

In the article of Denis H. Lynn and Wilhelm Foissner: The feet of *Pseudochlamydonellopsis plurivacuolata* (Ciliophora, Cyrtophorida) and a brief review of tentacle-like structures of ciliates [Europ. J. Protistol., 30, 423–430 (1994)] the species name throughout should have been *Chlamydonellopsis plurivacuolata* Blatterer and Foissner, 1990.

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The Feet of *Pseudochlamydonellopsis plurivacuolata* (Ciliophora, Cyrtophorida) and a Brief Review of Tentacle-like Structures of Ciliates

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SUMMARY

Pseudochlamydonellopsis plurivacuolata is a cyrtophorid ciliate that has 3 rows of club-shaped to cylindroid protuberances between the somatic kineties. These feet are apparently supported by a few microtubules, which are not organized into a complex array. Elongate cylindroid extrusomes are found in the middle of the foot and insert at its tip where their contents are released. The feet appear not to function either in adherence to the substrate or for food uptake. Their ultrastructure is discussed in relation to tentacle-like appendages of other ciliates, and it is concluded that the feet do not exhibit clear similarities to suctorian tentacles or other tentacle-like appendages.

Introduction

Ultrastructural studies have demonstrated that cyrtophorids, chonotrichs, rhynchodines, hypocomatines, and suctorians share features of the somatic kinetid, the oral microtubular ribbons [23], and distinct ontogenetic similarities [9]. This has resolved these taxa as a monophyletic assemblage, named the Class Phyllopharyngea [26]. However, it has not provided any evidence on the evolutionary relationships within the group, except to suggest that the cyrtophorids and chonotrichs are sister groups and the suctorians and rhynchodines/hypocomatines are sister groups [14]. There is no evidence of how the suctorians arose.

Recently, Blatterer and Foissner [3] described a cyrtophorid, *Pseudochlamydonellopsis plurivacuolata*, which has tentacle-like structures or feet whose function is not certain. It was of interest to discover whether these are, in fact, homologues of the tentacles of suctorians. This report provides evidence of the fine

structure of these feet and demonstrates that, although there are some similarities, the homologies are not strong.

Material and Methods

Pseudochlamydonellopsis plurivacuolata was collected from the type locality, the Amper River in Bavaria [3]. Cells were prepared for light and scanning electron microscopy as described by Foissner [7]. For transmission electron microscopy, cells were fixed for 2 h in 3.0% (v/v) glutaraldehyde in 0.05 M Na-cacodylate buffer, pH 7.0, rinsed in the same buffer, post-fixed for 1 h in 2.0% (w/v) OsO₄ in 0.025 M Na-cacodylate buffer, and then rinsed in the same buffer, and stored for some weeks in 3.0% glutaraldehyde as above. Then, cells were dehydrated and flat-embedded in Epon. Flat-embedded cells were removed from the Epon, oriented and mounted so that feet could be sectioned. Sections were stained with uranyl acetate and lead citrate and photographed in a JEOL 100S TEM.

Results

Light and Scanning Electron Microscopy

This elliptical, flattened ciliate measures 50–110 × 30–55 μm and has an average of 4 contractile vacuoles and 21 somatic kineties on the ventral side (Figs. 1, 2). On the ventral surface, there are 3 rows of club-shaped to cylindroid protuberances, which we will call feet. They originate between the ciliary rows and measure 3–5 × 1–2 μm *in vivo*. The feet are clearly recognizable *in vivo* and in the SEM (Figs. 2–5), but do not stain with silver nitrate or protargol. Although they are rather stiff, they can perform small movements, flexing anteriorly and posteriorly in the axis of the kinety. This ciliate crawls along a substrate, but apparently does not use the feet to cling firmly to it. It feeds possibly exclusively on small and large diatoms, which are ingested by the cyrtos: the feet have no function in food uptake. For a detailed descriptions of *P. plurivacuolata* refer to Blatterer & Foissner [3].

Transmission Electron Microscopy

The feet are elongated extensions of the cytoplasm, covered by an alveolar layer that is underlain by a dense, but thin epiplasm (Figs. 6–9). Beneath the epiplasm, microtubules are arranged irregularly (Figs. 8, 9), diverging from the base of the extension towards the tip (Figs. 6–9). The foot also contains some small vesicles and elongated dense bodies that are assumed to be extrusomes. These insert at the distal end in projections (Fig. 7) and apparently extrude their contents in the typical manner by membrane fusion (Fig. 12). It has not been possible to follow the movement of extrusomes into the feet. However, it appears that they begin as coiled bodies at the base of a foot (Figs. 10, 11). As they move into the foot, it is presumed that they change into the elongate cylinders observed (Figs. 6, 7, 12).

