References and Notes

1. N. E. Adelman, M. E. Hammond, S. Cohen, H. F. Dvorak, in *The Biology of the Lymphokines*, S. Cohen, E. Pick, J. J. Oppenheim, Eds. (Academic Press, New York, 1979), p. 13.
2. M. C. Cohen, A. Goss, T. Yoshida, S. Cohen, *J. Immunol.* **121**, 840 (1978).
3. M. C. Cohen, R. Zeschke, P. E. Bigazzi, T. Yoshida, S. Cohen, *ibid.* **114**, 1641 (1975); M. C. Cohen, in preparation.
4. T. Yoshida, T. Kuratsuji, A. Takada, Y. Takada, J. Minowada, S. Cohen, *J. Immunol.* **117**, 548 (1976).
5. T. Yoshida, in *Methods in Immunodiagnosis*, N. R. Rose and P. E. Bigazzi, Eds. (Wiley, New York, 1980), p. 27.
6. U. S. Ryan, E. Clements, D. Habliston, J. W. Ryan, *Tissue Cell* **10**, 535 (1978).
7. S. F. Gilbert and B. R. Migeon, *Cells* **5**, 11 (1975).
8. T. Yoshida and M. Watanuki, *Med. Biol. (Tokyo)* **77**, 131 (1968); J. Reeves, T. Yoshida, S. Cohen, *Immunol. Commun.* **2**, 287 (1973); N. Adelman, S. Cohen, T. Yoshida, *J. Immunol.* **121**, 209 (1978); N. Adelman, M. Hasson, N. Masih, M. C. Cohen, *J. Immunol. Methods* **34**, 235 (1980).
9. R. C. Graham and S. Shannon, *Am. J. Pathol.* **69**, 7 (1972); N. D. Anderson, A. O. Anderson, R. G. Wyllie, *ibid.* **81**, 131 (1975).
10. P. J. Polverini, R. S. Cotran, M. M. Sholley, *J. Immunol.* **118**, 529 (1977); P. J. Polverini, R. S. Cotran, M. A. Gimbrone, Jr., E. R. Unanue, *Nature (London)* **269**, 804 (1977).
11. M. E. Pliskin, *Transplantation*, in press.
12. Y. A. Sidky and R. Auerbach, *J. Exp. Med.* **141**, 1084 (1975).
13. J. Folkman, E. Merler, C. Abernathy, G. Williams, *ibid.* **133**, 275 (1971).

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Stephanopogon, a Phylogenetically Important "Ciliate," Shown by Ultrastructural Studies to Be a Flagellate

Abstract. *A benthic marine protist* (Stephanopogon) *with a homokaryotic nucleus has long been considered to be a gymnostome ciliate. It has been important in hypotheses concerning the origin of ciliates, the evolution and origin of the dual nuclear apparatus of contemporary species of the Ciliophora, and the origin of the multicellular Eumetazoa. Ultrastructural observations reveal that the organism should be reclassified as a flagellate, despite its superficial resemblance to ciliates.*

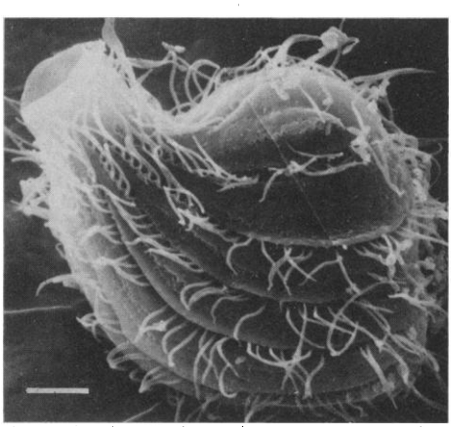
Protistologists have long been confronted with the challenges of explaining both the origin of the allegedly highly evolved ciliated protozoa, presumably from a flagellate progenitor, and the subsequent evolution of their dual nuclear apparatus (micro- plus macronucleus). Several theories implicate *Stephanopogon* not only in the phylogenetic origin of ciliates from a flagellate ancestry and in the evolvement of the duplex ciliate nucleus but even in the origin of the metazoa from a ciliate protozoan line.

