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Morphology and Morphogenesis of *Fuscheria terricola* n. sp. and *Spathidium muscorum* (Ciliophora: Kinetofragminophora)[1]

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ABSTRACT. The morphology and morphogenesis of the kinetofragminophoran soil ciliates, *Fuscheria terricola* n. sp. and *Spathidium muscorum* Dragesco & Dragesco-Kerneis, 1979, are described. Stained specimens (protargol) are characterized biometrically. The new species differs from the other species of the genus in its body size, body shape, number of kineties, length of extrusomes, and habitat. Both species have telokinetal stomatogenesis, which commences with a proliferation of kinetosomes at those kineties which bear the brosse. *Fuscheria terricola* does not have a complex perioral ciliature; indeed, it might be that this species has only monokinetids. Thus only a proliferation of kinetosomes and the separation of the kineties takes place in the prospective division furrow. In contrast, *S. muscorum* differentiates short dikinetid kinetofragments in the region of the division furrow, which are arranged to form the perioral kinety of the opisthe in the intermediate and late stages of the stomatogenesis. The right part of the perioral kinety develops first. This and other studies show that telokinetal stomatogenesis proceeds very differently depending on the differentiation of the oral ciliature; however, detailed studies on the morphogenesis of kinetofragminophoran ciliates are still too few in number for subtypes to be defined.

FEW detailed studies about the morphogenesis of free-living gymnostome ciliates are available (7, 14), perhaps because they are so difficult to culture. The stomatogenesis of the kinetofragminophoran ciliates is telokinetal (5). The published descriptions of stomatogenesis reveal that it proceeds in very different modes, indicating that this voluminous and probably artificial group is very heterogeneous. Telokinetal stomatogenesis obviously requires classification into subtypes (5), but this should not be done until more data are available. Toward this end, we have investigated the morphology and morphogenesis of two "lower" ciliates.

MATERIALS AND METHODS

Two populations of *Fuscheria terricola* were investigated. Population 1 occurred in the soil of a bottomland near Grafenwörth, Lower Austria. Population 2 was isolated from soil in the Schlossalm, Bad Hofgastein, Salzburg. Both populations were cultured by the method of Foissner (10). For revealing the infraciliature the protargol silver staining method according to Tuffrau (19) as modified by Foissner (13) was used. The silverline system was studied in specimens impregnated by the Chatton-Lwoff silver method (3).

Spathidium muscorum occurred in soil in the Schlossalm. Identification was made according to the descriptions of Dragesco & Dragesco Kerneis (6) and Foissner (12). As a culture medium, Eau de Volvic was used, with yeast and a species of the "*Tetrahymena pyriformis*" complex added as the food supply. The protargol method was used to reveal the infraciliature (13, 19).

All statistical procedures follow methods described in Sokal & Rohlf (18).