Discussion

Distribution of Tentacles and Tentacle-Like Structures

Tentacles *sensu stricto* are usually long, cytoplasmic extensions that ciliates use primarily for ingestion [4] while tentacle-like structures are cytoplasmic exten-

sions that have other functions, such as prey capture but not ingestion (e.g., *Actinobolina*). These structures have been observed in three classes of ciliates. In the Phyllopharyngea, they are found in the subclass Suctorina and subclass Cyrtophoria in the sense of Small and Lynn [26]. In the Litostomatea, they are restricted to the Haptoria in the sense of Small and Lynn [26]. In the class Colpodea, only the family Grossglockneriidae has been reported to have a cytopharyngeal apparatus that is analogous to the suctorian tentacle [1, 8].

In the Haptoria, Kahl [20] included illustrations showing that the following genera have tentacles or tentacle-like structures: *Actinobolina*, *Dactylochlamys*, *Legendrea*, and *Enchelyomorpha* (see also [2, 17]). Kahl [18, 19] considered the latter genus to be the swarmer of an unknown suctorian. However, he transferred it to the Actinobolinidae in 1935 [20].

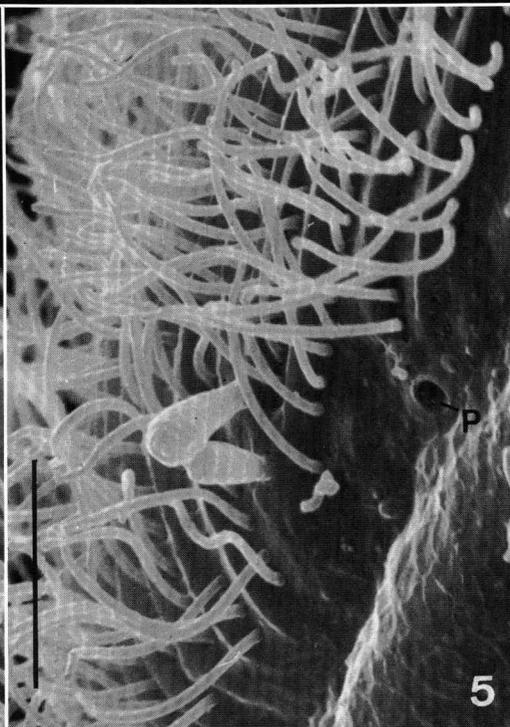
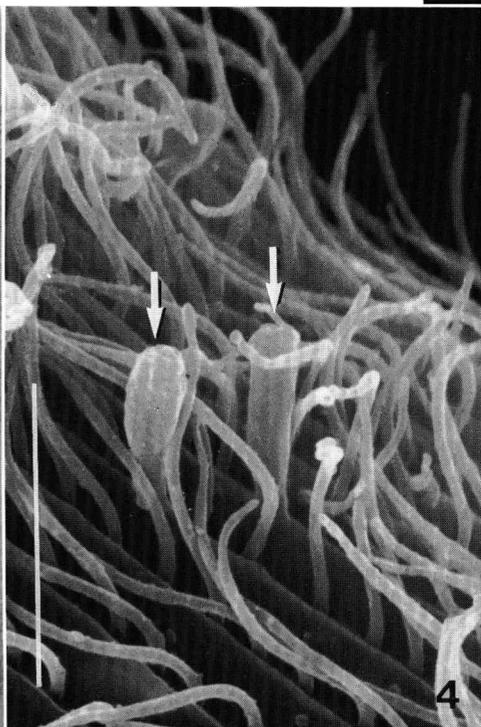
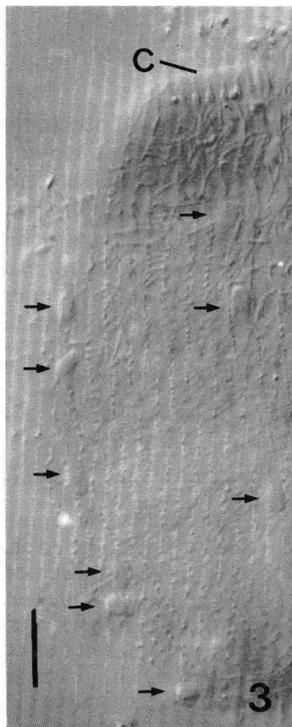
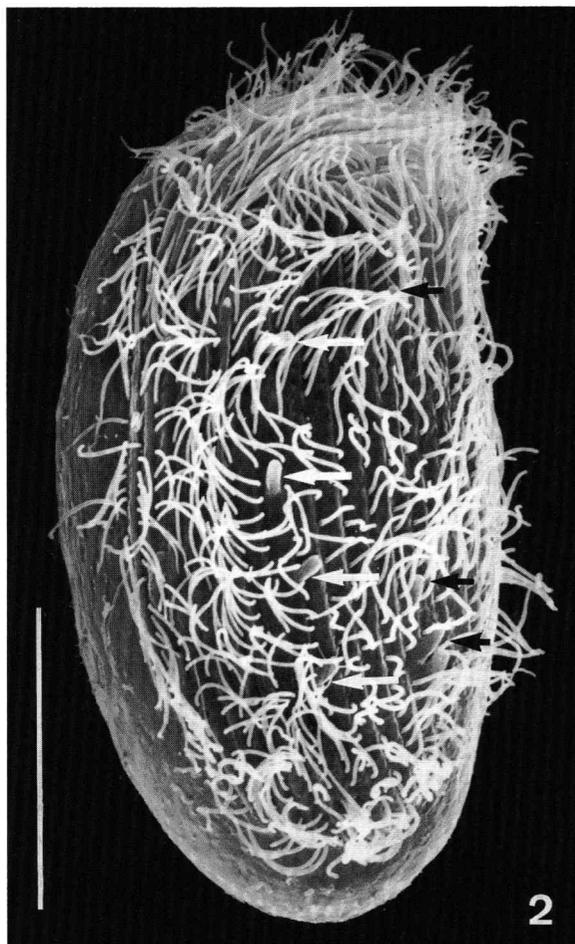
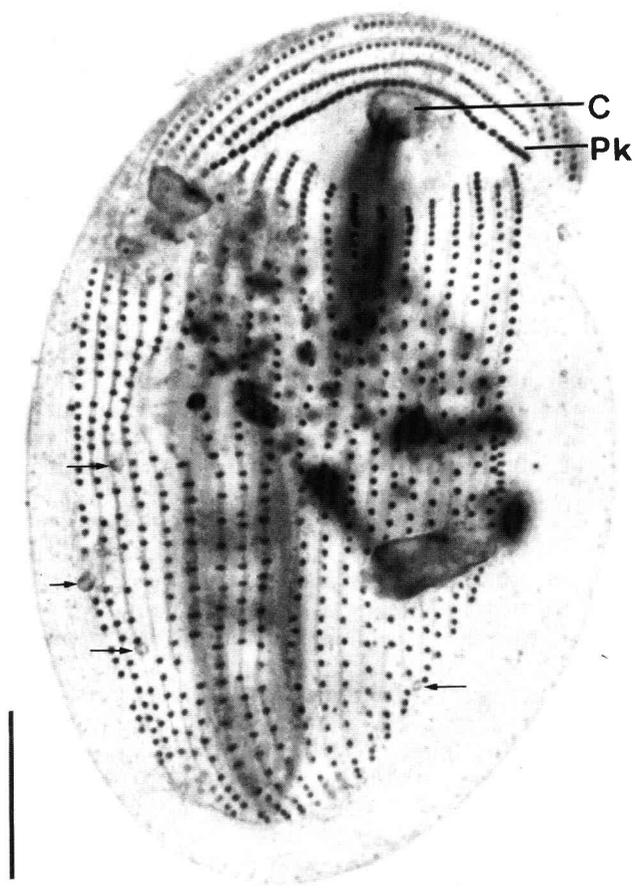
In the Cyrtophoria, the Rhynchodida and the Cyrtophorida have representatives with tentacles or tentacle-like structures. The rhynchodines/hypocomatines have tentacle-like cytopharyngeal apparatuses while the diversity of these structures is higher in the Cyrtophorida. Among the cyrtophorids, feet have been observed in the following genera: *Chlamydonella*, *Chlamydonellopsis*, *Trochilia*, *Parachilonella*, *Trochilioides*, and *Dysteria*, but these structures are apparently absent in chlamyodontines, which have a posterior somatic fixative organelle of a very different fine structure [21].

