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Corticocolpoda kaneshiroae N. G., N. Sp., a New Colpodid Ciliate (Protozoa, Ciliophora) from the Bark of Ohia Trees in Hawaii

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ABSTRACT. *Corticocolpoda kaneshiroae* n. g., n. sp. was discovered in the bark of Ohia trees (*Metrosideros polymorpha*) from the Hawaiian Archipelago. Its morphology and infraciliature were studied in live cells, with the scanning electron microscope, as well as in specimens impregnated with protargol, silver nitrate, and silver carbonate. The new genus, *Corticocolpoda,* belongs to the family Colpodidae and is unique in having a left oral ciliary field (polykinetid) composed of 13–20 equidistantly spaced, *paired* rows of monokinetids. The new species, *C. kaneshiroae,* measures 100–300 × 60–250 μm and has a huge vestibulum used to capture large food items, mainly other ciliates. The oral ciliary fields are within the vestibulum and of equal length; the right field is a paroral membrane composed of a single row of dikinetids. Several micronuclei are attached to the ellipsoid macronucleus. The mucocysts are inconspicuous and arranged around the somatic dikinetids. The resting cyst is globular and has a 1–2 μm thin, firm, yellowish inner wall and a 20-μm-thick, fragile, hyaline outer wall. Convergent evolutionary paths and a possible polyphyly of the Colpodidae are discussed. An improved key to the genera of the family Colpodidae is provided. Twenty-three species of ciliates and one flagellate (*Hemimastix amphikineta*), which occurred together with *C. kaneshiroae,* are new for the fauna of Hawaii and listed in the ecology section.

Supplementary key words. Bark fauna, Colpodea, Colpodidae, Hawaiian Archipelago, infraciliature, *Metrosideros polymorpha.*

SINCE I finished the revision of the class Colpodea in August 1991 [9], I have found 10 new species, four of which are types of new genera. This indicates that we are far from knowing the real size of this intriguing group of ciliates, which predominately inhabits terrestrial biotopes in the broadest sense (soil, litter, moss, etc.). The new species described here was discovered in the bark of Ohia trees from the Hawaiian Archipelago. Like many of my new taxa it is a "holiday species," i.e. was collected during a vacation on Big Island. *Corticocolpoda kaneshiroae* possesses the typical characteristics of members of the order Colpodida as defined by Foissner [9], but has a unique organization of the left oral ciliary field which consists, as in colpodids sensu lato (e.g. cyrtolophosidids), of equidistantly spaced, *paired* (grouped) kineties.

Large colpodids like *C. kaneshiroae* are difficult to identify. It is thus important to present new species by a multitude of figures and techniques.

MATERIALS AND METHODS

Corticocolpoda kaneshiroae was collected on 07.07.1992 from bark of Ohia trees (*Metrosideros polymorpha*; Myrtaceae) growing in the Bird Park (Kipuka Puaulu) of the Volcano National Park, Big Island, Hawaiian Archipelago, about 1,200 m above sea level (155° 20′ W, 19° 25′ N). This area harbours one of the richest concentrations of native plants in Hawaii. The old Ohia trees have a thick, fibrous bark, the outer, partially detached and dry layer of which was collected.

In the laboratory, the dry bark was saturated with distilled water according to the non-flooded petri dish method [2]. The rewetted bark had pH 4.5. *Corticocolpoda kaneshiroae* appeared five days after rewetting, when the distilled water was honey-colored by leached substances from the bark. Pure cultures were set up with a few individuals in diluted lettuce or soil extract [9] with either *Colpoda inflata, Tetrahymena pyriformis,* or *Paramecium aurelia* as food supply. Although the food items were readily ingested, *C. kaneshiroae* did not grow well. However, when the lettuce medium was supplemented with some brownish leachate from the bark, the cultures thrived much better. Most investigations were performed on cultured cells and all photographs show such material.

Cells were studied in vivo using video-microscopy, a high-power oil immersion objective and differential interference contrast [3]. Extrusomes were stained with methyl green-pyronin [7]. The silver methods used to reveal the infraciliature, the silverline system, and other cytological details were: protargol [5; protocol 2 = Wilbert's method], silver nitrate [6], and silver carbonate [4]. Preparation for scanning electron microscopy (SEM) was performed as described in [8].

Counts and measurements on silvered specimens were performed at a magnification of ×1,000. In vivo measurements were conducted at a magnification of ×250–1,000. Although these provide only rough estimates, it is convenient to give such data as specimens usually shrink in preparations or may even contract during fixation. Standard deviation and coefficient of variation were calculated according to statistics textbooks. Drawings of live specimens are based on video records, those of impregnated cells were made with a camera lucida.

Terminology is according to the monograph by Foissner [9].

Table 1. Morphometric data of *Corticocolpoda kaneshiroae*.[a]

Character	$\bar{x}$	M	SD	$SD_{\bar{x}}$	CV	Min	Max
Body, length	161.7	170	47.8	11.0	29.6	100	285
Body, width	117.5	115	34.8	8.0	29.6	65	220
Body, length (Chatton-Lwoff silver nitrate impregnation)	150.9	150	27.8	6.4	18.4	107	200
Body, width (Chatton-Lwoff silver nitrate impregnation)	113.8	112	20.2	4.6	17.8	75	148
Distance anterior end to distal end of left oral ciliary field	45.7	50	21.4	4.9	46.8	15	110
Distance anterior end to proximal end of left oral ciliary field	114.8	120	35.8	8.2	31.2	55	195
Distance anterior end to macronucleus	76.9	78	29.0	6.6	37.7	36	128
Distance posterior end to centre of excretory pore	16.6	17	6.4	1.5	38.3	7	27
Macronucleus, length	44.2	42	8.6	2.0	19.4	32	57
Macronucleus, width	31.6	32	7.0	1.6	22.0	20	45
Micronucleus, length	5.9	6	0.9	0.2	15.9	5	8
Micronucleus, width	3.0	3	0.4	0.1	13.0	2	4
Left oral ciliary field, length	69.4	68	15.6	3.6	22.8	44	90
Left oral ciliary field, width	6.1	6	1.2	0.3	19.6	4	8
Left oral ciliary field, number of kinety pairs	15.8	16	1.7	0.4	10.7	13	20
Left oral ciliary field, number of basal bodies in longest row	17.3	18	1.9	0.4	10.9	13	20
Somatic kineties, number	87.8	86	8.1	1.9	9.2	75	105
Vestibular kineties, number	17.2	17	2.2	0.5	13.0	13	20
Macronucleus, number	1.0	1	0	0	0	1	1
Micronucleus, number	2.9	3	0.7	0.2	22.7	2	4

[a] All data based, if not stated otherwise, on 19 randomly selected, protargol-impregnated and mounted specimens from an exponentially growing culture. All measurements in μm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; SD, standard deviation; $SD_{\bar{x}}$, standard deviation of the mean; $\bar{x}$, arithmetic mean.

RESULTS

Corticocolpoda n. g.

Diagnosis. Medium-sized to large, rapacious Colpodidae with huge, cave-like vestibulum occupying anterior half of cell. Left wall of vestibulum projects over right vestibular wall. Right oral ciliary field composed of single row of dikinetids. Left oral ciliary field consists of equidistantly spaced, paired rows of monokinetids.

Type species. *Corticocolpoda kaneshiroae* n. sp.

Etymology. Composite of "cortex" (Latin, living in bark) and "colpoda" (Greek, bosom). Feminine.

Description of *Corticocolpoda kaneshiroae* n. sp.

Diagnosis. In vivo 100–300 × 60–250 μm. *Krassniggia*- to *Colpoda*-shaped. Single macronucleus, several micronuclei. Eighty-six somatic and 17 vestibular kineties on average. Left oral ciliary field composed of 13–20 ($\bar{x}$ = 16) kinety pairs.

