK_{1-3}). The posterior most kinetosomes of DK_3 proliferate to develop into one caudal cirrus each in the proter and the opisthe (Figs 27, 29). The two dorsomarginal rows are formed from two primordia, arising near the right marginal primordia and later shift to the dorsal surface (Fig. 22).

DISCUSSION

Generic assignment of the new species

Rubrioxytricha indica n. sp. possesses a question mark shaped AZM, curved and intersecting UMs; 18 FVT cirri, posterior frontal cirri in "V" shaped pattern, postoral ventral cirri in dense cluster behind buccal vertex, two pretransverse ventrals and 5 transverse cirri; one right and left marginal row of cirri; three dorsal kineties, two dorsomarginal rows and one caudal cirrus. Brown coloured crystalline inclusions are present in cytoplasm. Green coloured cortical granules are uniformly distributed in clusters.

Comparison with related and similar species

Rubrioxytricha indica n. sp. shows marked differences from the already two described species namely

R. haematoplasma (Blatterer and Foissner 1990, Berger 1999) and *R. ferruginea* (Song and Wilbert 1989, Berger 1999).

It is much smaller, therefore shows corresponding differences in its morphometric features from the other two species (Table 3). It is also distinct by the presence of dark brown crystalline inclusions, which are present in substantial number in the cytoplasm.

The cortical granules in *R. indica* n. sp. are uniformly distributed in clusters, whereas in case of other two reported species, these are arranged in longitudinal rows.

Rubrioxytricha indica n. sp. possesses three dorsal kineties and two dorsomarginal rows. No variation was noticed in the population in this regard. In contrast, *R. haematoplasma* possesses either three dorsal kineties and one dorsomarginal row (Blatterer and Foissner 1990, Berger 1999) or four dorsal kineties and one dorsomarginal row (Shin and Kim 1993). Likewise, it also differs from *R. ferruginea*, which has four dorsal kineties and one dorsomarginal row (Song and Wilbert 1989, Berger 1999). One may also mention that out of several hundred specimens examined no variation was seen in the number of caudal cirri i.e. presence of a single caudal cirrus at the end of DK_3. In *R. ferruginea* one or two caudal cirri are present.

The *Cyrtohymena* species differ from *R. indica* n. sp. in most of the morphometric features, colour and arrangement of cortical granules, nature of crystalline cytoplasmic inclusions and most importantly in number of caudal cirri (Table 4).

The *Oxytricha* species are distinct from *R. indica* n. sp. by the pattern of UMs (Berger and Foissner 1997), dorsal rows, number of caudal cirri and arrangement of cortical granules (Table 5).

Variability of cirral pattern

In *R. indica* n. sp. morphometric analysis of a large sample size (n=400) shows ~14% cells possess one or two extra ventral or transverse cirri. Furthermore, nearly 12% of the clonal population of *R. indica* n. sp. shows a second LMC row with few cirri. Development of the second row shows that it is formed by splitting of the primordium from the first LMC row. Thus the development pattern of the second LMC row in the above mentioned cases is similar to that seen in *Pleurotricha curdsi* (Gupta *et al.* 2003).

It is not certain whether these variations are intrinsic traits of the isolate or have arisen due to the influence of other factor (s) such as the clonal age or presence of intracellular symbionts/parasites. Morphometric variations are also known to arise in oxytrichids by the presence of metals in the medium (Arora *et al.* 1999, Machwe *et al.* 2001). Since the present data has been obtained from the laboratory grown cells, the role of the last mentioned factor is ruled out.

Morphogenesis

Morphogenetic data of *R. indica* n. sp. clearly shows the involvement of cirrus V/3. The cirrus V/3 disaggregates much later in the course of development after formation of two sets of six FVT streaks when its kinetosomes merge with the posterior part of the undulating membranes of the opisthe. In an earlier study of morphogenesis in *R. haematoplasma* (Blatterer and Foissner 1990, Berger 1999), involvement of cirrus V/3 in morphogenesis could not be seen with certainty.

The divisional morphogenesis of *R. indica* n. sp. is essentially similar to that of *Oxytricha* except the nature of involvement of V/3 wherein it takes part in the formation of streaks IV, V and VI along with the participation of cirri IV/2 and V/4.

It is noteworthy that cirrus V/3 remains intact till late stages of development, a situation not seen in other genera of oxytrichinae. Thus this feature could well be considered specific for the genus *Rubrioxytricha*.

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The Moss Dwelling Testacean Fauna of the Strømness Bay (South Georgia)

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Summary. The study of 22 aquatic and 36 terrestrial moss samples of the Strømness Bay (South Georgia, sub-Antarctica) revealed 71 testate amoebae taxa (Protists) belonging to 21 genera. Twenty-eight taxa were reported for the first time, which resulted in a total of 87 testate amoebae taxa observed from South Georgia. A cluster and a correspondence analysis pointed out a clear difference between the aquatic and the terrestrial moss samples. Four assemblages of characteristic testate amoebae species with specific ecological preferences were distinguished. The moss dwelling testacean fauna of South Georgia was compared to other sub-Antarctic islands, such as Marion Island, Îles Kerguelen and Île de la Possession.

Key words: aquatic mosses, assemblages, biogeography, Île de la Possession, South Georgia, sub-Antarctica, terrestrial mosses, testate amoebae.

INTRODUCTION

Testate amoebae (Protists) are a group of free-living, heterotrophic protists with a world-wide distribution (Smith 1992). Recently a lot of attention has been given to the factors influencing the geographical distribution of these testate rhizopods. Most species are dispersed world-wide by wind and on the legs of birds or floating vegetation (Smith and Wilkinson 1986). An alternative hypothesis suggests the existence of geographical barriers for larger and heavier species. The lack of decent records however, may lead to hasty conclusions about the bio-geographical distribution of testate amoebae. Therefore, extensive research of testate amoebae habitats all over the world is necessary, especially on remote islands such as South Georgia.

The earliest records of testate amoebae on South Georgia were made by Richters (1908), who reported 5 taxa from moss samples of the Cumberland Bay and Royal Bay areas (Fig. 1). Sixteen years later, Sandon and Cutler (1924) observed 15 taxa in organic soil samples collected in the Grytviken area. Both studies should be considered as preliminary and reveal only a very small fraction of the real living testacean fauna of South Georgia. Not until late in the twentieth century,

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was the testate rhizopod fauna of the island more extensively studied by Smith (1982) and Beyens *et al.* (1995). These authors reported respectively 20 testate amoebae taxa from soils and peats (Smith 1982) and 46 taxa from freshwater habitats of the Strømness Bay (Beyens *et al.* 1995).

The moss dwelling testacean fauna of South Georgia remained unstudied however. Given the fact that mosses are the dominant vegetational life form in the whole Antarctic region (Putzke and Pereira 2001) and that testate amoebae are frequently observed from Antarctic mosses (e.g. Grospietsch 1971; Smith 1974, 1986; Vincke *et al.* 2004a), it is clear that the study of the moss habitats will complete the information about the testate amoebae fauna on South Georgia. Furthermore will the results of this study allow testing of some hypotheses regarding the biogeography of testate amoebae or the relationship between testacean diversity and latitude?

MATERIALS AND METHODS

Study site. The sub-Antarctic island of South Georgia is located in the Southern Ocean (54-55°S; 36-38°W), about 1300 km east-southeast of the Falkland Islands and 1930 km of Cape Horn (Chile, South America) (Fig. 1). The 3760 km^2 large island lies south of the Antarctic Convergence and belongs to the sub-Antarctic region of Holdgate (1964). The climate is cold, but a permanent maritime influence limits the variation of temperatures between +4.4 in January and - 1.5 in July (Smith 1978). Annual precipitation usually exceeds 1580 mm (Greene 1964) and prevailing wind directions are northwest and southeast (Smith 1978).

Much of the island is rugged and mountainous, with the highest point, Mount Paget, at 2915 m a.s.l. About 56% of the island is covered by glaciers that have been retreating during the last 17000 years, depositing millions of tons of moraine on the floor of the island's bays and surrounding ocean (Morley 2004). The vegetation of South Georgia consists mainly of grasses, mosses and lichens, while seabirds and seals dominate the animal life on the island.

Sampling. During the austral summer of 1992-1993, twenty-two aquatic moss and thirty aquatic sediment samples were taken in the region of the Strømness Bay on South Georgia. Results on testate amoebae in these aquatic habitats have already been published by Beyens *et al.* (1995). During the same period another 36 terrestrial moss samples were collected near the Strømness Bay, from which the diatom flora has been studied (Van de Vijver and Beyens 1997). To determine the moss-inhabiting testacean fauna of South Georgia, the 36 terrestrial moss samples of Van de Vijver and Beyens (1997) as well as the 22 aquatic moss samples of Beyens *et al.* (1995) were analysed. Eleven samples (out of 48) were withdrawn from further analysis, since they contained no or too little testate amoebae (less than 10 tests per slide).

The moisture content of the sampled mosses was determined with reference to the F-classification of Jung (1936): FI - submerged mosses; FII - free-floating mosses, partly submerged, partly floating; FIII - very wet-water drips from sample without pressure; FIV - wet-water drips after by slight pressure; FV - semi-wet-water drips after moderate pressure; FVI - moist-little water produced after high pressure); FVII - semi-dry-only a few drops of water can be squeezed out; FVIII - dry-no water (Meisterfeld 1977). Water pH was measured, when possible, with a Hanna water tester and the habitat type of the sampled mosses was determined as follows: S - stream, P - pool, L - lake and T - terrestrial environments. All moss material was fixated in 3% formaldehyde.

Identifications of moss species are based on Bell (1973, 1974, 1984), Clarke (1973), Frahm (1988), Greene (1968, 1973), Lightowlers (1985), Newton (1979, 1983), Ochyra (1998). An overview of the characteristics of the samples used in this study is given in Table 1.

Slide preparation and counting. Moss samples were thoroughly shaken and stirred for 5 min in an indefinite amount of distilled water. The suspension was passed through a sieve with a mesh diameter of 595 µm and concentrated by centrifugation (10 min at 2500 rpm). The colour Rose Bengal was added to the samples to distinguish dead from living tests (at the moment of sampling). Encysted testate amoebae were considered as being alive. In each moss sample 150 tests were counted using a Leitz Wetzlar® microscope. Morphological identifications of the testate amoebae are mainly based on works by Deflandre (1928, 1929, 1936), Grospietsch (1964), Decloître (1962, 1978, 1979, 1981), Ogden and Hedley (1980), Ogden (1983) and Hoogenraad and de Groot (1940).

Data analysis. For pairwise comparison of the testate amoebae fauna of South Georgia with other sub-Antarctic islands, the Community Coefficient of Sørensen (1948) was calculated. This index, based on the number of common taxa, has following formula: 2C/(A+B+2C), with A and B being the number of taxa exclusively observed in one place, whereas C is the number of taxa the 2 places have in common.

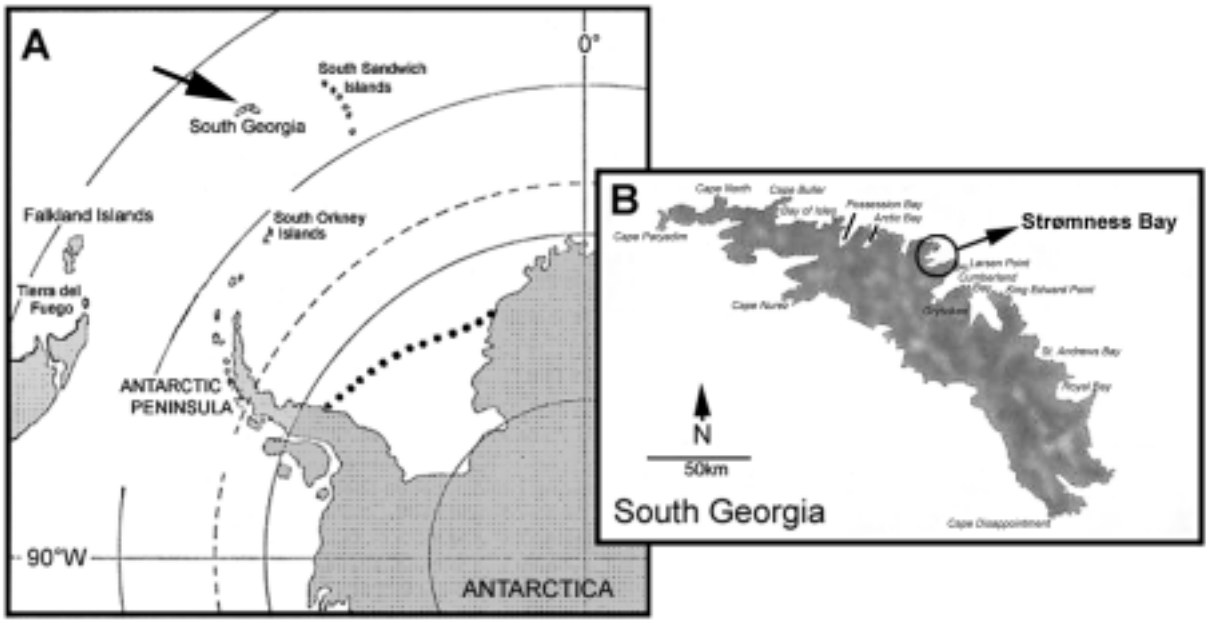
Diversity analysis [Shannon Wiener diversity index ($\log_{10}$-based)] was performed using the Multivariate Statistical Package (MVSP) (Kovach Computing Services, 2002). The Gini evenness measure was calculated because of his independence of the number of taxa per sample and therefore allowing a better comparison between the samples (Nijssen *et al.* 1998).

A hierarchic-agglomerative cluster analysis, based on a minimum variance strategy with the Squared Euclidian Distance as a dissimilarity measure, was carried out to classify the species data (MVSP) (Kovach Computing Services, 2002). Species data were log(e) transformed.

A correspondence analysis (CA) was performed to explore possible relationships between the moss dwelling testate amoebae fauna and the measured environmental variables (F-value, pH and habitat-type) using the computer program CANOCO version 4.0 (Ter Braak and Smilauer 1998). Species data were square-root-transformed in order to downweight dominant taxa. The statistical techniques used are described in full detail by Jongman *et al.* (1987).

RESULTS

Species composition. The microscopic analysis of 37 samples revealed a total of 71 testate amoebae taxa (species, varieties and forms), belonging to 21 genera.



Figs 1A, B. A - Sketch map of the southern Atlantic Ocean with the position of South Georgia. **B** - Map of South Georgia, with indication of the Strømness Bay where samples have been collected.

An alphabetical list of all observed taxa with their relative abundance is given in Appendix 1. This list contains 13 testate amoebae taxa (4.4% of all counted tests) which could not be identified up to species level. Identification of these taxa, using Scanning Electron Microscopy (SEM), will be the subject of another paper. Twenty-eight testate amoebae taxa (39%) are reported here for the first time from South Georgia. These are indicated with an * in Appendix 1.

The most abundant testate rhizopod taxa in the mosses of South Georgia were *Trinema lineare* Penard (16.6%), *Microchlamys patella* (Claparede and Lachmann) Cockerell (15.8%), *Corythion dubium* Taranek (14.6%), *Nebela collaris* Ehrenberg (11.1%) and *Difflugia pulex* Penard (10.1%). Twenty-five testate amoebae taxa had relative frequencies <1%.

Figure 2 shows the number of taxa encountered per genus and the relative abundance of the genus. The genera *Centropyxis* and *Difflugia* showed the highest species diversity, respectively 12 and 11 taxa, while *Trinema* was the most abundant genus.

Thirty-one percent of the testate amoebae fauna was alive at the moment of sampling. This number corresponds to a dead-living ratio of 2.2. The proportion of cysts was very low (0.4%). Encysted organisms belonged mostly to the taxon *Nebela collaris* (0.3 %), but also to *Difflugia globulosa* Dujardin, *Euglypha strigosa* Leidy and *Trinema lineare*.

The diversity analysis revealed a mean Shannon-Wiener diversity index (H') of 0.65 ± 0.05 and a Gini-evenness measure of 0.37 ± 0.01. The highest diversity was measured in sample M352 (H'=1.03), an FV moss sample (*Tortula robusta*) collected near a small brooklet at Tønsberg Point. The lowest diversity was observed in M332 (H'=0.02), a *Sphagnum fimbriatum* moss strongly dominated by *Nebela collaris*. The mean number of taxa per sample was 12 ± 1, with a maximum of 22 testate amoebae taxa in samples M352 and M326, and a minimum of 2 taxa in samples W395 and M332.

Community analysis. A hierarchic-agglomerative cluster analysis revealed 4 clusters, named after their most characteristic testate amoebae taxon (Fig. 3):

(1) *Nebela collaris* assemblage
(2) *Corythion dubium* assemblage
(3) *Microchlamys patella* assemblage
(4) *Difflugia bryophila* assemblage

Table 2 lists the most important characteristics of the 4 communities.

Samples of the *Nebela collaris* assemblage had very low diversity indices, due to the extreme dominance of *Nebela collaris* and *Euglypha strigosa*. Water pH-values were rather low (4.4 ± 0.1) compared to

Table 1. Overview of the characteristics of the samples used in the analysis.

Sample	Hab	F	pH[a]	Moss species
W365	S	II	8.2	*Brachytecium subplicatum* + *Orthotheciella varia*
W366	S	I	7.7	moss A
W367	P	II	6.1	*Warnstorfia sarmentosa* + *Warnstofia laculosa*
W370	P	I	5.6	*Warnstorfia laculosa*
W371	S	I	-	moss A
W383	S	II	7.6	moss A
W387	P	I	6.6	*W. sarmentosa* + *Sanionia uncinata* + *O. varia*
W388	L	I	5.7	*Warnstorfia laculosa*
W390	P	I	4.2	*Warnstorfia laculosa*
W395	P	II	4.3	*Warnstorfia laculosa*
W397	L	I	7.5	*Warnstorfia laculosa*
W399	P	I	4.6	*Warnstorfia laculosa*
W402	P	I	6.6	*Warnstorfia laculosa*
W407	P	I	8.1	*Sanionia uncinata* + *Warnstorfia laculosa*
W412	L	I	6.2	*Andreaea depressinervis* + *Warnstorfia laculosa*
M317	T	III	-	*Warnstorfia sarmentosa* + *Orthotheciella varia*
M318	T	III	7.3	Hepatic
M320	T	VI	-	*Tortula robusta*
M321	T	VII	-	*Tortula filaris*
M323	T	IV	-	*Warnstorfia sarmentosa*
M324	T	VIII	-	*Polytrichastrum alpinum*
M325	T	V	-	*Tortula robusta*
M326	T	IV	-	cfr. *Orthotheciella varia*
M329	T	VIII	-	*Tortula saxicola (?)* + *Polytrichum juniperinum*
M330	T	VII	-	*Conostomum pentastichum*
M331	T	VI	-	*Pohlia sp*
M332	T	IV	-	*Sphagnum fimbriatum*
M336	T	IV	4.4	*Campylium polygamum*
M337	T	III	6.2	*Brachytecium austrosalebrosum*
M338	T	III	6.6	*Sanionia uncinata*
M345	T	VII	4.6	*Campylium polygamum*
M347	T	VII	-	*Racomitrium striatipilum*
M348	T	VI	-	*Campylopus clavatus*
M349	T	V	6	*Warnstorfia sarmentosa*
M350	T	VII	-	*Camplylopus clavatus*
M351	T	VIII	-	*Racomitrium lanuginosum*
M352	T	V	-	*Tortula robusta*

Hab -habitat type: S - stream, P - pool, L - lake and T - terrestrial environments; F - classification of Jung (1936);[a] when measured.

neutral pH-values of the other assemblages. The terrestrial moss samples of the *Corythion dubium* assemblage had very low moisture contents (between FVI and FVII). These dry mosses were also characterised by taxa as *Assulina muscorum*, *A.* sp1 and *Euglypha compressa*. On the other hand, the *Microchlamys patella* assemblage grouped all aquatic mosses (FI and FII) from pools, lakes and streams. Next to *M. patella*, *Difflugia pulex* (and other *Difflugia* taxa), *Difflugiella crenulata* and *Euglypha tuberculata* typified this cluster. The three terrestrial moss samples appearing in this cluster (M317, M318, M337) were taken along fast flowing brooklets of meltwater. The very frequent washing over by the meltwater explains the FIII moisture values of these three moss samples and emphasises again the importance of humidity on the testacean species distribution. The terrestrial samples of the *Difflugia bryophila* assemblage had intermediate F-values and highest diversity indices. Testate amoebae taxa such as *Centropyxis aerophila* and *Nebela lageniformis* were characteristic for this assemblage.