Structure and Function of Tentacles and Tentacle-Like Structures

It is exceedingly difficult to determine the homology of these structures from light microscopic observation, and thus electron microscopy provides one means for resolving these issues. Nevertheless, only a few genera with these structures have been examined by electron microscopy (Fig. 13).

The suctorian tentacles were the first to be examined. These are composed of two “rings” of microtubules. The outer ring may or may not be complete and the inner ring is always composed of small ribbons of arm-bearing microtubules (Fig. 13A, B). Kahl’s original view [18] that the “haptorian” *Enchelyomorpha* is a suctorian swarmer that bears small tentacles (Fig. 13B) has been confirmed recently (Foissner, unpublished observations). These tentacles are used by suctorians both in prey capture and immobilization, through the use of the haptocysts, and ingestion. In ad-

Figs. 1–5. Light and scanning micrographs of *Pseudochlamydonellopsis plurivacuolata*. – Fig. 1. Ventral view after protargol impregnation, showing contractile vacuole pores (arrows), cytopharyngeal region (C), and perial kinety (Pk). Note that the feet are not visible (cf. Fig. 2). Scale bar = 20 μm. – Fig. 2. Ventral view. Note the two rows of feet (black and white arrows) distributed between the kineties. Scale bar = 20 μm. – Fig. 3. Ventral view *in vivo* showing the right and median rows of feet (arrows) in interference contrast. C = cytopharyngeal region. Scale bar = 10 μm. – Fig. 4. A club-shaped and a cylindroid foot of the median row (arrows). Scale bar = 5 μm. – Fig. 5. Lower left portion of a cell showing two club-shaped feet. Scale bar = 5 μm. P = contractile vacuole pore. Figs. 2, 5 have also appeared in [3] while Figs. 2, 3, 4, and 5 have appeared in [10]. ▶



dition to ingestory tentacles, some suctorians also have tentacles that are “prehensile” (Fig. 13C), that is, used solely for prey capture [12].

The rhynchodine “suctorial” cytopharynx was described by Lom and Kozloff [22]. Unlike the suctoria, the rhynchodines have only a series of arm-bearing microtubular ribbons (Fig. 13I), surrounding the toxicyst-bearing region through which ingestion also occurs. A similar arrangement occurs in the hypocomatines in the distal region of the cytopharynx (Fig. 13E). However, towards the proximal end (Fig. 13D), the hypocomatine cytopharynx has a structure very similar to the suctorian tentacle (Figs. 13A, B): a ring of microtubules surrounding curved and radially-oriented arm-bearing ribbons [15]. It has been concluded that the somatic kinetids of rhynchodines/hypocomatines and suctorians are homologous [15, 23], and one is led to believe that the oral structures are also homologous.

The “tentacle” of the colpodean *Grossglockneria* shows similarities to the rhynchodines (Fig. 13H). It is used in ingestion (i.e., as a cytopharyngeal structure) and is composed of ribbons of microtubules whose inner microtubule bears dense material [1]. Like the cytopharyngeal ribbons of many ciliates, these ribbons are probably derived from postciliary microtubules, but instead of being inverted to form a cytopharynx, they have become everted to form a “suctorial” or tube-like cytopharyngeal structure.

Aside from *Enchelyomorpha*, which is properly a suctorian, *Actinobolina* is the only tentacled haptorian to have been examined [17]. Its tentacle-like structures are used to deploy toxicysts that the ciliate uses in prey capture. The tentacle is supported usually by a complete ring of microtubules surrounding a spiral of one or more turns (Fig. 13G), which may enclose an elongate toxicyst. The microtubules of the inner spiral do not apparently bear arms. As with suctorian haptocysts, it is not known how the toxicysts are transported to the tip.

In the cyrtophorids, the cytopharyngeal apparatus has inner ribbons of arm-bearing microtubules, like the rhynchodines and hypocomatines, but these are surrounded by substantial nematodesmata [21, 24, 27]. Here, the arm-bearing microtubules enclose the

space in which the extrusomes are apparently transported. Tentacles sensu stricto have not been observed, but the posterior somatic fixative organelle and the feet have now been described. The fixative organelle of dysteriines like *Trochilia* and *Hartmannula* [5] and *Brooklynella* [21] is a specialized cortical region in which there are small vesicles, probably extrusomes, presumably used for attaching the ciliate to the substratum.

