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Cultellothrix velhoi gen. n., sp. n., a new Spathidiid Ciliate (Ciliophora: Haptorida) from a Brazilian Floodplain Soil

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Summary. *Cultellothrix velhoi* sp. n. was discovered in floodplain soil from the Paraná River near the town of Maringá, Brazil, South America. Its morphology was investigated using live observation and protargol impregnation. *Cultellothrix* belongs to the gymnostomatous ciliate family Spathidiidae, where it represents a new genus characterized by the lateral location of the dorsal brush. *Cultellothrix velhoi* sp. n. differs from the European congener, *C. lionotiformis* (Kahl, 1930a) comb. n. (basionym: *Spathidium lionotiforme* Kahl, 1930a), by the smaller body size, the possession of a postoral extrusome row, and the lack of ordinary somatic ciliary rows between the circumoral and brush kineties. It is argued that the redescription of *S. lionotiforme* Kahl, 1930a by Foissner (1984) is based on an unfortunate misidentification. Foissner's species is *Spathidium scalpriforme* Kahl, 1930a, which is assigned to the genus *Arcuospathidium* and lowered to subspecies rank: *Arcuospathidium cultriforme scalpriforme* (Kahl, 1930a) comb. n., stat. n.

Key words: *Arcuospathidium cultriforme scalpriforme* comb. n., stat. n., *Cultellothrix lionotiformis* comb. n., *Cultellothrix velhoi* gen. n., sp. n., litostomateous ciliates, South America, *Spathidium lionotiforme*, terrestrial Protozoa.

INTRODUCTION

Spathidium is one of the largest ciliate genera known. Kahl (1930b) already compiled nearly 100 species, and about the same number has been described later (Foissner, unpubl.). Unfortunately, many descriptions lack sufficient details, leaving doubt about the validity of the species. However, recent studies confirm that there are many distinct *Spathidium* species and even indicate a high number of still undescribed taxa (Dragesco and Dragesco-Kernéis 1979; Foissner 1984, 1996, 1999, 2000; Leitner and Foissner 1997; Foissner *et al.* 2002).

Dragesco and Dragesco-Kernéis (1979) and Foissner (1984) split *Spathidium* into the genera *Spathidium* (continuous circumoral kinety with somatic kineties still attached to circumoral kinety fragments; oral bulge elongate elliptical), *Protospathidium* (with circumoral kinety fragments distinctly separated from each other and attached to somatic kineties), *Arcuospathidium* (somatic ciliary rows directed dorsally at both sides of oral bulge and separated from continuous circumoral kinety; oral bulge cuneate), *Epispathidium* (somatic

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ciliary rows separated from continuous circumoral kinety and so strongly curved anteriorly that the circumoral kinety is seemingly doubled), and *Supraspathidium* (with many contractile vacuoles; poor generic feature). Recently, Foissner *et al*. (2002) added three further genera, all based on new species discovered in Namibian soils: *Apospathidium* (with nematodesmata-bearing somatic monokinetids), *Semispathidium* (with discoidal oral bulge and continuous circumoral kinety), and *Apertospathula* (circumoral kinety shortened at left end). Here, I add a further new genus, *Cultellothrix*, based on a new species discovered in floodplain soil from Brazil.

MATERIALS AND METHODS

The soil sample, which contained *Cultellothrix velhoi* sp. n., was collected in May 2001 by Dr. L. Felipe Machado Velho in the high Paraná River floodplain near the town of Maringá (53°15'W 22°40'S, altitude about 500 m), State of Mato Grosso do Sul, Brazil. It was taken from the Aurelio Lagoon, that is, a marginal lagoon associated with the Baia River, a tributary to the Paraná River. The dark, humic soil was mixed with much partially decomposed plant litter, had pH 5.1 (in water), was air-dried in the Salzburg laboratory for about one month, and then stored in a plastic bag. In November 2001, the about 300 g soil were put in a Petri dish (13 cm ø) and saturated, but not flooded with distilled water to obtain a "non-flooded Petri dish culture", as described in Foissner *et al*. (2002). About two weeks after rewetting, a small population of *C. velhoi* developed and was studied *in vivo* and with Foissner's protargol protocol (Foissner 1991).