RESULTS

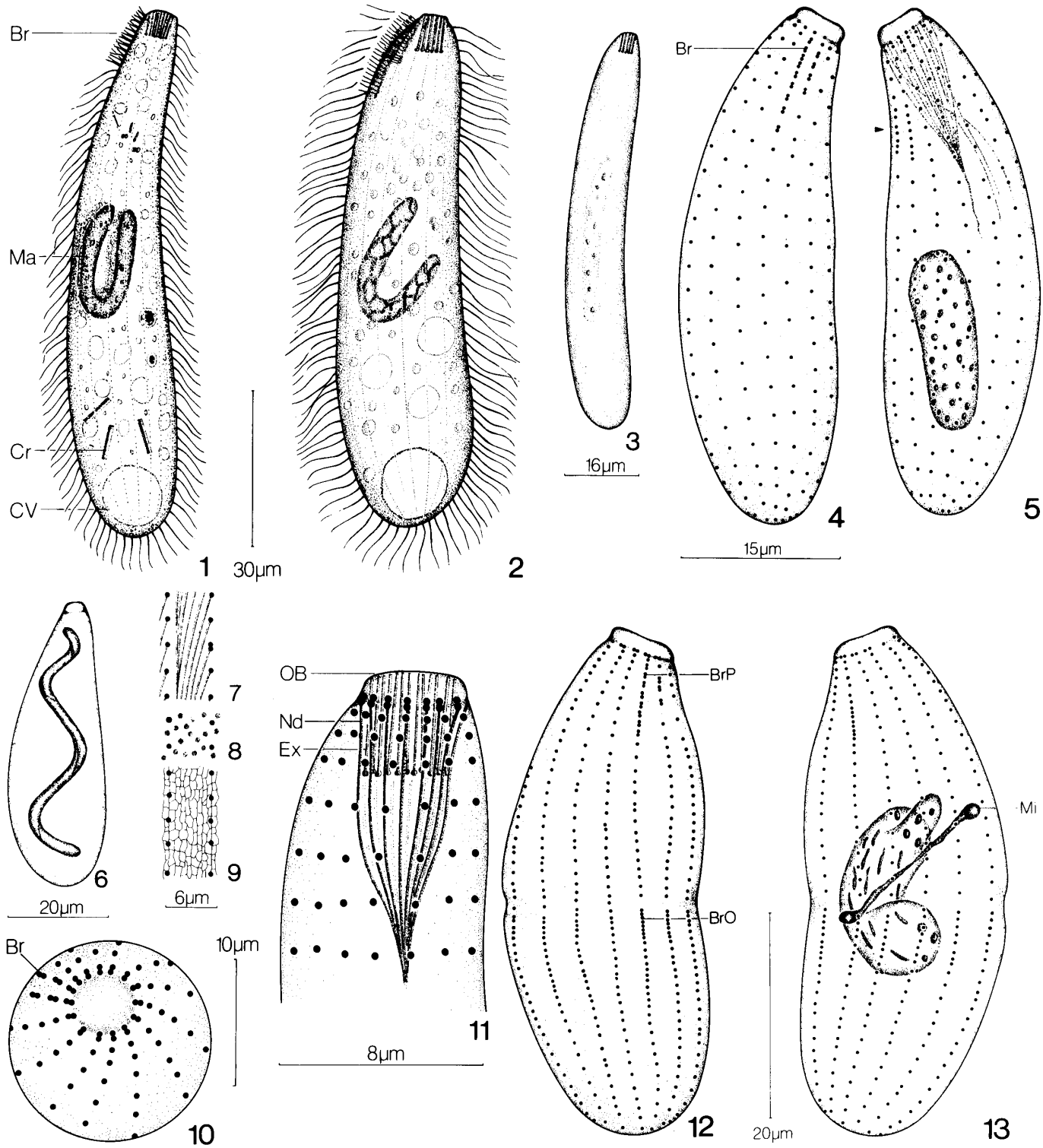
Fuscheria terricola n. sp.
(Figs. 1–17, Table I)

Diagnosis. In vivo ca. 80–100 μm in length and ca. 27 μm in width (n = 10)[2]. Body cylindrical to slightly bottle-shaped. Fifteen somatic kineties on the average. Many 5–7-μm-long extrusomes.

Type location. Moderately frequent in the soil of a bottomland near Grafenwörth, Lower Austria.

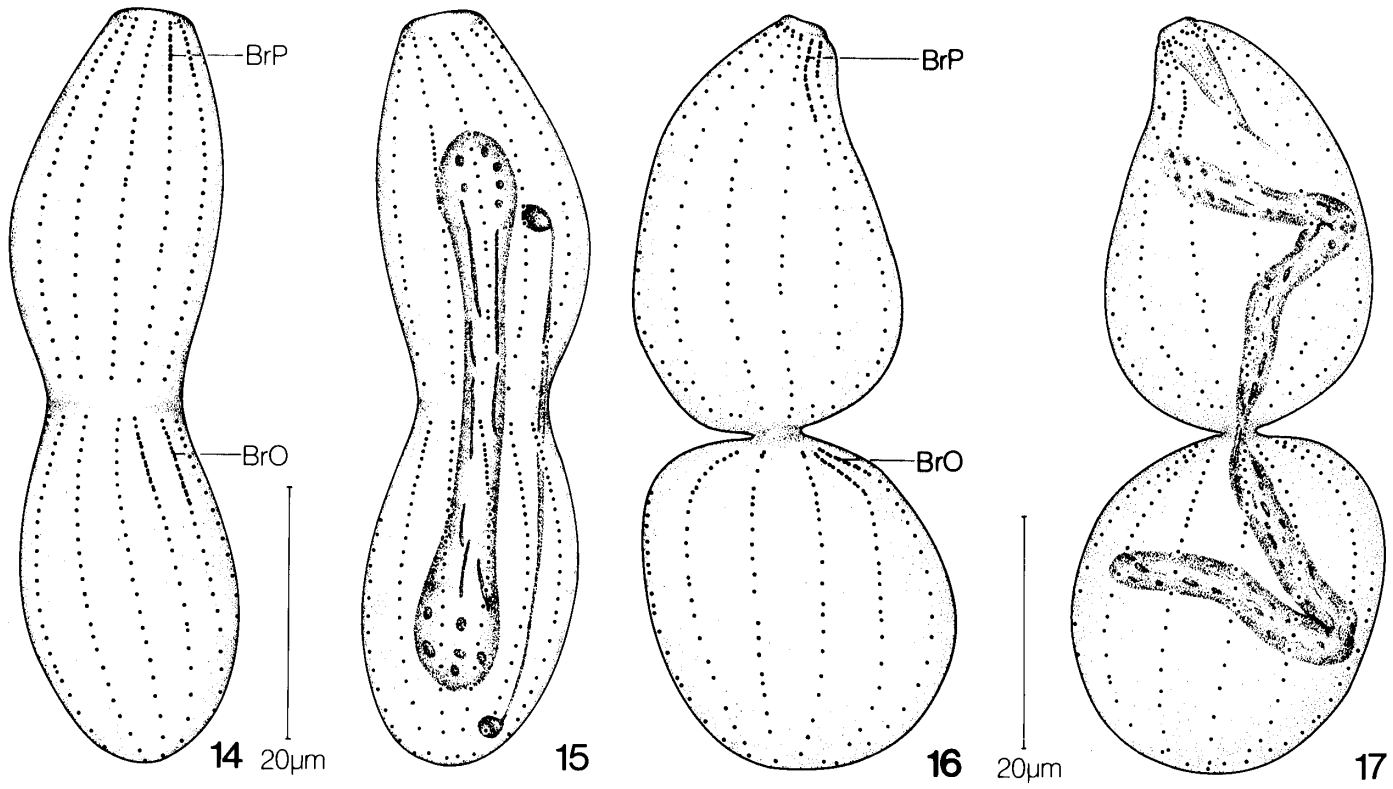
[1] The authors wish to express their thanks to the Austrian MaB-6 programme of the Austrian Academy of Science and the Bundesministerium für Gesundheit und Umweltschutz for financial support.