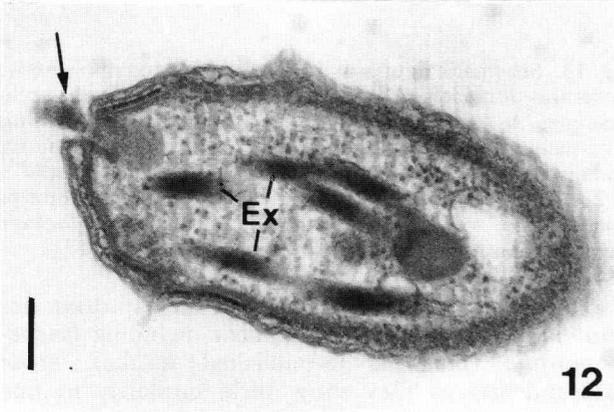
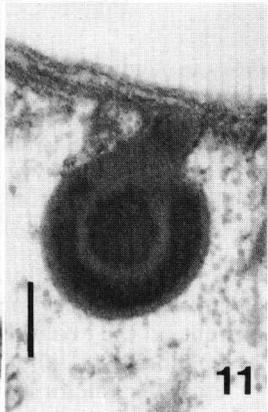
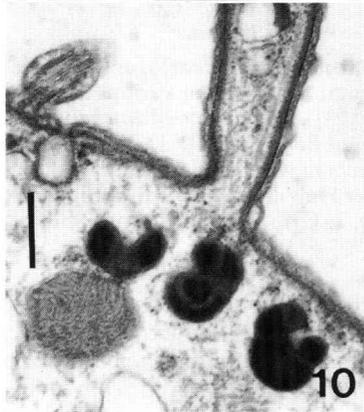
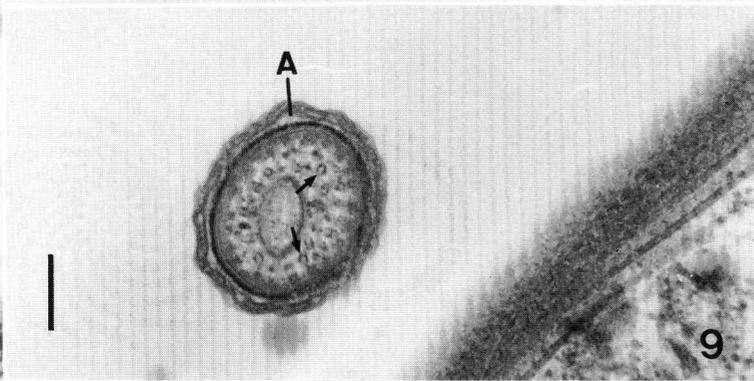
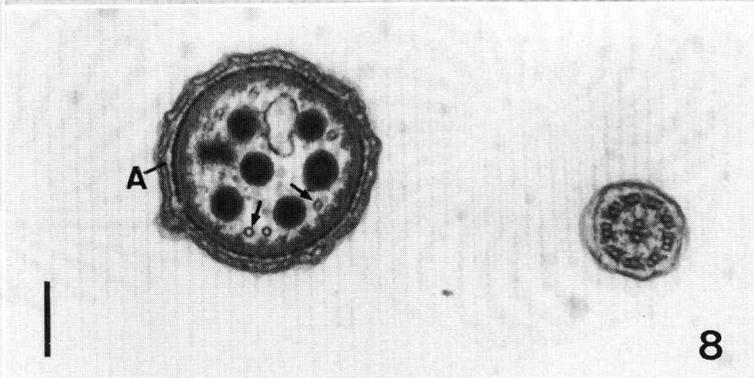
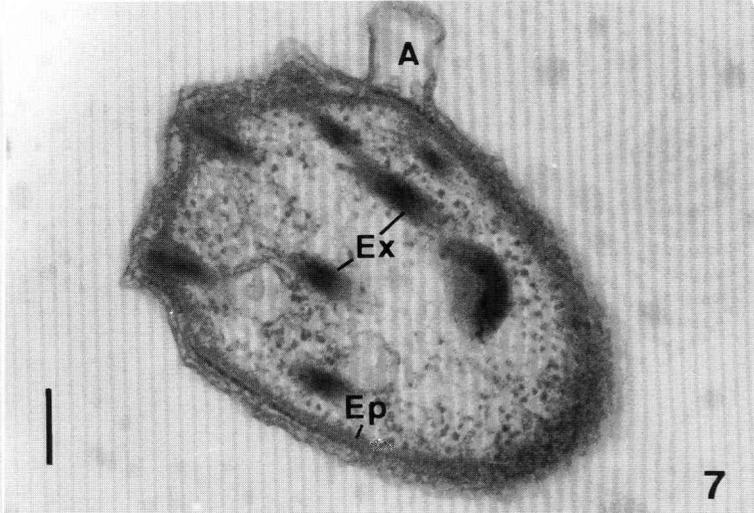
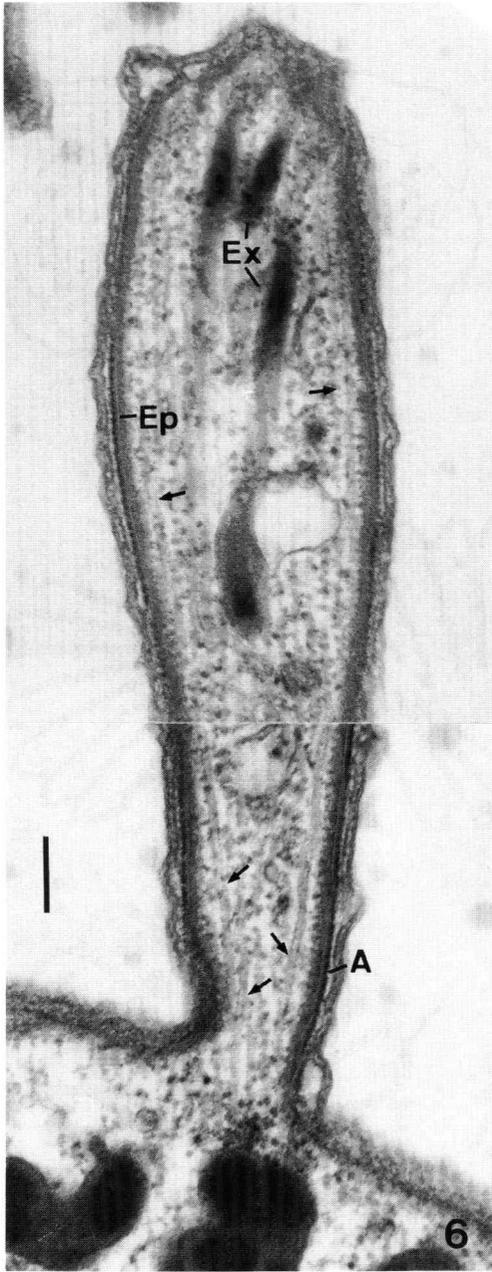
On the other hand, the feet, distributed over the ventral somatic surface and between kineties of *Pseudochlamydonellopsis*, are apparently not used for clinging to the substrate. These cell processes (Fig. 13F) are apparently supported by microtubules that are arranged beneath the epiplasm, originating proximally in the organelle and splaying out as the foot broadens towards its tip (see Results). Within the foot, elongate extrusomes are observed that are positioned as apparently coiled forms in the cortex outside the foot and become elongated upon entering the foot. Since the feet are seemingly not used in attachment, the function of these extrusomes, which do extrude their contents, is unclear. Again, it is unclear how these extrusomes are transported to the tip of the feet.

