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Biology and Fertility of Soils

Kuehneltiella terricola gen. nov., sp. nov. – a carnivorous ciliate (Protozoa, Ciliophora) from a sandy soil in Australia*

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Summary. The morphology and biology of the colpodid ciliate *Kuehneltiella terricola* gen. nov., sp. nov. has been investigated using living organisms, various silver impregnation methods, and scanning electron microscopy. The new species has been isolated in soil from central Australia and might be endemic to this continent. The new genus *Kuehneltiella* differs from its nearest relative, *Bresslaua*, in having a right oral polykinetid composed of a single row of dikinetids. A reinvestigation of Lynn's slides of *Bresslaua insidiatrix* showed that, contrary to the statement of Lynn (1979), this species has a typic colpodid right oral polykinetid, i.e., composed of many short, disordered kineties. A brief review of the literature suggests that simple, single-rowed, right oral polykinetids are apomorphic in the colpodids s. str. Further, this special character has obviously evolved independently several times within the class Colpodea and even within the colpodids s. str. An illustrated key to the genera of the family Colpodidae is provided.

Key words: *Kuehneltiella terricola* gen. nov., sp. nov. – Soil ciliates – Colpodidae – Systematics – Evolution – Australia

A great number of new soil ciliates have been described during the last decade (Foissner 1987a). Currently, about 300 species are known to inhabitat the soil, but certainly many more wait to be discovered. Many of these species are apparently restricted to terrestrial habitats; some might even be endemic to certain areas of the world. The most common soil ciliates belong to the class Colpodea, and Foissner (1987a) has designated the soil ciliate community as "Colpodetea". The evolutionary success of the Colpodea in terrestrial biotopes depends on their *r*-selected survival strategy, especially on their capability to produce four or more offspring in reproductive cysts.

The new species described in this paper belongs to the colpodids s. str., i.e., the family Colpodidae. Within this assemblage, two feeding strategies have been evolved. Members of the large genus *Colpoda* (about 30 reliable species) feed mainly on bacteria, and are comparatively small-sized species with a funnel-shaped mouth. These species obviously profit from the high number of microbes present in terrestrial biotopes. The three other genera of this family, *Bresslaua*, *Krassniggia*, and *Kuehneltiella* gen. nov., each consisting of only a few large species, have a huge oral apparatus occupying the anterior half of the cell. These species are rapacious carnivores, consuming the production of bacteria-feeding ciliates and flagellates. Lynn (1979) suggested that carnivorous colpodids evolved from bacteria-feeding ones and discussed several fine structural characters that might be related to carnivory.

Materials and methods

Kuehneltiella terricola was isolated from a sample of litter and soil (0–5 cm) collected on 3 February 1987 about 10 km south of Alice Springs (Northern Territory, Australia), near the Stuart Highway. The vegetation in this area belongs to the arid hummock grassland type with widely spaced trees and shrubs (Frith 1979). The soil is red, sandy, contains little humus, and has a pH (water) of 6.2.

A raw culture was set up from the air-dried sample with the "non-flooded petri dish method" of Foissner (1987a). From this stock, some cells were isolated and cultured in bottled spring water (Eau de Volvic, France), enriched with 1% cabbage-lettuce medium. A species of the *Tetrahymena pyriformis* complex and colourless flagellates, originating from the raw culture, were added as food organisms. The cultures (petri dishes, 15-cm diameter) were kept at room temperature, and the medium was changed about every week. Food must be added every day in well flourishing cultures, otherwise most cells will encyst.

Kuehneltiella terricola was examined in vivo and in silver-impregnated slides. The Chatton-Lwoff silver nitrate method as described by Corliss (1953) and the pyridinated silver carbonate method as modified by Augustin et al. (1984) were used to reveal the infraciliature and the silverline system. Preparation for scanning electron microscopy fol-

* Dedicated to the late Prof. Dr. W. Kühnelt

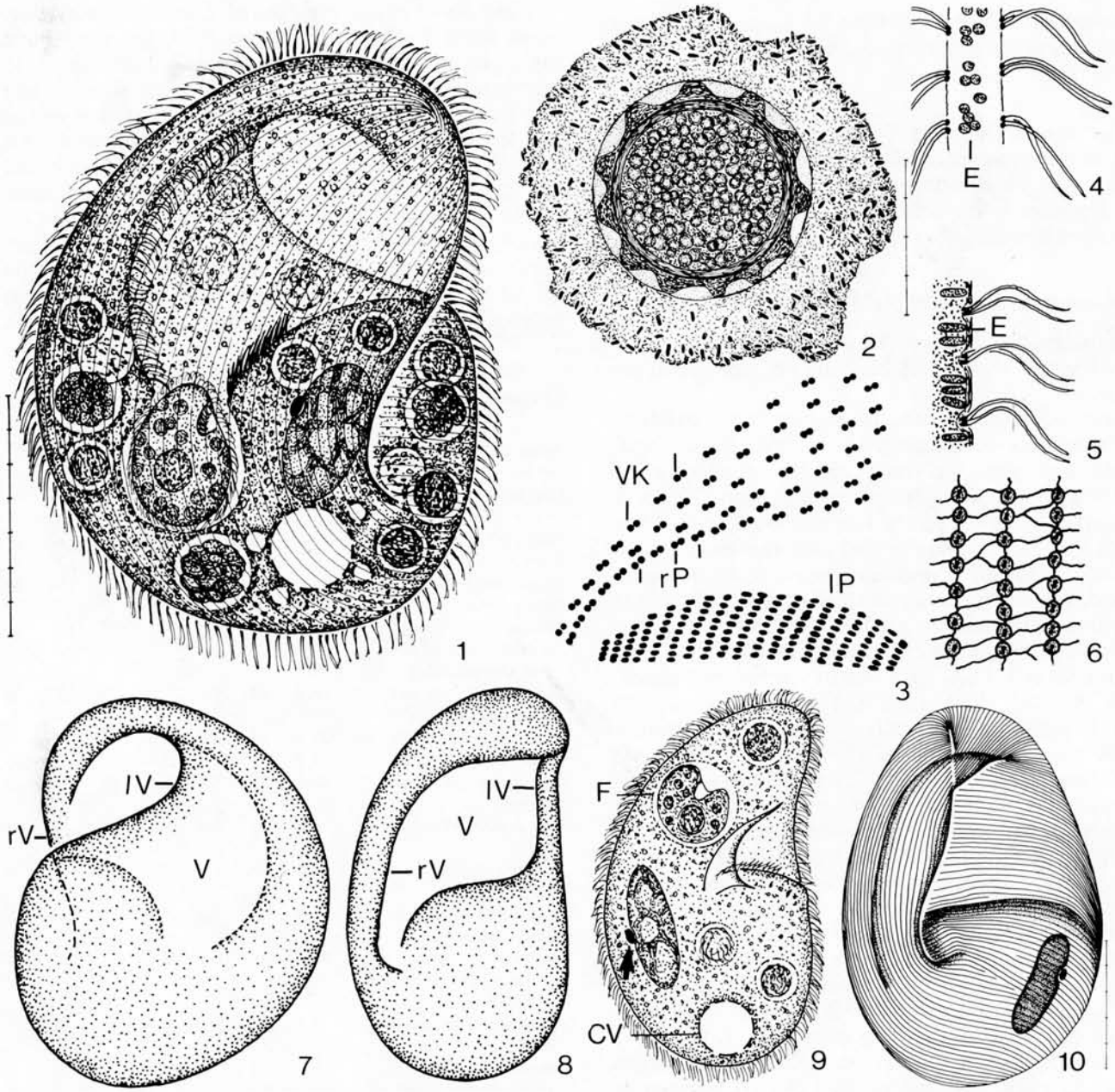
lowed the method of Ammermann and Schlegel (1983). All counts and measurements were taken at a magnification of 1000× on individuals from well fed cultures in the exponential growth phase. With the oil-immersion objective, one scale mark of the ocular micrometer corresponded to 1 µm. The statistical procedures followed methods described in Köhler et al. (1984).

