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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

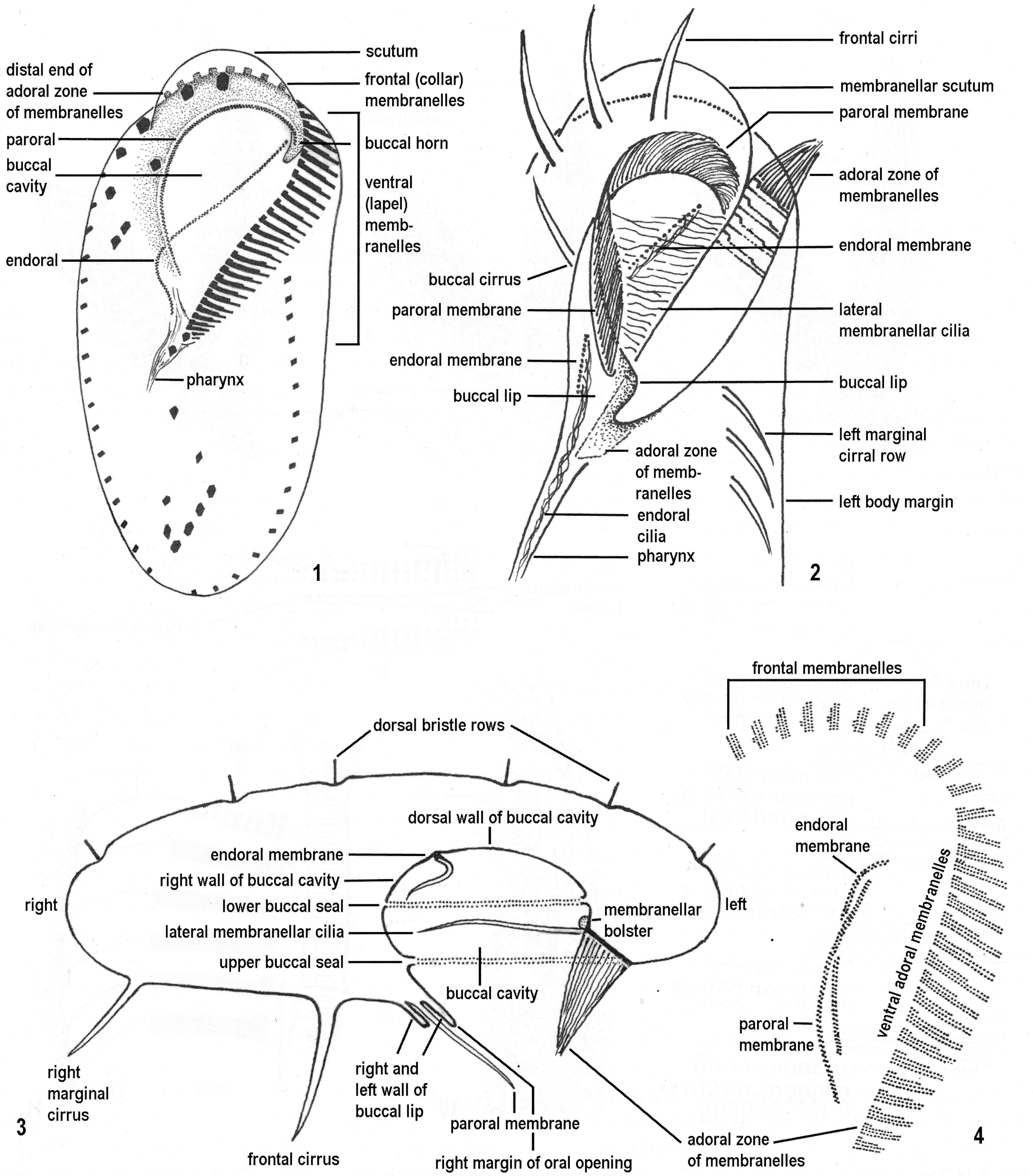
See Figures 1-8 for the terms used. They are based on Corliss (1979) and Berger (1999). Some new terms will be explained in the appropriate sections.