Counts and measurements on prepared specimens were performed at a magnification of x 1000. *In vivo* measurements were conducted at magnifications of x 100-1000. Although these provide only rough estimates, it is worth giving such data as specimens may change in preparations. Illustrations of live specimens were based on *in vivo* measurements and free-hand sketches, while those of prepared cells were made with a camera lucida. Terminology is according to Kahl (1930a, b) and Corliss (1979).

RESULTS

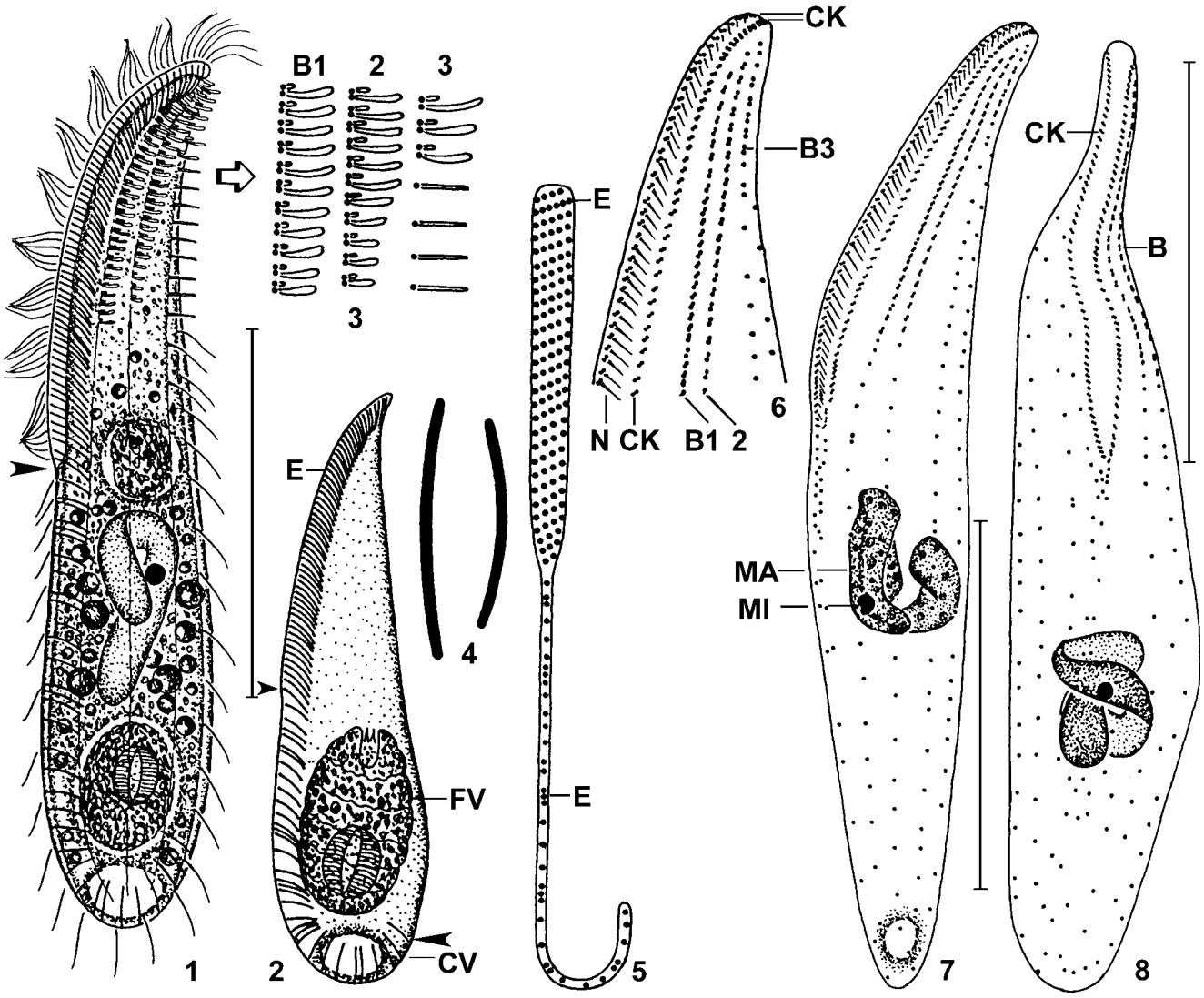
Cultellothrix gen. n.