The drawings of the impregnated specimens were made with the help of a camera lucida. Contours of the in vivo aspect were taken from photographs of specimens not covered by a cover slip, a method easily possible with this species because it often remains motionless for some minutes.

Results

Kuehneltiella gen. nov.

Diagnosis. Medium-sized to large rapacious Colpodidae with huge, cave-like vestibulum occupying anterior half of cell. Right wall of vestibulum overhangs semicircularly grooved left, forming large, bright, circular patch anteriorly. Right oral polykinetid consisting of short, single row of dikinetids.



Figs. 1–10. *Kuehneltiella terricola* from life **(Figs. 1, 2, 4, 5, 7–9)**, after silver carbonate impregnation **(Fig. 3)**, and after Chatton-Lwoff silver nitrate impregnation **(Figs. 6, 10)**. **Fig. 1** Right lateral view of representative trophont. **Fig. 2** Resting cyst. **Fig. 3** Oral structures. **Figs. 4, 5** Detail of cortex showing extrusomes (mucocysts) from outside of cell and in optical section. **Fig. 6** Detail of silverline system of left side. **Fig. 7** Left lateral view. **Fig. 8** Ventral view. **Fig. 9** Precystic specimen. *Arrow* marks micronucleus attached to macronucleus. **Fig. 10** Nuclear apparatus and pattern of ciliary rows in ventral view. *Scale bar divisions*, 10 µm. *CV*, contractile vacuole; *E*, extrusomes; *F*, food vacuole; *lP*, left oral polykinetid; *lV*, left wall of vestibulum; *rP*, right oral polykinetid; *rV*, right wall of vestibulum; *V*, vestibulum; *VK*, vestibular kineties

Type species. Kuehneltiella terricola sp. nov.

Derivatio nominis. The new genus is named in honour of the late (1905–1988) Professor Dr. Wilhelm Kühnelt, famous Austrian soil biologist. Feminine.

Kuehneltiella terricola sp. nov.

Diagnosis. In vivo about 110–170×80–120 µm, bean-shaped, macronucleus elliptical, about 70 somatic kineties. Left oral polykinetid composed of 16 kineties on average. Outer layer of resting cyst hexagonally sculptured.

Type location. Upper soil layer of an arid hummock grassland near Alice Springs, Northern Territory, Australia, 133 °E, 24 °S.

Type specimens. One slide of holotype specimens and two slides of paratype specimens (cultured material impregnated by the Chatton-Lwoff silver nitrate method) have been deposited in the collection of microscope slides of the "Oberösterreichisches Landesmuseum" in Linz (Austria).

Derivatio nominis. "Terricola", because of living in soil.

Morphology and biology (Figs. 1–37, Table 1). Morphometric data shown in Table 1 are not repeated in this section.