Table 2. Characteristics of the 4 clusters. Means are provided with standard errors.

	CL 1 *Nebela collaris*	CL 2 *Corythion dubium*	CL 3 *Microchlamys patella*	CL 4 *Difflugia bryophila*
Number of samples	6	10	15	6
Number of species	20	39	47	39
Shannon-Wiener Diversity	0.37 ± 0.12	0.60 ± 0.07	0.69 ± 0.07	0.91 ± 0.04
Gini Evenness Measure	0.39 ± 0.04	0.34 ± 0.02	0.35 ± 0.02	0.40 ± 0.02
Mean Species Richness	6.5 ± 1.6	10.2 ± 1.1	12.7 ± 1.2	17 ± 2
Mean F-range	FIII - FIV	FVI - FVII	FI - FII	FIV - FV
Habitat type (number of samples):				
Stream	0	0	4	0
Pool	3	0	5	0
Lake	0	0	3	0
Terrestrial	3	10	3	6
*Frequency of occurrence in samples (%) ** *Relative abundance in these samples (%)*				
Assulina muscorum	0 * 0	90 * 7	7 * 1	0 * 0
Centropyxis aerophila	0 * 0	30 * 6	80 * 4	100 * 11
Corythion dubium	50 * 1	100 * 50	40 * 2	83 * 4
Difflugia bryophila	0 * 0	10 * 1	20 * 1	100 * 10
Difflugia pulex	33 * 4	40 * 17	87 * 21	50 * 7
genus *Difflugia*	5 * 3	12 * 7	33 * 8	36 * 4
Difflugiella crenulata	0 * 0	0 * 0	40 * 9	17 * 1
Edaphonobiotus campascoides	0 * 0	10 * 1	47 * 5	50 * 2
Euglypha compressa	0 * 0	50 * 3	0 * 0	0 * 0
Euglypha rotunda	33 * 1	60 * 3	40 * 3	67 * 2
Euglypha strigosa	50 * 31	30 * 2	0 * 0	0 * 0
Euglypha tuberculata	0 * 0	0 * 0	13 * 16	0 * 0
Microchlamys patella	83 * 7	30 * 1	100 * 34	83 * 6
Nebela collaris	100 * 66	10 * 9	7 * 1	17 * 7
Nebela lageniformis	17 * 1	10 * 3	7 * 2	100 * 6
Pseudodifflugia fulva	17 * 3	30 * 1	67 * 3	33 * 2
Trinema enchelys	17 * 9	10 * 3	67 * 3	67 * 9
Trinema lineare	67 * 8	100 * 16	87 * 18	100 * 33

A correspondence analysis confirmed the assemblages formed by the cluster analysis (Fig. 4a). The Eigen values (l_1 = 0.60 and l_2 = 0.49) of the first two CA-axes accounted for only 24.8% of the cumulative variance in the testate amoebae data. This low percentage is typical for noisy data sets containing may zero values. Most probably the first axis corresponds to the pH of the samples, while the second axis relates to the moisture content of the moss samples. A CA-species plot is also shown (Fig. 4b) and indicated the same characteristic taxa for each assemblage. Species in the centre of the ordination, such as *Trinema lineare* and *Euglypha rotunda* (abundant in all 4 assemblages), have little ecological preferences and appear under highly variable moist conditions.

DISCUSSION

Species composition and communities

The study of the moss dwelling testacean fauna of South Georgia revealed 71 taxa, which is the highest number of testate amoebae taxa recorded from the island so far. Twenty-eight taxa are reported for the first time and comparing the species list with Sandon and Cutler (1924), Smith (1982) and Beyens *et al.* (1995), brings the total to 87 testate amoebae taxa on South Georgia (17 unidentified species not taken into account).

The cluster and the correspondence analysis point out a clear difference between the aquatic and the terrestrial

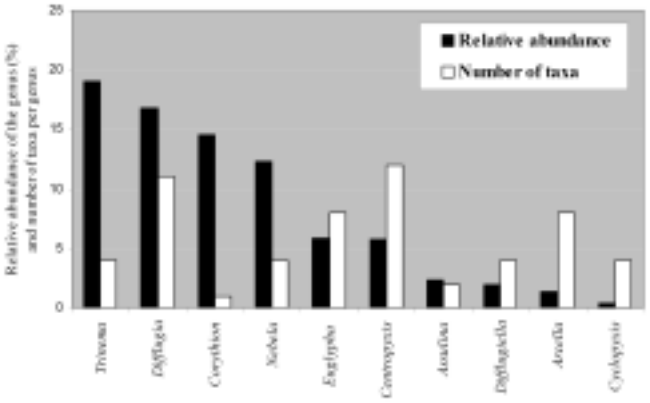


Fig. 2. Diagram showing the number of testate amoebae taxa per genus and the relative abundance (%) of the genus.

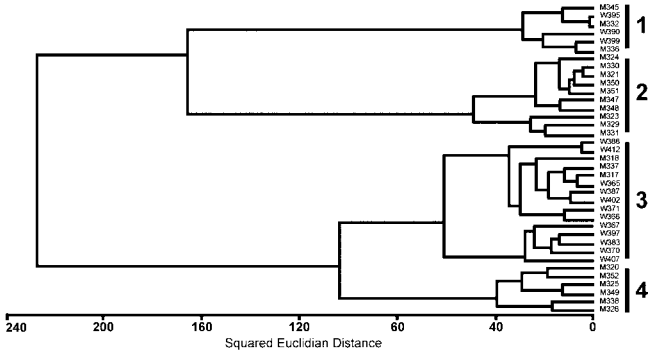
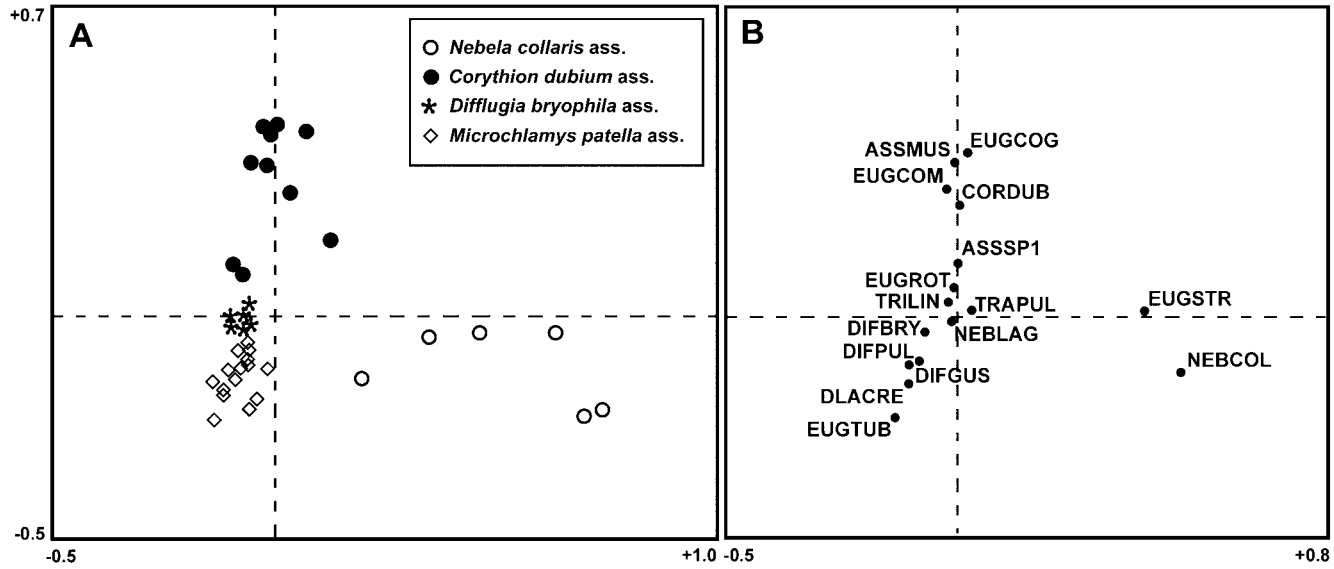


Fig. 3. A hierarchic-agglomerative cluster analysis showing the 4 clusters: (1) *Nebela collaris* cluster, (2) *Corythion dubium* cluster, (3) *Microchlamys patella* cluster and (4) *Difflugia bryophila* cluster



Figs 4A, B. A - CA ordination showing sample sites. Sites are labelled according to their correspondent cluster. **B** - CA species ordination. Taxon codes are explained in Appendix 1.

moss samples. The water surrounding the aquatic mosses has a significant influence on the testacean species distribution and therefore aquatic moss samples should be handled as aquatic samples rather than moss samples. The preference of *Microchlamys patella* for aquatic mosses (FI-FII), confirmed its ecological preference as observed on Île de la Possession (Vincke *et al*. 2004c). Similarly, *Difflugia*-taxa (especially *D. pulex*) were more abundant in aquatic habitats (Beyens *et al.* 1995; Vincke *et al.* 2004a,b), whereas *Nebela*-taxa were more bound to moist terrestrial mosses (e.g. *Nebela lageniformis* in the *Difflugia bryophila* assemblage). The species poor *Nebela collaris* assemblage was found in rather wet mosses (FIII - FIV) sampled from different localities in the Strømness Bay. The same assemblage was also described by Beyens *et al.* (1995),

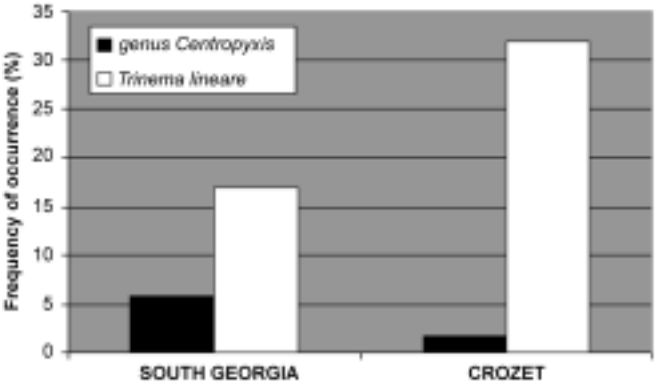


Fig. 5. Comparison of the relative abundance (%) of *Trinema lineare* (white bars) and individuals of the genus *Centropyxis* (black bars) on South Georgia and Crozet.

as characteristic for most acid waterbodies. Indeed, samples of this cluster had a mean pH of 4.4 ± 0.1 (SE) and were clearly distinctive from the neutral to slightly alkaline pH-values from the moss samples from the other assemblages.

Taxa such as *Corythion dubium, Assulina muscorum, Assulina* sp1 and *Euglypha compressa* were characteristic for the driest mosses sampled (FVI-FVII-FVIII). Moisture preferences of these taxa all correspond to the ones found on Île de la Possession (Vincke *et al.* 2004c). Smith (1982) found *A. muscorum* to be more characteristic of wet mosses, but results of this study clearly indicate *A. muscorum* to be associated to drier mosses (Table 2). This study confirmed the hygrophilous nature of *Centropyxis aerophila* found by Smith (1982). The highest abundances of this taxon were observed in the range from FIII to FV. *Difflugia bryophila*, typical for the third assemblage, had high frequencies in semi-wet moss samples (FIV-FV), confirming earlier records of Île de la Possession (Vincke *et al*. 2004c).

The observed dead-living ratio of 2.2 (31% living tests) of all observed tests may seem rather low compared to high ratios of the temperate regions (about 10), where empty tests dominate manifold above living and encysted tests (Balik 1994). Probably the penetration of water into empty tests, as suggested by Balik (1994), caused the destruction of empty tests in freeze-thaw cycles (as appear frequently on South Georgia).

Comparison with other sub-Antarctic islands

Smith (1982) compared the testate amoebae fauna of South Georgia with Marion Island (Grospietsch 1971: 53 taxa) and Kerguelen (Bonnet 1981: 50 taxa) and found at that time "a significantly greater species diversity on these wetter and less cold sub-Antarctic islands than on South Georgia". Comparing the actually known rhizopod fauna of South Georgia (87 taxa) with the same data of these sub-Antarctic islands (no additional data yet), results are just the other way round and therefore Smith's observed trend about pauperisation towards the South Pole seems to be overruled. However, this ostensible finding is more probably the result of insufficient research and lower sampling intensities on Kerguelen and Marion Island.

When the testacean fauna of South Georgia is compared to the one of sub-Antarctic Île de la Possession (Crozet Archipelago) (Smith 1975, Vincke *et al.* 2004a,b,c: 88 taxa + 34 unidentified taxa) however, both sub-Antarctic islands seem to have rather similar amounts of testate amoebae taxa. Taken into account the unidentified taxa on both islands (South Georgia: 87+17=104 taxa; Île de la Possession: 88+34=121 taxa), Smith's theory (1982) about pauperisation towards the South Pole is reconfirmed.

Nevertheless it's still possible that the number of testate amoebae taxa of South Georgia is higher than that of Île de la Possession. In contrast to the intensive sampling strategy on Île de la Possession (over 300 samples analysed from places all over the island by Richters 1907, Smith 1975 and Vincke *et al.* 2004a,b,c), the sampling on South Georgia was restricted (so far) to several bays along the north-east coast of the island (in total about 110 samples, Richters 1908, Sandon and Cutler 1924, Smith 1982, Beyens *et al.* 1995 and this study). Knowing that South Georgia (3760 km²) has about 24 times the surface of Île de la Possession (156 km²), it is most likely that the limited number of samples does not represent the total testacean diversity of the island. Moreover the diversity of microclimates, that influences the diversity of niches for different species, may be higher on South Georgia because of the lower exposure of the island compared to Île de la Possession. Even though these assumptions can't be proven at this point in time, it remains possible that the testacean fauna of South Georgia, although located at higher latitude, is indeed more divers than that of Île de la Possession. Therefore the pauperisation phenomenon towards the South Pole should be considered as a general trend rather than a strict rule.

The Sørensen similarity index between South Georgia and Île de la Possession (0.42) indicates that the composition of the testate amoebae fauna on both islands is

rather different. Both islands have 47 taxa in common, including 2 unidentified taxa, *Assulina* sp1 and *Difflugia* sp6, that are morphologically (genetic similarity unknown) identical on both places. *Trinema lineare* was the most dominating taxon on both islands, but its relative abundance differed significantly (17%: South Georgia; 32% Île de la Possession) (Fig. 5) (Vincke *et al.* 2004a,b,c). Another striking difference between the islands is the number of taxa of the genus *Centropyxis* (South Georgia: 17 taxa; Île de la Possession: 8 taxa) (Fig. 5) and the relative abundances of the *Centropyxis* taxa (moss samples South Georgia: 5.8% versus Île de la Possession: moss: 1.7% (Vincke *et al.* 2004c); aquatic: 1.3% (Vincke *et al.* 2004b), soils: 0.5% (Vincke *et al.* 2004a)). On Île de la Possession, *Trinema lineare* became more abundant when moisture was a limiting factor (24% aquatic habitats; 30% mosses; 41% soils) (Vincke *et al.* 2004a,b,c). It appears that samples of South Georgia were on average taken in wetter conditions than those of Île de la Possession and this would explain the higher relative abundance of the generally hygrophilous *Centropyxis*-taxa (de Graaf 1956) and lower relative abundance of *Trinema lineare*.

Despite the considerable geographical distance between the sub-Antarctic islands of South Georgia and Île de la Possession, and differences in their climatological and bryological characteristics, it is clear that similar habitats on both islands are colonised by rather similar testate amoebae faunas. The 42 rhizopod taxa both islands have in common (mostly cosmopolitan taxa) seem to have well-defined ecological preferences (especially for moisture) that are similar in different geographical locations. Besides this shared testate amoebae fraction, each island maintains a certain degree of uniqueness, expressed by a number of taxa occurring only on that specific island, when it comes to filling up the gaps in the ecological niches. The question remains if this uniqueness is due to the existence of biogeographical barriers that inhibit the distribution of certain species, or to the lack of specific environmental conditions and habitats required for the survival and development of population of these species. Therefore, every additional study on the diversity and ecology of testate amoebae, from places all over the world, will add indispensable information about the precise ecological optima and the biogeography of these free-living protists.

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APPENDIX 1. List of all observed testate amoebae taxa, including abbreviations used in figures and relative abundancies of the taxa. Taxa reported for the first time on South Georgia are indicated with *. The habitat type in which each taxon was found is also indicated with S (stream), P (pool), L (lake) and T (terrestrial samples).