Type location. Bark of Ohia trees (*Metrosideros polymorpha*) in the Bird Park of the Volcano National Park, Big Island, Hawaiian Archipelago, 155° 20′ W, 19° 25′ N.

Type specimens. Two holotypes and two paratypes of *C. kaneshiroae* as four slides of protargol- (Wilbert technique) and silver nitrate- (Chatton-Lwoff technique) impregnated cells, respectively, have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz, Austria. Accession numbers: 3–6/1993.

Etymology. I dedicate this new species to the present Editor-in-Chief of the Journal of Eukaryotic Microbiology, Dr. Edna S. Kaneshiro, a native of Hawaii. The name "Kaneshiro" means "golden castle."

Description. Morphometric data shown in Table 1 are not repeated in this section. *Corticocolpoda kaneshiroae* is, like other large colpodids, highly variable in most characters, as indicated by the high coefficients of variation (usually between 20% and 30%).

Size and shape highly variable, common and "typical" specimens about 170 × 120 μm, broadly elliptical, with distinct hump near center of ventral side formed by vestibular bottom which projects over rectangularly notched left vestibular wall (Fig. 1–4, 16, 19, 20, 22, 23, 28–32); no diagonal groove or postoral sack, both lateral surfaces thus slightly convex. Right vestibular wall neatly curved, extends utricle-like to mid-body in most specimens (Fig. 1, 9, 16, 22, 28), as in *Krassniggia auxiliaris* [9]; however, in about 20% of cells it is more or less distinctly semicircular as in an ordinary *Colpoda* (Fig. 12, 18, 23, 30). Largest and overfed cells often almost circular, only slightly flattened, appear dark at low magnification due to many food vacuoles and innumerable, refractile spherical inclusions (Fig. 17). Normal fed and starving specimens brownish to colorless and flattened up to 2:1 (Fig. 21).

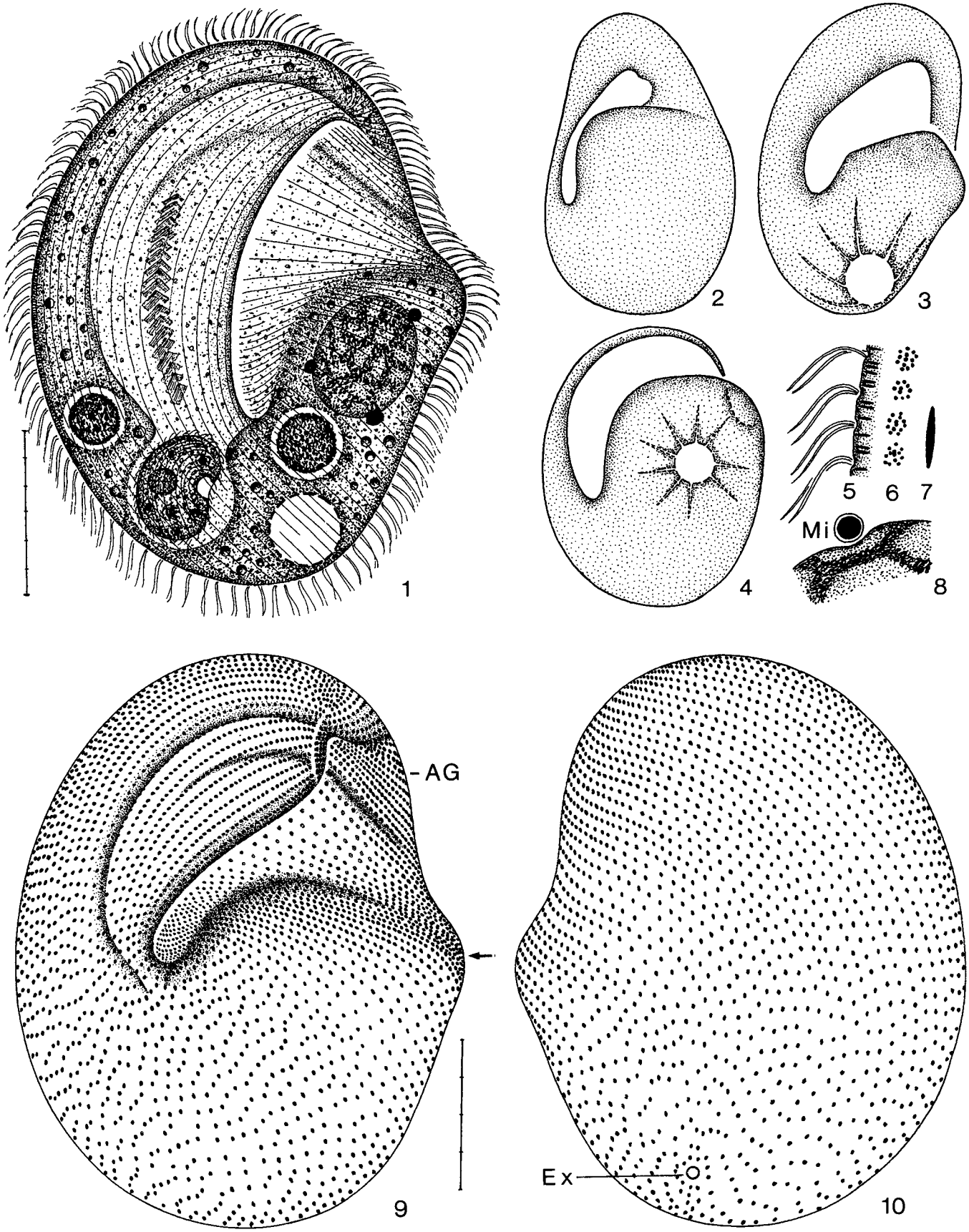
Macronucleus elliptical, usually in left posterior quadrant of cell; chromatine reticulate. Up to six large, lenticular to spherical micronuclei attached to macronucleus, surrounded by distinct membrane in living cells (Fig. 1, 8, 25, 36, 39, 43).

Contractile vacuole subterminally in median of cell or slightly shifted to ventral side, buckles cell surface when completely filled, associated with single excretory pore and many fine, anastomosing canals (Fig. 1, 3, 4, 10, 25, 32).

Extrusomes (mucocysts) numerous, mainly around somatic dikinetids, 1.5–2 × 0.5 μm in size, slightly fusiform, colorless but strongly refractive, embedded in 2-μm-thick layer of highly viscous ectoplasm; released and stained red after addition of methyl green-pyronin, elongate to up to 50-μm-long threads that form thin, tightly meshed layer around cell (Fig. 5–7, 26, 27).

Cytoplasm colorless, viscous, usually full of 1–5 μm sized, greasily shining, colorless to yellowish globules; no crystals. Very rapacious, in raw cultures feeding on *Colpoda steinii, C. inflata* and *C. lucida,* in pure cultures on *Tetrahymena pyriformis* and even on the large *Paramecium aurelia* (Fig. 1, 17, 20, 21, 25, 29). Prey is transported into the huge vestibulum by the powerful swirl produced by the cilia of the large vestibular bottom, and enters cell at proximal end of oral ciliary fields. Swims leisurely in wide spirals, often resting with right side on bottom of culture dish, eagerly devouring food items.

General plan of somatic and oral infraciliature as in other members of family and order [9]. Somatic cilia approximately 12-μm long, paired throughout, densely spaced in anterior body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half, more loosely arranged in posterior body portion and in upper half of vestibular bottom. A stripe of condensed ciliature, evidently homologous to the diagonal (somatic) groove of *Colpoda* spp., is found on the ridge that extends horizontally from the left oral ciliary field to the distal end of the vestibular bottom, i.e. the ventral hump. Kineties of right and left side descend horizontally from anterior, external portion of preoral suture, which is inconspicuous and extends from keel to distal end of oral ciliary fields (Fig. 1, 9–12, 22–24, 28–33, 42–45).