Diagnosis: Spathidiidae with dorsal brush on left side and ciliature in *Arcuospathidium* pattern.

Table 1. Morphometric data on *Cultellothrix velhoi* sp. n.

Characteristics[a]	x	M	SD	SE	CV	Min	Max	n
Body, length	105.7	103.0	18.1	4.4	17.1	77.0	140.0	17
Body, width	29.2	25.0	9.7	2.4	33.4	21.0	50.0	17
Anterior body end to end of circumoral kinety (mouth length), distance	53.8	55.0	10.4	2.5	19.3	38.0	75.0	17
Mouth, width in anterior third	3.8	4.0	0.6	0.2	16.6	3.0	5.0	10
Anterior body end to macronucleus, distance	56.3	58.0	12.1	2.9	21.5	41.0	80.0	17
Dorsal brush row 1, length[b]	32.2	32.0	7.6	1.9	23.7	20.0	43.0	17
Dorsal brush row 1, number of dikinetids	29.0	28.0	7.3	1.8	25.1	18.0	40.0	17
Dorsal brush row 2, length[b]	32.7	35.0	8.0	2.0	24.6	20.0	47.0	17
Dorsal brush row 2, number of dikinetids	23.2	24.0	4.4	1.1	18.8	16.0	29.0	17
Dorsal brush row 3, length[b]	9.2	10.0	1.5	0.4	16.0	7.0	12.0	17
Dorsal brush row 3, number of dikinetids	7.1	7.0	1.3	0.3	19.1	5.0	10.0	17
Macronuclear figure, length	26.9	22.0	10.7	2.6	39.7	17.0	56.0	17
Macronucleus, length (spread)[c]	40.7	40.0	-	-	-	25.0	56.0	17
Macronucleus, width	5.8	6.0	1.2	0.3	20.8	4.0	9.0	17
Macronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	17
Micronucleus, length	2.9	3.0	0.4	0.1	14.6	2.2	4.0	17
Micronucleus, width	2.6	2.5	0.5	0.1	20.8	2.0	4.0	17
Micronucleus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	17
Somatic kineties, postoral number[d]	11.7	12.0	1.1	0.3	9.4	10.0	14.0	17
Kinetids in a right side kinety, number	27.2	28.0	5.0	1.2	18.5	18.0	35.0	17
Dorsal brush rows, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	17

[a] Data based on mounted, protargol-impregnated (Foissner's method), selected (cells with large rotifers ingested strongly inflated and thus excluded) specimens from a non-flooded Petri dish culture. [b] Distance from circumoral kinety to last brush dikinetid. [c] Approximate values. [d] Including dorsal brush rows. Measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SE - standard error of arithmetic mean, x - arithmetic mean.