Size moderately variable, usually about 140×100 µm, large trophonts occasionally up to 250 µm in length; starved and precystic cells usually smaller, about 80–120 µm long. Shape fairly constant, bean-like, dorsal side markedly convex, ventral side concave at level of bottom of vestibulum, anterior and posterior ends broadly rounded. Right wall of vestibulum semicircular to slightly sigmoid, extends to posterior third of cell. Left wall of vestibulum commences at level of the bottom of vestibulum, extends semicircularly dorsad, to meet cowl-like anterior end. Thus, right wall of vestibulum distinctly longer than left (Figs. 1, 7, 8, 11–15, 20, 21, 25, 26, 29–31). Right side markedly convex, left side flat to slightly convex, no diagonal groove; flattening usually lost during preparation (Figs. 8, 10). Similarly, left wall of vestibulum often becomes more sigmoid in prepared specimens because vestibulum collapses more or less during preparation (Figs. 26, 27, 29, 30). Such individuals resemble *Krassniggia auxiliaris* (cp. Foissner 1987b). Macronucleus elliptical (about 2:1), usually located in posterior half of body. Nucleolus net-like. Single, elliptical micronucleus attached to macronucleus (Figs. 1, 9, 10, 22, 37). Contractile vacuole in posterior third of left side of cell; during diastole surrounded by many smaller collecting vesicles, single excretory pore (Figs. 1, 9, 22, 31). Pellicle soft, flexible. Many subpellicular mucocysts, about 1.5×1 µm, arranged in small clusters between somatic kineties, give cells brownish colour at low magnification (Figs. 4, 5). Mucocysts become extruded after addition of methylgreen-pyronin, form spongy envelope around cell consisting of 5–10 µm long threads. Cytoplasm colourless, in well-nourished cells densely filled with about 1 µm roughly spherical, yellowish crystals and 1–10 µm greasily shining globules. Many small and large food vacuoles containing few to hundreds of small, colourless flagellates or one to few individuals of various species of ciliates (*Tetrahymena pyriformis*, *Colpoda* spp., *Drepanomonas* sp., *Sathrophilus muscorum*, swarmers of *Vorticella* sp.). In starved cultures, cannibalism may even occur (Figs. 13–15). Movement slowly gliding on right side or rotating about main body axis, remains nearly motionless during feeding. *Kuehneltiella terricola* waits for prey, which is captured in huge vestibulum and then apparently sucked in by strong cytoplasmic cyclosis (Figs. 13–15).

Cilia about 10 µm long, paired. Somatic infraciliature as in genus *Bresslaua* (cp. Lynn 1979, Foissner 1985), highly complicated due to large vestibulum. About 15 kineties of ventral side project onto inner side of right vestibular wall, forming vestibular kineties. Other ventral and left lateral somatic kineties cover bottom and inner side of left wall of vestibulum. Vestibular, ventral, and left lateral kineties adjoin at dorsal wall of vestibulum, forming long suture (homologous to "keel" of genus *Colpoda*), which extends anteriorly on ventral side of cell. No diagonal groove, but ciliature is slightly condensed on left of left oral polykinetid where shortened kineties not projecting onto left side frequently occur (Figs. 1, 10, 20–27, 29–31, 33–35).

Vestibulum occupying anterior half of cell, cone-shaped, very large. Oral polykinetids near centre of cell,

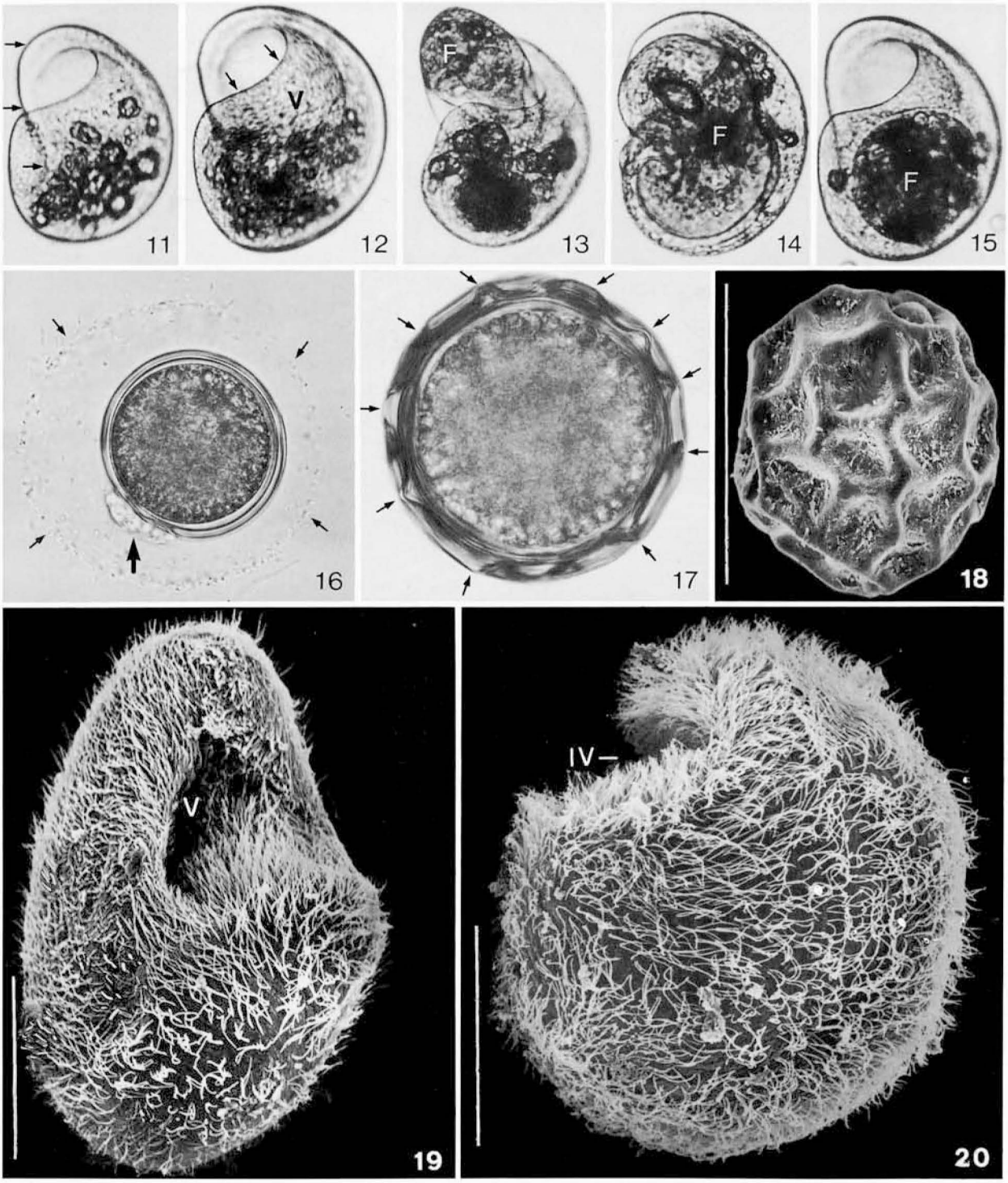
Table 1. Morphometric characterization of *Kuehneltiella terricola*