Vestibulum very large, occupies anterior half of body, dorsal margin semicircular, ventral margin (= left vestibular wall) almost rectangularly notched, projects distinctly over sigmoid right vestibular wall. Inner surface of left vestibular wall bipartite by small ridge which extends cornucopia-like from anterior left body margin to dorsal posterior half of vestibulum producing a narrow channel in anterior fifth of large oral cavity (Fig. 1–4, 9, 11, 12, 16, 20, 25, 29, 36, 43). This "second vestibulum" is recognizable only in the largest specimens and less pronounced than that found in *Bresslauides discoideus* [9].

Seventeen vestibular kineties on average on inner surface of right vestibular wall, about half abut to right oral ciliary field and have no connection to somatic kineties; other (ventral) half formed by right lateral somatic kineties which turn into posterior vertex of vestibular opening and spread fan-like to anterior ventral half of vestibulum to end at internal (vestibular) portion of preoral suture. Vestibular bottom and left vestibular wall covered by postoral kineties which commence near left oral ciliary field and at preoral suture (Fig. 13, 24, 25, 29, 36–38, 43–46).

Right oral ciliary field follows semicircular dorsal vestibular curvature, of same length as left oral ciliary field, composed of single row of dikinetids; distance between dikinetids gradually increases from proximal to distal (Fig. 13, 25, 37, 46–48). Left oral ciliary field parallel to main body axis and dorsal margin of cell, in center of oral cavity and thus far from right oral ciliary field. Space between oral ciliary fields without cilia, appears wrinkled in protargol slides (Fig. 1, 13, 25, 29, 36, 37, 43).

Left oral ciliary field crescentic since slightly curved as a whole and length of kineties decreases gradually at both ends, consists of pairs of slightly curved kineties; pairs at ends of field sometimes composed of single kinety only (Fig. 1, 13, 25, 36, 37, 43, 46, 48). Cilia remarkably short, viz. about 5 μm.

Cytostome at proximal end of oral ciliary fields, i.e. in posterior body half and slightly right of cell median. Pharyngeal fibres inconspicuous, most originate from proximal portion of right oral ciliary filed (Fig. 1, 13, 21, 25, 29, 36, 37, 43).

Silverline system colpodid, i.e. composed of quadratic to rectangular meshes connecting somatic dikinetids (Fig. 14, 34, 35); meshes rather irregular in more sparsely ciliated postoral half of cell. Extrusomes usually do not impregnate with silver nitrate, except in oral cavity where they form an irregular pattern of argyrophilic granules usually obscuring somatic infraciliature and silverline system (Fig. 29).

Reproduction and morphogenesis not yet studied in detail. Division occurs in reproductive cysts covered by a thin, structureless membrane.

Most cultured cells died without forming resting cysts, indicating suboptimal conditions, at least for encystment. The resting cysts observed had the following structure (Fig. 15, 40, 41): spherical to slightly ellipsoid, diameter 120–180 μm ($\bar{x}$ = 146, n = 5), black-brown at low magnification, surrounded by two distinct walls. Outer wall (ectocyst) about 20 μm thick, covered by detritus and bacteria, fragile, hyaline and colorless, composed of many thin layers (membranes?) with some granular inclusions (extruded chromatin?). Inner wall (meso- and endocyst) 1–2 μm thick, firm and flexible, yellowish. Extrusomes retained, form distinct seam under pellicle.

Occurrence and ecology. As yet found only at type location, together with the following species that are also new for the fauna of Hawaii: *Bryometopus pseudochilodon* Kahl, 1932, *B. triquetus* Foissner, 1993, *Cinetochilum margaritaceum* (Ehrenberg, 1830), *Colpoda inflata* (Stokes, 1885), *C. steinii* Maupas, 1883, *Cyclidium muscicola* Kahl, 1931, *Cyrtolophosis mucicola* Stokes, 1885, *Drepanomonas muscicola* Foissner, 1987, *D. pauciciliata* Foissner, 1987, *Epispathidium terricola* Foissner, 1987, *Frontonia depressa* (Stokes, 1886), *Grossglockneria acuta* Foissner, 1980, *Hausmanniella discoidea* (Gellért, 1956), *Holosticha sylvatica* Foissner, 1982, *Kahlilembus fusiformis* (Kahl, 1926), *Leptopharynx costatus* Mermod, 1914, *Nivaliella plana* Foissner, 1980, *Platyophrya vorax* Kahl, 1926, *Pleuroplites australis* Foissner, 1988, *Pseudoplatyophrya nana* (Kahl, 1926), *P. terricola* Foissner, 1985, *Urosomoida agiliformis* Foissner, 1982, and *Vorticella astyliformis* Foissner, 1981. Another remarkable species found in this sample is *Hemimastix amphikineta*, a rare heterotrophic flagellate described only recently from soils of Australia and Chile [11]. The ciliate fauna in the bark of Ohia trees is obviously dominated by r-selected colpodids (*Bryometopus* spp., *Colpoda* spp., *Cyrtolophosis, Grossglockneria, Hausmanniella, Nivaliella, Platyophyra, Pseudoplatyophyra* spp.), indicating that it is an extreme biotope [1, 9].

Corticocolpoda kaneshiroae must be a rare species since I did not find it in about 1,000 other soil, moss, and bark samples collected worldwide; it is probably endemic to the Hawaiian Archipelago. For sumptuous growth it needs substances contained in the bark, as mentioned in the material section. This distinguishes it from *Colpoda cavicola*, which thrives well in ordinary lettuce medium on bacteria or baker's yeast [9], although it has been found in nature almost exclusively in tree holes.

DISCUSSION

Classification and comparison of *Corticocolpoda kaneshiroae* with related taxa. The order Colpodida contains five families (Colpodidae, Hausmanniellidae, Marynidae, Bardeliellidae, Grandoriidae) with a total of 16 genera and 74 bonafide species [9]. All have the left oral polykinetid composed of single, equidistantly spaced rows of monokinetids. The equidistantly spaced

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Fig. **1–10**. *Corticocolpoda kaneshiroae* n. sp. (Fig. 1, 3–8, from life, shape of cells from video records; 2, drawn after a scanning electron micrograph; 9, 10, Chatton-Lwoff silver nitrate impregnation). **1**. Right lateral view of a *Krassniggia*-shaped trophont (cp. Fig. 22). The center of the large vestibulum is occupied by the left oral ciliary field; at its posterior end a large food vacuole with a trapped *Colpoda lucida* pinches off. **2-4**. Ventral, ventro-lateral, and posterior polar views. Inconspicuous canals surround the contractile vacuole. **5, 6**. Side and surface view of the cortex showing extrusomes surrounding paired somatic cilia. **7**. Resting extrusome, length 1.5 μm. **8**. Micronucleus attached to macronucleus. **9, 10**. Infraciliature of right and left side of a *Krassniggia*-shaped, representative specimen (cp. Fig. 22). Arrow marks a stripe of condensed ciliature extending from the posterior portion of the left oral ciliary field to the left lateral surface of the cell. AG, flat groove in anterior portion of vestibulum; Ex, excretory pore of contractile vacuole; Mi, micronucleus. Scale bar division = 10 μm.