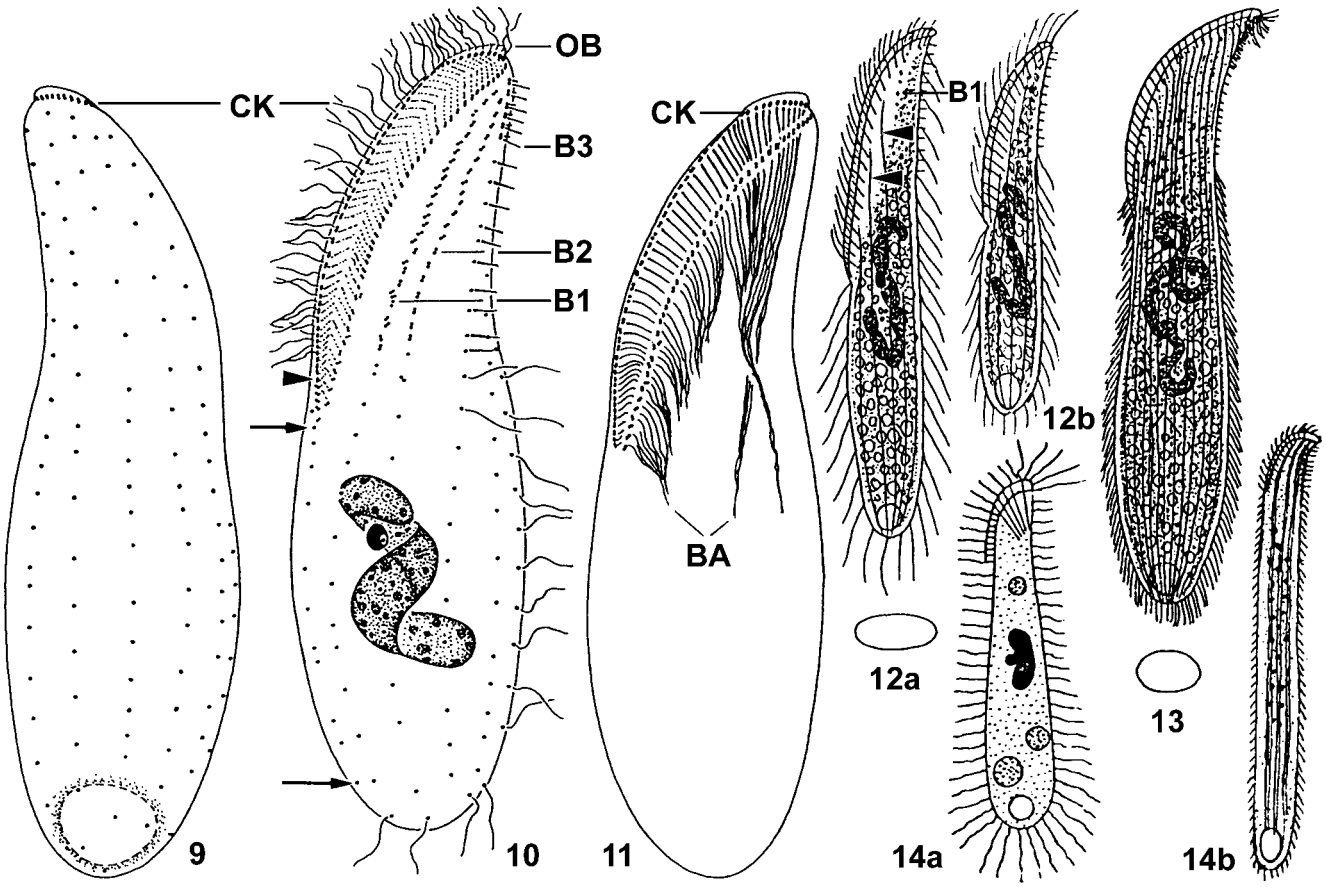
Figs 1-8. *Cultellothrix velhoi* sp. n. from life (1-5) and after protargol impregnation (6-8). **1 -** left side view of a representative, knife-shaped specimen with dorsal brush occupying anterior left side of cell. The oral apparatus extends to mid-body (arrowhead); **2 -** left side view of a broadly knife-shaped specimen having just ingested a rotifer (FV). Note the postoral extrusome row (arrowheads), which extends onto the dorsal side of the cell and is difficult to recognize *in vivo* because the individual extrusomes are fine and often hidden by cell inclusions (Fig. 1); **3 -** posterior portion of dorsal brush with bristles up to 5 µm long. Note monokinetidal bristle tail of row 3; **4 -** extrusomes are curved rods with a size of about 6-7 x 0.3 µm; **5 -** arrangement of extrusomes, which form short, oblique rows in the oral bulge and a single, postoral row extending to dorsal side of cell; **6, 7 -** ciliary pattern of left side. Note lack of an ordinary somatic ciliary row between circumoral kinety and dorsal brush, a main difference to *C. lionotiformis*, which has at least two such rows (Figs 12a, b). Dorsal brush rows 1 and 2 are distinctly more narrowly spaced than the other ciliary rows; **8 -** ventral view showing the parallel-sided circumoral kinety with cuneate proximal portion. B - dorsal brush; B1, 2, 3 - dorsal brush rows; CK - circumoral kinety; CV - contractile vacuole; E - extrusomes; FV - food vacuole with a rotifer; MA - macronucleus; MI - micronucleus; N - nematodesmata (oral basket rods). Scale bars 50 µm.