Abbreviation	Testate amoebae taxon	Rel. Abund. (%)	Habitat
ARCARE	*Arcella arenaria* Greeff	0.02	T
ARCBAT	* *A. bathystoma* Deflandre	0.02	T
ARCROT	*A. rotundata* Playfair	0.36	T/P/L
ARCRSU	* *A. rotundata* v. *stenostoma* f. *undulata* Deflandre	0.09	P
ARCSP1	*A.* sp1	0.07	T
ARCSP2	*A.* sp2	0.09	T
ARCSP3	*A.* sp3	0.47	T
ARCVUL	*A. vulgaris* Ehrenberg	0.29	T/S
ARCHS1	*Archerella* sp1	0.02	P
ASSMUS	*Assulina muscorum* Greeff	1.80	T/S
ASSSP1	*A.* sp1	0.56	T
CENACU	*Centropyxis aculeata* Stein	0.18	T/L
CENAER	*C. aerophila* Deflandre	3.53	T/P/S/L
CENAEM	*C. aerophila* v. *minuta* Chardez	0.05	T
CENASP	*C. aerophila* v. *sphagnicola* Deflandre	0.54	T/P/S/L
CENASY	* *C. aerophila* v. *sylvatica* Deflandre	1.06	T/S
CENCAS	* *C. cassis* Deflandre	0.04	T/S
CENELO	*C. elongata* (Penard) Thomas	0.02	P
CENGIB	* *C. gibba* Deflandre	0.02	P
CENMIN	* *C. minuta* Deflandre	0.02	S
CENORB	* *C. orbicularis* Deflandre	0.05	P/L
CENPLA	* *C. platystoma* Penard	0.22	T/PS/L
CENSP1	*C.* sp1	0.04	S
CORDUB	*Corythion dubium* Taranek	14.56	T/S/P
CYCARC	*Cyclopyxis arcelloides* (Penard) Deflandre	0.02	P
CYCARM	* *C. arcelloides* v. *minima* Van Oye	0.22	T
CYCEUR	* *C. eurystoma* Deflandre	0.09	T
CYCEUP	* *C. eurystoma* v. *parvula* Bonnet & Thomas	0.11	T/P
DIFBRY	* *Difflugia bryophila* (Penard) Jung	1.68	T/S/P
DIFGLA	*D. glans* Penard	0.45	T/S/P
DIFGSA	*D. globulosa* Dujardin	0.99	T/P/S/L
DIFGUS	*D. globulus* Hopkinson	0.86	T/S/P
DIFLUC	*D. lucida* Penard	0.11	T/P
DIFPAR	* *D. parva* (Thomas) Ogden	0.02	P
DIFPUL	*D. pulex* Penard	10.07	T/P/S/L
DIFSP1	*D.* sp1	1.23	T/PS/L
DIFSP2	*D.* sp2	0.02	T
DIFSP6	*D.* sp6	1.26	T/P/S/L
DIFTEN	* *D. tenuis* (Penard) Chardez	0.13	T/P
DLACRE	* *Difflugiella crenulata* Playfair	1.46	T/P/L
DLACRG	* *D. crenulata* v. *globosa* Playfair	0.02	P
DLAOVI	* *D. oviformis* (Penard) Bonnet & Thomas	0.27	T/P/S/L
DLAOVF	* *D. oviformis* v. *fusca* (Penard) Bonnet & Thomas	0.18	T/S/P
EDACAM	* *Edaphonobiotus campascoides* Schönborn, Foissner & Meisterfeld	1.14	T/S/P
EUGCIL	* *Euglypha ciliata* (Ehrenberg) Perty	0.04	T
EUGCOM	* *E. compressa* Carter	0.40	T
EUGCOG	* *E. compressa* v. *glabra* Cash	0.34	T
EUGPOL	* *E. polylepis* Bonnet	0.14	T
EUGROT	*E. rotunda* Wailes	1.35	T/S/P
EUGSTR	*E. strigosa* Leidy	2.67	T/P
EUGSTG	* *E. strigosa* v. *glabra* Wailes	0.07	T
EUGTUB	*E. tuberculata* Dujardin	0.85	P/L
HELSYL	* *Heleopera sylvatica* Penard	0.02	T
HYAMIN	* *Hyalosphenia minuta* Cash	0.02	P
HYASP1	*H.* sp1	0.04	T
HYASP2	*H.* sp2	0.16	T

Appedix 1.

MICPAT	*Microchlamys patella* (Claparede & Lachmann) Cockerell	15.80	T/P/S/L
MICSP1	*Microcorycia* sp1	0.11	P
NEBCAU	* *Nebela caudata* Leidy	0.02	T
NEBCOL	*N. collaris* (Ehrenberg) Leidy	11.14	T/L
NEBLAG	*N. lageniformis* Penard	1.08	T/S
NEBVAS	*N. vas* (Certes)	0.14	T
NEBWAI	*N. wailesi* Deflandre	0.07	T
PHRACR	*Phryganella acropodia* (Hertwig & Lesser) Hopkinson	0.22	T/S
PSEFUL	* *Pseudodifflugia fulva* Penard	1.06	T/P/S/L
TRAPUL	*Trachelocorythion pulchellum* (Penard) Bonnet	0.41	T/S/P
TGPSP1	*Trigonopyxis* sp1	0.38	T/S
TRIALO	*Trinema alofsi* Stepanek	0.20	T
TRICOM	*T. complanatum* Penard	0.22	T
TRIENC	*T. enchelys* Leidy	2.05	T/P/S/L
TRILIN	*T. lineare* Penard	16.59	T/P/S/L

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Morphology, Biometry, Ecology and Biogeography of Five Species of *Difflugia* Leclerc, 1815 (Arcellinida: Difflugiidae), from Tiete River, Brazil

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Summary. *Difflugia corona* Wallich, 1864; *D. gramen* Penard, 1902; *D. lanceolata* Penard, 1890; *D. claviformis* Penard, 1899 and *D. gigantea* Chardez, 1967 collected at the Tiete River Ecological Park (Brazil) are redescribed and compared to previous descriptions regarding morphology, biometry, ecology and geographical distributions. The biometrical measures and analysis based on a large number of individuals shows that natural populations of testate amoebae may be highly variable. Distinguishing traits for each species are discussed. The five *Difflugia* species inhabit quite a large range of micro habitats. The geographic distribution for each species is reviewed.

Key words: biometry, ecology, *Difflugia*, geographic distribution, morphology

Abbreviations used: ad - aperture diameter, al - number of apertural lobes, desc - description of the taxon, dim - dimensions of the test, eco - ecological associations, geo - geographic distribution, LM - light microscopy, ns - number of spines, SEM - scanning electron microscope, sl - spine length, syn - synonymic list, td - test diameter, tl - test length.

INTRODUCTION

The genus *Difflugia* Leclerc, 1815 is well defined as a genus of testate amoebae (Protista), but there is considerable uncertainty as to what distinguishes the species. There are descriptions of about 300 species and 200 subspecific and infrasubspecific taxa (Ogden 1983, Meisterfeld 2000). These descriptions were made mostly by light microscopy which does not allow detailed comparisons of test structure, form and composition (Ogden and Živković 1983). The identity of species is unclear partly because few species have types. Gauthier-Lièvre and Thomas (1958), in the most complete survey of the genus (Ogden 1983), described 129 species based on African specimens. Forty-five taxa were new to science, but none had a designated type. Few lists of synonyms have been compiled.

There have however been a number of improvements. The application of electron microscopy to protistology from the early 1960's, has improved the descriptions of protists (Patterson 1994, 1999; Patterson and

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Brugerolle 1988). Scanning electron microscopy allows more detailed examination of many characters of testate amoebae and more consistent definitions at the species level (Ogden 1983, Ogden and Živković 1983, Meisterfeld 2000). *Difflugia* has benefited from this approach (Ogden 1979, 1980a, b, 1983, 1990; Ogden and Fairman 1979; Ogden and Hedley 1980; Ogden and Živković 1983; Ogden and Meisterfeld 1989; Dekhtyar 1993). Finally, biometric analysis has been used increasingly to characterize species (Schönborn *et al.* 1983, Bobrov *et al.* 1999, Bobrov and Mazei 2004).

Good taxonomic work, redescribing and establishing the synonymies of poorly described species (Bobrov and Mazei 2004), is important to understand other aspects of the biology of these organisms such as their distribution, dispersal, ecology, and will help solve issues such as species concepts (Schlegel and Meisterfeld 2003, Bobrov and Mazei 2004).

Studies of testate amoebae to date emphasize species from Europe and Africa. Yang and colleagues have extended the observations to China (e.g.: Yang *et al.* 2004, Yang and Shen 2005) applying ultrastructural characters, morphometry and ecology. There are few studies on testate amoebae from South America and most have been conducted using LM only (e.g.: Boltovskoy 1956; Boltovskoy and Lena 1966, 1971; Dioni 1970; Vucetich 1970, 1972, 1973, 1978). There have been a few studies on the genus *Difflugia* from Brazil (Velho and Lansac-Tôha 1996, Lansac-Tôha *et al.* 2001).

The present paper adds data on five species of the genus *Difflugia* from the much polluted waters of the Tiete River. These observations extend species circumscriptions, and bring new light to bear on the morphology, biometry, ecology and distribution of *Difflugia*.

MATERIALS AND METHODS

Samples were taken from two localities in the Ecological Park of the Tiete River, Sao Paulo - Brazil in February (summer) and August (winter) 2004. The first locality was in the River itself (23°29'374"S 46°31'500"WO), a lotic environment, and the second was at a marginal lake (23°29'055"S 46°30'939"WO), a lentic environment. In both localities, two sampling methods were used: for organisms associated to the bottom sediment, 2 liters of the top 5 cm of the bottom sediment and water were collected and fixed using 4% neutralized formaldehyde. For organisms associated to the roots of aquatic plants, the method of Dioni (1967, 1970) was adapted in which 2 liters of water containing *Azolla* sp. and *Salvinia* sp. were collected, roots removed and fixed with 4% neutralized formaldehyde.

Collected materials were filtered through a series of sieves and the 40 µm to 500 µm size fraction was preserved in alcohol 70% for analysis. Sub-samples were placed in Petri dishes and stained with Bengal's Rose. Testate amoebae were separated from sediment under a stereomicroscope. Detailed analysis and measures were obtained using dissecting and compound microscopes. All procedures were done by the same person, following the recommendations of Patterson and Lee (2000) and Finlay *et al.* (2001).

The scanning electron microscopy procedures follow Ogden and Hedley (1980) and Yang *et al.* (2004). A diversity of individuals were selected and cleaned by successive transfers through distilled water and placed on a glass slide to dry. The individuals were transferred, using the static electricity of a single hair brush, to an aluminum-stub covered with adhesive from commercial adhesive tape dissolved in chloroform. The cells were oriented as required. Specimens were covered by a thin gold layer. The materials were analyzed and photographed using a Zeiss DSM 940 scanning electron microscope operating at 10 kV, at the Electronic Microscopy Laboratory, Institute of Biosciences, University of Sao Paulo (IBUSP). Materials are deposited at Laboratorio de Malacologia (IBUSP).

The terminology in testate amoebae is poorly established (Foissner and Korganova 2000). The present paper uses the following standard for descriptions: form of the test, composition of the test, pattern of the cementing organic matrix, color of the test, texture of test's surface, apertural complex (structures that delimit and surrounds the aperture). Polarity: apertural region and abapertural region (Fig. 1) is suggested terminology to substitute the use of oral and aboral. The present paper also uses anterior to refer to the apertural end of the cell, and posterior, regarding abapertural.

The review of the literature includes data previously reported. Some of this has been reported under different names. The abbreviations in parenthesis indicate the nature of the information given. Comprehensive tables regarding morphometry, localities and ecology have been compiled by the authors and are available upon request.

For all five species, three morphometric characters, as indicated in Fig. 1, were measured: test length, test diameter and aperture diameter. For *Difflugia corona*, two additional characters were measured: number of spines (ns) and spine length. Statistics were performed using the program STATISTICA 6.0.

RESULTS

Difflugia corona Wallich, 1864

Synonyms: *Difflugia proteiformis* Ehrenberg subspecies *globularis* Dujardin variety *corona* Wallich 1864, *Difflugia corona ecornis* Gauthier-Lièvre et Thomas, 1958 (published as *Difflugia corona* var. *ecornis*) *syn. nov.*

Data previously reported: *Difflugia proteiformis* Ehrenberg *globularis* Dujardin var. *corona* Wallich 1864:240-241 pl. 15, figs. 4b, c (desc); *Difflugia corona* Daday 1905:13-14 (desc, dim, geo); Deflandre 1926:523-525 (desc, dim, geo, syn); Štepánek 1952:16-17 (desc,

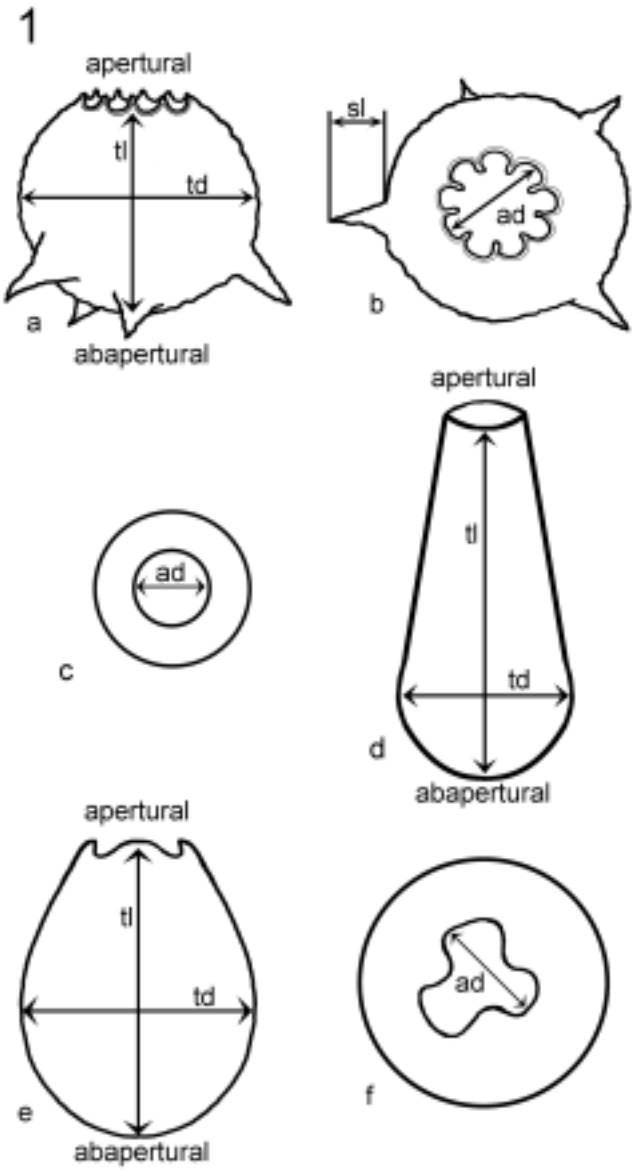


Fig. 1. Schematic outline of tests, showing position of measured axis and polarity. Not drawn in scale. *Difflugia corona* a - side view, b - apertural view; generalized pyriform *Difflugia*, c - apertural view, d - side view; *Difflugia gramen* e - side view, f - apertural view. tl - test length, td - test diameter, ad - aperture diameter, sl - spine length.

dim); Boltovskoy 1956:307-308 fig. 5 (desc, geo, syn); Gauthier-Lièvre and Thomas 1958:254-256, fig. 2a (desc, dim, eco, geo); Closs and Madeira 1962:8-9 (desc, dim, geo, syn); Closs and Madeira 1967:14 (desc, dim, geo); Vucetich 1972:277-278 (desc, dim, eco, geo, syn); Vucetich 1973:301 (desc, dim, eco, geo, syn); Ogden and Hedley 1980:128-129, figs. A-D (desc, dim, geo, SEM); Ogden and Živković 1983:346-348, fig. 4a-d (desc, dim, eco, geo, SEM); Medioli and Scott 1985:27 fig. 2a, b (desc, syn); Dekhtyar 1993:11-13, figs 4a-f (not *Protocucurbitella coroniformis* Gauthier-Lièvre and Thomas 1960, desc, dim, eco, geo, syn, SEM); Torres and Jebram 1994:72 (desc, dim, eco); Velho and Lansac-Tôha 1996:179-181 figs. 1, 1a (desc, dim, geo); Blanco 2001 (desc, dim); Zapata *et al.* 2002:24 (desc, dim, geo); Bobrov and Mazei 2004:136 (dim); *Difflugia corona ecornis* - Gauthier-Lièvre and Thomas 1958:256-258, fig. 3 (published as *Difflugia corona* var. *ecornis*)

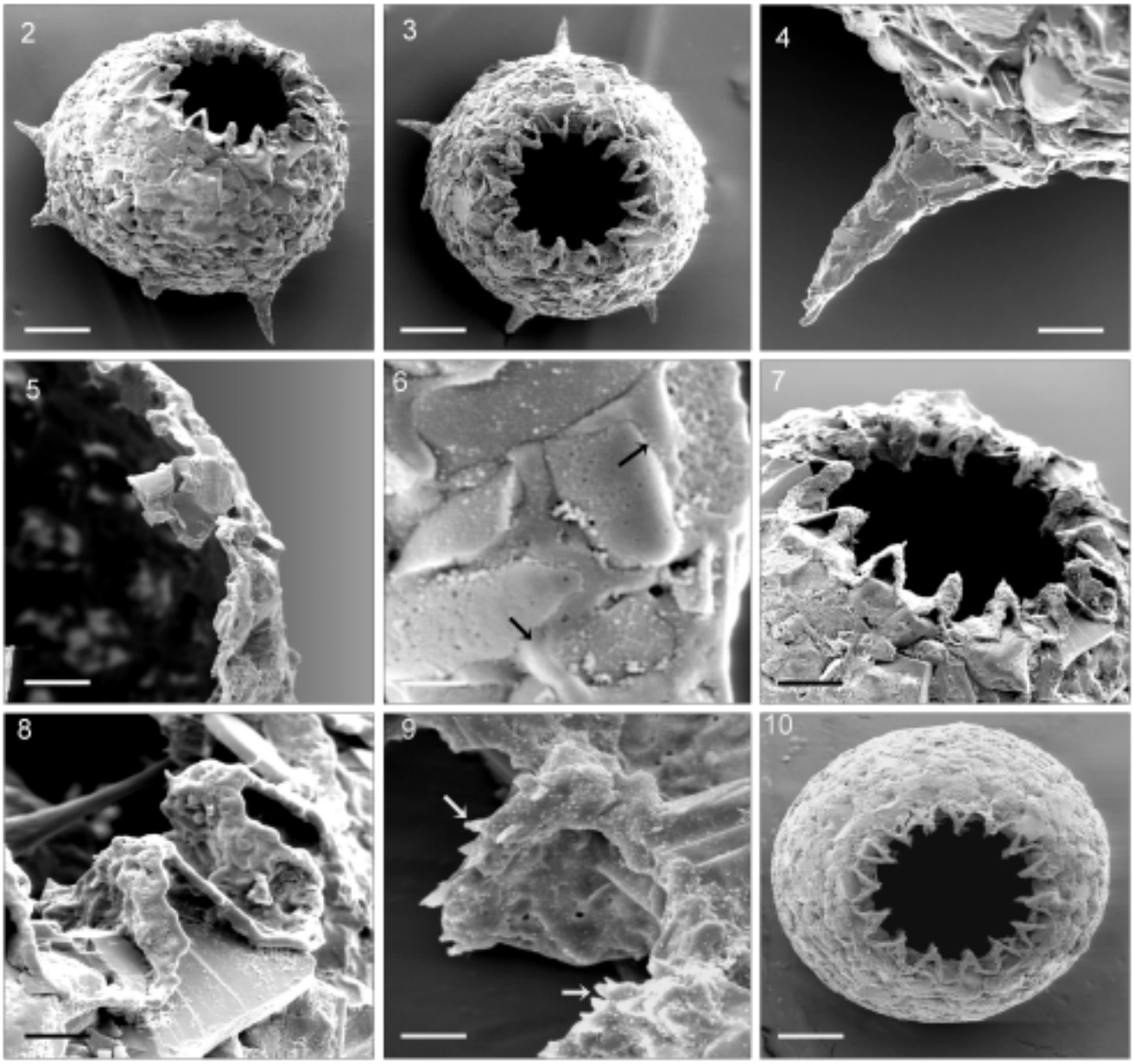
Examined material: 683 specimens by LM, 26 by SEM; 42 micrographs taken by Colin G. Ogden deposited at the Natural History Museum, London (NHM)

Morphology. Test sub-spherical to spherical (Figs 1a, 2). Circular in apertural view (Figs 1b, 3). Variable number (0-10) of conical hollow spines (Fig. 4) inserted from the mid-line to the abapertural end (Figs 2, 3). Surface of test smooth (Fig. 5), component aggregated particles flat, rarely breaking the circular contour. Test walls even in thickness (Fig. 5), except for a thicker aperture. Whole test composed of fine sand granules, rounded particles of quartz and cylindrical structures of exogenous nature (Figs 5, 6) all agglutinated by smooth organic cement (Fig. 6). Inside of test covered by organic matrix.