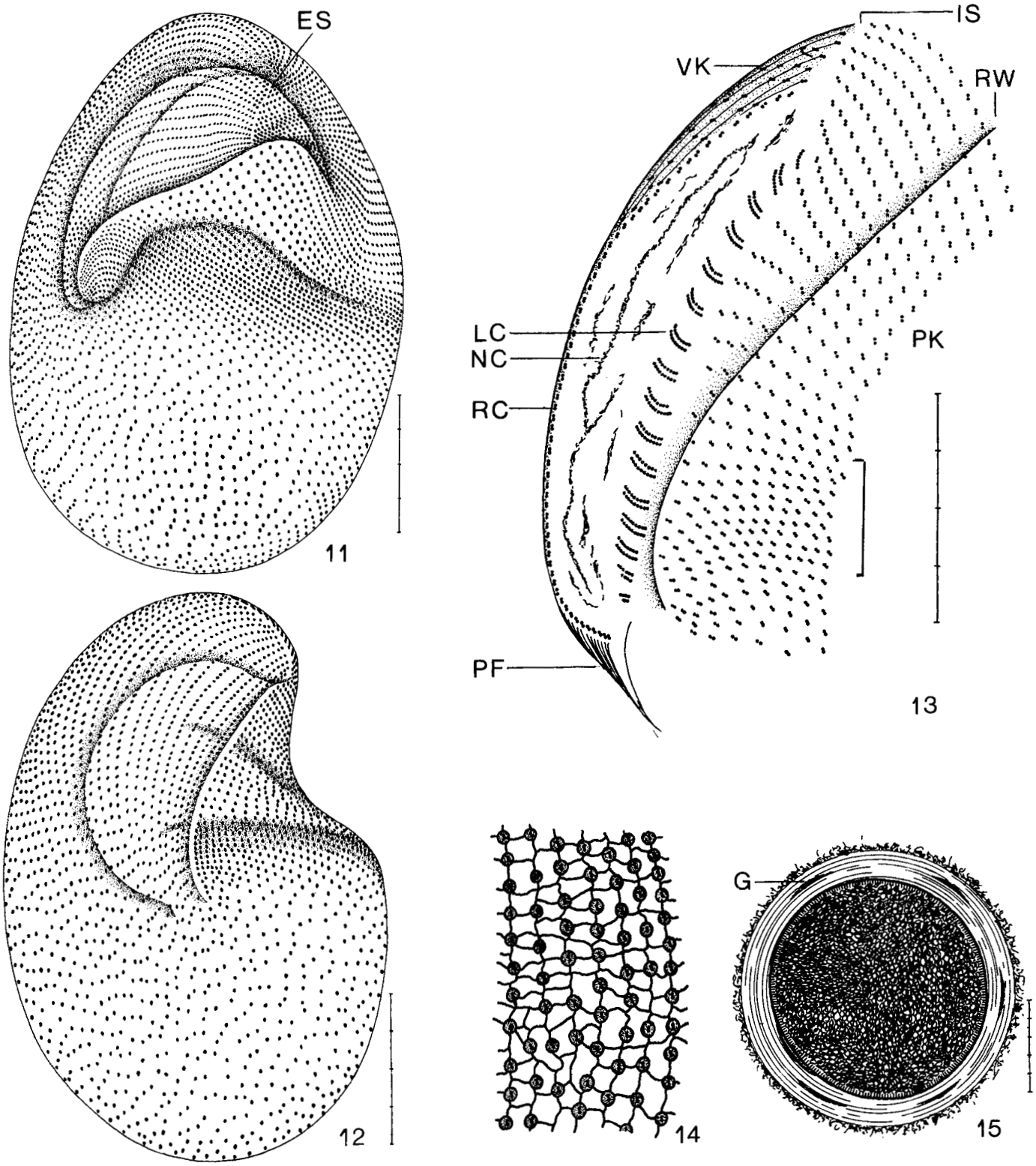

Fig. **11–15**. *Corticocolpoda kaneshiroae* n. sp. (Fig. 11, 12, 14, Chatton-Lwoff silver nitrate impregnation; 13, protargol impregnation; 15, from life). **11**. Ventral view of a *Krassniggia*-shaped trophont. The preoral suture extends to the left oral ciliary field (cp. Fig. 13). **12**. Right lateral view of a *Colpoda*-shaped trophont (cp. Fig. 23). **13**. Oral infraciliature. Most of the vestibular kineties, which extend on the inner surface of the right vestibular wall and abut on the right oral ciliary field, are not shown (cp. Fig. 25, 44). Bracket marks stripe of condensed ciliature extending from the left oral ciliary field to the left lateral surface of the cell. **14**. Silverline system. **15**. Resting cyst. The very hyaline ectocyst is covered by detritus and bacteria. ES, external portion of the preoral suture; G, granular inclusions in ectocyst; IS, internal portion of preoral suture; LC, left oral ciliary field (polykinety); NC, non-ciliated area between right and left oral ciliary field; PF, pharyngeal fibres; PK, postoral kineties; RC, right oral ciliary field (paroral membrane); RW, edge of right vestibular wall; VK, vestibular kineties. Scale bar division = 10 μm.

paired rows of monokinetids found in *C. kaneshiroae* are thus exceptional and might justify establishing a new family. However, the remaining organization of *C. kaneshiroae* is very similar to that of other genera of the Colpodidae, especially *Kuehneltiella* and *Krassniggia*. Furthermore, there is an indistinct grouping of the monokinetidal rows of the left oral ciliary field in *Ilsiella*, a marynid colpodid [9]. Grouped adoral organelles are common in other orders of the Colpodea, especially the Cyrtolophosidida. The discovery of such an organization in a colpodid s.str. thus further supports a monophyly of colpodids s.str. and colpodids s.l. (e.g. cyrtolophosidids).

Corticocolpoda kaneshiroae has the typical characteristics of members of the family Colpodidae as defined by Foissner [9], i.e. the vestibular opening is in the anterior half of the body and the right and left oral ciliary field are approximately equal in length. The right vestibular wall of *C. kaneshiroae* usually extends utricle-like posteriad, as in *Krassniggia* (Fig. 50), whose right oral ciliary field is, however, composed of disordered monokinetids [9]. The right oral ciliary field of *C. kaneshiroae* consists of a single row of dikinetids as in *Kuehneltiella* [9]. This is an important difference to the other genera of the family and might indicate that *Corticocolpoda* and *Kuehneltiella* are a distinct taxon, i.e. that the Colpodidae are polyphyletic. However, similar exceptions are known from the Hausmanniellidae (*Avestina*) and Marynidae (*Ilsiella*), and most (all?) *Colpoda* species have an ordered row of dikinetids associated with the disordered basal bodies of the right oral ciliary field [9, 12].

The colpodids sensu lato, e.g. *Platyophryides*, are considered to represent the ancestral organization of the colpodids [9]. They have, like *C. kaneshiroae*, grouped adoral organelles and a simple right oral ciliary field (paroral membrane) composed of a single row of ciliated dikinetids. It is tempting to speculate that *C. kaneshiroae* retained this ancestral organization and that the complex right oral ciliary field of *Colpoda* and related genera is a new acquisition (Fig. 49). This would suggest two highly convergent evolutionary lines (*Colpoda, Bresslaua, Maryna, Hausmanniella* etc. on the one hand, and *Corticocolpoda, Kuehneltiella, Ilsiella,* and *Avestina* on the other). However, the last mentioned genera are rather specialized, both morphologically and ecologically, suggesting that their simple right oral ciliary field evolved by a secondary loss of the disordered ciliary field found in most genera of these families. This assumption seems more parsimonious than several convergent evolutionary lines. I thus suggest placing *Corticocolpoda* in the family Colpodidae.

No other species have been found in the literature that could be identical with *C. kaneshiroae*. However, there are several species that superficially resemble *C. kaneshiroae*, viz. *Krassniggia* sp., *Bresslaua* spp., *Bresslauides* spp., and *Kuehneltiella* spp. In spite of these, *C. kaneshiroae* is easily determined, even in vivo, by the paired ciliary rows of the left oral ciliary field, which are well recognizable with bright field and especially with interference contrast microscopy. It is thus not necessary to use silver impregnations for a correct identification of this species, although such methods are recommended for revealing the inconspicuous right oral ciliary field.