RESULTS

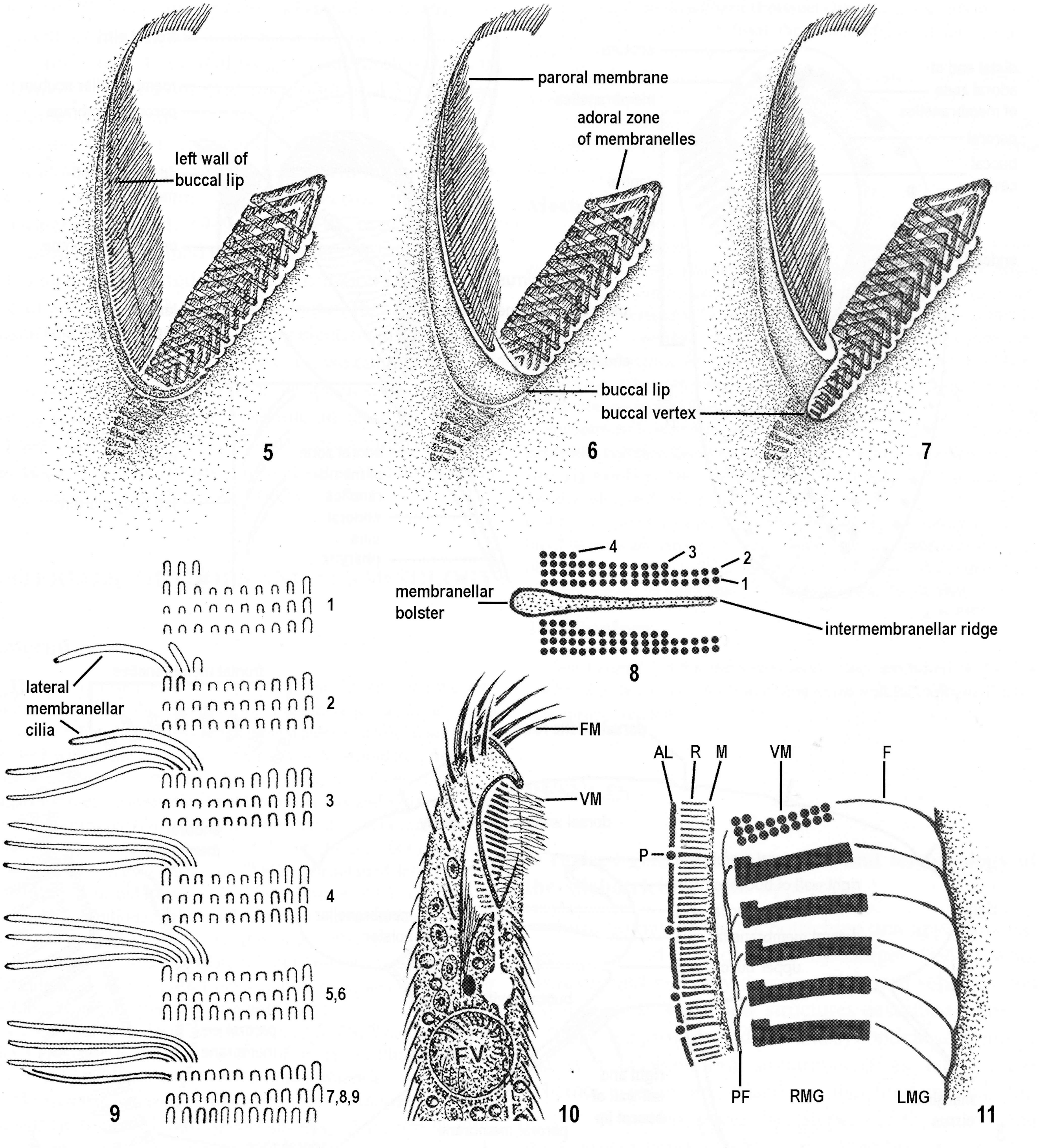
A revised general organization and terminology of the stichotrichine oral apparatus

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).