Aperture terminal, circular, with 9 to 15 denticulate lobes (Fig. 7). Apertural complex delimited by a particulate outline of the test and a rim of organic cement. Particles delimit a somewhat circular border, and an organic matrix covers this free edge to form lobes (Fig. 8). Apertural cover thick, each lobe is a hollow structure at the outer most part. Each lobe extends to the immediate inner surface of the test as a solid column, observed by LM as rod-like structures perpendicular to the outline of the aperture. Using SEM, it is possible to see that each lobe is provided, on the edge of each column, with one or two rows of spiny protuberances. These vary in number, shape and length (around 3-4 µm) from lobe to lobe (Fig. 9). The protuberances can be randomly distributed, not necessarily organized in rows.

Biometry. All measurements (Table 1) are highly variable (CV = 25.2-67.0). The number of conical spines presents the lowest standard error of the mean (0.11).

Size frequency distribution analyses indicate that *D. corona* has a main-size class and a large size-range for all characters. The smaller tests are less than a third of the size of bigger ones. The test height varies from 80 to 330 µm, but 58% are within the limits of 160 to 240 µm. The frequency analysis of test diameter gives



Figs 2-10. SEM micrographs of *Difflugia corona*. **2** - oblique apertural view of *D. corona*, showing test and spine form, and apertural outline; **3** - apertural view of *D. corona*, showing circular outline of the aperture; **4** - detail of one of the conical spines of *D. corona*; **5** - detail of test wall, this specimen was intentionally broken to show that walls are even in thickness; **6** - detail of agglutination in one of the spines, exactly as in all other areas of the test. Arrows indicate areas where the cement organic matrix is visible; **7** - detail of the aperture, completely outlined by organic material; **8** - detail of a denticulate lobe. Notice that the aperture is roughly defined by particle deposition, and the fine outline is made only by organic matrix; **9** - edge of denticulate lobe as seen from the inside of the test. Arrows indicate the spiny protuberances that are inserted at the outer-most edge of the lobe column; **10** -apertural view of *Protocucurbitella coroniformis ecornis*, showing test form, aperture outline and lack of spines. Scale bars: 60 µm (2, 3); 15 µm (4); 10 µm (5,8); 2 µm (6); 30 µm (7); 5 µm (9); 45 µm (10).

similar results. In regard to aperture diameter, the main-size class range is even smaller, where 67% of measurements are within the limits of 70-120 µm. The size of the conical spines varies from 20 to 40 µm in 70% of the analyzed tests. The number of spines varies from 0 to 10, but in 86.4 % of the tests is restricted to 3-5.

All measurements of *D. corona* are positively correlated to each other (Table 2), except for number of

Table 1. Biometric characterization of the investigated *Difflugia* species from Tiete River. Characters are as designated in Fig 1. Measurements in µm. $\overline{X}$ - arithmetic mean, M - median, SD - standard deviation, SE - standard error of the mean, CV - coefficient of variation in %, Min - minimum, Max - maximum, n - number of investigated specimens.

Characters	$\overline{X}$	Median	SD	SE	CV	Min	Max	n
Difflugia corona								
tl	192.1	200.0	48.36	1.85	25.2	80.0	380.0	683
td	189.8	200.0	48.76	1.87	25.7	80.0	360.0	683
ad	91.6	100.0	26.22	1.00	28.6	30.0	200.0	683
sl	37.5	40.0	12.07	0.49	32.2	10.0	100.0	607
ns	3.0	4.0	2.03	0.11	67.0	0.0	10.0	337
Difflugia gramen								
tl	108.0	100.0	20.21	1.46	18.7	80.0	160.0	192
td	92.3	80.0	29.07	2.10	31.5	50.0	160.0	192
ad	38.0	40.0	10.94	0.79	28.7	20.0	80.0	192
Difflugia lanceolata								
tl	174.8	180.0	26.37	2.16	15.1	80.0	240.0	149
td	75.1	80.0	13.83	1.13	18.4	40.0	120.0	149
ad	39.6	40.0	6.84	0.56	17.3	20.0	80.0	149
Difflugia claviformis								
tl	226.5	220.0	65.05	6.12	28.7	110.0	380.0	113
td	88.0	80.0	24.27	2.28	27.6	60.0	200.0	113
ad	49.3	40.0	11.83	1.11	24.0	40.0	90.0	113
Difflugia gigantea								
tl	380.0	420.0	78.10	26.03	20.6	280.0	460.0	9
td	204.4	200.0	32.83	10.94	16.1	180.0	280.0	9
ad	86.7	80.0	14.14	4.71	16.3	80.0	120.0	9

Table 2. Correlation coefficient between morphometric characteristics in the studied *Difflugia* species from Tiete River. Characters are as designated in Fig 1. n - number of studied specimens, NA - non applicable.

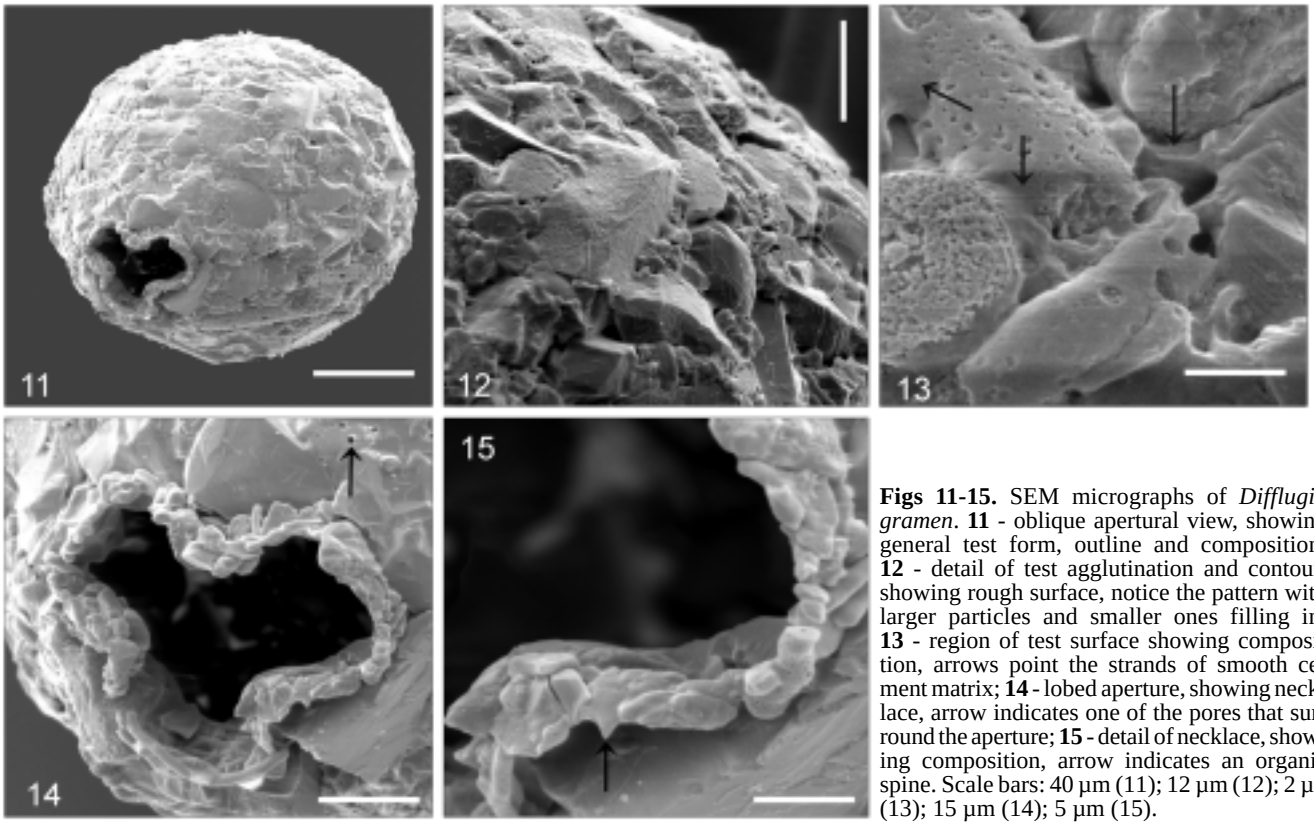
Species	tl-td	tl-ad	td-ad	sl-tl	sl-td	sl-ad	ns-tl	ns-td	ns-ad	ns-sl
Difflugia corona (n=♣)	0.93*	0.84*	0.85*	0.41*	0.44*	0.42*	0.16**	0.12^{NS}	0.17**	0.18^{NS}
Difflugia gramen (n=192)	0.72*	0.50*	0.58*	NA	NA	NA	NA	NA	NA	NA
Difflugia lanceolata (n=149)	0.66*	0.45*	0.75*	NA	NA	NA	NA	NA	NA	NA
Difflugia claviformis (n=113)	0.70*	0.72*	0.58*	NA	NA	NA	NA	NA	NA	NA

♣ - for characters tl-td, tl-ad and td-ad n=683; for sl-tl, sl-td and sl-ad n=608; for ns-tl, ns-td, ns-ad and ns-sl n=261. * $p < 0.001$, ** $p < 0.01$. NS - not significant

spines, which is not significantly correlated to test diameter and spine length. Table 3 shows morphometric ratios.

Remarks. These observations agree with recent studies (Ogden and Hedley 1980, Ogden and Živković 1983, Dekhtyar 1993) regarding test form and composition of this species. Although previous workers had not reported the apertural complex architecture described here, the authors of the present paper had access to Colin G. Ogden's micrographs of *D. corona* deposited in the Natural History Museum (NHM) Protist Collection, and the specimens registered are in agreement with the description given above. Some of the specimens registered by Ogden showed an apertural rim constructed mostly with agglutinated particles, while the ones encountered in the Tiete River always made it exclusively with organic cement material.

Compared with previous studies *D. corona* world-wide has a wide size range. The present work encountered a larger variation within the same population, and we attribute this to the size of sample (n=683).



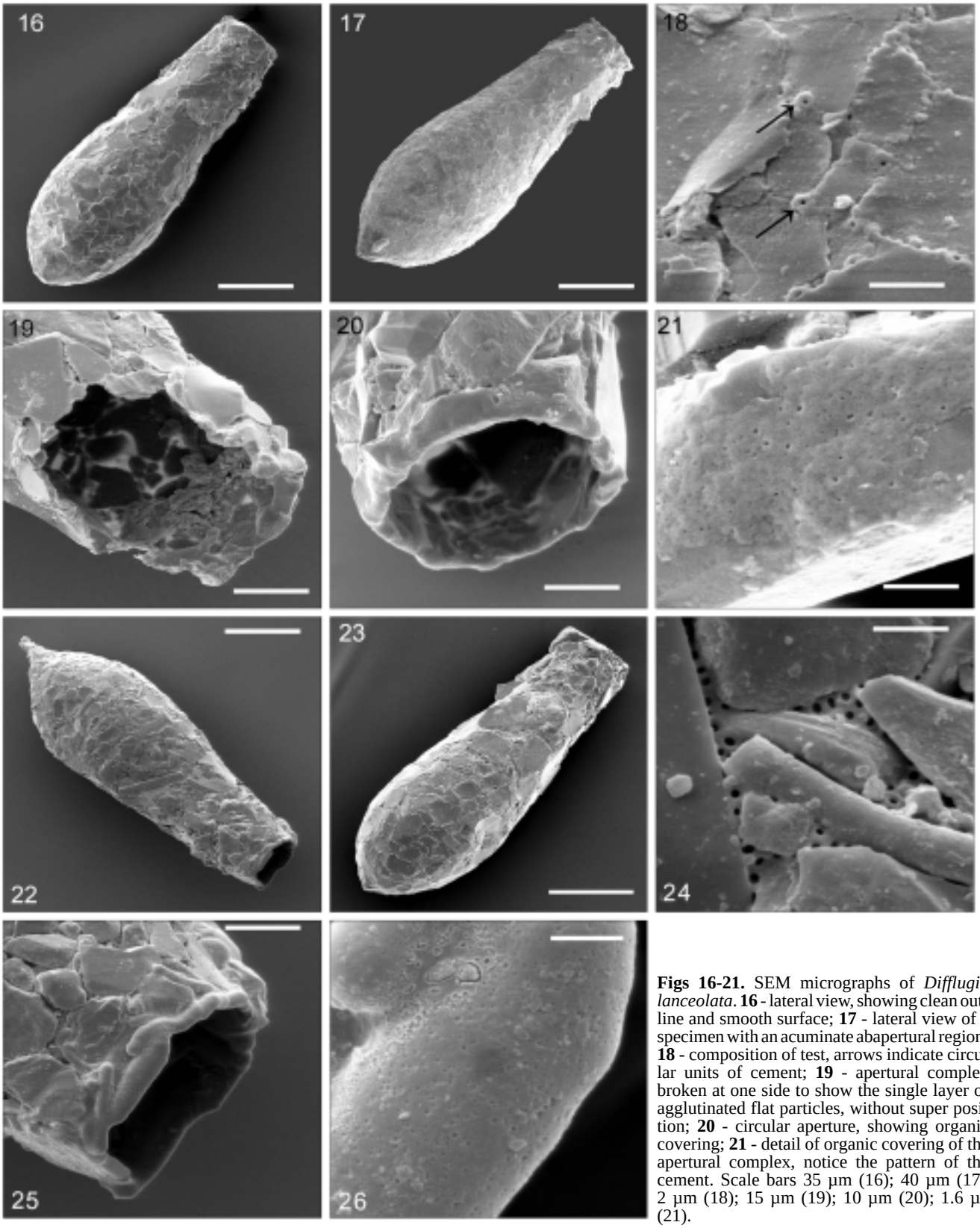
Figs 11-15. SEM micrographs of *Difflugia gramen*. **11** - oblique apertural view, showing general test form, outline and composition; **12** - detail of test agglutination and contour, showing rough surface, notice the pattern with larger particles and smaller ones filling in; **13** - region of test surface showing composition, arrows point the strands of smooth cement matrix; **14** - lobed aperture, showing necklace, arrow indicates one of the pores that surround the aperture; **15** - detail of necklace, showing composition, arrow indicates an organic spine. Scale bars: 40 µm (11); 12 µm (12); 2 µm (13); 15 µm (14); 5 µm (15).

Table 3. Size ratios between morphometric characters in the studied populations of *Difflugia* from Tiete River. Characters are as designated in Fig 1. SD - standard deviation, n - number of studied specimens.

Species	td/tl (SD)	ad/tl (SD)	ad/td (SD)
Difflugia corona (n=683)	0.99 (0.11)	0.48 (0.08)	0.49 (0.08)
Difflugia gramen (n=192)	0.85 (0.20)	0.35 (0.09)	0.43 (0.11)
Difflugia lanceolata (n=149)	0.43 (0.07)	0.23 (0.04)	0.53 (0.08)
Difflugia claviformis (n=113)	0.40 (0.10)	0.23 (0.06)	0.58 (0.13)
Difflugia gigantea (n=9)	0.55 (0.11)	0.24 (0.05)	0.43 (0.08)

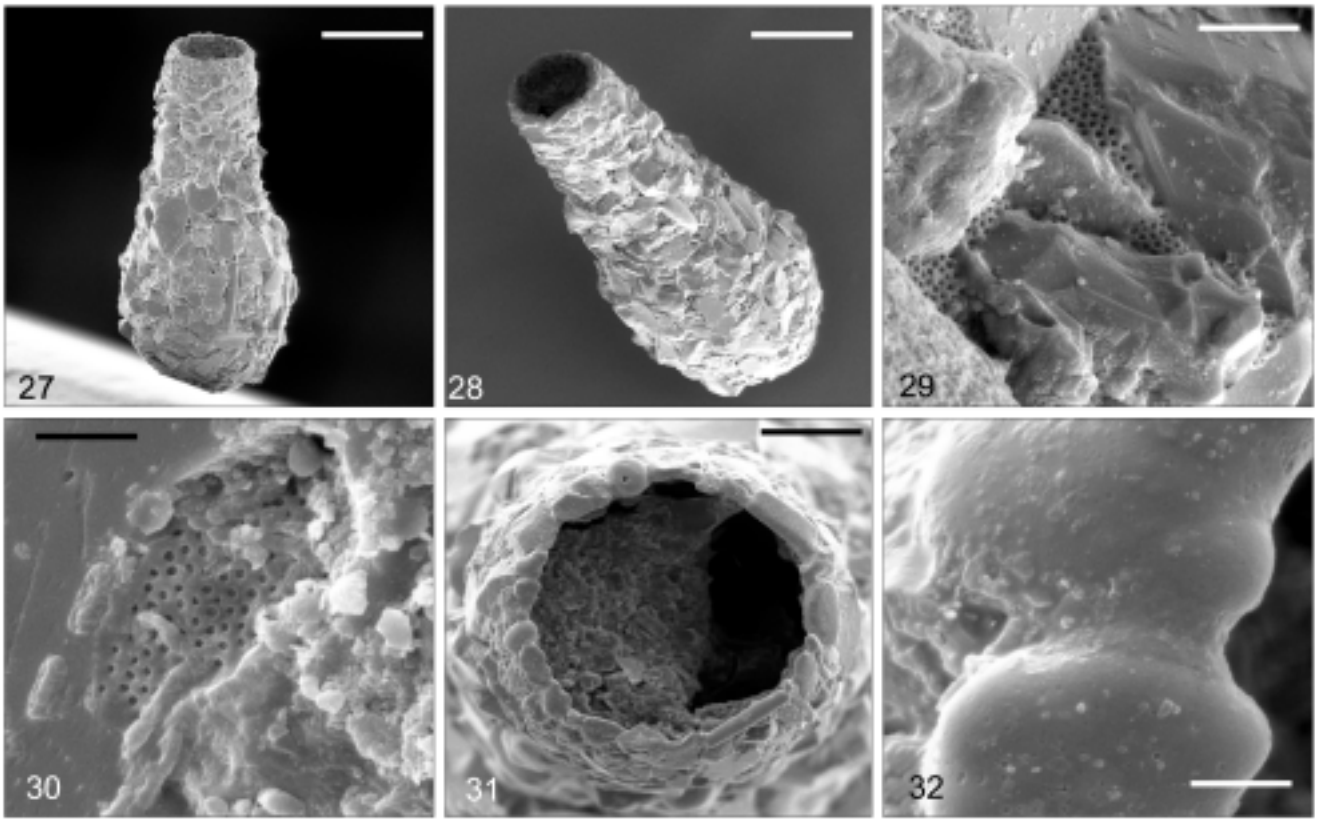
Table 4. Habitat exploration of *Difflugia* from Tiete River. Numbers in %. LS - lake sediment, LR - lake roots, RS river sediment, RR - river roots.

Species	LS	LR	RS	RR
Difflugia corona	26.06	46.76	9.15	18.03
Difflugia gramen	40.51	22.05	3.08	34.36
Difflugia lanceolata	0.00	3.57	9.29	87.14
Difflugia claviformis	5.60	6.40	64.00	24.00
Difflugia gigantea	33.33	33.33	33.33	0.00



Figs 16-21. SEM micrographs of *Difflugia lanceolata*. **16** - lateral view, showing clean outline and smooth surface; **17** - lateral view of a specimen with an acuminate abapertural region; **18** - composition of test, arrows indicate circular units of cement; **19** - apertural complex broken at one side to show the single layer of agglutinated flat particles, without super position; **20** - circular aperture, showing organic covering; **21** - detail of organic covering of the apertural complex, notice the pattern of the cement. Scale bars 35 µm (16); 40 µm (17); 2 µm (18); 15 µm (19); 10 µm (20); 1.6 µm (21).