Key to the genera of the family Colpodidae (Fig. 50). The following key is an adapted and refined version of those previously published [9, 10], which include a detailed discussion of all genera.

1. Vestibulum large, occupies front half of cell which thus has conspicuous, bright patch anteriorly. Rapacious carnivores ... 5
– Vestibulum inconspicuous, i.e. usually small compared to size of cell. No bright patch anteriorly. Microphages usually feeding on bacteria ... 2

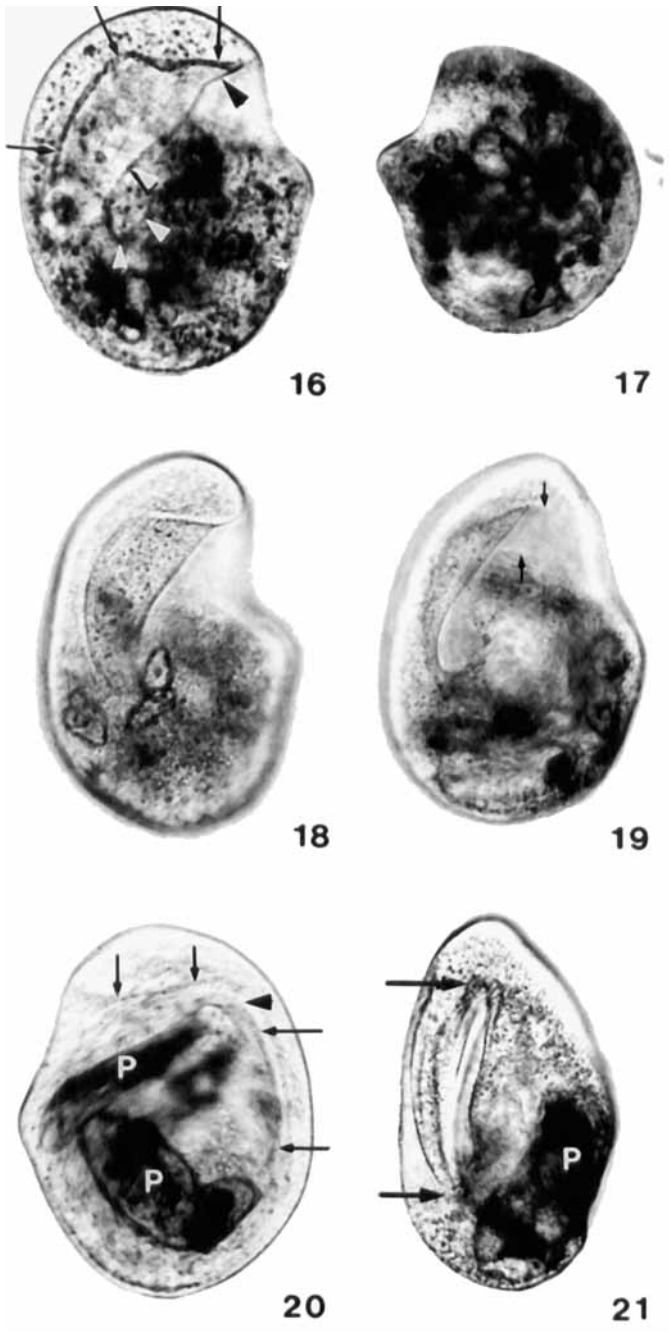

Fig. **16–21**. *Corticocolpoda kaneshiroae* n. sp., bright field micrographs of living cells, length 140–300 μm. **16**. Right lateral view of a common, *Krassniggia*-shaped trophont. Arrows mark dorsal wall of vestibulum, arrowheads mark edge of right vestibular wall. **17**. Left lateral view of a large, overfed, *Bresslauides discoideus*-shaped cell. **18**. Right lateral view of a *Colpoda*-shaped specimen. **19**. Ventro-lateral view of a *Krassniggia*-shaped cell. Arrows border the flat groove in the anterior portion of the vestibulum. **20**. Left lateral view of a *Krassniggia*-shaped specimen ingesting two *Paramecium aurelia* (P). Arrows border dorsal wall of huge vestibulum, arrowhead marks flat groove in anterior portion of vestibulum. **21**. Ventral view and focused to center of cell. Arrows border vestibulum. The large food vacuole contains a *Paramecium aurelia* (P).

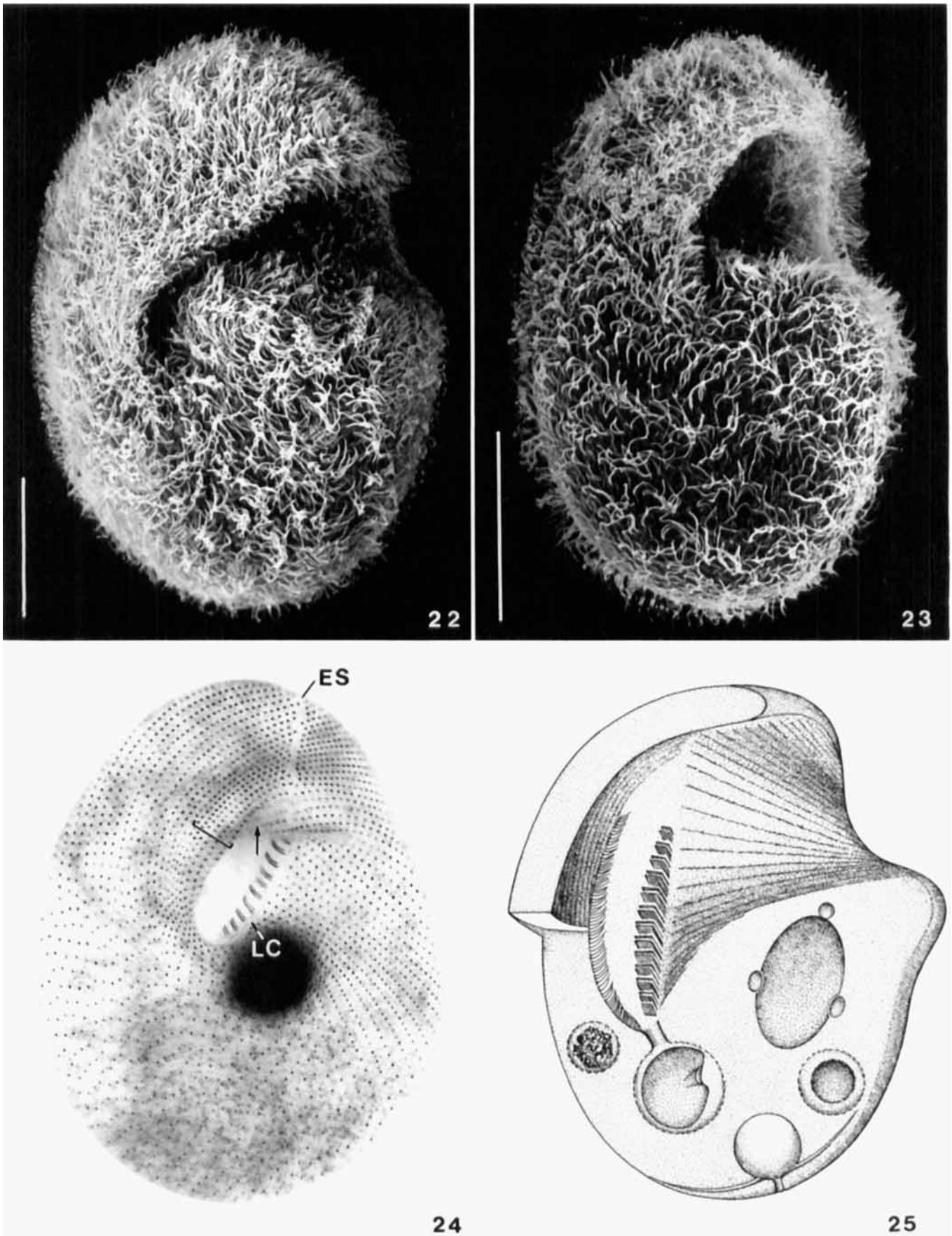

Fig. 22–25. *Corticocolpoda kaneshiroae* n. sp. (Fig. 22, 23, SEM micrographs; 24, silver carbonate impregnation; 25, three-dimensional reconstruction). **22.** Ventro-lateral view of a *Krassniggia*-shaped specimen; bar = 30 μm. **23.** Ventro-lateral view of a *Colpoda*-shaped specimen; bar = 30 μm. **24.** Ventral view of a squashed (thus without scale bar), unmounted specimen. Bracket encompasses upfolded portion of right