[2] Protargol impregnation generally causes a shrinkage of about 20–30%.



Figs. 1–17. Morphology and morphogenesis of *Fuscheria terricola* n. sp. Fig. 1, population 2; Figs. 2–17, population 1; Figs. 1–3, 8, from life; Figs. 4–7, 10–17, infraciliature after protargol impregnation; Fig. 9, after Chatton-Lwoff silver method; Figs. 1–11, non-dividing specimens; Figs. 12–17, morphogenesis. Legend: Br, brosse; BrO, brosse of the opisthe; BrP, brosse of the proter; Cr, crystal; CV, contractile vacuole; Ex, extrusome; Ma, macronucleus; Nd, nematodesmata; OB, oral bulge.

Figs. 1–13. 1, 2, 3. Right lateral views. 4. Dorsal view. 5. Ventral view of a specimen with an ellipsoidal macronucleus. The arrow indicates the dense group of basal bodies, which in this case appears on two kineties. Same scale as 4. 6. Right lateral view of a specimen with a helical macronucleus. 7. Argentophilic fiber system. 8. Granules close beneath the pellicle. 9. Part of the silverline system. 10. View of the anterior pole. 11. Right lateral view of the anterior region. 12, 13. An early morphogenetic stage in dorsal and ventral view.



Figs. **14–17**. **14**, **15**. *Fuscheria terricola*. Intermediate morphogenetic stage in dorsal and ventral view. **16**, **17**. Late morphogenetic stage in dorsal and ventral view.

Type-specimens. One slide of holotype specimens and one slide of paratype specimens have been deposited in the collection of microscopic slides of the Upper Austrian Museum in Linz.

Description. Body shape in vivo moderately variable, slightly bottle-shaped to cylindrical. Little or no flattening. Anterior distinctly narrowed, apical truncated, posterior widely rounded. The side with the brosse is convex, especially at the anterior region. The opposite ventral side is concave in the front third. Thus the anterior part seems to be curved (Figs. 1, 2). Slightly contractile. Cilia of the somatic kineties ca. 7–10 µm long, those of the brosse ca. 3 µm. Pellicle slightly indented by the ciliary rows. Close beneath the pellicle, many tiny granules, probably not mucocysts because they were not ejected when the ciliate was stimulated by mechanical pressure or Methyl Green-Pyronin (9). Between the kineties a longitudinal fiber system, presumably postciliary microtubules (Figs. 7, 8). Cytostome circular in oral view, lies polar on a ca. 1-µm-high hyaline oral bulge (Figs. 10, 11). Nematodesmata in vivo not recognizable, after staining with protargol very delicate. They form a funnel-shaped structure, filled with the genus-specific extrusomes (Figs. 5, 11, 17) that also appear in the cytoplasm. Additionally, in the cytoplasm there sometimes appear extrusomes enlarged in their midregion. Macronucleus usually horseshoe-shaped, unusually stretched or helical, lies centrally. Numerous, irregularly distributed nucleoli, sometimes forming a reticular structure (Figs. 1–6). Micronucleus spherical, in vivo not recognizable. Contractile vacuole terminal, several slightly subpolar contractile vacuole pores (Figs. 1, 2). Endoplasm colorless, generally densely filled with many colorless, refractile 1–5-µm inclusions and numerous dumbbell-shaped, ca. 2-µm-long particles (bacteria?). Posterior region sometimes contains a few ca. 7-µm-long, staff-shaped crystals (Figs. 1, 2). Movement moderately rapid with rotation around the longer axis of the body. Feeds in nature on ciliates (*Vorticella astyliformis* and *Colpoda* sp.).