Figs 22-26. SEM micrographs of *Difflugia claviformis*. **22** - lateral view showing general test form, outline and composition; **23** - another individual, to show variation on spine morphology; **24** - detail of test composition, showing particles embedded in a network like cement; **25** - circular aperture, showing organic covering; **26** - detail of the organic covering of the apertural complex, notice the pattern of the cement. Scale bars: 35 µm (22, 23); 2 µm (24); 15 µm (25); 2 µm (26).



Figs 27-32. SEM micrographs of *Difflugia gigantea*. **27** - lateral view, showing general test form, outline and composition; **28** - oblique apertural view shows the lack of distinction between the fundus and the neck; **29** - composition of test; **30** - detail of cement organization, notice the smooth layer underlying the perforations; **31** - circular aperture, showing organic covering; **32** - detail of the organic covering of the apertural complex, notice the pattern of the cement. Scale bars: 100 µm (26); 90 µm (27); 4 µm (28); 2 µm (29); 25 µm (30); 2 µm (31).

Ecology and geographic distribution. *Difflugia corona* has been reported from Africa, Asia, Europe, North and South America (Ogden and Hedley 1980, Ogden and Živković 1983). Records were made from plankton, sediment and periphyton samples (Table 4). In this study, the majority of organisms (46.76%) were in periphyton environments, such as the roots of aquatic plants in the River, a lotic habitat, confirming previous observations.

Taxonomic remarks about *Difflugia corona* var. *ecornis* Gauthier-Lièvre *et* Thomas, 1958. According to the ICZN (Ride *et al*. 1999), *Difflugia corona* var. *ecornis* must be regarded as *Difflugia corona* subspecies *ecornis*, because it was described before 1961 as a variety and is deemed to be treated as subspecies (article 45.6.4).

We are unable to distinguish *Difflugia corona ecornis* from the individuals observed here (and elsewhere) as *Difflugia corona*. The present survey found 76 specimens without spines, 15 of them were examined by SEM. All morphological features (Fig. 10) and biometrical values (Table 1) are otherwise the same as in *D. corona*. Our view is that the spineless specimens are part of a continuum within *Difflugia corona* and the names *Difflugia corona* var. *ecornis*, *Difflugia corona ecornis* and *Difflugia ecornis* are synonymized with *D. corona corona* (created through the principle of co-ordination, article 43.1).

We suspect this is indicative of a more general trend. Many early descriptions are inadequate to distinguish taxa to todays standards (Meisterfeld 2000). Small sample sizes will underestimate the biometric range in

Table 5. Morphometric comparisons among different surveys that used large sample sizes of the investigated *Difflugia* species. Characters are as designated in Fig 1. Measurements in µm.CA - character absent, ? - not informed.

References	td	Tl	ad	al	sl	ns	N
Difflugia corona							
Blanco (2001)	50-230	50-220	20-90	?	?	0-7	200
Bobrov and Mazei (2004)	132.5-184.5	141-210	8.5-86.1	?	?	?	65
present work	80-330	80-380	30-200	9-15	10-100	0-10	683
Difflugia gramen							
Stepanek and Jiri (1958)	53.4-64.7	70.8-92.4	17.5-28	3	CA	CA	100
Ogden (1983)	42-75	61-97	18-33	3-4	CA	CA	44
Bobrov and Mazei (2004)	39.5-77.5	57.0-85.5	15.9-36.0	3	CA	CA	40
present work	50-160	80-160	20-80	3-4	CA	CA	192
Difflugia lanceolata							
Ogden (1983)	56-92	108-155	22-32	CA	CA	CA	38
Bobrov and Mazei (2004)	40.5-84.5	87.5-189.5	21-54	CA	CA	CA	82
present work	40-120	80-240	20-80	CA	CA	CA	149
Difflugia claviformis							
Ogden and Hedley (1980)	97-196	247-393	33-62	CA	CA	CA	12
present work	60-200	110-380	40-90	CA	CA	CA	113
Difflugia gigantea							
Ogden and Fairman (1979)	168-231	341-480	55-84	CA	CA	CA	7
present work	180-280	280-460	80-120	CA	CA	CA	9

populations. As larger numbers of cells are included, the size ranges and ranges for other attributes within a species will increase. The concept of the species will expand, and taxa previously deemed to be distinct as varieties or subspecies will lose their identity.

Difflugia gramen Penard, 1902

Data previously reported: *Difflugia gramen* - Penard 1902:281; Deflandre 1926:518 (eco, syn); Štepánek 1952:17 (desc, dim, eco); Gauthier-Lièvre and Thomas 1958:257 Pl I figs a-e (desc, dim, eco, geo, syn); Štepánek and Jiří 1958:139, fig. 1 (desc, dim, eco, syn); Green 1975:550 (eco, geo); Vucetich 1970:45 (syn), 1972:275, fig. 4 (desc, dim, eco, geo, syn), 1973:302, fig. 25 (desc, dim, eco, geo, syn), 1978:81 (eco); Velho and Lansac-Tôha 1996:182 Pl I figs 6, 6a (desc, dim, eco, syn); Ogden 1980b:125 figs 1-6 (desc, eco, dim, SEM), 1983:50 (desc, eco, dim); Ogden and Meisterfeld 1989:121 figs 5, 6, 23, 24 (desc, dim, eco, geo, syn, SEM)

Examined material: 192 specimens by LM, 16 by SEM.

Morphology. Test spherical to ovoid (Figs 1e, 11), slightly tapered at apertural end. Circular in apertural view. Contour regular, sometimes broken by a larger agglutinated particle (Fig. 12). Whole test composed of some large and many medium sized mineral grains, mostly particles of quartz and between these are smaller ones, usually spherical, cemented by an organic matrix (Fig. 13). Surface of test slightly rough. Smooth cement matrix between the particles (Fig. 13) sometimes with perforations.

Aperture terminal, with 3 to 4 petal-shaped lobes (Fig. 14), delimited by a slightly raised irregular ridge (necklace), made of small agglutinated particles - small sand grains or cylindrical structures - and smooth organic matrix, evidenced by spiny protuberances (Fig. 15). A ring of pores in the organic matrix, outside the raised ridge, surrounds the aperture (Fig. 15).

Biometry. All characters exhibit much variability (Table 1, CV between 18.7 and 31.5). A low standard error of the mean is observed only in aperture diameter (0.79). Size frequency analysis shows that all measurements have a large size range, however, all present a main size class: for test length, 54.6% of observed measures are within the limits of 90 to 110 µm; for test diameter, 52.58% are within 60-100 µm; for aperture diameter 77.04% are within 20-40 µm. Table 2 shows that all characters are positively correlated to each other.

Remarks. *Difflugia gramen* was redescribed by Ogden (1980b). His observations agree with those of the

present paper and other works (Penard 1902; Gauthier-Lièvre and Thomas 1958; Štepánek and Jiří 1958; Vucetich 1972, 1973; Ogden and Hedley 1980; Ogden 1983; Ogden and Meisterfeld 1989; Velho and Lansac-Tôha 1996). Nevertheless, there is some morphological variance in the Tiete River population.

Ogden (1980b) and Ogden and Meisterfeld (1989) observed that texture and composition of the shell varies from a smooth outline composed of fine sand granules to a rough outline, composed of medium sized granules, sometimes breaking the contour of the test, whilst the present study observed an intermediate situation where the test is composed of both small and medium granules, and when the test does present a rough texture, the contour is rarely broken.

The apertural ridges observed here are more clearly delineated than previously described (Ogden 1980b, 1983; Ogden and Hedley 1980; Ogden and Meisterfeld 1989), and composed of both small grains and organic cementing material. The pores surrounding the aperture are smaller and sparser than those observed by Ogden (1980b).

The greatest variation observed regards cementing material. Ogden (1980b) and Ogden and Meisterfeld (1989) show cement matrix arranged in a mesh network, while the present work revealed a very smooth texture for the observed individuals.

The current study includes more individuals than previous studies. Most previous observations provide measurements that fall within the size range observed for all characters, except for Gauthier-Lièvre and Thomas (1958) and Ogden (1983), who observed smaller individuals. The present study extends the maximum size for this species. Ogden (1983) observed that a common feature for two British populations in Norfolk (Ogden 1980b, 1983) was the ratio aperture diameter/test length (0.32 ± 0.04 and 0.34 ± 0.04), the present study concurs with that ratio: 0.35 ± 0.09 (Table 3).

Ecology and geographic distribution. *Difflugia gramen* is recorded for all continents (Ogden and Hedley 1980). Species can be found in plankton, periphyton and in the sediment. In South America, records have been made by Deflandre (1926), Decloitre (1955), Vucetich (1972, 1973, 1978). In Brazil, records are for Rio de Janeiro (Wailes 1913, Cunha 1913), Mato Grosso (Green 1975), Minas Gerais (Dabés 1995), and Mato Grosso do Sul (Velho and Lansac-Tôha 1996) where the species was associated with the roots of aquatic plants.

Most organisms (Table 4) were in lentic habitats (62.58% of total), preferably associated to the sediment (40.53%). Previous workers have not observed this. An association with periphyton was indicated by Vucetich (1973), Ogden (1980b) and Ogden and Meisterfeld (1989).

Difflugia lanceolata Penard, 1890

Data previously reported: *Difflugia lanceolata*: Penard 1890:145-146, pl. IV, figs 59,60; Decloitre 1954:107-108, fig. 25 (dim); Vucetich 1973: 311, pl. V, fig. 43 (desc, dim, geo); Ogden and Hedley 1980:140, pl 59, figs A-D (desc, dim, geo, SEM); Ogden 1983:11, figs 6a-6f (desc, dim, geo, syn, SEM)

Examined material: 149 specimens by LM, 10 by SEM.

Morphology. Test pyriform, elongate (Fig. 16). Circular in apertural view. Abapertural region varies from regular semi-circumferential to acuminate (Figs 16, 17), tapering evenly from widest diameter at the posterior third towards the aperture. Tapering angle varies, in some cases slender, conical tear-shaped, in others the diameter is maintained along the test and individuals are more cylindrical. Test contour completely regular. Test made of flat particles of quartz (Fig. 18) and flat diatom frustules, arranged in a puzzle way, so that no superposition occurs (Fig. 19), hence, the test is thin and surface smooth, with polished appearance. Particles are agglutinated by circular units of organic cement (Fig. 18), seen between test particles.

Aperture terminal, circular. Complex constructed with flat or rounded quartz particles agglutinated and covered by several layers of organic cement (Fig. 20). Pattern of organic matrix is conserved indicating that this cover is made with the same cementing material (Fig. 21).

Biometry. Details are given in Table 1. All characters are variable (CV between 15.1 and 18.4), high standard error of the mean (SE = 0.56-2.16). Size frequency analyses show that all characters have a wide size range, but a main size classes can be defined as follows: test diameter, 63.75% of measures are within the limits of 80 to 90 µm; test length, 70.46% are within 180-200 µm; and aperture diameter, 73.82% are within 30-40 µm. Table 2 shows that all measured characters are high and positively correlated to each other.

Remarks. The characteristics of the test are in complete agreement with the diagnostic features of *D. lanceolata* described in Ogden (1983) and Ogden

and Hedley (1980). Variations have been observed in the Tiete River population in regard to the general test form and abapertural region. Even though these variations have been reported in the literature, they have not previously been reported within the same population.

The smallest and also the largest known individuals for this species were found in this study, extending the known size-range.

Ecology and geographic distribution. *D. lanceolata* has been reported from Africa, Asia, Europe, North and South America. In South America, there are reports from Argentina (Vucetich 1973), Venezuela (Grospietsch 1975) and Brazil for Mato Grosso (Hardoim and Heckman 1996) and Goiás (Lansac-Tôha *et al.* 2000). In the present study 87.2% of individuals associated to the roots of aquatic plants in the River (Table 4).

Difflugia claviformis Penard, 1899

Synonyms: *Difflugia pyriformis* var. *claviformis* Penard, 1899; *Difflugia oblonga* var. *claviformis* Cash, 1909; *Difflugia (oblonga) claviformis* Štepánek, 1952.

Data previously reported: *Difflugia pyriformis* var. *claviformis* - Penard, 1899 figs. 12-14; 1902: 218 fig. 3, 4; *Difflugia oblonga* var. *claviformis* - Cash *et* Hopkinson, 1909; Chardez 1967:594 Pl II fig 18 (desc, dim, syn); *Difflugia* (*oblonga*) *claviformis* - Štepánek 1952: 25 (desc, syn); 1967: 39 fig. 8. 6,8 (syn); *Difflugia claviformis* - Ogden 1979:145, 146, 148 fig. 4 (desc, SEM); Ogden and Hedley 1980:126 Pl. 52 figs A-D (desc, dim, geo, SEM)

Examined material: 113 specimens examined by LM, 12 by SEM.

Morphology. Test pyriform, elongate. Circular in apertural view. The abapertural region ends in a conical spine that varies in size and shape (Figs 22, 23), tapering evenly. Test contour regular, outline rarely broken by a larger particle. Apertural end composed of rounded particles of quartz and cylindrical structures, so the surface is slightly rough, while the abapertural end is composed of flatter particles of quartz, so the surface is smoother (Figs 22, 23). In both cases, particles are embedded in a network-like organic cement matrix (Fig. 24).

Aperture terminal, circular. Apertural complex made of cylindrical and spherical particles smaller than those in other regions of the test, agglutinated and covered by organic cement matrix (Fig. 25). Several layers of organic matrix are deposited on the free edge, this covering looks smooth, but still some of the meshed pattern can be seen (Fig. 26).

Biometry. All characters are very variable (Table 1, CV = 24.0-28.7), with a high standard error of the mean (1.11-6.12). Size frequency analysis shows that all measurements have a large size range, however, all have main size classes: 67.24% of all measured test diameter are within the range of 80 to 100 µm; 56.59% of all test lengths are within 180 to 240 µm; 91.13% of all aperture diameters are within 40 to 60 µm. Table 2 shows that all characters are positively correlated to each other.

Remarks. The morphology of tests observed here agree with *D. claviformis* as described by Ogden and Hedley (1980) and (Ogden 1979), except for additional observation of an apertural organic cover, and larger variation on conical spine size and shape. Changes of circumscription in this species are mainly due to variation in spine form. Cash and Hopkinson (1909) restricted *D. claviformis* to those provided with a nipple-like abapertural spine. However, Ogden (1979) and Ogden and Hedley (1980) lump different spine morphologies under this taxon - sometimes the spine is reduced or even absent - based on ultrastructural identity of the cement, which agrees to specimens observed here. Specimens from Tiete River, opposing to European populations, are mostly provided with a tubular conical spine, but other forms are also present. Differential diagnosis from similar species, namely *D. acuminata* Ehremberg, 1838, *D. elegans* Penard, 1890 and *D. curvicaulis* Penard, 1899, is given elsewhere (Ogden 1979). The population of Tiete River shows more variation in size than all previous studies.

Ecology and geographic distribution. *Difflugia claviformis* has been reported from Europe and North America (Ogden and Hedley 1980), from Venezuela (Grospietsch 1975), and from bottom samples in coastal lagoons of Rio Grande do Sul state, Brazil (Madeira-Falcetta 1974). In The present study 88% of all individuals were found in the River, 64% associated with the roots of aquatic plants and 24% associated with the sediment (Table 4).

Difflugia gigantea Chardez, 1967

Synonym: *Difflugia oblonga* v. *gigantea* Chardez, 1967

Data previously reported: *Difflugia oblonga* v. *gigantea* - Chardez 1967:593 fig. 2 (dim, syn);

Difflugia gigantea - Ogden and Fairman 1979:375 figs 12-15 (desc, dim, SEM).

Examined material: 9 specimens examined by LM, 5 by SEM.

Morphology. Test elongate, pyriform (Figs 27, 28). Circular in apertural view. Abapertural region spherical. Sides taper evenly towards the aperture from middle of total test length (Fig. 28). Test contour smooth, outline sometimes broken by larger particles. Test composed of agglutinated quartz particles and diatom frustules, so the test has an irregular outline (Fig. 29). Particles embedded in network-like organic cement with a meshed pattern (Fig. 30). The depressions that make up the mesh do penetrate through the cement, as there is an underlying continuous layer where smaller perforations can be seen.

Aperture terminal, circular. Apertural complex made of cylindrical and spherical particles agglutinated together and covered by organic cement matrix (Fig. 31). Several layers of organic matrix are deposited on the free edge so it is smooth but the meshed pattern can be seen (Fig. 32).

Biometry. All characters are variable (Table 1, CV between 16.1 and 20.6) and with a high standard error of the mean (4.71-26.03).

Remarks. *Difflugia gigantea* was originally proposed by Chardez (1967) as *Difflugia oblonga* v. *gigantea*, and elevated to species status by Ogden and Fairman (1979). In *D. oblonga* the test outline is more irregular due to the large particles agglutinated, the test surface, therefore, is rough. There is a well defined neck, characteristic of the species, and the organic matrix is only seen as a strand binding the particles together (Ogden and Fairman 1979, Ogden and Hedley 1980), both characteristics differ in *D. gigantea*. The specimens studied are generally more slender, especially in the anterior region, but for other characteristics are in complete agreement to those studied by Ogden and Fairman (1979). Population of Tiete River shows greater variation in size than reports in the literature.

Ecology and geographic distribution. Reported from England and North America, *Difflugia gigantea* is rare. The present work surveyed many samples where other pyriform species were abundant (*D. claviformis* and *D. lanceolata*) and found only nine specimens. Ogden and Fairman (1979) also found small numbers associated with *Sphagnum* mosses. Table 4 shows that the specimens were associated with the bottom sediment of the marginal lake (lentic environment), with the roots of aquatic plants in the same lake and with the sediment of the Tiete River.

DISCUSSION

As most prior studies of *Difflugia* lack type material and involve small sample sizes (see introduction), the identities of species is not clear. The most proximate genus is *Netzelia* Ogden, 1979, species of which build their test with autogenous material (idiosomes), or with siliceous structures found in nature (Ogden 1979, Anderson 1987, Meisterfeld 2000). *Netzelia* tests have regular contours, and a smooth surface. The particles that make up the test of *Difflugia* are picked up from the environment.

In this study we distinguished among spherical species by their form, size, and apertural complex architecture. *D. corona* can be distinguished by the presence of spines. *D. gramen* may be confused with some other testacea, such as *Difflugia lobostoma*, but can be distinguished by nuclear organization (*D. gramen* has a vesicular nucleus while *D. lobostoma* has an ovular nucleus with many nucleoli).

Distinctions between pyriform *Difflugia* are not easy to make (Chardez and Decloitre 1973; Ogden 1979, 1980a, b, 1983; Ogden and Fairman 1979). *D. claviformis* and *D. lanceolata* have similar LM appearances, with almost the same dimensions (Table 1) and are indistinguishable by size ratios (Table 3). The most important difference is the patterning of the organic matrix. It is treated as a reliable distinguishing character for species (Ogden 1983, 1990; Ogden and Živković 1983; Meisterfeld 2000). In *D. lanceolata* the cement is composed of circular, ring-like units, which connect the flattish agglutinated particles at some points. In *D. claviformis*, the cement is a network-like matrix where the particles are embedded. *Difflugia gigantea* can be distinguished by its larger size, but once again, the pattern of the organic cement matrix is also unique. At the outer side of the test, there is a perforated layer, and subjacent to it there is another one with smaller pores. Any uncertainties about this character would undermine the distinctions among these pyriform species.