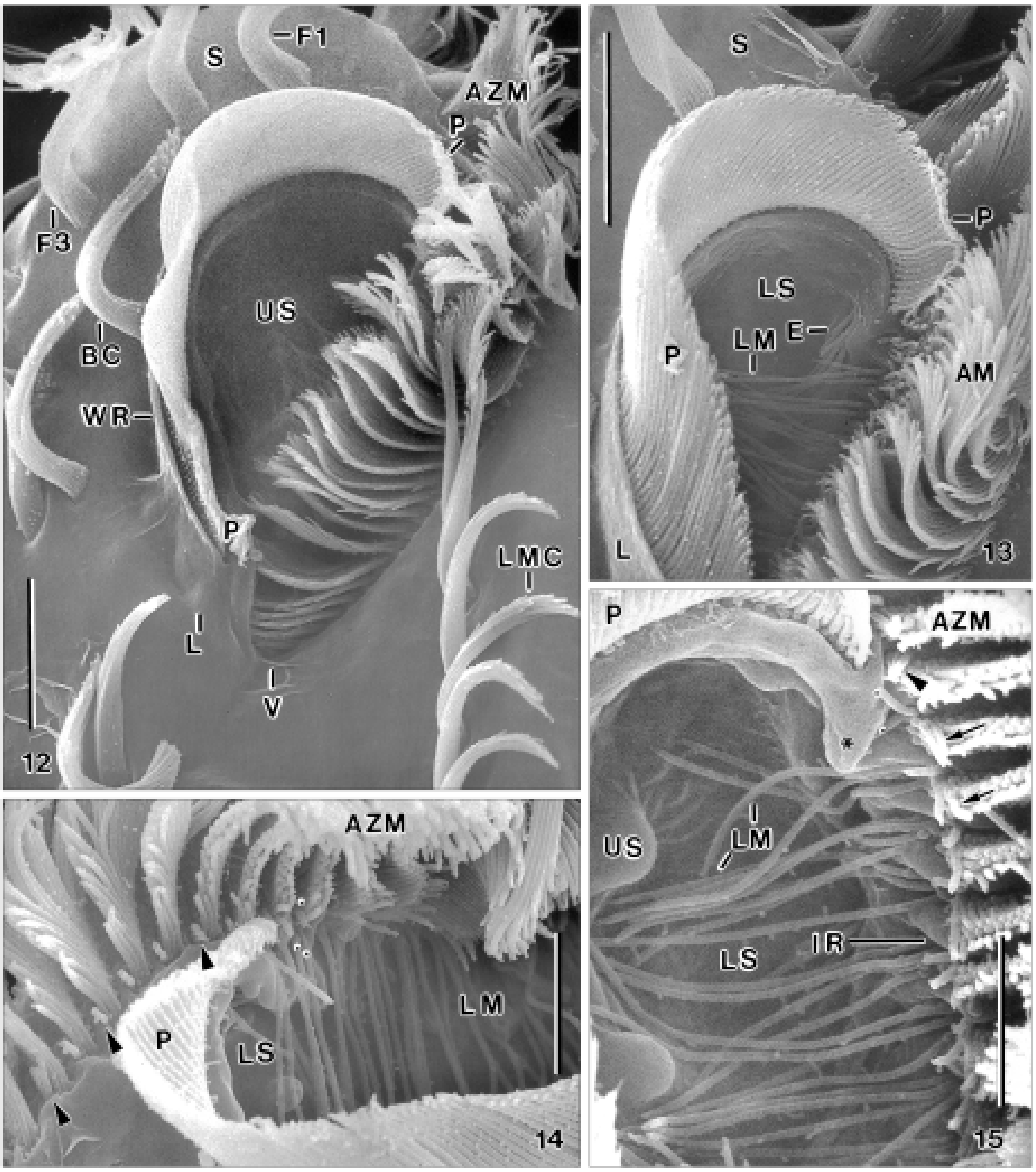
It was a great surprise when we recognized that the buccal cavity and thus the oral opening was covered by a membranous sheet in excellently prepared specimens (Figs 12, 13, 28, 29). Further investigations and a re-evaluation of the literature showed this structure, which we call "buccal seal" (denotes 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).