Type species: *Cultellothrix velhoi* sp. n.

Etymology: composite of the Latin noun *cultellus* (small knife) and the Greek noun *thrix* (hair ~ cilium ~ ciliate), referring to the conspicuous knife-shape of the two species known. Feminine gender.

***Cultellothrix velhoi* sp. n.**

Diagnosis: size about 120 x 25 µm *in vivo*. Knife-shaped with blade (oral portion) approximately half of body length. Macronucleus about 40 µm long, usually



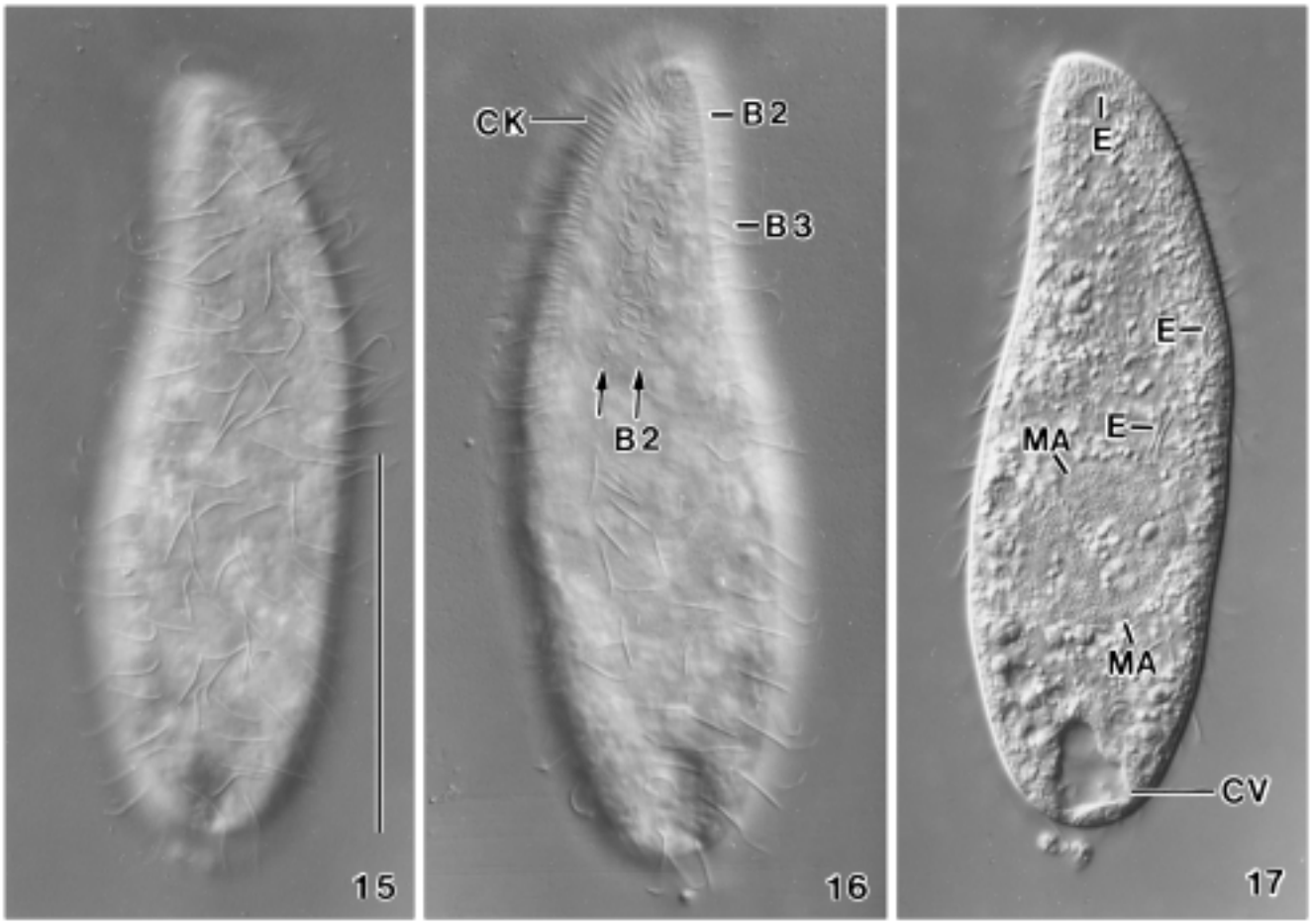
Figs 9-14. *Cultellothrix velhoi* sp. n. (9-11) and related species (12-14). **9-11 -** right and left side ciliary pattern (9, 10), nuclear apparatus (10), and oral basket (11) of a small (length 77 µm), stout specimen of *C. velhoi* after protargol impregnation. Note lack of cilia in proximal portion of circumoral kinety (arrowhead) and postoral basal body row (arrows). Dorsal brush row 3 (B3) has a monokinetidal bristle tail. The oral bulge (OB) is cuneate, hardly set off from body proper, and contains fibres originating from the circumoral dikinetids. Oral basket rods (nematodesmata) likely originate only from the right half of the circumoral kinety. Note lack of ordinary somatic ciliary rows between circumoral kinety and dorsal brush, a main difference to *C. lionotiformis*, which has at least two such rows (Figs 12a, b); **12a, b -** left side view and postoral transverse