The morphometric variation found in the Tiete River is the most extensive ever reported. Table 5 shows surveys with largest sample sizes to date, as well as the biometrical data obtained. High variations have also been observed for some species in another biometrical

survey (Bobrov and Mazei 2004). They observed that the most variable character is the aperture diameter. The present study differed, with a lower variance coefficient for this character when compared to other characters. Bobrov and Mazei (2004) used a dataset from numerous localities and the variation they observed may reflect selection pressures of many ecological factors. A single population, such as the one found in Tiete River, may therefore show less variation in highly selected features.

The high variation observed is in part a consequence of the large number of individuals investigated. It can also be attributed to ecological factors such as micro-habitats and great environmental ranges, since the Tiete River is highly polluted. Consequently more niche adaptation would follow. Attributes of each species show normal distributions. A main-size class is always present, and studies that rely on fewer cells are likely to report individuals with a limited size range. This will give good estimates of the mean, but underestimate the range.

The increase in range for attributes leads to increased overlap between nominal species described earlier, and should lead us to question the validity of some of those taxa.

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Isolation and Molecular Identification of Vahlkampfiid Amoebae from an Island (Tenerife, Spain)

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Summary. Four vahlkampfiid amoebae were isolated from freshwater samples taken on the island of Tenerife. Ribosomal DNA sequence analysis identified two isolates to belong to the genus *Naegleria*, representing novel species with their closest relatives *N. gallica and N. philippinensis* respectively. The two other isolates belong to the genus *Vahlkampfia* and represent also novel species, with as their closest relatives *V. avara* and *V. inornata* respectively. The new *Vahlkampfia* spp. are more divergent from their nearest relatives than the new *Naegleria* spp. are to their closest relatives. The latter is probably due to the already high number of described *Naegleria* spp. in contrast to the few known *Vahlkampfia* spp. There are no morphological features in these new species of either genus that allow their identification. Only molecular typing can discriminate species within the vahlkampfiids.

Key words: 5.8S rDNA, cyst morphology, internal transcribed spacers, *Naegleria canariensis* n. sp., *N. tenerifensis* n. sp., *Vahlkampfia ciguana* n. sp., *V. orchilla* n. sp

INTRODUCTION

Since the discovery that *Naegleria fowleri* can cause fatal disease in man a lot of searches to find this free-living pathogen in the environment has led to the recognition of a high diversity of species in the genus *Naegleria* (for an overview see De Jonckheere 2002, 2004). Although large parts of the world have been sampled to look for the presence of *Naegleria* spp. still a lot of geographic areas have not been covered. In one of these areas not covered, Madagascar, a recent case of PAM indicates *N. fowleri* is also present on this island (Jaffar-Bandjee *et al*. 2005).

Also other vahlkampiids have acquired an interest because of their possible implication in keratitis. However, amoebic keratitis is due to *Acanthamoeba* spp. and the implication of other free-living amoebae in the disease remains controversial (De Jonckheere 2002).

Recently a report was published on the occurrence of *Acanthamoeba* strains in Tenerife, Canary Islands, Spain (Lorenzo-Morales *et al*. 2005). There is no information on the presence of *Naegleria* on this island, and no cases of PAM are known from this place. I have, therefore, tried to isolate vahlkampfiids from freshwater on this island. Such an investigation has become quite

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easy because of the quick and accurate identification of new isolates using ITS and 5.8S rDNA sequences for the identification of *Naegleria* (De Jonckheere 1998) and other vahlkampfiids (De Jonckheere and Brown 2005).

MATERIALS AND METHODS

Samples of freshwater sediments were taken at different sites on the island of Tenerife, Spain, beginning of March 2005. Water bodies sampled were three agricultural water reservoirs in concrete, one disused and almost empty barrage, a small river and a water display near the swimming pool of a hotel (Table 1). Kept for a week at room temperature, the samples were inoculated onto plates with non nutrient (NN) agar plates coated with *Escherichia coli* (Page 1988). The sealed plates were incubated at room temperature (RT), 37°C and 42°C.

Only isolates that showed the typical vahlkampfiid morphology were further investigated. To be sure to have pure cultures the isolates were subcultured several times by cutting a piece of agar with the migrating ring of amoebae and placing it upside down on a fresh plate. Isolates grown from 37°C plates were tested for growth at 42°C. Flagellate formation was tested in distilled water (DW) at RT.

DNA was isolated from pelleted trophozoites using the UNSET method (Hugo *et al*. 1992). The ITS1, 5.8S and ITS2 rDNA were PCR-amplified using the ITS forward primer and the ITS reverse primer, corresponding to the 3' end of the SSU rDNA and the 5' end of the large subunit (LSU) rDNA, respectively (De Jonckheere 2004). Two pairs of ITS primers were employed, one pair designed for amplifying from *Naegleria* spp. specifically (De Jonckheere 1998) and a second less specific pair for amplifying from vahlkampfiid species in general (De Jonckheere and Brown 2005). The PCR product was sequenced (each strand in opposite direction) with the amplification primers without cloning with a Beckman CEQ2000 sequencer using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter Inc., Fullerton, CA, USA).

The 5.8S rDNA sequences of vahlkampfiid isolates other than *Naegleria* were aligned with those of published vahlkampfiid genera (De Jonckheere 2004, De Jonckheere and Brown 2005) using the programme ClustalX (Thompson *et al*. 1997). The total ITS1, 5.8S and ITS2 sequences of the *Naegleria* isolates were aligned with those of published sequences of other *Naegleria* which have identical 5.8S sequences. The alignment was manually adjusted. Phylogenetic trees were constructed using the DNAPARS (parsimony), DNADIST (distance matrix), NEIGHBOR (neighbor-joining and UPGMA), FITCH, KITSCH, DNAML and SEQBOOT (bootstrapping) programs of the PHYLIP (version 3.572c) package (Felsenstein 1989).

The complete ITS sequences have been deposited in EMBL with the accession numbers AJ973124 till AJ973127.

RESULTS

Isolation and morphological genus identification. There were so many amoeba isolates overgrowing each other on the plates at RT that they could not be cultured separately for identification. At 37°C two *Naegleria* isolates were recovered and one other vahlkampfiid genus (Table 1). At 42°C one isolate, belonging to another vahlkampfiid genus than *Naegleria*, was recovered. The vahlkampfiids were isolated from the samples of an agricultural water reservoir in concrete, the barrage and the small stream. Subculture at 42°C of the strains isolated at 37°C yielded growth for strain T3(37), not for strains TE1(37) and TE4(37). The two *Naegleria* isolates TE1(37) and TE3(37) transformed readily into flagellates when suspended in DW, the two other vahlkampfiid isolates did not.

Molecular identification. The two *Naegleria* isolates, TE1(37) and TE3(37), have identical 5.8S rDNA sequence which correspond to that of *N. gruberi* senso strictu, *N. pringsheimi*, *N. italica*, *N. clarki*, *N. australiensis*, *N. tihangensis*, *N. philippinensis*, *N. mexicana*, *N. gallica*, *N. endoi* and *N. laresi* (De Jonckheere 2004). But it is the ITS2 sequence that determines the species (De Jonckheere 2002, 2004). Strain TE1(37) has the same sequence length of 170 bp for the ITS2 (Table 2) as *N. gallica* (De Jonckheere 2004) and *N. dunnebackei* (Visvesvara *et al.* 2005). It differs in the ITS2 sequence by two bp substitutions from *N. gallica* (Table 3) and four bp substitutions from *N. dunnebackei*. I propose the name *N. canariensis*, denoting the name of the Canary Islands, Spain, from where the strain was isolated. The ITS2 sequence length of 110 bp (Table 2) in strain TE1(37) is not detected in any described *Naegleria* sp. Its ITS2 sequence differs by 5 bp from *N. philippinensis* (Table 4), 6 bp from *N. endoi* and *N. byersi*, 7 bp from *N. mexicana* and 13 from *N. gruberi* sensu stricto. I propose the name *N. tenerifensis*, denoting the name of the Isle of Tenerife, Spain, from where the strain was isolated.

Strain TE3(42) represents a new vahlkampfiid with unique ITS1, 5.8S and ITS2 sequences (Table 2). Its closest relative is *V. avara* (Table 5) with which it clusters with all methods used in trees based on the 5.8S rDNA sequences (Fig. 1), but there is a lot of sequence difference in all parts sequenced (Table 5). I propose the name *V. ciguana*, denoting the name of the place from where the strain was isolated, Embalso de Ciguana in Tenerife, Spain. Strain TE4(37) represents a new vahlkampfiid with unique ITS1, 5.8S and ITS2 sequences (Table 2). Its closest relative is *V. inornata* (Table 6), with which it clusters with all methods used in trees based on the 5.8S rDNA sequences (Fig. 1), but there is an enormous sequence difference in ITS1 and

Table 1. Amoeba isolation at 37°C, and morphological vahlkampfiid identification in samples from Tenerife.

Sample	Place	Amoeba growth	vahlkampfiid identification	Enflagellation
TE1	Buenavista del Norte (WR)	+	*Naegleria*	+
TE2	Buenavista del Norte (WR)	-	NA	NA
TE3	Embalso de Ciguana	+	*Naegleria**	+
TE4	Rio Orchilla	+	vahlkampfiid	-
TE5	San Miguel (WR)	+	-	NA
TE6	Adeje (hotel water display)	+	-	NA

WR - agricultural water reservoir in concrete.
* also a *vahlkampfiid* isolate at 42°C.

Table 2. Lengths (in bp) of ITS1, 5.8S and ITS2 rDNA sequences of strains and proposed new species names.

Isolate	ITS1	5.8S	ITS2	Total	Closest relative	Proposed new species
TE1(37)	33	175	170	378	*N. gallica*	*N. canariensis* n. sp.
TE3(37)	33	175	110	318	*N. philippinensis*	*N. tenerifensis* n. sp.
TE3(42)	31	155	161	347	*V. avara*	*V. ciguana* n. sp.
TE4(37)	141	153	368	662	*V. inornata*	*V. orchilla* n. sp.

Table 3. Sequence lengths (in bp) of *N. gallica* and strain TE1 (37) (differences from *N. gallica* in parenthesis).

	N. gallica Capd60	*N. canariensis* n. sp strain TE1 (37)
ITS1	33	33 (0)
5.8S	175	175 (0)
ITS2	170	170 (2 substitutions)

Table 4. Sequence lengths (in bp) of *N. philippinensis* and strain TE3 (37) (differences from *N. philippinensis* in parenthesis).

	N. philippinensis RJTM	*N. tenerifensis* n. sp. strain TE3 (37)
ITS1	33	33 (0)
5.8S	175	175 (0)
ITS2	113	110 (2 substitutions, 3 deletions)

Table 5. Sequence lengths (in bp) of *V. avara* and strain TE3 (42) (differences from *V. avara* in parenthesis).

	V. avara CCAP1588/1A	*V. ciguana* n. sp. strain TE3 (42)
ITS1	28	31 (5 substitutions, 3 insertions)
5.8S	155	155 (13 substitutions)
ITS2	152	161 (32 substitutions, 9 insertions)

Table 6. Sequence lengths (in bp) of *V. inornata* and strain TE4 (37) (differences from *V. inornata* in parenthesis).

	V. inornata CCAP1588/2	*V. orchilla* n. sp. strain TE4 (37)
ITS1	320	141 (almost no overlap)
5.8S	154	153 (36 substitutions, 6 indels)
ITS2	185	368 (almost no overlap)

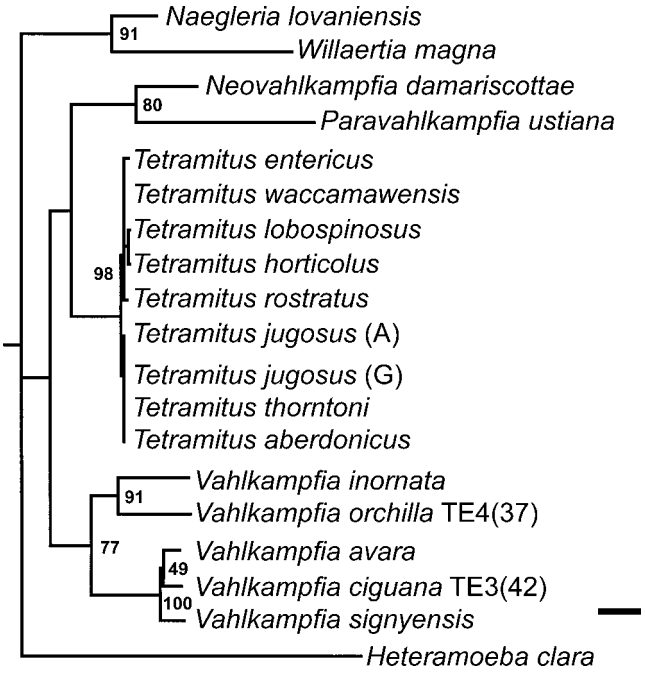
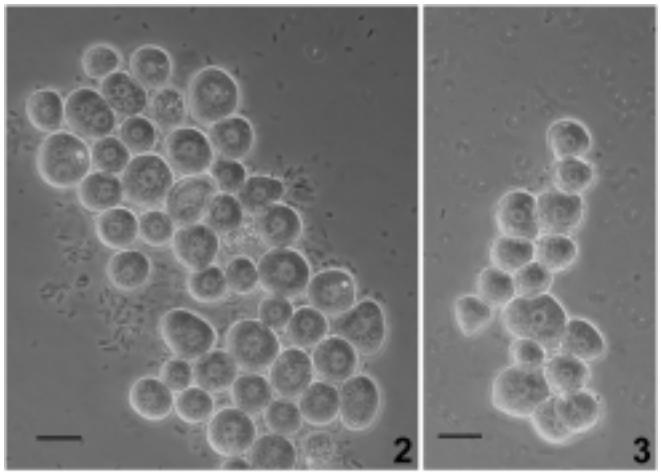


Fig. 1. Phylogenetic tree inferred from comparisons of the 5.8S rDNA sequences using the Kimura two-parameter correction and the neighbor-joining method. Only bootstrap values higher than 50% are indicated at the nodes, except for *Vahlkampfia avara - V. ciguana*. The distance corresponding to 10 changes per 100 nucleotide positions is indicated by the bar.



Figs 2, 3. 2 - cysts of *Vahlkampfia ciguana* n. sp. with phase contrast; **3** - cysts of *Vahlkampfia orchilla* n. sp. with phase contrast. Scale bars: 10 µm.

ITS2 (Table 6). I propose the name *V. orchilla*, denoting the name of the place from where the strain was isolated, Rio Orchilla in Tenerife, Spain.

Light microscopy of the two *Naegleria* isolates. Both isolates T1(37) and T3(37) have typical *Naegleria* cysts, round with pores (not shown). No angular type cysts are observed. The cysts become empty and non-viable very quickly on the agarose plates. When grown at 42°C (strain TE3(37) only) the cysts are much more variable in shape and size.

Light microscopy of the two *Vahlkampfia* isolates. Isolate T3(42) has mostly circular cysts (diameter 8.0-16.5 µm) with a smooth outer wall to which bacteria stick (Fig. 2). Cysts are often loosely aggregated, embedded in detritus and bacteria. Amoebae sedimented by centrifugation show a pink colour. Isolate TE4(37) has cysts (diameter 8.0-20 µm) that are more variable in form, circular and reniform, to angular, and size (Fig. 3) with a smooth outer wall. In the biggest cysts two or three nuclei are frequently observed. Cysts are often loosely aggregated. Amoebae sedimented by centrifugation show a pink colour.

Naegleria canariensis n. sp.

Diagnostic summary. The trophozoites have the typical appearance of *Naegleria* spp. and they can be induced to transform into flagellates. The cysts have a round shape with pores in the walls as for most other described *Naegleria* spp. The strain grows at 37°C but not at 42°C.

Because of the morphological similarity of the cysts with those of other *Naegleria* spp. molecular identification is required. The species can be identified from the ITS2 sequence, which differs by 2 bp substitutions from that *N. gallica.* The ITS1 and 5.8S rDNA sequence is identical to that of *N. gallica*. The sequence is available from EMBL under accession N° AJ973124.

The type strain TE1(37) was isolated from a water reservoir in Tenerife, one of the islands of the Canary Islands, from which the name was chosen.

Naegleria tenerifensis n. sp.

Diagnostic summary. The trophozoites have the typical appearance of *Naegleria* and they can be induced to transform into flagellates. The cysts have a round shape with pores in the walls as for most other described *Naegleria* sp. The strain grows at 42°C.

Because of the morphological similarity of the cysts with those of other *Naegleria* spp. molecular identification is required. The species can be identified from the ITS2 sequence, which differs by 2 bp substitutions and

3 bp deletions from that *N. philippinensis*. The ITS1 and 5.8S rDNA sequence is identical to that of *N. philippinensis*. The sequence is available from EMBL under accession N° AJ973125.

The type strain TE3(37) was isolated from a water reservoir in Tenerife, from which the name was chosen.

***Vahlkampfia ciguana* n. sp.**

Diagnostic summary. The trophozoites have the typical appearance of vahlkampfiid amoebae and could not be induced to transform into flagellates. The cysts have a round shape without pores in the walls. The strain grows at 42°C. A pink colour is observed.

Because morphology does not allow identification to genera within the Vahlkampfiidae, except for *Naegleria*, molecular identification is required. The species can be identified from the ITS1, 5.8S and ITS2 sequences. The 5.8S rDNA sequence demonstrates the strain belongs to the genus *Vahlkampfia* and that its closest relative is *V. avara*. The sequence is available from EMBL under accession N° AJ973126.

The type strain TE3(42) was isolated from a water reservoir in Tenerife, Embalso de ciguana, from which the name was chosen.

***Vahlkampfia orchilla* n. sp.**

Diagnostic summary. The trophozoites have the typical appearance of vahlkampfiid amoebae and they can not be induced to transform into flagellates. The cysts have a round to irregular shape without pores in the walls. The strain grows at 37°C but not at 42°C. A pink colour is observed.

Because morphology does not allow identification to genera within the Vahlkampfiidae, except for *Naegleria*, molecular identification is required. The species can be identified from the ITS1, 5.8S and ITS2 sequences. The 5.8S rDNA sequence demonstrates the strain belongs to the genus *Vahlkampfia* and that its closest relative is *V. inornata*. The sequence is available from EMBL under accession N° AJ973127.

The type strain TE4(37) was isolated from a river in Tenerife, Rio Orchilla, from which the name was chosen.

DISCUSSION

It has been shown that in the genus *Naegleria* strains within the same species have identical ITS2 sequences (De Jonckheere 2004). Only in two species, *N. byersi* and *N. andersoni*, a difference of one bp is detected in the ITS2 of one strain in each species. Two *Naegleria* spp. have been described which differ by only two bp in the ITS2 sequence. *Naegleria australiensis* has a closely related species, which was called originally a sister species, later the spa variant, and then a subgroup of *N. australiensis*. It was finally given species status, *N. tihangensis* , and differs by an insert of only two T's in the ITS2 compared with that of *N. australiensis* (De Jonckheere 2002). Unlike *N. australiensis*, which is pathogenic in experimental animals, there is no indication that *N. tihangensis* is pathogenic.