Superficially, *Stephanopogon* species, which are small (20 to 50 μm by 10 to 20 μm) and relatively inconspicuous, resemble ciliates (Fig. 1), and they live and behave like various benthic marine gymnostomes (*1*). They move by means of "cilia," arranged in several rows; and, possessing a conspicuous cytostome-cytopharyngeal apparatus, they are actively phagotrophic, feeding on bacteria, various diatoms, and small flagellates. Lwoff's (*2*) discovery of their homokaryotic status, with its phylogenetic implication of primitiveness, was long ignored. Raikov and Corliss (*3*) revived and expanded Lwoff's idea, hypothesizing that the nuclear condition characteristic of ciliates today (diploid micronucleus plus independent polyploid macronucleus) evolved from the single-nucleated condition (with *Stephanopogon* as a lone survivor) via the intermediate (diploid micronucleus plus nondividing diploid macronucleus) "karyorelictid" ciliates. Corliss (*4*) further utilized this phylogenetic theory in his recent major revision of the classification scheme for the Ciliophora, the system essentially adopted by the international Society of Protozoologists (*5*). Hanson (*6*) identified *Stephanopogon* as his (homokaryotic) ciliate progenitor of the first Eumetazoa (for him, the acoelous turbellarians).

We discovered several nonciliate characteristics in *Stephanopogon apogon* Borror, 1965 (*7*), by use of electron microscopy. Our material was taken from Rehoboth Bay, Delaware, in 1977. Specimens were isolated in filtered (Millipore, 0.45 μm) seawater (30 per mil) with a micropipette and fed on unidentified bacteria grown in the culture dishes by addition of a split pea. We have substantiated Lwoff's observation that the organism, while having multiple nuclei (2 to 12 in our species; others may have as many as 16), is homokaryotic; all nuclei are vesicular, each having a single large endosome (Fig. 2). During mitosis an intranuclear spindle forms, the nuclear envelope remains intact, and the endosome divides without dedifferentiation or dissolution. The nucleus and its pattern of acentric mitosis are distinctly trypanosome-like (*8*).

As concerns the "infraciliature" underlying the eight sparsely distributed rows (six on the ventral surface plus two short ones dorsally) of flagella, the most significant aspect of our transmission electron micrograph sections is the complete absence of the kinetidal system characteristic of ciliates (*4*). While an unusually short (~ 0.25 μm) kinetosome exists at the base of every flagellum, neither a kinetodesma (or any homolog) nor ribbons of transverse and postciliary microtubules are present (Figs. 3 and 5). Furthermore, there are no pellicular alveoli, parasomal sacs, contractile vacuole (or pore), or cytoproct in *Stephanopogon*. Spherical microbodies, of unknown function, are found in the peripheral cytoplasm next to the cell membrane.

Although *Stephanopogon* has a functional and well-developed cytostome-cytopharyngeal apparatus (as do a number of flagellates and ciliates), the 32 bundles of microtubules supporting it show an unusual quadratic packing (Fig. 4) and a curious origin from fibrous material. The area enclosed by these long microtubules



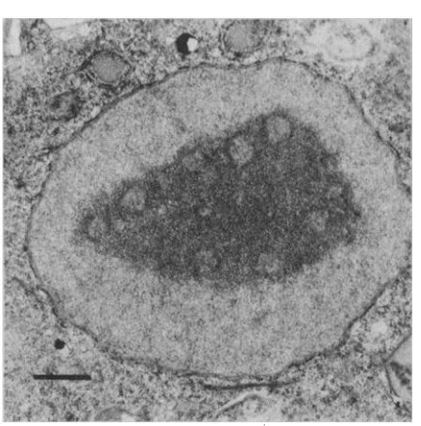


Fig. 1 (left). Scanning electron micrograph of *Stephanopogon apogon*. Ventral rows of flagella and the opening of the cytostome-cytopharyngeal complex have caused this unusual genus of flagellates to be incorrectly classified as a ciliate. Scale bar, 10 μm. Fig. 2 (right). Transmission electron micrograph of a section through the nucleus. There are two or more such identical nuclei in every organism, each measuring 3.5 to 4.0 μm in diameter, with a large central endosome. The chromatin appears finely granular and evenly dispersed. Scale bar, 1.0 μm.