Infraciliature genus-specific. The somatic kineties are oriented longitudinally, rarely very slightly left-spiralled. The distance between the kinetosomes of a kinety is smaller anteriorly than posteriorly (Figs. 4, 5, 10). The anterior end of the kineties usually has a basal body (Figs. 4, 5, 14, 15). In some specimens the first and second basal body are arranged very closely, giving the impression of a basal body pair (Figs. 10, 11, 16). Unusually two basal bodies lay side by side (Figs. 12, 13). The brosse consists of two rows, occasionally with a third, very short row (Figs. 4, 10). About 50% of population 1 has a dense group of 7 kinetosomes on the average in the anterior part of the third somatic kinety to the left of the brosse (Figs. 5, 13, 15, 17). In some specimens this group appears on the second or on the fourth kinety. The mesh-size of the silverline system is 0.2–0.6 µm; it is oriented longitudinally (Fig. 9).

Morphogenesis. The process commences with the proliferation of kinetosomes at the site of the division furrow, which already appears in this early stage (Figs. 12, 13). Proliferation only occurs close beneath the division furrow. It is very pronounced at the primordium of the brosse of the opisthe, which originates from the kineties bearing the brosse of the proter (Fig. 12). The macronucleus is condensed. The nucleoli in the ends of the macronucleus are spherical, those of the middle part are stretched (Fig. 13). This stretching increases up to the middle stage (Fig. 15). The micronucleus, which is not impregnated in non-dividing specimens, stains very intensely now and starts to divide (Fig. 13).

An intermediate stage of fission is shown in Figs. 14, 15. The cell and the macronucleus are distinctly dumbbell-shaped. The

TABLE I. *Biometrical characterization of* Fuscheria terricola *n. sp.*[a]

Character	$\bar{x}$	SD	SE	CV	Range	n
Body, length	[b]55.8	8.3	1.8	14.9	44.0–78.0	21
	[c]60.2	9.3	1.9	15.5	44.0–83.0	25
Body, width	18.2	5.0	1.1	27.3	12.0–28.0	21
	[d]29.2	5.4	1.1	18.5	22.0–42.0	25
Width of the oral bulge	4.5	0.6	0.1	12.5	3.3–5.3	21
	4.8	0.7	0.1	15.5	4.0–6.0	25
Height of the oral bulge	1.3	0.2	0.1	17.0	1.0–2.0	21
	1.2	0.4	0.1	29.7	1.0–2.0	9
Length of the right brosse kinety	9.2	2.0	0.4	21.6	6.6–15.0	21
	9.0	1.8	0.4	19.8	5.0–12.0	25
Length of the left brosse kinety	4.7	0.9	0.2	19.4	3.5–6.6	21
	—	—	—	—	—	0
Number of basal body pairs of the right brosse kinety	10.2	1.8	0.4	17.7	7.0–15.0	21
	10.5	2.0	0.5	19.4	5.0–13.0	18
Number of basal body pairs of the left brosse kinety	4.6	1.2	0.3	25.1	3.0–7.0	21
	—	—	—	—	—	0
Macronucleus, length	26.5	8.0	1.7	30.2	12.0–40.0	21
	29.0	7.4	1.5	25.3	18.0–43.0	25
Macronucleus, width	4.4	0.8	0.2	17.6	3.2–6.0	21
	4.8	0.8	0.2	16.6	4.0–6.0	25
Number of kineties	14.5	1.7	0.4	11.8	12.0–19.0	21
	17.1	3.0	0.6	17.3	13.0–24.0	21
Number of basal bodies in the kinety right of the brosse	29.4	7.2	1.6	24.5	21.0–50.0	21
	34.0	7.0	1.5	20.7	22.0–46.0	23

[a] All data are based on protargol-impregnated specimens. All measurements in μm. Legend: $\bar{x}$, mean; SD, standard deviation; SE, standard error of the mean; CV, coefficient of variation in %; n, sample size.
[b] Upper line; population 1.
[c] Lower line; population 2.
[d] Some specimens were inflated at the posterior region due to the protargol impregnation.