It is, therefore, through the sequence of the ITS2 that it can be concluded that the two *Naegleria* isolates from the island of Tenerife are new species, as they differ from described species by 2 bp (1.2%) and 5 bp (4.5%) respectively. Both have round cysts with pores which are not distinguishable from those of their closest relatives *N. gallica* strain Capd60 (Pussard and Pons 1979) and *N. philippinensis* strain RJTM (Matias *et al.* 1990) respectively. The cysts of strain Capd60 were also reported to need frequent subculture in order to survive, just as these two new isolates from Tenerife. The max. growth temperature of *N. gallica* is 37°C, and also the new species *N. canariensis* grows at this temperature, not at 42°C. The max. growth temperature of *N. philippinensis* is 40°C, while the new species *N. tenerifensis*, its closest relative, grows at 42°C.

Also in the case of the two new *Vahlkampfia* isolates it is very difficult to see the differences in cyst morphology between the new isolates and those of the three established *Vahlkamfia* spp., i.e. *V. avara*, *V. inornata* (Page 1967) and *V. signyensis* (Garstecki *et al*. 2005). Page (1967) reported that the cysts of *V. avara* collect more debris, as is found in the related *V. ciguana*, and that the cysts of *V. inornata* are more variable in shape, as is found in the related *V. orchilla*. Only the sequence results disclose that these two new isolates are to be considered two new species. Based on rDNA sequences, *V. avara* and *V. inornata* were the only two *Vahlkampfia* spp. of the 7 investigated, which remained within the genus (De Jonckheere and Brown 2005). The cysts of the only other molecular valid *Vahlkampfia* sp., *V. signyensis*, are always spherical and are often loosely aggregated and/or embedded in detritus (Garstecki *et al*. 2005). *Vahlkampfia avara* grows at 37°C while the new species *V. ciguana*, strain TE3(42), grows at 42°C, but *V. signyensis*, within the same cluster, grows at 20°C only. The latter was isolated from Antarctica, which probably explains this low temperature tolerance.

In the cluster of *V. avara*, *V. ciguana* and *V. signyensis* all have a 5.8S rDNA length of 155 bp and the latter two differ by almost the same number of bp from *V. avara* (8.5% sequence divergence). In the other *Vahlkampfia* cluster *V. inornata* has a 5.8S rDNA length of 154 bp and *V. orchilla* 153 bp (27.5% sequence divergence). There is also a lot more sequence difference in the ITS1 and ITS2 between the latter two species than there is between *V. ciguana* and *V. avara*. In general, the new *Vahlkampfia* spp. are more divergent from their nearest relatives than the new *Naegleria* spp. are to their closest relatives. The latter is probably due to the already high number of described *Naegleria* spp. in contrast to the low number of *Vahlkampfia* spp. discovered until now

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ACTA
PROTOZOOLOGICA

Myxidium elmatboulii n. sp. and *Ceratomyxa ghaffari* n. sp. (Myxozoa: Myxosporea) Parasitic in the Gallbladder of the Red Sea Houndfish *Tylosurus choram* (Rüppell, 1837) (Teleostei: Belonidae) from the Red Sea, Egypt

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Summary. Two new myxosporean species, *Myxidium elmatboulii* n. sp. and *Ceratomyxa ghaffari* n. sp., are described from the gallbladder of the Red Sea houndfish *Tylosurus choram* (Ruppell, 1837) from the Gulf of Suez, Red Sea, Egypt. Diagnostic features of *Myxidium elmatboulii* n. sp.: plasmodia disporous, spherical in shape. Spores s-shaped with smooth surface and measures 20.7 (19.0-23.0) × 10.6 (9.0-12.0) × 10.4 (8.5-12.0) µm. Polar capsules pyriform with 9-10 polar filaments, measuring 9.10 (8.0-10.0)× 3.8 (3.5-4.0) µm. Spores of *Ceratomyxa ghaffari* n. sp. slightly crescent-shaped, measuring 7.6 (6.0-9.0) × 29.9 (25.0-33.0) µm. Polar capsules typically rounded, 3.3 (3.0-4.0) µm in diameter with 5 filament turns.

Key words: *Ceratomyxa elmatboulii* n. sp., Egypt, houndfish, *Myxidium elmatboulii* n. sp., Myxosporean, Myxozoa, Red Sea.

INTRODUCTION

Myxosporea constitute a major group of fish parasites and their impact on wild and cultured fishes is significant (Kent *et al*. 2001) and are thus of both economic and ecological importance. Yet, little is known about the myxosporeans of the Red Sea fishes. Research on the parasites of the Red Sea fauna has been sporadic and inadequate considering the high fish biodiversity in the area. Few studies have been carried out on parasites of the Red Sea fishes as those of Paperna and Overstreet (1981), Ali *et al*. (2001, 2003) and Diamant *et al*. (2004). In view of the recognized pathogenicity inflicted by many fish parasites on commercial fish, it is desirable to obtain data as complete as possible on the protistan parasites of fish from this region. During a recent survey on the protistan parasites from selected Red Sea fish, two myxosporean parasites were recorded. *Myxidium elmatboulii* n. sp. and *Ceratomyxa ghaffari* n. sp. are described as new species from the gallbladder of the Red Sea houndfish *Tylosurus choram* (Ruppell, 1837).

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MATERIALS AND METHODS

Fresh caught fishes were purchased from fishermen at Gulf of Suez during visits between November 2000 and February 2002. Visits were carried out every two months and about 15 fish were examined during each visit. A total of 108 *Tylosurus choram* fish were examined; the fish range from 28-40 cm in total length. Fish were necropsied and all organs and body fluids were examined for myxosporean infection. Fresh spores were measured and photographed using Zeiss Axiovert 135 microscope with Contax Camera. Descriptions and measurements of spores followed the guidelines of Lom and Arthur (1989). Measurements were based on 30 fresh spores and data were presented as: mean ± SD (range). All measurements in µm. Drawings were made with the aid of a camera Lucida. For permanent preparation, air-dried smears were stained with Giemsa after fixation in acetone-free absolute methanol.

RESULTS AND DISCUSSION

Myxidium elmatboulii n. sp. (Figs 1-4, 7a)

Vegetative stages: The bile contained floating plasmodia of *Myxidium* mixed with *Ceratomyxa* forms (Fig. 2). Plasmodia were spherical and contained two tightly packed spores (Fig. 1). The diameter of the plasmodia was 27 ± 1.4 (25-30).

Spores: Spores typical of the genus *Myxidium*. Mature spores were smooth, sigmoid or s-shaped in the frontal view with prominent acuminated tips (Figs 3, 4, 7a). Sutural line was thin and slightly curved. Polar capsules pyriform; equal in size and tapering anteriorly to form prominent necks opening at the end of the spore tips. Polar filament with 9-10 coils was perpendicular to the longitudinal axis of the polar capsule. Single binucleated sporoplasm was filling the entire extracapsular spore cavity. Spore dimensions: 20.7 ± 1.2 (19.0-23.0) in length × 10.6 ± 0.9 (9.0-12.0) in width × 10.4 ± 0.8 (8.5-12.0) in thickness. Polar capsules: 9.10 ± 0.6 (8.0-10.0) × 3.8 ± 0.2 (3.5-4.0).

Taxonomic affinities: The spore shape and/or measurements of the present species showed some similarities with other *myxidium* sp. As the present spores have smooth surfaces, species with ridged surfaces are excluded from the comparison. Of those species with smooth surface, only the following resemble *M. elmatboulii*: *Myxidium incurvatum* Thelohan, 1892 (from Lom and Dykova 1992); *M. sphaericum* Thelohan, 1895 (Moser *et al.* 1989); *M. giganteum* Dolfein, 1898 (Kpatch *et al.* 1996); *M. baurei* Kovaleva *et* Gaevskaya, 1982 (after Kalavati *et al.* 1996); *M. trachinorum* Canning *et al.* 1999 and *M. tuanfegensis* Gong *et al.* 2003 (Table 1). *M. incurvatum* is smaller than *M. elmatbouli* and there are only 5-7 turns to the polar filament. *Myxidium sphaericum* has spores of comparable length; however; its polar capsules are about half of the present species and have lower number of polar filament coils (5-8 *vs.* 9-10). Among the species infecting the African fishes, *M. giganteum* differs from the present species in having quite smaller polar capsules and less polar filament turns (6 *vs.* 9-10). *M. baueri* has more straight spores than the present species, quite smaller polar capsules and less number of filament coils (5*vs.* 9-10). Spores of *M. trachinorum* are smaller in all body measurements and possess prominent kink in the spore middle. The spore dimensions of *M. tuanfegensis* very close to the present species, however it appears distinctly different from our species in having quite smaller rounded polar capsules with a lower number of polar filaments (5-6 *vs.* 9-10).

Regarding the fish host, no myxosporean infection is mentioned in the literature to this fish species. The comparison of the present parasite revealed no *Myxidium* species recorded thus far is identical. Therefore, we propose to establish the present myxosporean as *Myxidium elmatboulii* n. sp.

Host: *Tylosurus choram* (Ruppell, 1837)

Locality: Gulf of Suez (Lat. 30° N and Long. 32.5° E)

Location in the host: Gall bladder

Prevalence: 18/108 (16.6%).

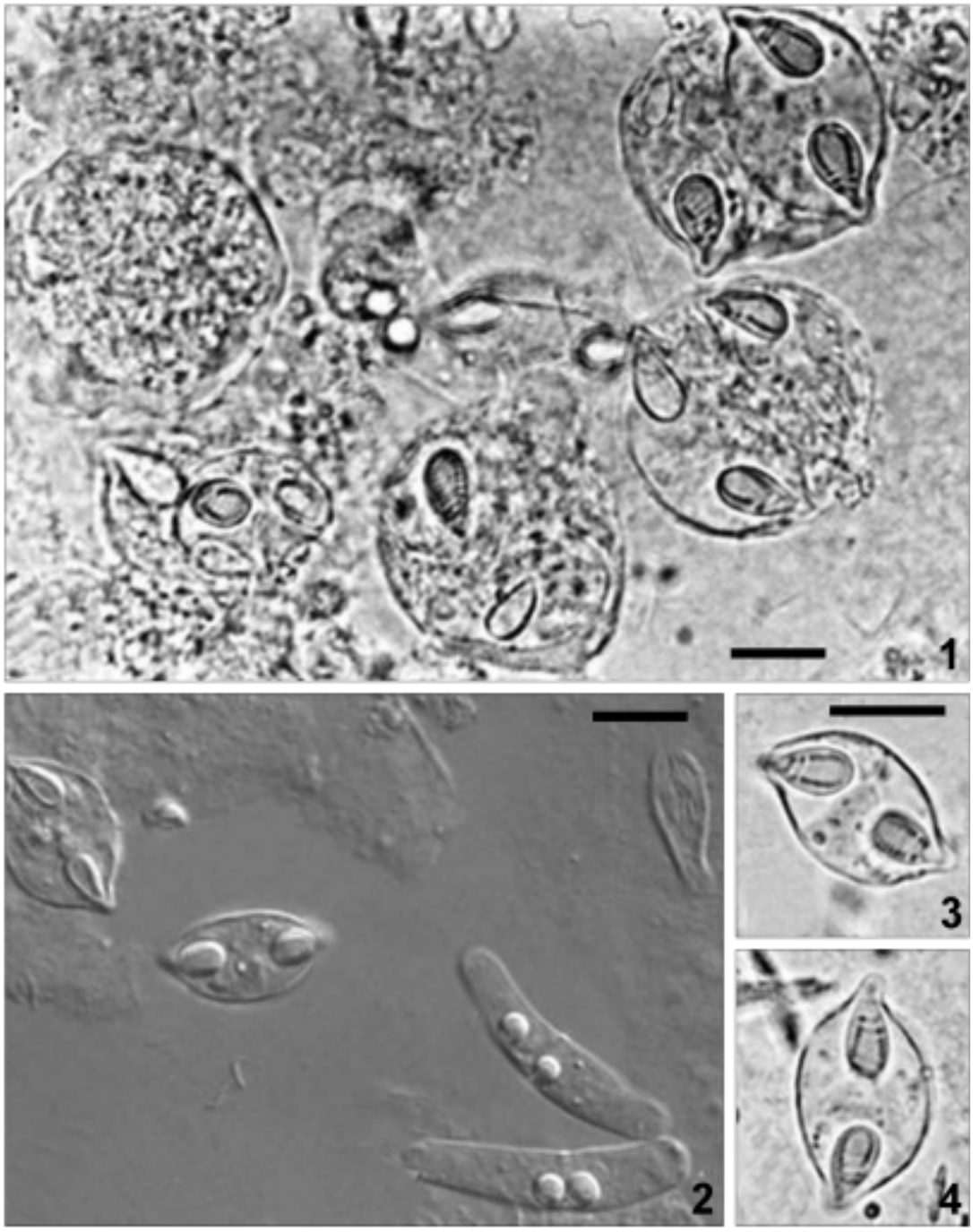
Type material: Syntypes on slid no. Myx.-15 is deposited at the Museum of Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Etymology: The parasite is named for Prof. Mansour El-Matbouli in recognition of his valuable contribution to myxozoan research.

Ceratomyxa ghaffari n. sp. (Figs 5-6, 7b)

Vegetative stages: The vegetative forms of this parasite were not observed. Free spores, mostly mature ones, were densely floating in the bile. The infection was mixed with *Myxidium* forms (Fig. 2).

Spores: Spores typical of genus *Ceratomyxa*. Mature spores were slightly crescent-shaped, the anterior margin was arched and the posterior margin was nearly straight or with very slight concavity. Valves were equal,



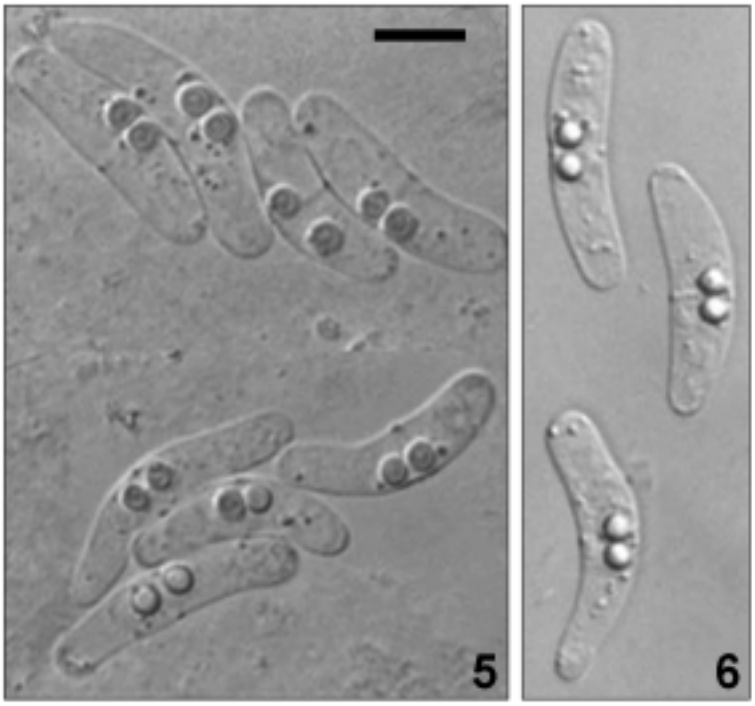
Figs 1-4. *Myxidium elmatboulii* n. sp. from the gallbladder of *Tylosurus choram*. **1** - disporic plasmodia; **2** - fresh spores of *Myxidium elmatboulii* and *Ceratomyxa ghaffari* (DIC); **3, 4** - spores of *Myxidium elmatboulii* in sutural and frontal views, respectively. Scale bars: 10 µm.

Table 1. Comparative descriptive measurements (µm) of *Myxidium elmatboulii* n. sp. with morphologically similar species.

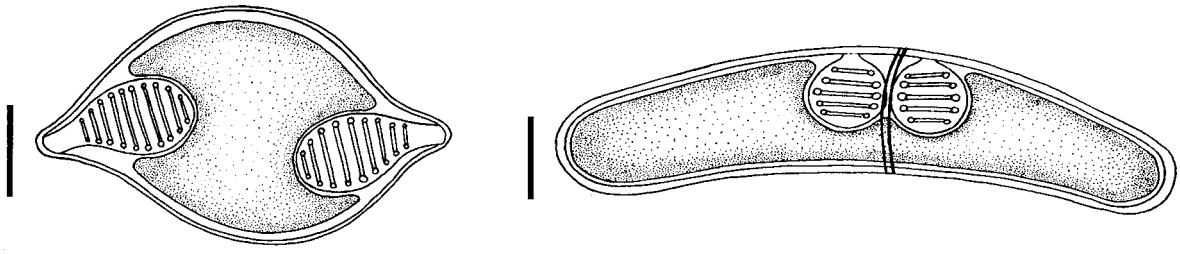
Species	Host	Site of infection	Locality	Spore		Polar capsule	
				length	width	length	width
M. incurvatum Thelohan, 1892 (from Lom and Dykova 1992)	*Syngnatus acus*	Gallbladder	Mediterranean coast, France	8.0-20.0	4.0-8.2	3.0-5.5	2.4-2.7
M. sphaericum Thelohan, 1895 (Moser *et al.* 1989)	*Trachinotus blochi*	Gallbladder	Heron Island, Australia	17.5 (15.0-20.0)	7.5 (6.0-8.0)	4.5 (4.0-6.0)	3.7 (3.0-5.0)
M. giganteum Dolfein, 1898 (Kpatch *et al.* 1996)	*Raja miraletus*	Gallbladder	Coast of Senegal	19.9 ± 0.2 (19.0-20.0)	9.5 ± 0.8 (8.0-10.0)	4.7 ± 0.6 (4.0-5.4)	2.9 ± 0.2 (2.0-3.0)
M. baueri Kovaleva *et* Gaevskaya, 1982 (Kalavati *et al*. 1996)	*Patagonatothen sima*	Gallbladder	Falkland Islands, Southwest Atlantic	22.4 ± 1.9 (19.0-24.0)	9.9 ± 1.0 (9.0-11.0)	5.2 ± 0.2 (4.5-5.5)	3.7 ± 0.2 (3.0-4.0)
M. trachinorum Canning *et al*., 1999	*Echiichthys vipera*	Gallbladder	South coast of UK at Plymouth and Weymouth	17.2	8.8	6.5	3.7
M. tuanfengensis Gong *et al*., 2003	*Letobotia taeniops*	Liver, intestine	China	19.5 ± 0.8 (18-20)	9.7 ± 0.6 (9.1-10.4)	6.8 ± 0.5 (5.7-7.8) (diameter)	
M. elmatbouli n. sp.	*Tylosurus choram*	Gallbladder	Gulf of Suez, Red Sea	20.7 ± 1.2 (19.0-23.0)	10.6 ± 0.9 (9.0-12.0)	9.1 ± 0.6 (8.0-10.0)	3.8 ± 0.2 (3.5-4.0)

Table 2. Comparative descriptive measurements (µm) of *Ceratomyxa ghaffari* n. sp. with morphologically similar species.