Character[a]	x̄	M	SD	CV	Min	Max	n
Body, length in vivo	136.4	130	18.2	13.3	115	170	11
Body, width in vivo	100.0	100	13.6	13.6	80	120	11
Body, length	135.7	138	11.6	8.6	95	150	20
Body, width	103.5	106	10.3	9.9	73	117	20
Distance anterior end to proximal end of left oral polykinetid	90.1	90	13.4	14.8	70	125	20
Distance anterior end to macronucleus	66.9	63	19.0	28.4	23	100	20
Macronucleus, length	32.9	34	4.4	13.5	22	38	20
Macronucleus, width	14.9	15	2.4	15.8	10	20	20
Left oral polykinetid, length	20.4	20	3.5	17.0	13	28	20
Left oral polykinetid, width	4.2	4	0.4	10.1	4	5	18
Number of somatic kineties	71.5	70	9.3	13.0	58	95	20
Number of kineties in left polykinetid	16.4	16	2.4	14.9	12	21	20
Diameter of resting cyst (with mucous sheath) in vivo[b]	92.3	87	14.5	15.7	72	125	11
Diameter of resting cyst (without mucous sheath) in vivo[b]	62.5	62	9.4	15.0	48	82	11

[a] All data are based, if not stated otherwise, on the investigation of randomly selected Chatton-Lwoff silver nitrate impregnated specimens originating from cultures in the exponential growth phase; all measurements in µm. CV, coefficient of variation (%); M, median; Max, maximum; Min, minimum; n, number of specimens investigated; SD, standard deviation; x̄, arithmetic mean.

[b] Data from cysts about 4 weeks old

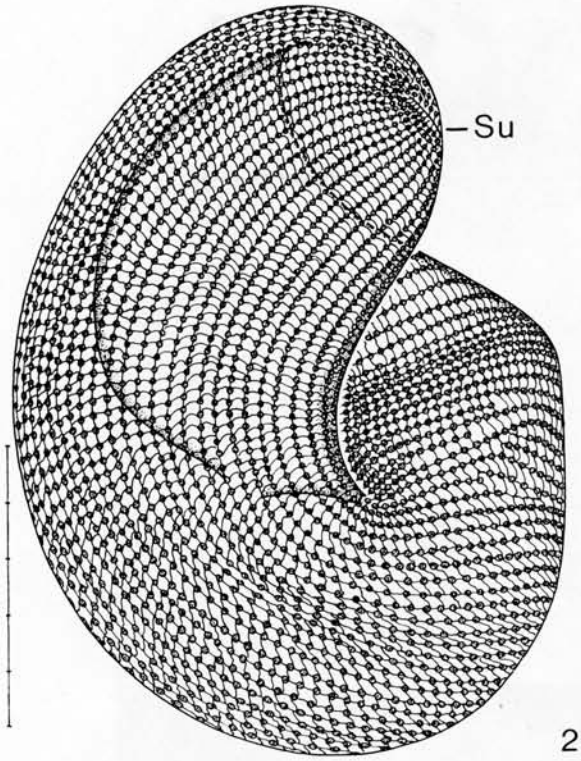
do not project out of vestibulum; very small compared with size of cell. Left polykinetid horizontally orientated, banana-shaped, consists of 16 rather distantly spaced rows, length of which gradually decreases to ends of kinetid. Right polykinetid consists of single row of densely spaced dikinetids. In about 30% of individuals investigated, even this row lacking or at least hardly distinguishable from neighbouring vestibular kineties (Figs. 1, 3, 7, 8, 11, 12, 15, 21–27, 29–31, 33, 35, 36).