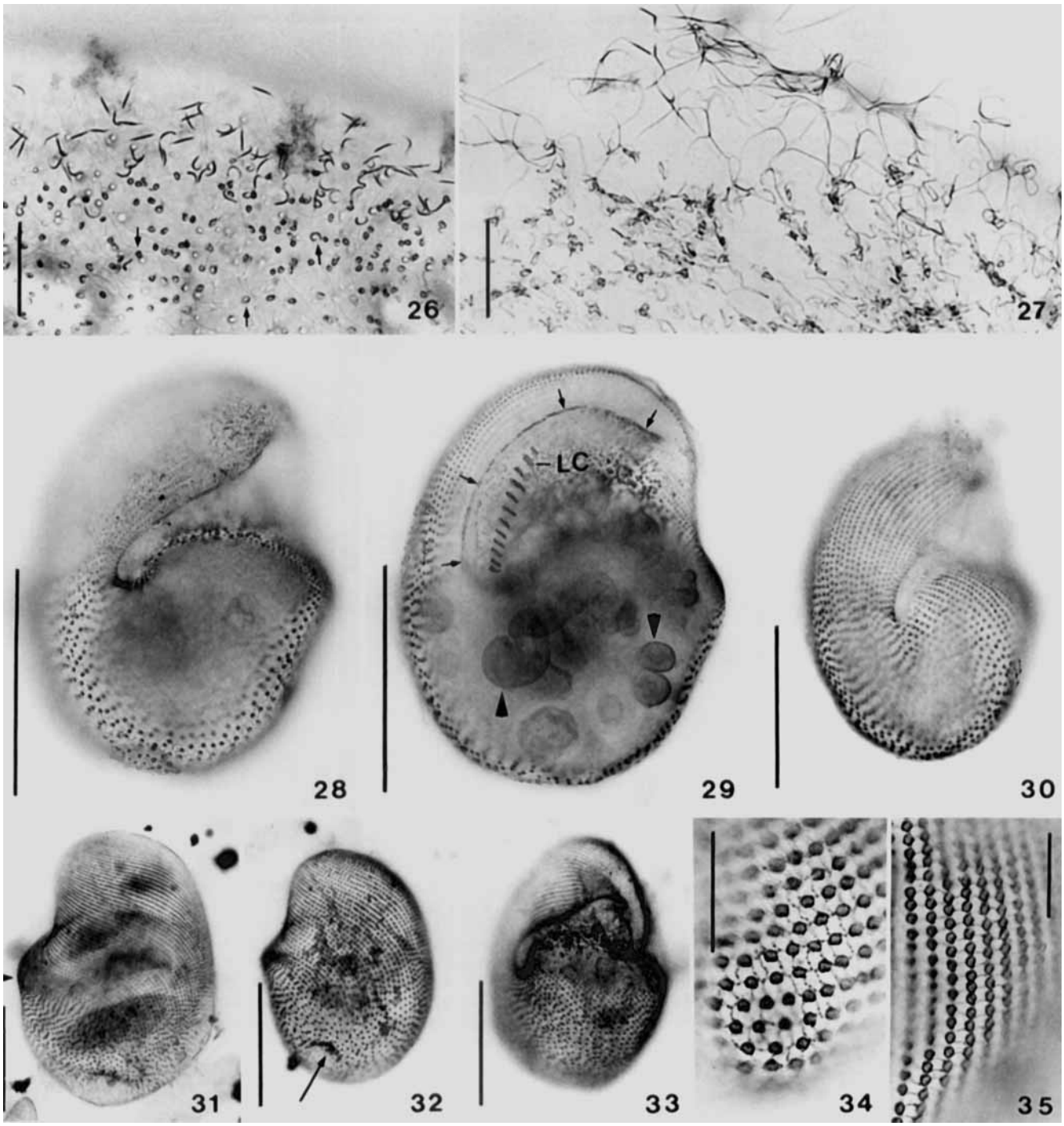

Fig. 26–35. *Corticocolpoda kaneshiroae* n. sp. (Fig. 26, 27, methyl green-pyronin stain; 28–35, Chatton-Lwoff silver nitrate impregnation). **26, 27.** Mucocysts are released after addition of the stain and swell to 3–5-μm-long rods (Fig. 26), which later elongate to long threads forming a more or less dense reticulum around the cell (Fig. 27). Arrows mark incompletely swollen mucocysts, which are semicircular in shape. **28, 29.** Ciliary pattern on right side and oral infraciliature of a *Krassniggia*-shaped specimen focused to surface and center of cell. Arrows mark dorsal wall of vestibulum; arrowheads mark food vacuoles containing *Colpoda* and *Tetrahymena*. **30.** Ciliary pattern on the right side of a *Colpoda*-shaped specimen. **31, 32.** Ciliary pattern on the left side of a large (Fig. 31) and a medium-sized (Fig. 32) specimen. Arrow in Fig. 31 marks condensed ciliary stripe extending from left oral ciliary field to left lateral surface; it is much less pronounced in the medium-sized specimen shown in Fig. 32. Arrow in Fig. 32 indicates excretory pore of contractile vacuole. **34, 35.** Infraciliature and silverline system in the postoral area and on the outer surface of the right vestibular wall, where the dikinetids are very densely spaced and the silverline pattern is very regular. LC, left oral ciliary field. Fig. 26, 27, 34, 35, bars = 15 μm; 28–33, bars = 80 μm.

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vestibular wall covered with vestibular kineties; arrow marks vestibular kineties abutting on right oral ciliary field. **25.** "Cut-away" view showing huge vestibulum, oral ciliary fields, non-ciliated area between oral ciliary fields, nuclear apparatus, contractile vacuole, and some food vacuoles (cp. Fig. 13). ES, external portion of preoral suture; LC, left oral ciliary field.