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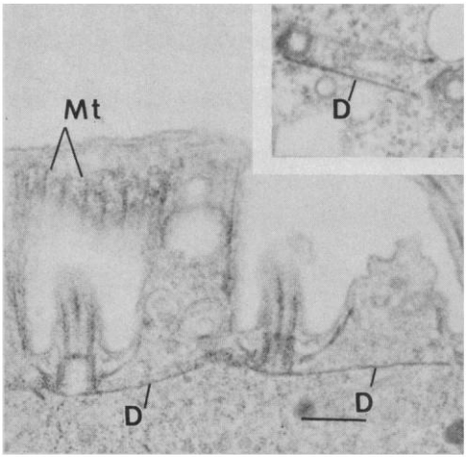

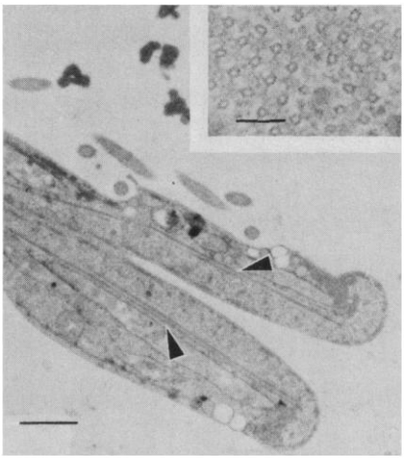

Fig. 3 (left). Transmission electron micrograph of kinetosome and associated structures. Longitudinal section showing desmose (*D*) and basket-like arrangement of microtubules (*Mt*). Scale bar, 0.5 μm. (Inset) Cross section showing two-pronged nature of desmose. Fig. 4 (right). Transmission electron micrograph of microtubular bundles surrounding the cytopharynx; longitudinal section: arrows indicate bundles. Scale bar, 5.0 μm. (Inset) Cross section through a bundle showing individual microtubules. Scale bar, 0.1 μm.

is rich in phagoplasmic vesicles. Subpellicular microtubules (in a sheet) and muciferous bodies are abundant, but both of these structures are quite widely found in euglenoid flagellates, and the former is seen in trypanosomatids (*9*).

Among features particularly distinctive of species assignable to many groups of flagellates and also noted in our organism, in addition to the monomorphic nucleus, are the lamellar (in *Stephanopogon*, discoidal) cristae of its mitochondria. Ciliates have tubular cristae, although a major branch of phytoflagellates do too, along with opalinids and certain other protozoan groups (*10*). A nonstriated amorphous rootlet or desmose arises from electron-dense material surrounding the proximal end of each kinetosome and extends anteriorly, in a two-pronged configuration (Figs. 3 and 5), toward the next basal body in a given row of flagella; this is rarely seen in other protozoa, although it has been described in a few zooflagellates of uncertain taxonomic status (*11*). As in euglenoid and kinetoplastid flagellates (*9*, *12*), microtubules also arise from this material and extend, basket-like, up to the cell surface where they terminate (Figs. 3 and 5).

The flagella of *S. apogon* have the usual nine-plus-two pattern of microtubules in cross section (Fig. 5). No swelling or paraxial rod is present, and no flagellar hairs or mastigonemes have been seen. In the basal bodies, the transition fibers do not form a stellate pattern, nor is there a transitional helix (*13*). Of course, there are no plastids in our organism. The cell membrane is essentially unsupported (Fig. 5) in *Stephanopogon*; there are no thickening layers of proteinaceous material, inside or out, and no secreted extrapellicular substances or cellulose wall.

The complex life cycle of *Stephanopogon* involves multiple fission of a large multinucleated individual within a cyst; the divisions are of a longitudinal (better, symmetrogenic) kind, typical of flagellates. Features characteristic of the phenomenon include morphogenetic movements of nuclei, of newly appearing basal bodies (parental ones having been resorbed), and of microtubules that will constitute the bundles surrounding the new cytopharynges. At the conclusion of the remarkable process, small binucleate tomites (the number depending on the precise number of nuclei in the previously encysted parental organism—in our species commonly six to eight) emerge from the ruptured cyst wall to enter a fresh period of growth, ultimately reaching the large multinucleated trophont stage again.