TABLE II. *Biometrical characterization of* Spathidium muscorum[a]

Character	$\bar{x}$	SD	SE	CV	Extremes	n
Body, length	93.0	8.5	1.7	9.1	78.0–105.0	25
Body, width close beneath the peristome[b]	27.7	5.4	1.1	19.6	18.0–37.0	25
Body, maximum width[b]	40.3	5.6	1.1	13.9	29.0–50.0	25
Length of the peristome bulge[c]	45.5	7.5	1.5	16.6	31.0–55.0	25
Length of the first brosse kinety[d]	12.0	1.4	0.3	11.8	10.0–15.0	25
Length of the second brosse kinety	12.5	1.7	0.3	13.7	9.0–16.0	25
Length of the third brosse kinety	9.1	1.3	0.3	14.6	7.0–12.0	25
Macronucleus, width	6.2	1.0	0.2	15.4	5.0–9.0	25
Number of kineties	21.1	2.8	0.6	13.4	16.0–27.0	25
Diameter of the cyst[e]	36.7	3.1	0.6	8.4	32.5–43.0	24

[a] All data are based on protargol-impregnated specimens. All measurements in μm. Legend: $\bar{x}$, mean; SD, standard deviation; SE, standard error of the mean; CV, coefficient of variation in %; n, sample size.
[b] Measured on laterally oriented specimens.
[c] Measured was the chord of the peristome arc.
[d] Lies nearest to the ventral side.
[e] From life.

brosse of the opisthe is completed and the somatic kineties are separated in the cytokinetic plane. The distance between the two macronuclei, which are still connected by a fiber, is very large.

Figures 16, 17 show a very late morphogenetic stage. Proter and opisthe will be separated soon. In the opisthe, there is still no recognizable cytopharynx. The cytostome can only be formed after division, because the daughters are connected at this site until separation. The nucleoli of the macronucleus acquire the shape typical of a non-dividing specimen. The micronuclei did not stain at this stage. After division both filial products have a rounded shape and small size.

Spathidium muscorum Dragesco & Dragesco-Kerneis, 1979 (Figs. 18–28, Table II)

Non-dividing specimen (Fig. 18, Table II). After protargol staining, distinctly sack-shaped, rarely slim. Oral bulge scalpel-shaped, approximately half of the body length. Somatic kineties meridional, with densely arranged kinetosomes. Brosse generally with 3, rarely with 4 rows. Angle between the somatic kineties and the perioral kinety on the left side greater than on the right. Basal body pairs of the perioral kinety arranged very densely. Nematodesmata bundled, about 30 μm long. Macronucleus long and usually twisted, with many small, spherical nucleoli.

Cyst (Fig. 28, Table II). Spherical, with smooth cover surrounded by a 5–10-μm-thick mucous layer.

Morphogenesis (Figs. 19–27). Morphogenesis commences with the proliferation of kinetosomes on the widest part of the animal. The kineties that bear the brosse proliferate a little earlier and more strongly than the others (Figs. 19). The kinetosomes at this site become arranged in pairs and form the primordia of the perioral kinety (Figs. 20, 21). In the next stage these kinetofragments curve to the right (Fig. 23). During these early morphogenetic stages, the macronucleus is ring-shaped and has spherical nucleoli (Figs. 20–22). When the division furrow appears, the macronucleus begins to stretch into the filial products and nucleoli become thread-like (Figs. 23–25). Small contractile vacuoles appear above the division furrow in the proter.

In the intermediate stages of morphogenesis, the primordia of the perioral kinety are separated from the somatic kineties at their anterior ends and turn to the right. Thus an undulating kinety originates, which surrounds the cell. The single kinetofragments are closely spaced (Fig. 23). The brosse of the opisthe is completed and occupies the same kineties as in the proter (Fig. 24).

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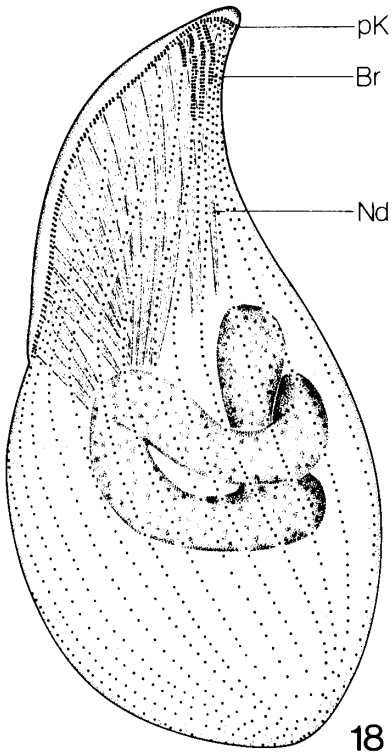
Figs. **18–28.** Morphology and morphogenesis of *Spathidium muscorum*. Figs. **18–27**, infraciliature after protargol impregnation; Fig. **28**, from life; Figs. **19–27**, morphogenesis; Figs. **18–24**, same scale. Legend: Br, brosse; BrO, brosse of the opisthe; BrP, brosse of the proter; Nd, nematodesmata; pK, perioral kinety; pKO perioral kinety of the opisthe; pKP, primordium of the perioral kinety.