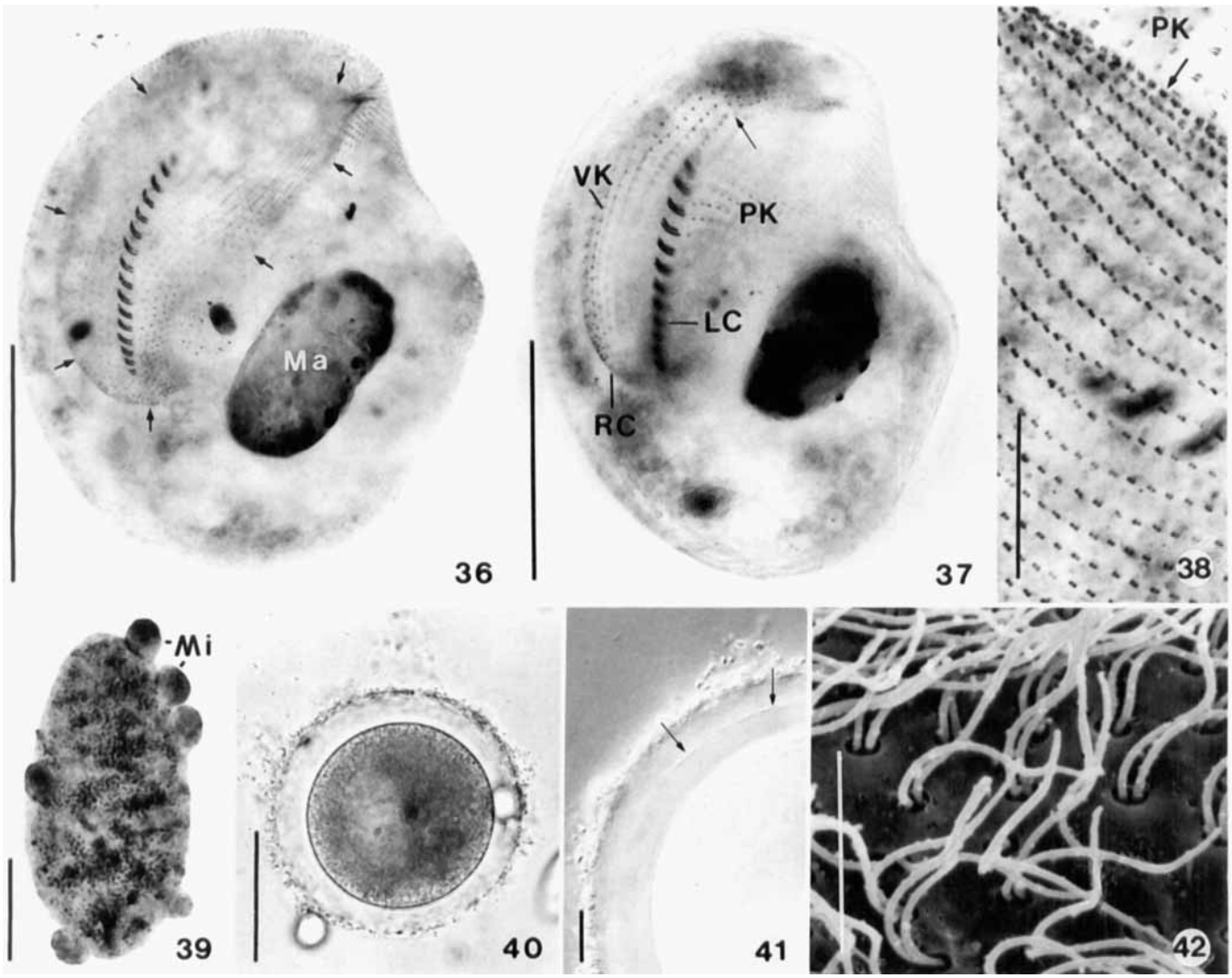

Fig. **36–42**. *Corticocolpoda kaneshiroae* (Fig. 36–38, protargol impregnation; 39, silver carbonate impregnation; 40, 41, bright field and interference contrast light micrographs of live cells; 42, SEM-micrograph). **36**. Right lateral view of a slightly squashed, unmounted, *Krassniggia*-shaped specimen having a huge vestibulum (bordered by arrows). **37**. Ventro-lateral view of a slightly squashed, unmounted, *Colpoda*-shaped specimen showing location of main cell organelles. Arrow marks internal portion of preoral suture. **38**. The somatic basal bodies are paired (dikinetids) and much more densely spaced on the external surface of the right vestibular wall than in the postoral kineties; note that dikinetids are oriented obliquely to main axis of the kinety. **39**. Six micronuclei are attached to the macronucleus. **40**, **41**. The resting cyst has a thick, hyaline ectocyst containing granular inclusions (arrows). **42**. The somatic cilia are paired and protrude from distinct cortical pits. LC, left oral ciliary field; Ma, macronucleus; Mi, micronucleus; PK, postoral kineties; RC, right oral ciliary field; VK, vestibular kineties. Fig. 36, 37, 40, bars = 80 μm; 38, 39, 41, bars = 15 μm; 42, bar = 5 μm.

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Fig. **43–48**. *Corticocolpoda kaneshiroae* (Fig. 43–47, protargol impregnation; 48, silver carbonate impregnation). **43–45**. Overview and details of the infraciliature of the right side and of the oral apparatus of a *Krassniggia*-shaped specimen. Arrows in Fig. 43 border dorsal margin of vestibulum. Brackets in Fig. 43 and 45 encompass stripe of condensed dikinetids (cilia) extending from posterior portion of left oral ciliary field to right side of cell. Arrows in Fig. 44 mark somatic kineties on external surface of right vestibular wall; arrowheads mark vestibular kineties on the inner surface of the right vestibular wall. Arrows in Fig. 45 border edge of right vestibular wall. **46**. Right lateral view showing details in posterior portion of oral apparatus. The right half of the vestibular kineties abut to the right oral ciliary field (paroral membrane; arrows), the left half is formed by postoral somatic kineties which extend anteriorly. **47**, **48**. The right oral ciliary field consists of a single row of dikinetids to which vestibular kineties abut. LC, left oral ciliary field; LVK, left half of vestibular kineties; Ma, macronucleus; PK, postoral kineties; RC, right oral ciliary field (paroral membrane); RVK, right half of vestibular kineties. Figures purposely without scale bars since the applied techniques (squashed, unmounted specimens) lead to unavoidable distortions of the cells.


Ma
43
LC
PK
44
PK
45
LC
RVK
LVK
46
LC
RVK
RC
47
RC
LC
RC
48

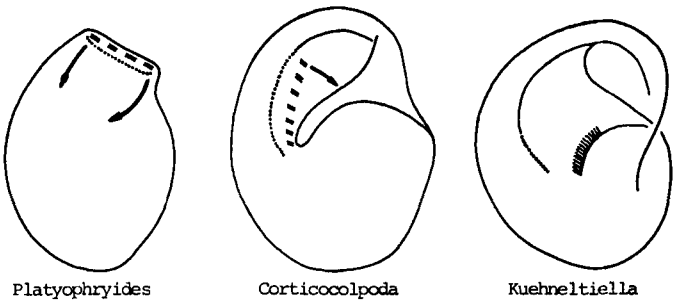

Fig. 49. A possible evolutionary line from a sorogenid colpodid (*Platyophryides*) to *Corticocolpoda* and *Kuehneltiella*. This path cannot be excluded, although it is less convincing than a secondary loss of the disordered kinetids in the right oral ciliary field of *Colpoda*.

2. Right wall of vestibulum and oral opening on right or right ventro-lateral side of cell 3
– Right wall of vestibulum and oral opening on left side of cell ... . *Idiocolpoda*
3. Surface smooth or with tooth-like processes 4
– Surface ornamented ladder-like by conspicuous cortical ridges .. .. *Cosmocolpoda*
4. Anterior cell half helmet-like due to distinct preoral ridge, the proximal edge of which forms the right vestibular wall and is aligned with a membranoid structure produced by the lowermost left lateral somatic kinety ..... *Apocolpoda*
– Anterior cell half not helmet-like (usually rounded or acute) and without preoral ridge ..... *Colpoda*
5. Left oral polykinetid composed of equidistantly spaced, single kineties 6
– Left oral polykinetid composed of equidistantly spaced pairs of kineties ..... *Corticocolpoda*
6. Right wall of vestibulum overhangs semicircularly notched left vestibular wall; cell thus possesses conspicuous, bright, circular patch anteriorly 7
– Right wall of vestibulum extends utricle-like posteriad on right side of cell, i.e. does not overhang left vestibular wall .... *Krassniggia*
7. Right oral polykinetid composed of many short, disordered kineties (use silver carbonate impregnation) ..... *Bresslaua*
– Right oral polykinetid inconspicuous, composed of single row of dikinetids (use silver carbonate impregnation) .. *Kuehneltiella*