Although the feet of *Pseudochlamydonellopsis* do bear microtubules, their arrangement gives no clue as to how likely a candidate they are as the “ancestor” for the suctorian tentacle. These microtubules do enclose extrusomes, as the suctorian tentacles do, and the feet are not apparently used for attachment, but it is a long jump to the organized ingestory structure of the suctoria.

Evolutionary Origin of Suctoria and Suctorian Tentacles

Given the diversity of tentacles and tentacle-like structures of ciliates described above, it is obvious that, in a number of lineages, there has been selection pressure for the independent evolution of such structures. It is undoubtedly the case that the “suctorial” cytopharynx of the colpodean *Grossglockneria* and the rhynchodines/hypocomatines arose independently, even though they are structurally similar. It is

Figs. 6–12. Transmission electron micrographs of *Pseudochlamydonellopsis plurivacuolata*. – Fig. 6. Longitudinal, composite section of a foot. Note the elongate extrusomes (Ex) inserting at the distal end of the structure and the microtubules aligned near the base and distally (arrows). A = alveolus, Ep = epiplasm. Scale bar = 0.2 μm . – Fig. 7. Oblique section of the distal end of a foot, demonstrating how the cell surface protrudes where the extrusomes (Ex) insert. A = alveolus, Ep = epiplasm. Scale bar = 0.2 μm . – Fig. 8. Transverse section near the middle of a foot. Note the microtubules arranged around the periphery of the organelle (arrows) and the extrusomes in the centre. A = alveolus. Scale bar = 0.2 μm . – Fig. 9. Transverse, proximal section of a foot, demonstrating the denser packing of the microtubules (arrows). A = alveolus. Scale bar = 0.2 μm . – Fig. 10. Three extrusomes at the base of a foot, appearing to be coiled. Scale bar = 0.4 μm . – Fig. 11. A detail of an extrusome, suggesting that it is a coiled cylinder. Scale bar = 0.2 μm . – Fig. 12. Oblique section of the distal end of a foot, demonstrating the extrusion of material (arrow) from one of the distal extrusomes (Ex). Scale bar = 0.2 μm .