Spores	Host	Site of infection	Locality	Spore		Polar capsule	
				length	thickness	length	width
C. inaequalis Doflein, 1898 (Lubat *et al.* 1989)	*Symphodus mediterraneus* and *S. tinca*	Gallbladder coast, Italy	Mediterranean	5.5 (5.0-6.0)	25.0 (23.5-31.0)	2.5 (diameter)	
C. elegans Jameson, 1929 (Jurakhno 1993)	*Scorpaena porcus*	Gallbladder	Black Sea	6.7- 8.0	26.0-31.5	2.4-2.5	2.2-2.4
C. sprenti Moser *et al.*, 1989	*Chaeodon rainfordi*, *C. trifascaiatus*, *C. aureofasciatus*, *Choerodon venustus*	Gallbladder Australia	Heron Island,	5.7 (4.0-8.0)	16.3 (14.0-23.0)	2.4 (2.0-3.0)	-
C. dissimilaris Narasimhamurti *et al.*, 1990	*Nemipterus mesoprion*	Gallbladder	Bay of Bengal, India	10.4 (9.2-11.8)	41.6 (36.4-45.8)	4.8 (3-5.2) 3.5 (2.4-3.8)	large small
C. peculiaria Jurakhno, 1991	*Spicara flexuosa*	Gallbladder	Black Sea	6.5-8.5	21.0-29.3	2.4-2.7	1.9-2.4
C. aspera Aseeva, 2003	*Limanda aspera*, *L. herzensteini*	Gallbladder	Japan Sea	8.0-10	34-42.5	4.0-4.5 (diameter)	
C. ghaffari n. sp. (Present paper)	*Tylosurus choram*	Gallbladder	Gulf of Suez, Red Sea	7.6 ± 1.1 (6.0-9.0)	29.9 ± 3.6 (25.0-33.0)	3.3 ± 0.4 (3.0-4.0) (diameter)	



Figs 5, 6. Fresh spores of *Ceratomyxa ghaffari* n. sp. from the gallbladder of *Tylosurus choram* (DIC). Scale bar: 10 µm.



Figs 7a, b. 7a - line diagrams. Spore of *Myxidium elmatboulii* n. sp. in frontal view. **7b -** sutural view of *Ceratomyxa ghaffari* sp. n spore. Scale bars: 5 µm.

cylindrical, thin and smooth with rounded ends. Straight sutural line was clearly seen between the two valves. Polar capsules were spherical and equal size. Polar filament with 5 coils was perpendicular to the longitudinal axis of the capsule. Single binucleated sporoplasm was filling the entire extracapsular spore cavity. Spore Dimensions: 7.6 ± 1.1 (6.0-9.0) in length x 29.9 ± 3.6 (25.0-33.0) in thickness. Polar capsules 3.3 ± 0.4 (3.0-4.0) in diameter.

Taxonomic affinities: *C. ghaffari* is superficially similar to *Ceratomyxa inaequalis* Dolfein, 1898 (Lubat *et al.* 1989); *C. elegans* Jameson, 1929 (Jurakhno 1993); *C. sprenti* Moser, Kent *et* Dennis 1989; *C. dissimilaris* Narasimhaurti, Calavati, Anuradha *et* Dorothy, 1990; *C. peculiaria* Jurakhno, 1991 and *C. aspera* Aseeva, 2003 (Table 2).

Comparatively, *C. inaequalis* can be distinguished from the present species by having unequal valves and

unequal polar capsules. In addition, *C. inaequalis* has shorter spores with smaller polar capsule. Similarly, *C. elegans* differs in having quite smaller polar capsules with lower number of filament coils (3-4 *vs*. 5). C*eratomyxa sprenti* can be separated from the present species by its quite thinner spores, which are nearly half of the present species. The asymmetrical spore valves with bent ends, unequal polar capsule and polar filament can distinguish *C. dissimilaris*. Moreover, *C. dissimilaris* has quite thicker spores. Similarly, *C. peculiaria* can be differentiated by its smaller spore dimensions; lower number of filament coils (3 *vs*. 5) and its pyriform polar capsules compared to typically rounded ones characterizing the present species. *C. aspera* appears distinctly different from the present species in having quite thicker spores with asymmetrical spore valves.

The above comparison revealed that the present species is distinctive in its feature from other recorded species. In addition, no myxosporean infections have been recorded in the present fish host. Therefore, the present species is considered new and the name proposed is: *Ceratomyxa ghaffari* n. sp.

Host: *Tylosurus choram* (Ruppell, 1837)

Locality: Gulf of Suez (Lat. 30° N and Long. 32.5° E)

Location in the host: Gall bladder

Prevalence: 15/108 (13.8%).

Type material: Syntypes on slide no. Myx.-16 is deposited at the Museum of Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Etymology: The parasite is named for Prof. Fathy Abdel-Ghaffar, Professor of Parasitology, Cairo University, Egypt.

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Short Communication

Blood Parasites in Passerine Birds in Slovakian East Carpathians

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Summary. Wild birds in two adjacent locations of the Bukovské Vrchy Hills in the East Carpathians (Slovakia) were investigated for blood parasites in the post-breeding period. In 2001, 595 birds were investigated at Ruské. In 2003, 289 birds were investigated in Kurników Beskid. In Ruské, parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* were found at prevalences of 21 %, 6 % and 0.5 %, respectively. In Kurników Beskid, the prevalences of infections with the parasites of genera *Haemoproteus*, *Leucocytozoon* and *Trypanosoma* were 17 %, 0.7 % and 0.3 %, respectively. Parasite species found in blood smears of the birds included *Haemoproteus attenuatus, H. balmorali, H. belopolskyi, H. fringillae, H. picae, H. zosteropis, Leucocytozoon dubreuili, L. fringillinarum, L. majoris* and occasionally also *Plasmodium* sp. and *Trypanosoma* sp. Except for *H. fringillae*, all found species of hematozoa are priority findings for Carpathians. This is the first report of finding of *H. zosteropis* in *Hippolais icterina*.

Key words: *Haemoproteus, Leucocytozoon,* Passeriformes, *Plasmodium, Trypanosoma*.

INTRODUCTION

Blood parasites (hematozoa) are a heterogeneous group of organisms that typically live in the blood of the host during at least some of the stages of their development. Hematozoa have been found in 68 % of birds examined to date (Krone *et al*. 2001). Although hematozoa are wide-spread geographically, their prevalences in different regions are very different. The parasites found most frequently in peripheral blood smears include protozoa of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Peirce 1989). Besides this group, a number of other protozoan blood parasites from the genera *Trypanosoma, Babesia*, *Atoxoplasma* and *Hepatozoon* and larval stages of nematodes (microfilariae) have also been described in birds. Findings of these genera in peripheral blood smears are, however, rare (Kučera 1982).

Blood parasites in passerine birds were investigated in Slovakia in the 1970s (Kučera 1981a, b, c, 1982). These reports, however, do not specify the locations where the investigations were made, individual species of birds or the determination of individual species of blood parasites. In the present study, blood parasites in birds from the Slovakian part of the East Carpathians were investigated with the aim to determine the spec-

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trum of the parasite species, their prevalence and the intensity of infections.

MATERIALS AND METHODS

The birds examined were mist-netted for the purpose of ornithological research in two locations in the Bukovské Vrchy Hills. The area is in the north-east Slovakia, and it borders with Poland and the Ukraine. In 2001, investigations were made at the lower border of the forest in the abandoned village of Ruské in the Ruská Kotlina Valley (49° 07´ N, 22°21´ E). The location lies 500 m above sea level. A total of about 200 m of mist-nets were used. The netting and collection of blood samples took place from the end of June to mid-September. In 2003, Kurników Beskid Mountain at the Slovak-Polish border (1000 m above sea level) about 3 km from Ruské was selected for bird monitoring. The nets were installed along a narrow grassy meadow on a ridge surrounded with natural woody growths with a dominant representation of the beech (*Fagus sylvatica*). A total of about 200 m of mist-nets were used. The netting and collection of blood samples took place in the second half of August and in September. In Ruské, a total of 595 birds were examined (499 juveniles, 96 adults) of 33 species, and 289 birds (265 juveniles, 24 adults) of 26 species were examined in Kurników Beskid.

Blood samples used for smears were collected from *vena ulnaris cutanea*. The blood smears were left to dry, then fixed by dipping in methanol, and dried again. In the laboratory, the smears were stained with a combination of stains May-Grünwald and Giemsa-Romanowski using a method according to Pappenheim (Lucas and Jamros 1961).

The smears stained were examined microscopically for the presence of blood parasites. At least 100 000 erythrocytes were examined

Table 1. Blood parasites in wild passerine birds in summer 2001 in Ruské. (H - *Haemoproteus* spp., L - *Leucocytozoon* spp., P - *Plasmodium* spp.)

Host species	Positive/examined	Blood parasites in total	Prevalence (%) of H	L	P
Acrocephalus palustris	0/2	0.0			
Aegithalos caudatus	0/13	0.0			
Anthus trivialis	0/13	0.0			
Coccothraustes coccothraustes	1/1	100.0	100.0	0.0	0.0
Certhia familiaris	0/3	0.0			
Dendrocopos major	0/2	0.0			
Emberiza citrinella	1/8	12.5	12.5	0.0	0.0
Erithacus rubecula	26/118	22.0	14.4	9.3	0.0
Ficedula albicollis	0/2	0.0			
F. hypoleuca	0/2	0.0			
F. parva	0/2	0.0			
Fringilla coelebs	3/5	60.0	60.0	0.0	0.0
Garrulus glandarius	2/2	100.0	100.0	0.0	0.0
Hippolais icterina	4/7	57.1	57.1	0.0	0.0
Lanius collurio	4/16	25.0	6.3	12.5	6.3
Locustella fluviatilis	0/4	0.0			
Luscinia luscinia	3/4	75.0	75.0	0.0	0.0
Muscicapa striata	1/2	50.0	50.0	0.0	0.0
Parus ater	0/1	0.0			
P. caeruleus	0/21	0.0			
P. major	2/54	3.7	0.0	3.7	0.0
P. montanus	0/11	0.0			
Phoenicurus phoenicurus	0/2	0.0			
Phylloscopus collybita	3/84	3.6	1.2	2.4	0.0
P. sibilatrix	2/6	33.3	33.3	0.0	0.0
P. trochilus	1/21	4.8	4.8	0.0	0.0
Prunella modularis	0/5	0.0			
Pyrrhula pyrrhula	2/11	18.2	18.2	0.0	0.0
Saxicola rubetra	0/1	0.0			
Sitta europaea	0/9	0.0			
Sylvia atricapilla	83/125	66.4	60.8	15.2	0.0
S. communis	1/21	4.8	4.8	0.0	0.0
Turdus merula	12/17	70.6	58.8	5.9	11.8
Total	151/595	25.4	21.2	6.2	0.5

Table 2. Blood parasites in wild passerine birds in summer 2003 in Kurników Beskid. (H - *Haemoproteus* spp., L - *Leucocytozoon* spp., T - *Trypanosoma* spp.)

Host species	Positive/examined	Blood parasites in total	Prevalence (%) of H	L	T
Anthus trivialis	0/5	0.0			
Certhia familiaris	0/1	0.0			
Dryocopus martius	0/1	0.0			
Erithacus rubecula	28/143	19.6	19.6	0.0	0.0
Ficedula hypoleuca	0/8	0.0			
F. parva	0/2	0.0			
Fringilla coelebs	2/4	50.0	50.0	0.0	0.0
Garrulus glandarius	1/1	100.0	100.0	0.0	0.0
Glaucidium passerinum	0/1	0.0			
Parus ater	1/1	0.0			
P. caeruleus	0/1	0.0			
P. montanus	0/2	0.0			
Phylloscopus collybita	0/11	0.0			
P. trochilus	1/4	25.0	25.0	0.0	0.0
Prunella modularis	4/21	19.0	14.3	0.0	4.8
Pyrrhula pyrrhula	5/10	50.0	30.0	20.0	0.0
Saxicola rubetra	0/3	0.0			
Sitta europaea	1/5	20.0	20.0	0.0	0.0
Sturnus vulgaris	0/1	0.0			
Sylvia atricapilla	9/21	42.9	42.9	0.0	0.0
S. communis	1/13	7.7	7.7	0.0	0.0
S. curruca	0/1	0.0			
Troglodytes troglodytes	0/7	0.0			
Turdus merula	0/4	0.0			
T. philomellos	0/12	0.0			
T. torquatus	0/6	0.0			
Total	52/289	18.0	17.0	0.7	0.3

Table 3. Intensity of infections of birds with blood parasites in Ruské and Kurników Beskid. (L - low intensity, M - medium intensity, H - high intensity)

	Haemoproteus spp. intensity of infection (% of infected)				*Leucocytozoon* spp. intensity of infection (% of infected)			
	n	L	M	H	n	L	M	H
Ruské	126	40	38	22	37	65	32	3
Kurników Beskid	49	23	23	54	2	100	0	0

in each of the smears (Garvin *et al.* 1993) under magnification of 400×. The quantity of erythrocytes was estimated by calculating their numbers in three squares of the eyepiece grid across the smear (Reauz *et al.* 1999) over an area where the erythrocytes formed a uniform layer without overlapping each other, and by calculating the number of squares. If some parasites were found, their number was calculated in a similar manner per 10,000 erythrocytes. Intensity of infection was expressed in three degrees (Lederer 2000): Infections with species *Haemoproteus* and *Plasmodium* were classified as low if fewer than 0.1 % erythrocytes were affected, as medium if 0.1-0.5% erythrocytes were affected and as high if over 0.5 % erythrocytes were affected. Infections with species *Trypanosoma* and *Leucocytozoon* were classified as low if fewer than 0.02 % erythrocytes were affected, as medium if 0.02-0.1% erythrocytes were affected and as high if the number of erythrocytes affected exceeded 0.1 %.

Table 4. Species of blood parasites found in wild birds in Ruské and Kurników Beskid.

Host	Ruské parasite	Kurników Beskid parasite
Coccothraustes coccothraustes	*Haemoproteus fringillae*	-
Emberiza citrinella	*H. fringillae*	-
Erithacus rubecula	*H. balmorali*	*H. balmorali*
Erithacus rubecula	*H. attenuatus*	*H. attenuatus*
Erithacus rubecula	*Leucocytozoon* sp.	-
Fringilla coelebs	*H. fringillae*	*H. fringillae*
Garrulus glandarius	*H. picae*	*Haemoproteus* sp.
Hippolais icterina	*H. zosteropis*	-
Lanius collurio	*Haemoproteus* sp.	-
Lanius collurio	*L. majoris*	-
Lanius collurio	*Plasmodium* sp.	-
Luscinia luscinia	*H. attenuatus*	-
Muscicapa striata	*Haemoproteus* sp.	-
Parus major	*Leucocytozoon* sp.	-
Phylloscopus collybita	*Haemoproteus* sp.	-
Phylloscopus collybita	*Leucocytozoon* sp.	-
Phylloscopus sibilatrix	*Haemoproteus* sp.	-
Phylloscopus trochilus	*H. fringillae*.	*Haemoproteus* sp.
Prunella modularis	-	*H. fringillae*
Prunella modularis	-	*Trypanosoma* sp.
Pyrrhula pyrrhula	*Haemoproteus* sp.	*H. fringillae*
Pyrrhula pyrrhula	-	*L. fringillinarum*
Sitta europaea	-	*Haemoproteus* sp.
Sylvia atricapilla	*H. belopolskyi*	*H. belopolskyi*
Sylvia atricapilla	*L. dubreuili*	-
Sylvia comunnis	*Haemoproteus* sp.	*Haemoproteus* sp.
Turdus merula	*Haemoproteus* sp.	-
Turdus merula	*L. dubreuili*	-
Turdus merula	*Plasmodium* sp.	-

The genus and species of blood protozoa were determined upon their morphological traits according to Valkiunas (1997). Parasite morphology was studied under 1000× magnification. Measurements were made visually using the eyepiece gauge. In some cases, it was impossible to determine the species because of artificial deformations of cells in the smear.

RESULTS

Blood parasites were found in 25% of the 595 birds examined in Ruské. Parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* were found in 21 %, 6 % and 0.5 % of the birds, respectively (Table 1). In Kurników Beskid, blood parasites were found in 22 % of the 289 birds examined there. Parasites of the genera *Haemoproteus*, *Leucocytozoon* and *Trypanosoma* were found in 17 %, 0.7 % and 0.3 % of the birds, respectively (Table 2).

Intensity of blood parasite infections with individual genera of blood parasites are shown in Table 3. The highest intensity of infection with haemoprotea was found in a juvenile *Erithacus rubecula* netted in Ruské on 4 September 2001 (7.8 % erythrocytes infected) and a juvenile *E. rubecula* netted in Kurników Beskid on 28 August 2003 (5.8 % erythrocytes infected). The highest intensity of leucocytozoon infection was found in an adult *Sylvia atricapilla* netted in Ruské on 14 August 2001, where 244 gametocytes per 100 000 erythrocytes were found. The highest intensity of plasmodia was found in a juvenile *Turdus merula* from Ruské netted on 10 August 2001 (0.9 % erythrocytes infected).

The list of species found includes 6 species of the genus *Haemoproteus* and 3 species of the genus *Leucocytozoon* (Table 4). It was not possible to classify the species of any of the parasite belonging to the genera *Plasmodium* and *Trypanosoma*.

DISCUSSION

Wild birds all over the world except Antarctica are commonly the host to haemoprotozoa of the genera *Haemoproteus*, *Leucocytozoon*, *Plasmodium* (Valkiunas 1997) and *Trypanosoma* (Rintamäki *et al.* 1999). In some seasons their prevalence is very high. In winter, however, when there is no transmission of infection by vectors, blood smears will identify infection in a few birds only, and those infections are of a low intensity (Kučera 1981a, b, c; Valkiunas 1997; Hauptmanová *et al.* 2002). Our summer investigations showed that the most frequently found parasites in both of the locations were those of the genus *Haemoproteus*.

Infection prevalences were different in different bird species. Differences in prevalences between bird species have also been described in other locations (Kučera 1981 a, b, c; Shurulinkov and Golemansky 2002). Such variability may be caused by the specificity of individual species of parasites for specific species, or groups of species, of hosts (Deviche *et al.* 2001), differences in environmental demands of vectors and different exposure of the bird hosts to vectors. The exposure may depend on the time of daily activities of individual species, their type of trophic behaviour, selection of the place for nesting, rest, etc.

We assume that most of the infected birds contracted the infection at the site where they were netted because most of the birds examined were juveniles, who stay around for some time after leaving the nest. However, there is no method available that would allow the determination of the ratio between local birds and those just passing through. Autumn migration of most of bird species examined in the study reaches its peak in the second half of September (Ferianc 1973).

For the Carpathians, all the blood protozoa determined are a priority findings, except for *H. fringillae*, reported from Bieszczady by Dymowska and Żukowski (1968). *H. zosteropis* was found for the first time in *Hippolais icterina* (Sylviidae). Only birds from the family Zosteropidae have been recorded as host for this species to date (Valkiunas 1997). According to Valkiunas (1997), *H. zosteropis* has a wide range of distribution includig Afrotropical, Indomalaysian and Palaearctic realm. It is likely that this species can invade other groups of passerine hosts, e.g. species from the family Sylviidae.

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INSTRUCTIONS FOR AUTHORS

Acta Protozoologica is a quarterly journal that publishes current and comprehensive, experimental, and theoretical contributions across the breadth of protistology, and cell biology of lower Eukaryote including: behaviour, biochemistry and molecular biology, development, ecology, genetics, parasitology, physiology, photobiology, systematics and phylogeny, and ultrastructure. It publishes original research reports, critical reviews of current research written by invited experts in the field, short communications, book reviews, and letters to the Editor. Faunistic notices of local character, minor descriptions, or descriptions of taxa not based on new, (original) data, and purely clinical reports, fall outside the remit of *Acta Protozoologica.*

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Articles from books: Neto E. D., Steindel M., Passos L. K. F. (1993) The use of RAPD's for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. In: DNA Fingerprinting: State of Science, (Eds. S. D. J. Pena, R. Chakraborty, J. T. Epplen, A. J. Jeffreys). Birkhäuser-Verlag, Basel, 339-345

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