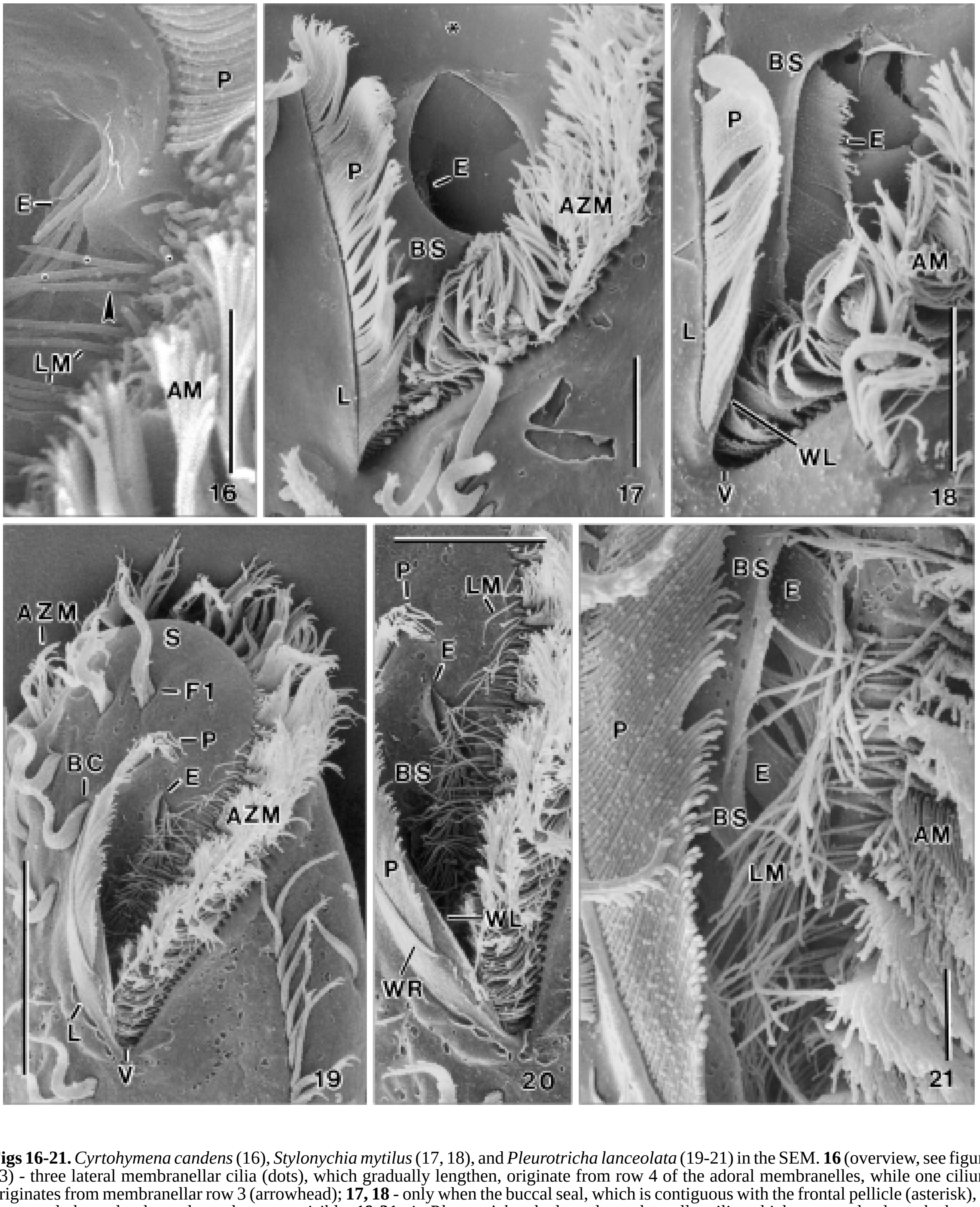
The detailed analysis of the species mentioned above revealed three modes of buccal seal position. In the first mode, the seal covers not only the oral opening but also the lateral membranellar cilia and the proximal portion of the adoral membranelles. Thus, the buccal area is very smooth and devoid 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.