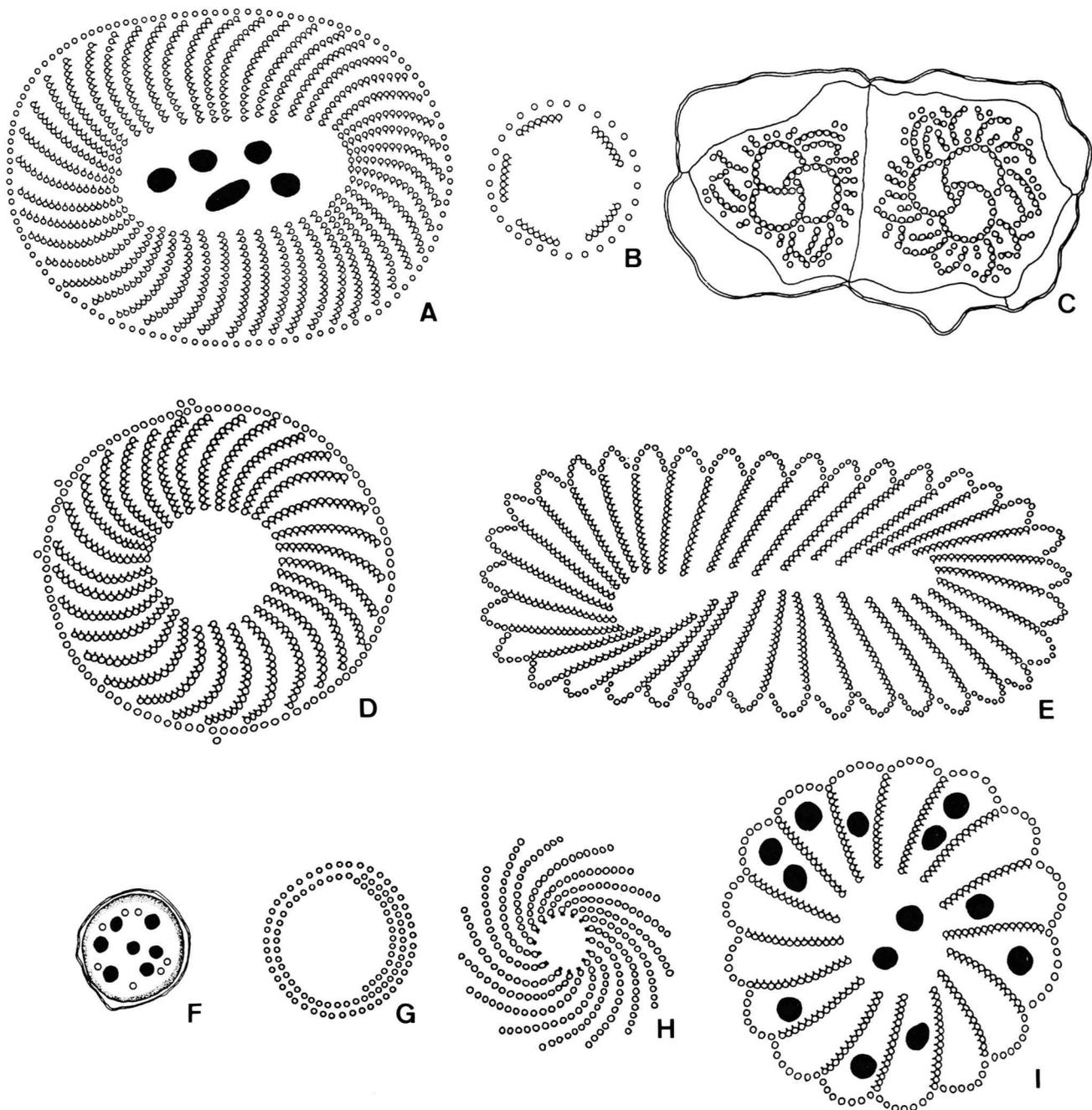


Fig. 13. Schematic figures of the microtubular components and/or surrounding cortical structures of tentacles and tentacle-like structures of ciliates. All figures are drawn to the same scale. Black dots within the structures represent various kinds of extrusomes. A: Ingestatory tentacle of the suctorian, *Acinetopsis* (after Grell and Meister [13]). B: Tentacle of *Enchelyomorpha* (Foissner, unpublished observations). C: "Prehensile" tentacle of *Ephelota* (from Grell and Benwitz [11]). D, E: Mid-level (D) and distal level (E) sections of the cytopharyngeal "tentacle" of the hypocomatine, *Hypocoma* (Grell and Meister [13]). F: Foot of *Pseudochlamydonellopsis* (see Results). G: Tentacle-like process of *Acintobolina* (after Holt et al. [14]). H: Cytopharyngeal "tentacle" or feeding tube of the colpodean, *Grossglockneria* (after Aescht et al. [1]). I: Cytopharyngeal "tentacle" of the rhynchodine, *Ignotocoma* (after Lom and Kozloff [17]).

also unarguable that the tentacles of the haptorian *Acintobolina* and the suctorians, now including *Enchelyomorpha* (Foissner, unpublished results), arose independently as they show little similarity in fine structure.

It was the original hope of this study to shed some light on the evolutionary origin of suctorians and suctorian tentacles. If the Class Phyllopharyngea is monophyletic, then suctorians must have derived from a ciliated ancestral form, a form that is basically retained by their

supposed sister taxon, the rhynchodines/hypocomatines. This form is presumably “recapitulated” in each life cycle as the swarmer, which shows strong morphological similarity to the cyrtophorid body plan in a number of suctorian genera. But, how might the suctorians have originated?

There are at least two possibilities. On the one hand, the suctorians and rhynchodines/hypocomatines may have diverged from the phyllopharyngean ancestral stock before the origin of the cyrtophorids and chonotrichs [14]. Both rhynchodines/hypocomatines and suctorians have similar ingestatory structures and they have homomerous macronuclei, like the majority of ciliates, while the cyrtophorids and chonotrichs share the derived character of heteromerous macronuclei. In this case, the rhynchodine/hypocomatine oral apparatus