Figs. **18–24.** **18.** Left lateral view of a non-dividing specimen. **19.** Left lateral view of the division zone of a very early morphogenetic stage. The arrows indicate the kineties bearing brosse rows. **20–22.** Early morphogenetic stages in left lateral view, ventral view and left lateral view of the division zone. **23, 24.** Intermediate morphogenetic stages in ventral and dorsal view.



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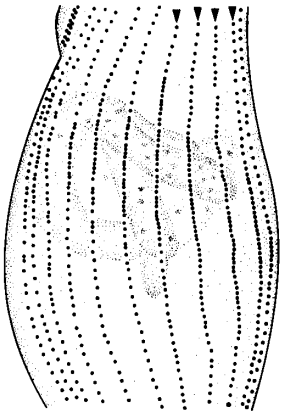

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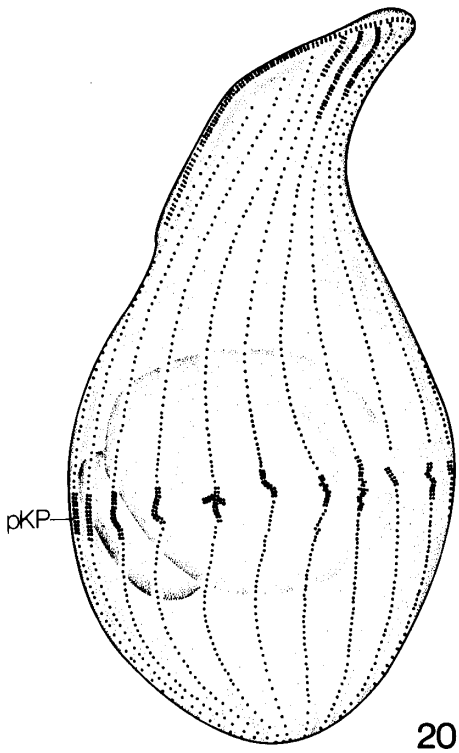
30µm



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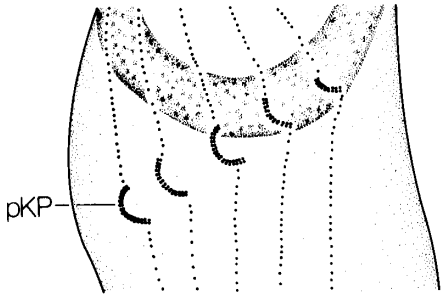

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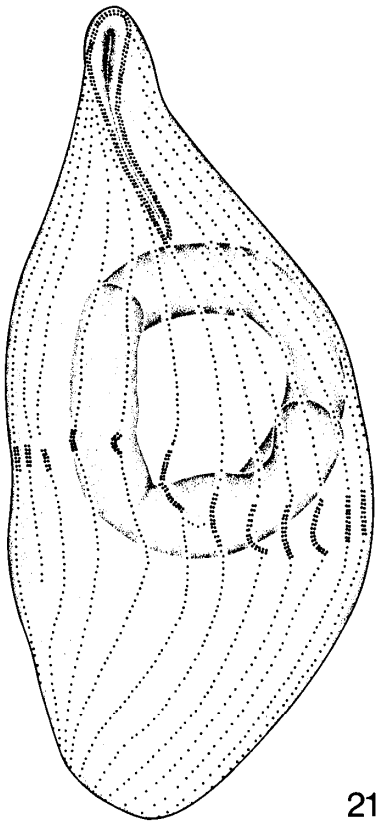

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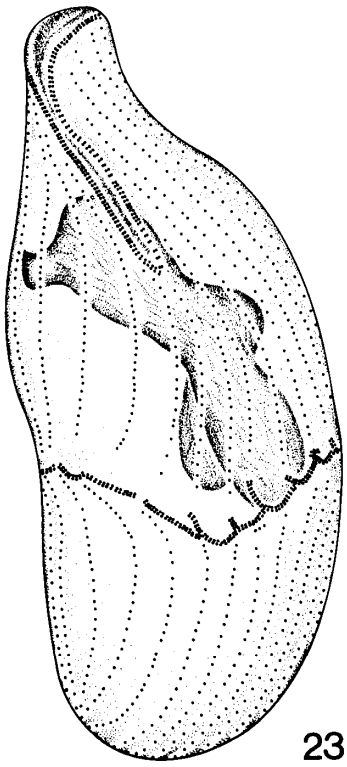