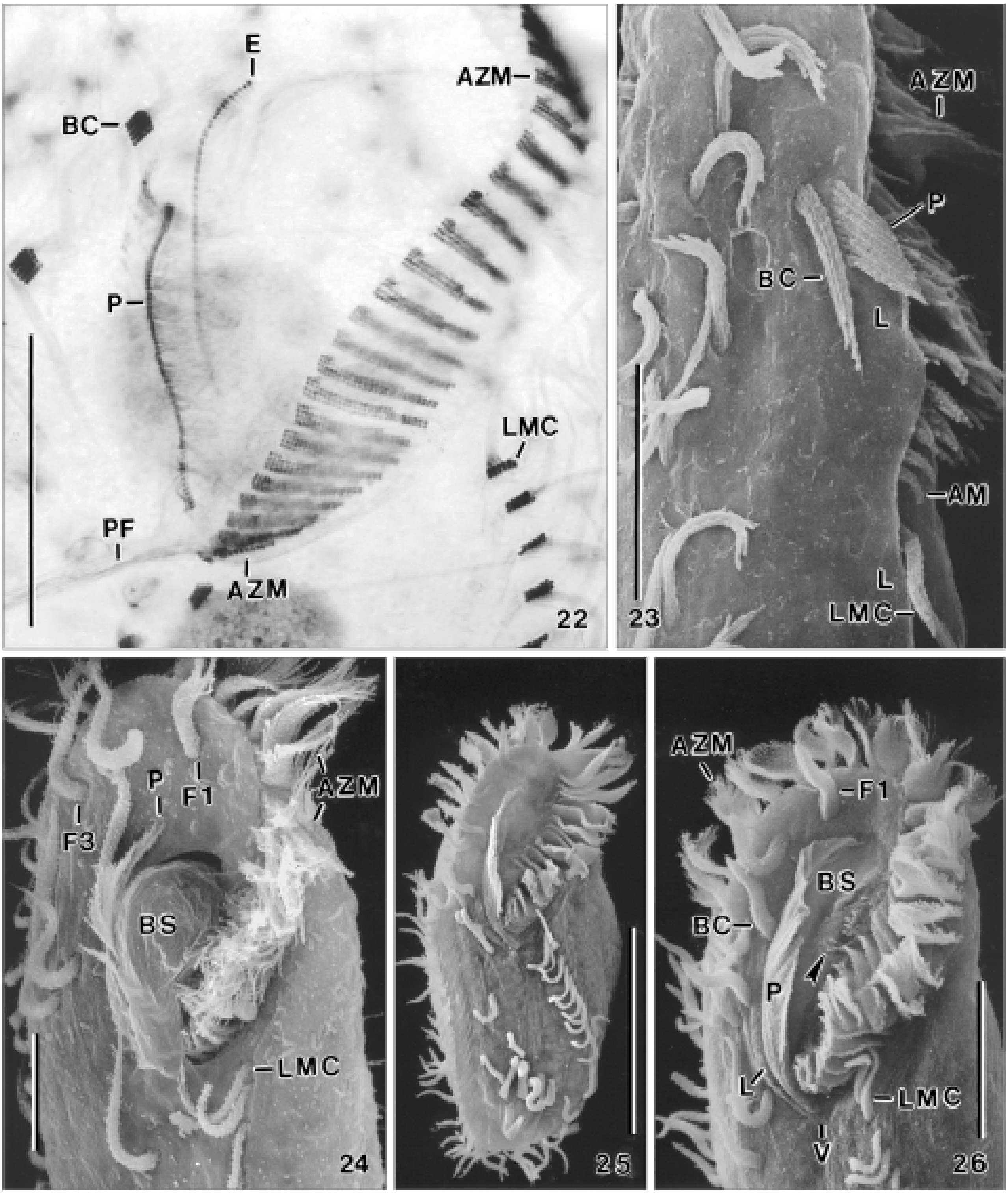
In vivo, in protargol preparations (Fig. 22), in the scanning electron microscope (Figs 14, 15, 33), and during light microscopical ontogenesis (Foissner *et al*. 2002), the lateral membranellar cilia and their basal bodies look like those of the rest of the membranelle. However, transmission electron 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