and suctorian tentacle must be considered homologous. Suctorians could be considered truly polystomous “rhynchodine”/“hypocomatine” ciliates that had lost their somatic ciliature and transformed the extrusomes into haptocysts. There are problems with this scenario. First, most contemporary rhynchodines and hypocomatines are “parasitic” while most suctorians are not: suctorians would have to be considered secondarily free-living, an unusual evolution for a “parasitic” form, or derived from an unknown free-living “rhynchodine”/“hypocomatine” ancestor. Second, while it is probable that the rhynchodine/hypocomatine oral apparatus develops in association with “somatic” kinetosomes, suctorian tentacles certainly develop in a highly organized way in association with a subcortical kinetosome [16]. The conclusion that these two groups are sister taxa would be considerably strengthened by ultrastructural evidence on the development of the rhynchodine/hypocomatine oral apparatus.

On the other hand, and less probable, the homomerous macronucleus of suctorians and rhynchodines/hypocomatines may be secondarily derived, and their oral structures may be convergent. The rhynchodines/hypocomatines and suctorians then may have originated independently from cyrtophorid ancestors with a heteromerous macronucleus. In this case, suctorians may have lost the “primary” oral apparatus and evolved tentacles from somatic appendages. Here, they might be considered pseudopolystomous, consistent with the development of tentacles in association with subcortical kinetosomes [16]. Thus, a cyrtophorid ancestor with somatic tentacle-like appendages might indeed be their putative ancestor. However, the feet of *Pseudochlamydonellopsis* at least do not show clear homologies with the suctorian tentacle.

Although there are substantial genetic distances separating the major clades or “classes” of ciliates [6, 11, 25], it has been possible to establish the branching structure within these clades. This provides hope that through molecular techniques it will be possible to discover the ancestral stock within the class Phyllopharyngea from which the suctorians derived, even if ultrastructural studies may not discover the ancestral tentacle-like structure.

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