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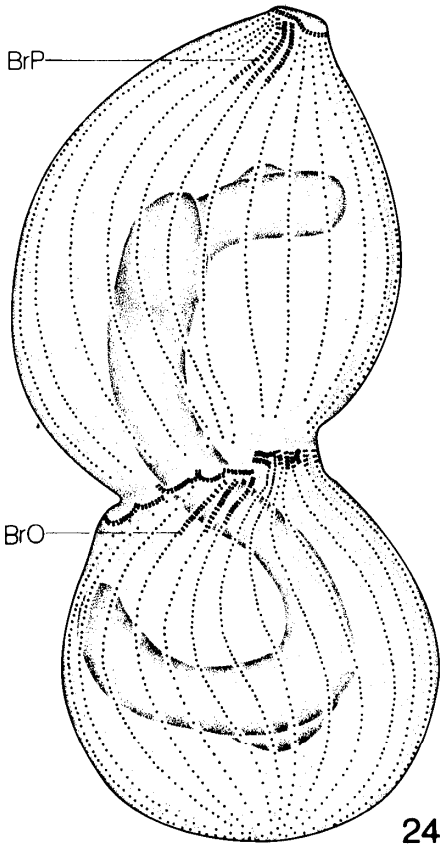

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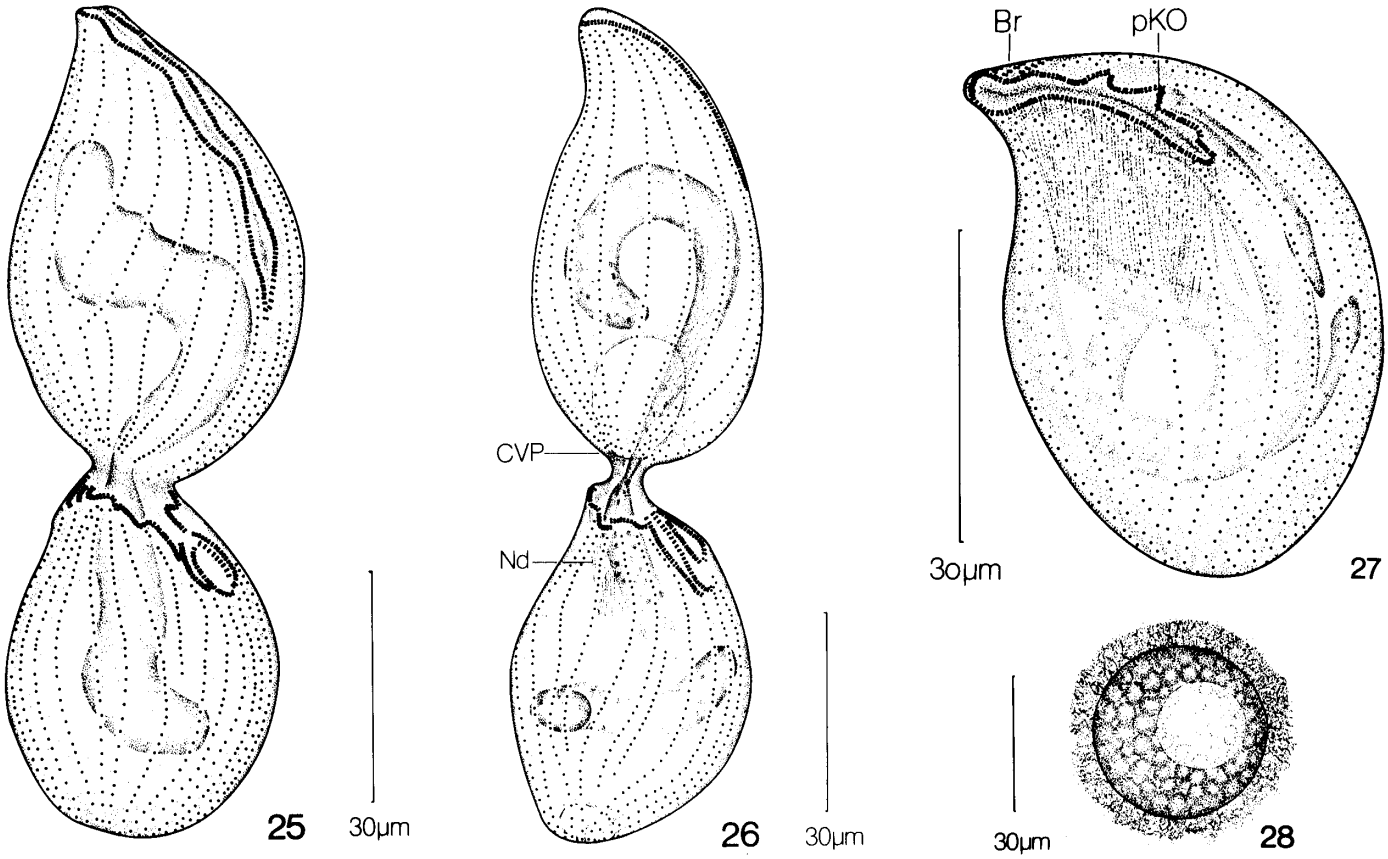

Figs. 25–28. **25**. *Spathidium muscorum.* Right lateroventral view of a late morphogenetic stage. **26**. Right lateral view of a very late morphogenetic stage. **27**. Right lateroventral view of the opisthe after division. **28**. Cyst.