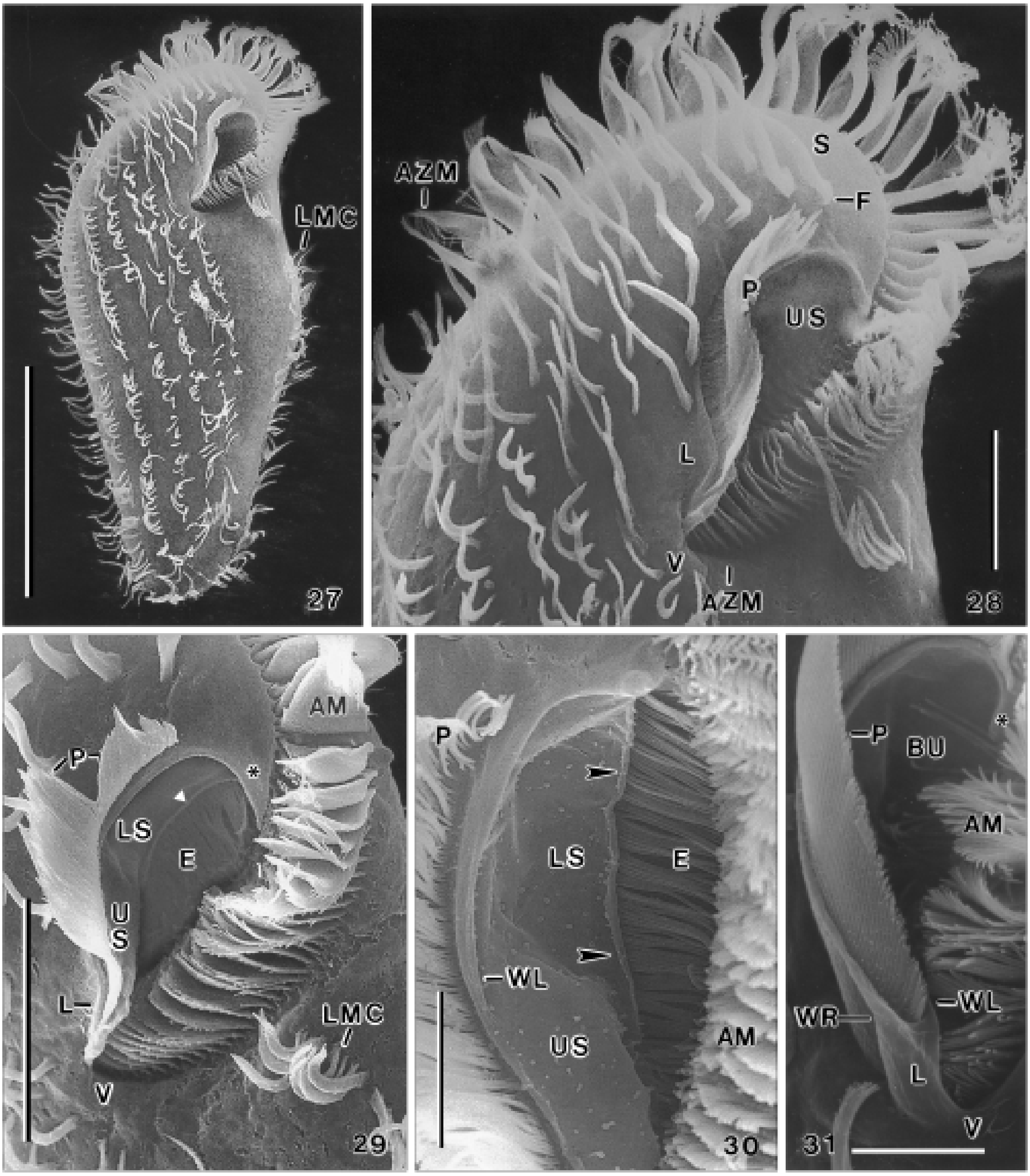
The left margin of the stichotrichine oral (buccal) opening is delimited by the ventral portion of the adoral zone of membranelles, while the right is defined by the edge of the buccal lip, a hyaline structure well recognizable in the scanning electron microscope (Figs 2, 3, 12, 20, 31, 34, 35, 39), 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).