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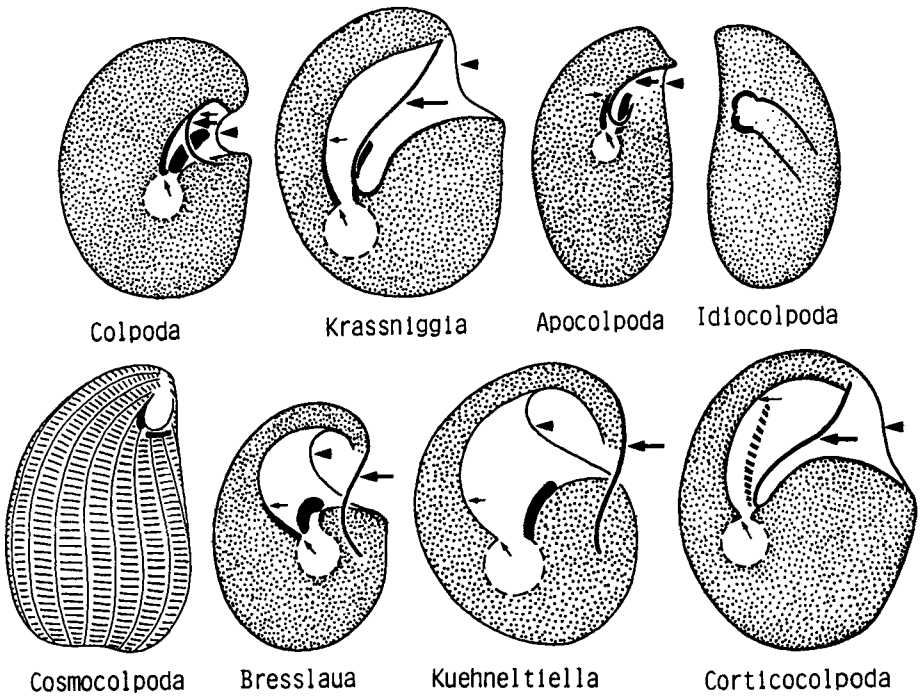

Fig. 50. Genus distinction in the family Colpodidae by the location of the oral apparatus (at left or right side of cell), the structure of the cortex (conspicuous ridges in *Cosmocolpoda*), the shape of the vestibulum (funnel-like in *Colpoda, Apocolpoda, Idiocolpoda* and *Cosmocolpoda*, cave-like in others), the shape of the right (large arrows) and left (arrowheads) vestibular wall, the left oral ciliary field (equidistantly spaced pairs of kineties in *Corticocolpoda*, single rows in others), and the right oral ciliary field (small arrows), which consists of a single row of dikinetids in *Kuehneltiella* and *Corticocolpoda* (from Foissner [9, 10]; supplemented). Vestibulum and descending food vacuole unstippled, oral polykinetids shaded black (except of *Corticocolpoda*), ends of right oral polykinetid marked by small arrows.

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Purification and Characterization of Phospholipase C Preferentially Hydrolysing Phosphatidylcholine in *Tetrahymena* Membranes

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ABSTRACT. A phospholipase C (PLC) activity that preferentially hydrolyses phosphatidylcholine to diacylglycerol and phosphorylcholine was found to be present in *Tetrahymena pyriformis*, strain W and most of its activity was recovered in the membrane fraction. This enzyme was extracted with 1% Triton X-100 from the membrane fraction and purified to apparent homogeneity by sequential chromatographies on Fast Q-Sepharose, hydroxyapatite HCA-100S, Mono Q and Superose 12 gel filtration columns. The purified enzyme had specific activity of 2083 nmol of diacylglycerol released/mg of protein/min for dipalmitoylphosphatidylcholine hydrolysis. Its apparent molecular mass was 128 kDa as determined by SDS-polyacrylamide gel electrophoresis and was 127 kDa by gel filtration chromatography, indicating that the enzyme is present in a monomeric form. The enzyme exhibited an optimum pH 7.0 and the apparent K_m value was determined to be 166 μM for dipalmitoylphosphatidylcholine. A marked increase was observed in phosphatidylcholine hydrolytic activity in the presence of 0.05% (1.2 mM) deoxycholate. Ca^{2+} but not Mg^{2+} enhanced the activity at a concentration of 2 mM. This purified phospholipase C exhibited a preferential hydrolytic activity for phosphatidylcholine but much less activity was observed for phosphatidylinositol (~ 9%) and phosphatidylethanolamine (~ 2%).

Supplementary key words. Ciliated protozoa, lysosome, thermal adaptation, triacylglycerol biosynthesis.

IT has been shown that the ciliated protozoan *Tetrahymena* is a potentially useful eukaryote for investigation of lipid metabolism [11, 18]. This cell has been known to contain various types of phospholipases distinguishable with respect to pH optimum, Ca^{2+}-dependency, substrate specificity and positional specificity. We first reported that the phospholipase A_1 (PLA_1) and phospholipase C (PLC) activities were predominantly present in lysosomes of *Tetrahymena pyriformis* NT-1 [2]. The lysosomal phospholipases had acidic pH optimum from 4 to 5 and hydrolysed both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) without Ca^{2+}. A phospholipase A activity hydrolysing PE was also found in the *Tetrahymena* membrane, which had an optimum pH at 9 and was dependent on Ca^{2+}. The lysosomal PLA_1 and PLC were found to be released into the culture medium during their growth [3]. Furthermore, the released PLC were of two different types in molecular weight and substrate specificity. However, these phospholipases have not yet been highly purified.

Recently, we found evidence in *T. pyriformis* W for the presence of a membrane-bound PLC preferentially hydrolysing phosphatidylcholine (PC-PLC), which is distinct from lysosomal PLC. The physiological role of this enzyme remains unknown. Some recent studies have suggested that the enzyme PC-PLC may be involved in proliferation of mammalian cells [7, 12]. In the present study, as an initial step to gain insight into the functional role of PLC in *Tetrahymena* cells, we have undertaken purification of the PLC from *Tetrahymena* membrane fraction that shows a preferential selectivity for phosphatidylcholine and characterized its biochemical properties.

MATERIALS AND METHODS

Materials. Fast Q-Sepharose, Mono Q (HR 5/5) and Superose 12 (HR 10/30) were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). Hydroxyapatite HCA-100S was from Mitsui Tohatsu (Tokyo, Japan). Phosphatidylinositol (PI, soybean) was from Serdary Research Laboratories (London, U.K.). 1,2-Dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) was from Avanti Polar-Lipids (Alabama). Dioleoylphosphatidylethanolamine (DOPE) was from Sigma Chemical Company (St. Louis, MO). Dipalmitoyl[choline-methyl-^{3}H]phosphatidylcholine ([^{3}H]choline-DPPC, 40.0 Ci/mmol) and 1-palmitoyl-2-[9,10-^{3}H]palmitoyl-sn-glycero-3-phosphatidylcholine ([^{3}H]palmitoyl-DPPC, 50.0 Ci/mmol) were from New England Nuclear (Boston, MA). 1,2-Dioleoyl-3-phosphatidyl[2-^{14}C]ethanolamine ([^{14}C]DOPE, 54 mCi/mmol) and 3-phosphatidyl[2-^{3}H]inositol ([^{3}H]PI, 23.2 Ci/mmol) were from Amersham Corp. (Buckinghamshire, U.K.).

Cell and cell culture. *Tetrahymena pyriformis* strain W was grown with shaking at 28° C in an enriched medium containing 2% proteose-peptone as previously described [19]. At early stationary phase the cells were harvested and kept at −80° C until sufficient material was obtained for purification.

Extraction of phospholipase C from *Tetrahymena* membrane. The frozen *Tetrahymena* cells were thawed and suspended in 20 mM Tris-HCl buffer (pH 7.0), containing 2 mM EDTA and

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