In the later stages of morphogenesis, longer rows of basal body pairs are formed by shifting and fusion of single kinetofragments. They are transported toward and arranged around the new cytostome, which has the same position as the cytostome of the proter (Fig. 25). Nematodesmata develop from the kinetosomes of the immature perioral kinety of the opisthe. The macronuclei, which are still connected by a thin fiber, again contain spherical nucleoli (Fig. 26). The differentiation of the perioral kinety of the opisthe is completed after cytokinesis. It is remarkable that the left part of the perioral kinety is always considerably delayed in its development (Fig. 27). The filial products are small and round.

DISCUSSION

Species comparison. The genus *Fuscheria* is characterized by the very small, button-shaped swelling at the posterior end of the extrusomes that fill the cytopharynx (11). To date only one species, *F. nodosa,* has been described (11). It inhabits small bodies of water. *Fuscheria terricola* differs from this species in body size, body shape, number of kineties, length of extrusomes, and habitat. In our opinion these differences justify the establishment of a new species. The body shape of *F. terricola* resembles that of *Sorogena stoianovitchae* (1), which is now considered to be a Colpodida because it has dikinetids (17).

The morphology of *S. muscorum* largely agrees with the descriptions of Dragesco & Dragesco-Kerneis (6) and Foissner (12). The larger dimensions are probably the consequence of the good conditions during culture. The great variability of different biometrical characteristics and the body shape in *Spathidium* have been documented impressively by Wenzel (20).

The cyst was not described by the two authors (6, 12). Its surface is smooth, in contrast to that of *S. stammeri* (20).

Morphogenesis. Stomatogenesis of *F. terricola* proceeds very simply, obviously because it does not possess a real perioral kinety like *Spathidium.* The oral ciliature of *F. terricola* consists, in its typical appearance, of a monokinetid on the end of each kinety. Such a situation seems to be very rare in "lower" gymnostomes (16); however, this must be verified by ultrastructural investigations.

As yet no exact description of the morphogenesis of a species of the family Enchelyidae exists. Only single stages of the morphogenesis of different species had been illustrated (6), so that there are no earlier data with which to compare our results. By the classification of Corliss (4, 5), the stomatogenesis of *F. terricola* is telokinetal. Fryd-Versavel et al. (14) mentioned that this is also the case in *Chaenea vorax,* but no description was given.

The stomatogenesis of *S. muscorum* is more complicated than that of *F. terricola* although it, too, is telokinetal (4, 5). During the formation of the perioral kinety marked proliferations of basal body pairs take place. These pairs of kinetosomes form fragments which migrate into the division furrow. In other genera of the Spathidiidae as well—for example, *Bryophyllum* and *Homalozoon* (14)—the perioral kinety develops from kinetofragments. Then the fragments rotate from their vertical site to a horizontal one, reminiscent of the Cyrtolophosidina (2, 8).

Although the kineties which bear the brosse begin to proliferate only slightly in advance of the others in *S. muscorum,* nevertheless, this proliferation is the first visible sign of a division in all species investigated. Because of the constant distance between the kineties in *S. muscorum,* the single kinetofragments are approximately of the same length. The kinetofragments of *Homalozoon vermiculare* are of different lengths, evidently because of the unequal distance between the kineties (14). Although in *S. muscorum* proliferation begins almost simultaneously in all kineties, at the end of morphogenesis the left part of the perioral kinety is distinctly delayed in differentiation. The significance of this phenomenon is not clear.

With respect to the oral ciliature, the stomatogenesis of *Lagynophrya multinucleata* (6, 12) lies between that of *F. terricola* and *S. muscorum* because the anterior ends of the somatic kineties of the opisthe are only bent and are not separated.

Fryd-Versavel et al. (14) describe the stomatogenesis of *Urotricha puytoraci* (7) as parakinetal and that of *Amphileptus pleurosigma* (14) as buccokinetal. This is inconsistent with the definition of Corliss (4, 5). Probably the two species belong to different subtypes of telokinetal stomatogenesis which were already mentioned by Corliss (5), but not described in the literature. *Fuscheria, Lagynophrya,* the Colpodida, and *Spathidium* together with *Bryophyllum* and *Homalozoon* are probably four other subtypes (6, 14, 15). But these groups should not be defined and recognized until there are more detailed studies available.

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