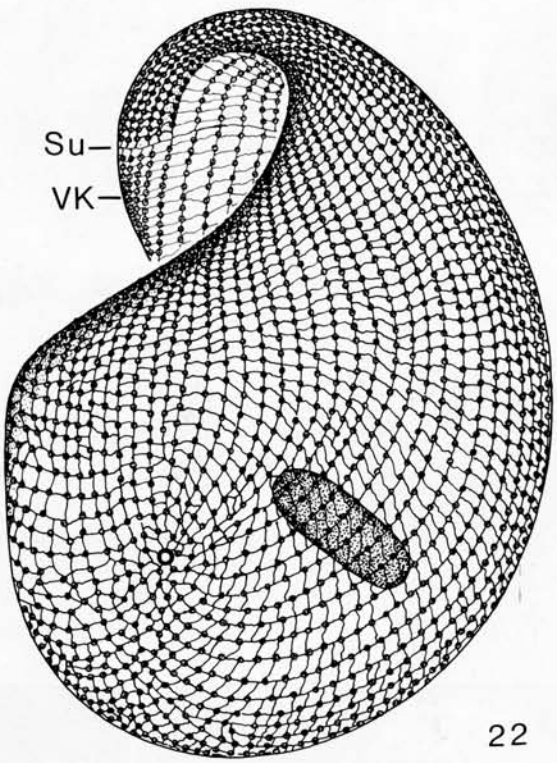


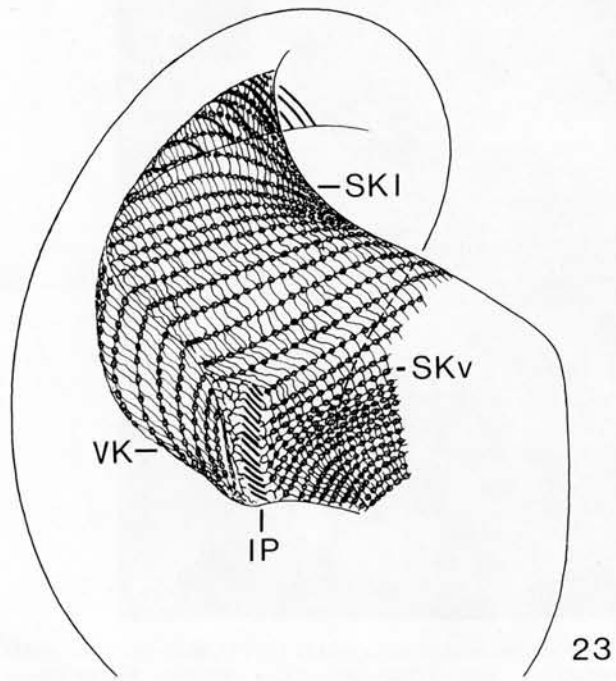
Figs. 11–20. *Kuehneltiella terricola*, living specimens in bright field **(Figs. 11–17)** and fixed individuals in the scanning electron microscope **(Figs. 18–20). Fig. 11** Left lateral view. *Arrows* mark sigmoid right wall of vestibulum. **Fig. 12** Left lateral view. *Arrows* mark semicircular left wall of vestibulum. **Figs. 13–15** A cannibalistic specimen during feeding. **Fig. 16** Developing resting cyst. *Large arrow* marks extruded material. *Small arrows* mark mucous sheath. **Figs. 17, 18** Completed, 4-week-old resting cysts. *Arrows* mark crests of cyst membrane. **Fig. 19** Ventrolateral view of precystic specimen. **Fig. 20** Left lateral view of trophont. *Scale bars*, 50 µm. *F*, food vacuole forming around ingested prey; *IV*, left wall of vestibulum; *V*, vestibulum

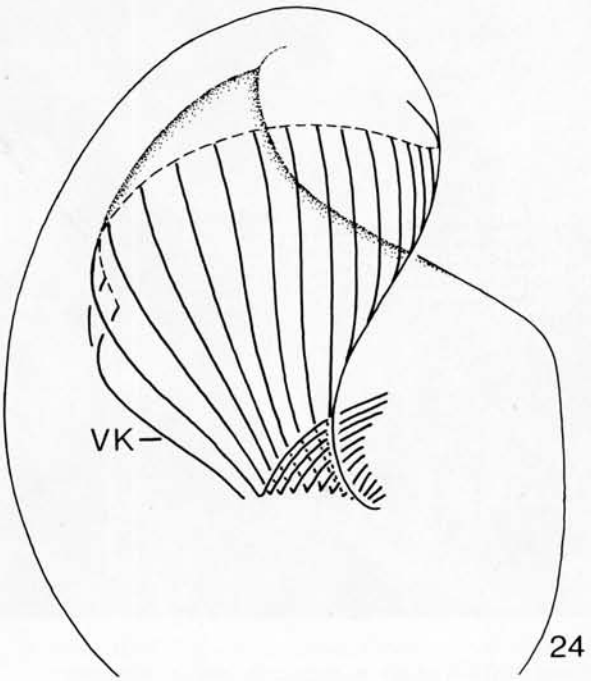
Silverline system colpodid, without peculiarities. Y-shaped silverlines rather frequent. Extrusomes usually do not impregnate with applied silver methods (Figs. 6, 21, 22, 28).

Reproduction within cysts, usually four tomites. Membrane of reproductive cysts thin (about 1 μm), surrounded by mucous envelope about 8 μm thick. Before establishing a resting cyst, cells become smaller and *Colpoda maupasi*-shaped, because their vestibulum flattens out and the vestibular walls become nearly straight (Figs. 9, 10, 19, 32). Such cells hardly comparable with trophonts, easily mistaken for separate species! After cells have rounded up, approximately 2-μm thick, honey-coloured membrane, probably later mesocyst, and volu-









Figs. 21–24. *Kuehneltiella terricola*, infraciliature and silverline system of a Chatton-Lwoff silver nitrate impregnated specimen. **Fig. 21** Right lateral view. **Fig. 22** Left lateral view. **Fig. 23** Right vestibular wall removed to show infraciliature on inner side of left vestibular wall. **Fig. 24** Course of kineties on inner side of right vestibular wall. *Scale bar divisions*, 10 μm. *IP*, left oral polykinetid; *SKl*, somatic kineties of left side; *Skv*, somatic kineties of ventral side; *Su*, suture; *VK*, vestibular kineties