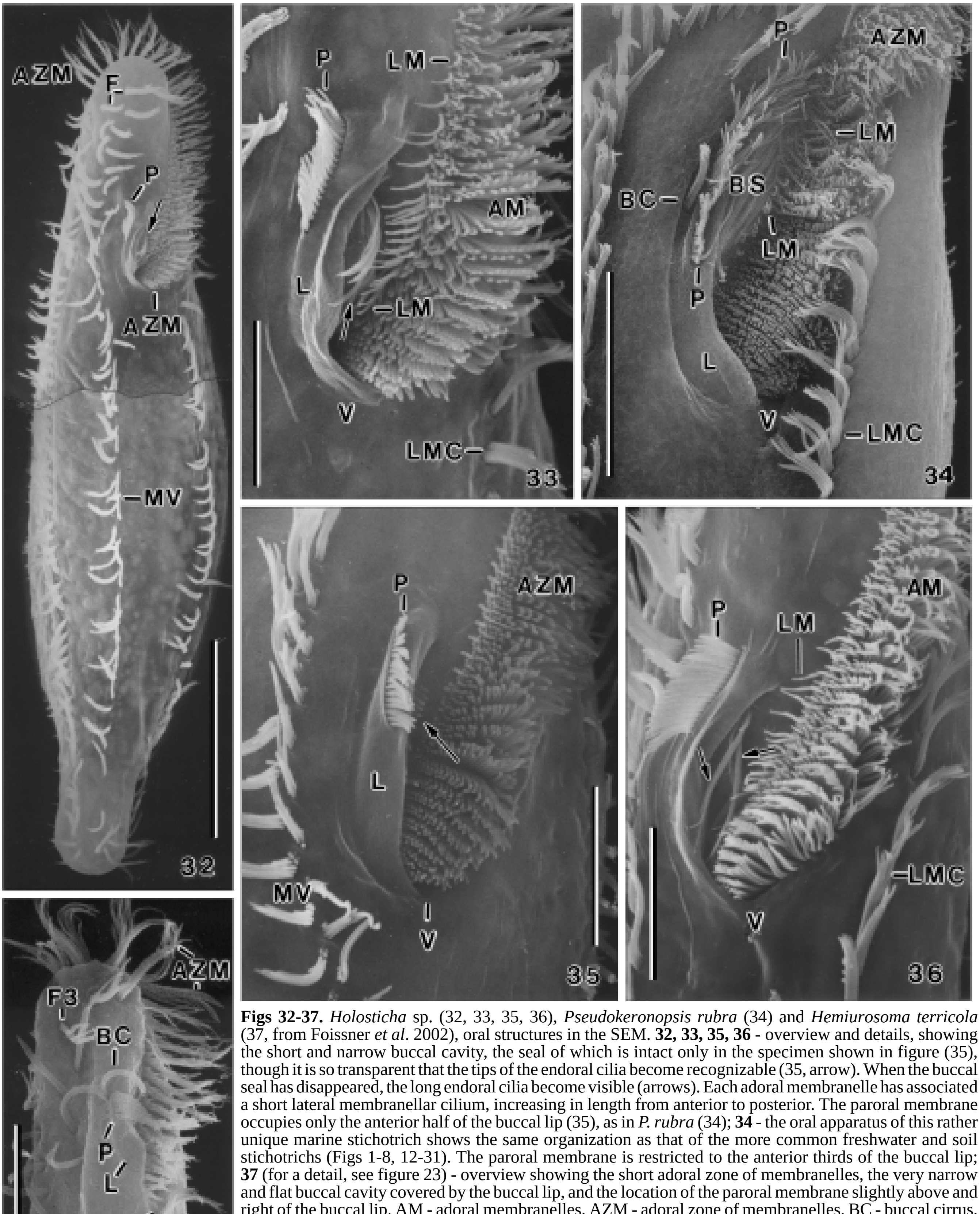
The endoral and paroral membrane can be distinguished by their location, viz., on the dorsal wall of the buccal cavity vs. outside the cavity on or near to the buccal lip. In some species, the cilia of the endoral membrane gradually lengthen from anterior to posterior, for instance, in *Bakuella granulifera* 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

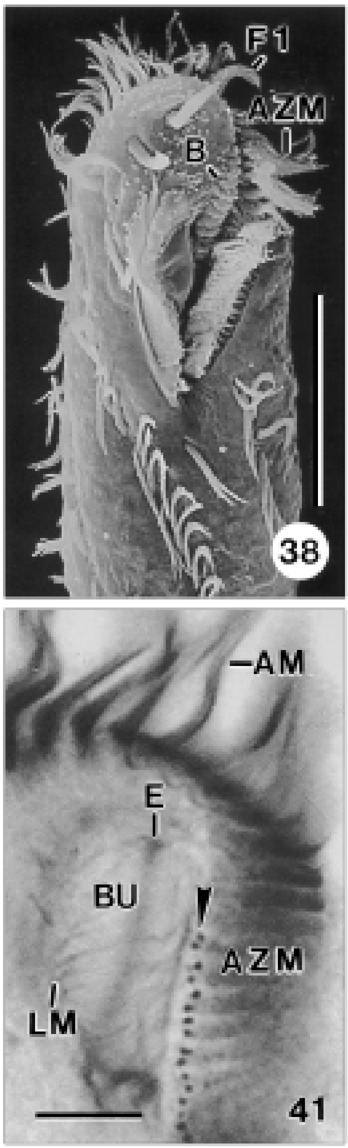
The food vacuoles of wild stichotrich populations show that they can feed on large prey, such as other ciliates, testate amoebae, resting cysts, and so on (Foissner *et al*. 