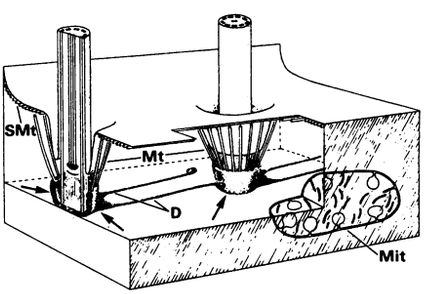

Fig. 5. Schematic drawing, based on transmission electron micrographs, of subsurface organelles and structures. Electron-dense material (arrows) surrounds base of every kinetosome, and basket-arranged microtubules (*Mt*) and two-pronged desmose (*D*) arise from it. A sheet of microtubules (*SMt*) runs under the pellicle. Ultrastructural features characteristic of the kinetids of ciliates are absent; there are no postciliary or transverse microtubules, kinetodesmal fibers, pellicular alveoli, or parasomal sacs. The mitochondria (*Mit*) have cristae that are discoidal rather than tubular.

Thus *Stephanopogon* must now be recognized as a flagellate (*14*). We propose the establishment of a new taxonomic order to contain the unusual protist genus, comparing it by numerical methods to representatives of other taxa of unicellular "algae," "protozoa," and "lower fungi" (*15*). Like the opalinid flagellates, also once considered as protociliates (*16*), *Stephanopogon* is far from the main trunk line on any phylogenetic tree that depicts the origin of the ciliate branch from some flagellate group. The use of this protist in deriving the Eumetazoa from a ciliate ancestry (*6*) is, therefore, no longer acceptable. With respect to hypotheses invoking origin of heterokaryotic ciliates from a homokaryotic progenitor (*17*), it is still possible that such an evolutionary event did occur; but this assumption must no longer depend on species of the flagellate *Stephanopogon* as its living example of such an "eociliate."

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References and Notes

1. A. Kahl, in *Die Tierwelt Deutschlands*, F. Dahl, Ed. (Fischer, Jena, 1930), vol. 1, part 18; T. Fenchel, *Ophelia* **5**, 73 (1968); J. O. Corliss and E. Hartwig, *Mikrofauna Meeresboden* **61**, 65 (1977).
2. A. Lwoff, *Acad. Sci.* **177**, 910 (1923); *Arch. Zool. Exp. Gen.* **78** (Notes et Rev.), 117 (1936).
3. I. B. Raikov, in *Research in Protozoology*, T.-T. Chen, Ed. (Pergamon, New York, 1969), vol. 3, pp. 110–114; J. O. Corliss, *BioSystems* **7**, 338 (1975).
4. J. O. Corliss, *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature* (Pergamon, New York, ed. 2, 1979).
5. N. D. Levine *et al.*, *J. Protozool.* **27**, 37 (1980).
6. E. D. Hanson, *The Origin and Early Evolution of Animals* (Wesleyan Univ. Press, Middletown, Conn., 1977).
7. A. C. Borror, *Trans. Am. Microsc. Soc.* **84**, 550 (1965).
8. K. Vickerman and T. M. Preston, *J. Cell Sci.* **6**, 365 (1970).
9. G. F. Leedale, *BioSystems* **10**, 183 (1978); K. Vickerman and T. M. Preston, in *Biology of the Kinetoplastida*, W. H. R. Lumsden and D. A. Evans, Eds. (Academic Press, New York, 1976).
10. F. J. R. Taylor, *BioSystems* **10**, 67 (1978).
11. D. J. Hibberd, *J. Protozool.* **23**, 374 (1976).
12. G. F. Leedale, *Euglenoid Flagellates* (Prentice-Hall, Englewood Cliffs, N.J., 1967); J. P. Mignot, *Protistologica* **3**, 5 (1967).
13. D. J. Hibberd, *BioSystems* **11**, 243 (1979).
14. D. L. Lipscomb and J. O. Corliss, *J. Protozool.* **25**, 7A (1978); J. O. Corliss, *ibid.* **26**, 56A (1979); D. L. Lipscomb, *ibid.* **27**, 29A (1980).
15. J. O. Corliss and D. L. Lipscomb, *ibid.*, in press.
16. J. O. Corliss, *ibid.* **2**, 107 (1955); *Ceylon J. Sci. Biol. Sci.* **13**, 65 (1979); H. S. Wessenberg, in *Parasitic Protozoa*, J. P. Kreier, Ed. (Academic Press, New York, 1978), vol. 2.
17. J. O. Corliss, *Am. Zool.* **19**, 894 (Abstr.) (1979).
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