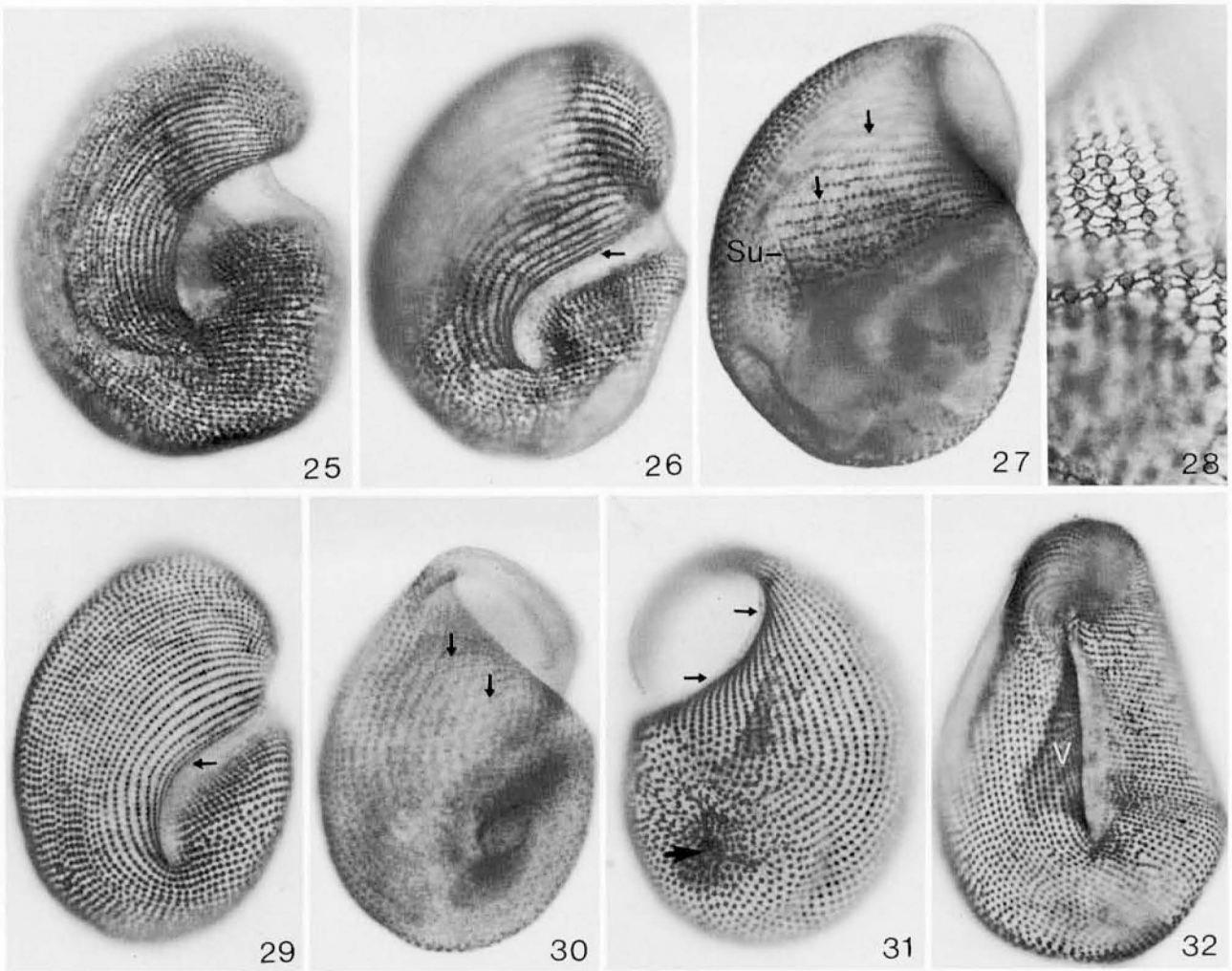
minous mucous sheath is secreted. Material (cytoplasmic? macronuclear?) extruded, remains outside cyst membrane (Fig. 16). Old (completed) resting cysts still possess mucous sheath and three membranes about 6 µm thick. Colourless endocyst about 2 µm thick and smooth. Meso- and ectocyst distinctly honey-coloured (yellowish to brownish), about 4 µm thick and hexagonally sculptured (Figs. 2, 17, 18). Mucous sheath surrounding cyst always densely populated with bacteria and colourless flagellates (Figs. 2, 16). Colourless content of resting cyst contains many greasily shining globules 2–4 µm in diameter. Unlike some species of *Colpoda* (Foissner, unpublished data), no brownish spheres attached to ectocyst.

Kuehneltiella terricola has been found only at the type location and at a site nearby. There is a high probability that *Kuehneltiella terricola* is endemic to the Australian continent or at least to Gondwanaland, because it has not been found in any of more than 1000 soil samples from many other regions of the world.

Discussion

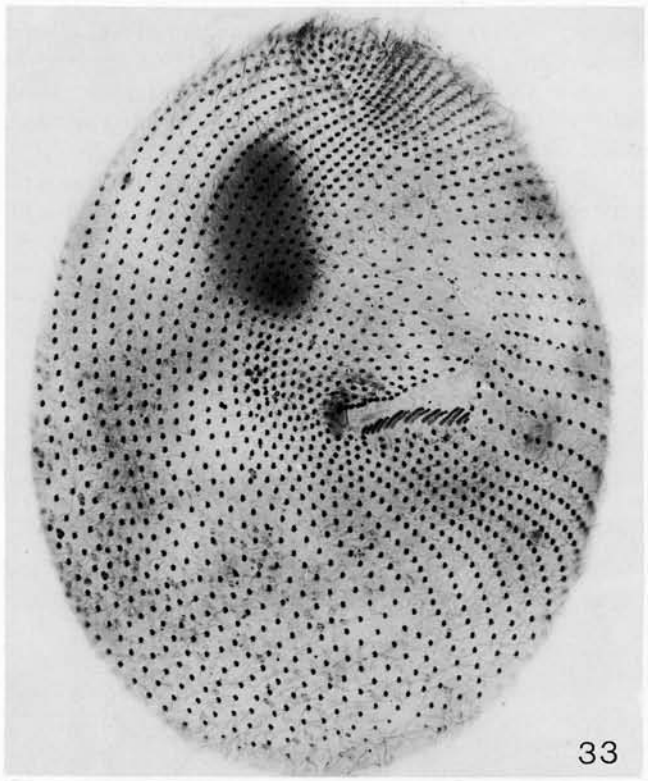
Comparison of Kuehneltiella terricola with related genera and species

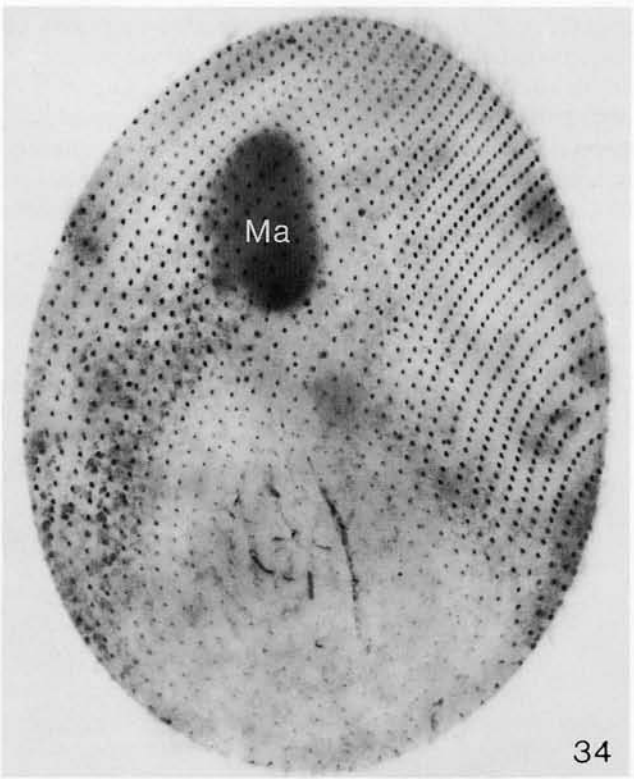
The family Colpodidae comprises the genera *Colpoda* Müller, 1773, *Bresslaua* Kahl, 1931, and *Krassniggia* Foissner, 1987. I now consider four other genera (*Tillina* Gruber, *Pseudocolpoda* Ilowaisky, *Repoma* Novotny, *Paracolpoda* Lynn; for dating of these genera see Foissner 1985) as junior synonyms of *Colpoda*. *Kuehneltiella* is obviously closely related to *Bresslaua*. In both, the right wall of the vestibulum overhangs the semicircularly grooved left vestibular wall forming a large, bright, circular patch anteriorly (Figs. 1, 7, 11, 12, 15, 20, 31). The important difference between *Bresslaua* and *Kuehneltiella* concerns the structure of the right oral polykinetid. It is a rather large field of disordered, short kineties in *Bresslaua* (Fig. 38) and a single row of dikinetids in *Kuehneltiella* (Figs. 33, 35, 36). I checked this spe-