1991, Foissner 1998). This applies also to the species used in this study, except *Holosticha* sp., which feeds mainly on bacteria.

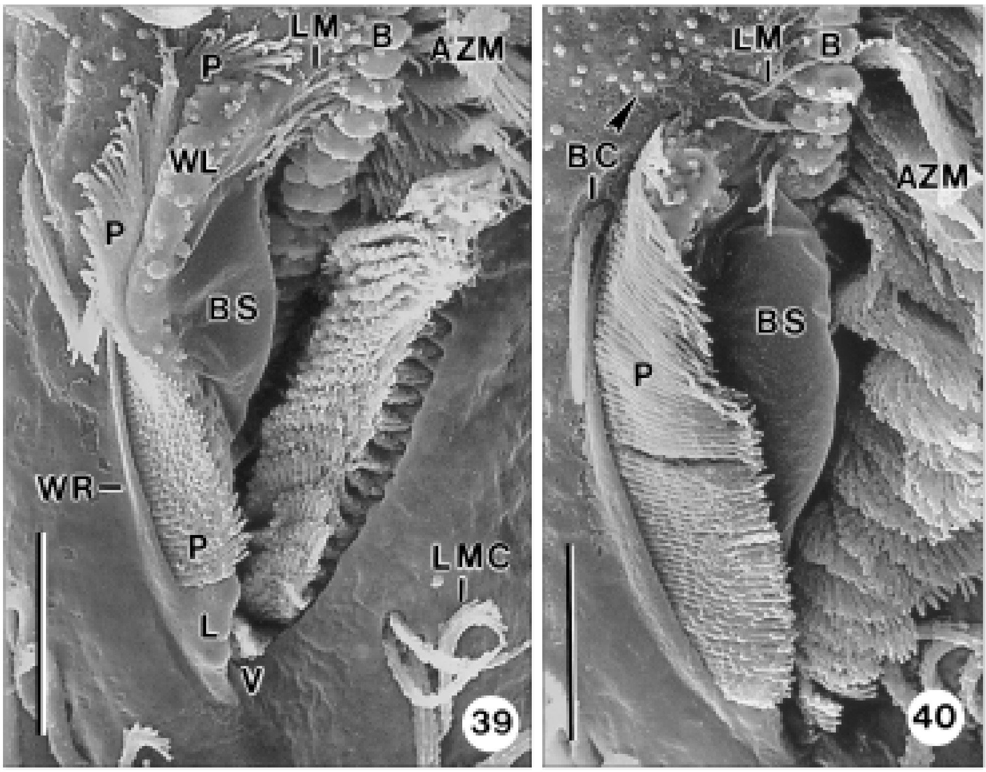
Unfortunately, detailed investigations on feeding in stichotrichine spirotrichs are rare (see Discussion). Thus, we report our anecdotal observations, which show that the predator attaches to ciliate prey with its frontal and oral area, whereby the buccal cavity becomes wider and the victim is engulfed 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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