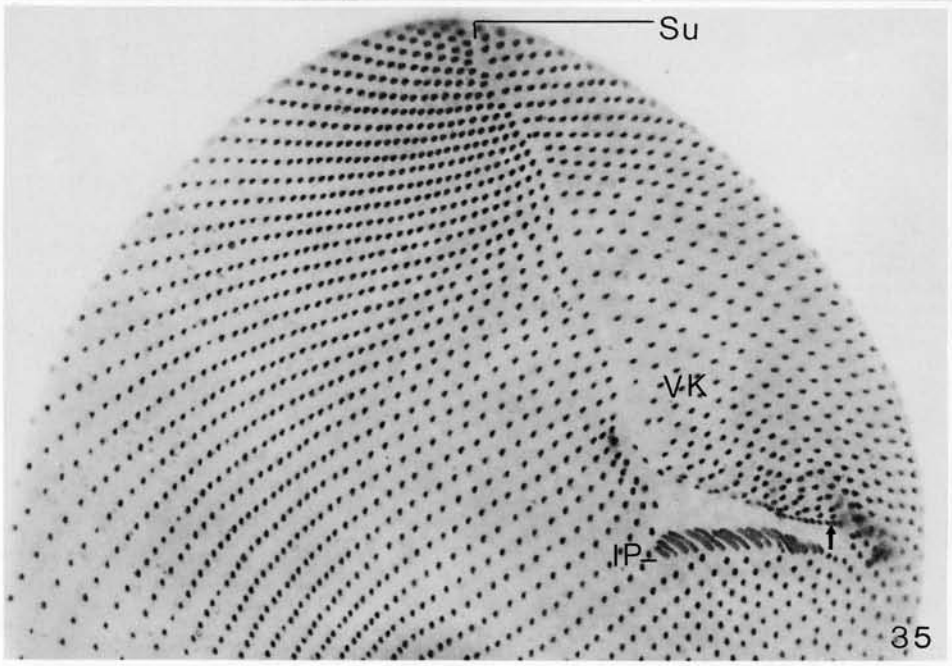


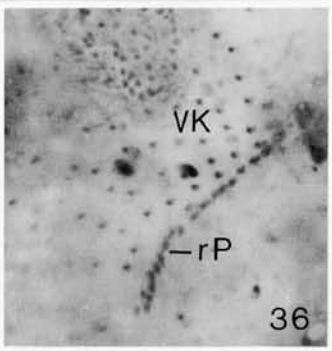
Figs. 25–32. *Kuehneltiella terricola*, infraciliature and silverline system after Chatton-Lwoff silver nitrate impregnation. **Fig. 25** Right lateral view of a specimen in which the vestibulum has not been collapsed during preparation. **Figs. 26, 27, 29, 30** Right lateral views of two specimens with slightly collapsed vestibula. The right vestibular wall (*arrow*) is in focus in **Figs. 26** and **29**; the kineties on the inner side of the left vestibular wall are focused in **Figs. 27** and **30** (*arrows*). **Fig. 28** Detail of silverline system of left side. **Fig. 31** Left lateral view showing semicircular left vestibular wall (*small arrows*) and excretory pore of contractile vacuole (*large arrow*). **Fig. 32** Ventral view of a precystic specimen. *Su*, suture; *V*, vestibulum

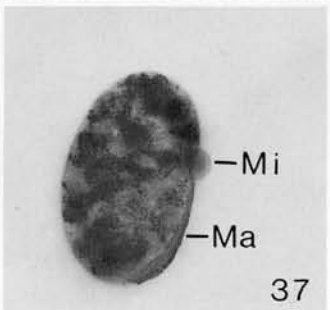
cial structure of the right polykinetid of *Kuehneltiella* very carefully because it is highly uncommon in colpodids s. str. However, within the Marynidae, a family closely related to the Colpodidae, there is also one genus, *Ilsiella* Foissner, 1987, whose right oral polykinetid consists of a single row of dikinetids only. Both *Kuehneltiella* and *Ilsiella* look rather specialized, suggesting that their simple right oral polykinetid is apomorphic. A right oral polykinetid composed of an ordered row of dikinetids is, however, common in the colpodid order Cyrtolophosidida (Foissner 1985). This indicates that simple, single-rowed, right oral polykinetids have evolved independently several times in the Colpodea and even within the colpodids s. str.











Figs. 33–37. *Kuehneltiella terricola*, specimens prepared with the silver carbonate method and strongly squeezed to show the details of the somatic and oral infraciliature. **Figs. 33,34** Infraciliature of ventral and dorsal side. **Fig. 35,36** Infraciliature of ventral anterior half and of oral apparatus at higher magnification. *Arrow* marks right oral polykinetid. **Fig. 37** Nuclear apparatus. *Ma*, macronucleus; *Mi*, micronucleus; *lP*, left oral polykinetid; *rP*, right oral polykinetid; *Su*, suture; *VK*, vestibular kineties

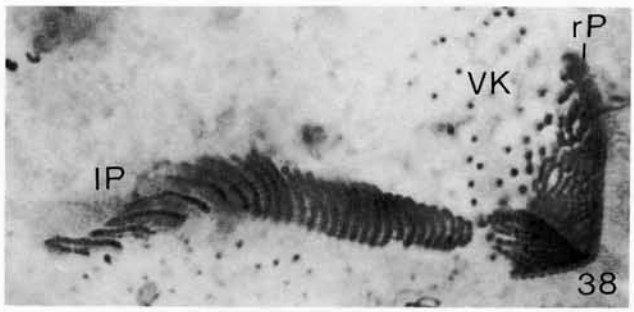


Fig. 38. *Bresslaua vorax* (population from Mittenwald, FRG). Infraciliature of oral apparatus after silver carbonate impregnation. *lP*, left oral polykinetid; *rP*, right oral polykinetid; *VK*, vestibular kineties

Lynn (1979) claimed that *Bresslaua insidiatrix* lacks a right oral polykinetid. He suggested that this is an adaptation to carnivory. However, this observation was doubted by Foissner (1985, 1987b), who found a well-developed right oral polykinetid in *B. vorax* and *B. terricola*. Thus, I restudied the Chatton-Lwoff silvered specimens of Lynn. A few well orientated specimens show very clearly, in my opinion, that *B. insidiatrix* has a right oral polykinetid similar in size and structure to that of *B. vorax* (Fig. 38). Thus, *B. insidiatrix* does not belong to the new genus *Kuehneltiella*.

No other species have been found in the literature which might be identical with *Kuehneltiella terricola*. Superficially, *Krassniggia auxiliaris* Foissner, 1987, and *Bresslauides australis* Blatterer and Foissner, 1988, resemble *Kuehneltiella terricola*. However, both have a typic colpodid or hausmanniellid right oral polykinetid and the ectocyst of their resting cysts is smooth (Blatterer and Foissner 1988; Foissner unpublished data). Likewise, the resting cysts of *Bresslaua* spp. are smooth.

Key to the genera of the Colpodidae (Fig. 39)

The separation of the colpodid genera is not easy, as indicated by the above-listed synonyms of *Colpoda*. I am, however, now able to provide a reliable key, based on the present results and on a monograph about colpodid ciliates that is in preparation.

1. Right wall of vestibulum overhangs semicircularly grooved left vestibular wall. Cells, thus, possess conspicuous, bright, circular patch anteriorly. Vestibulum is very large. Rapacious carnivores 3
– Right wall of vestibulum does not overhang left one. No bright, circular patch anteriorly 2
2. Vestibulum small to rather large, funnel-shaped; its right wall arched or semicircular. Anterior part of body is not cowl-like. Bacteria feeders ... *Colpoda*
– Vestibulum is very large, its right wall extends utricle-like posteriad. Anterior part of body is cowl-like. Rapacious carnivores ..... *Krassniggia*
3. Right oral polykinetid is composed of many short, disordered kineties (must be checked by silver carbonate impregnation) ..... *Bresslaua*
– Right oral polykinetid is inconspicuous, composed of single row of dikinetids (must be checked by silver carbonate impregnation) ..... *Kuehneltiella*

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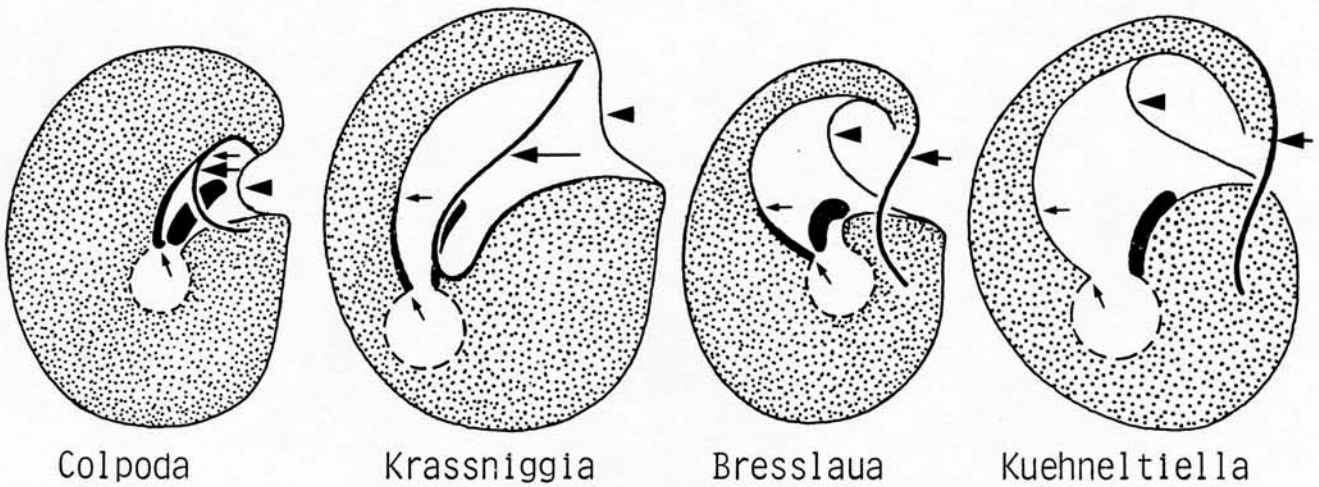

Fig. 39. Differentiation of the genera of the family Colpodidae by means of the shape of the vestibulum, the shape of the right (*large arrows*) and left (*arrowheads*) wall of the vestibulum, and the oral structures (right polykinetid of *Kuehneltiella* consists of single row of dikinetids only). The vestibulum and the developing food vacuole are unstippled; the oral polykinetids are solid. The ends of the right polykinetid are marked by *small arrows*

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