section of *Spathidium* (now *Cultellothrix*) *lionotiforme* from life, length 140-220 µm (from Kahl 1930a, b). Arrowheads mark two ordinary somatic ciliary rows, lacking in *C. velhoi* (Figs 7, 8, 10), between circumoral kinety and brush row 1 (B1); **13 -** left side view and postoral transverse section of *Spathidium* (now *Arcuospathidium*) *scalpriforme* from life, length 250-330 µm (from Kahl 1930a). Foissner (1984) misidentified this species as *Spathidium* (now *Cultellothrix*) *lionotiforme*; **14a -** left side view of *Cranotheridium stilleri* from life, length 80 µm (from Lepsi 1959); **14b -** left side view of *Litonotus dubius* from life, length 160 µm (from Vuxanovici 1962). B1, 2, 3 - dorsal brush rows; BA - oral basket; CK - circumoral kinety; OB - oral bulge.

tortuous. Extrusomes approximately 6 x 0.3 µm, form short, oblique rows in oral bulge and a long postoral row extending to dorsal side of cell. On average 12 ciliary rows, those left of circumoral kinety anteriorly modified to dorsal brush; brush row 3 distinctly shortened consisting of an average of 7 dikinetids.

Type location: floodplain soil of Paraná River in Brazil, near the town of Maringá, 53°15'W 22°40'S.

Dedication: dedicated to Dr. Luiz Felipe Machado Velho, Universidade Estadual de Maringá, Brazil, who provided the sample containing this and other new species.

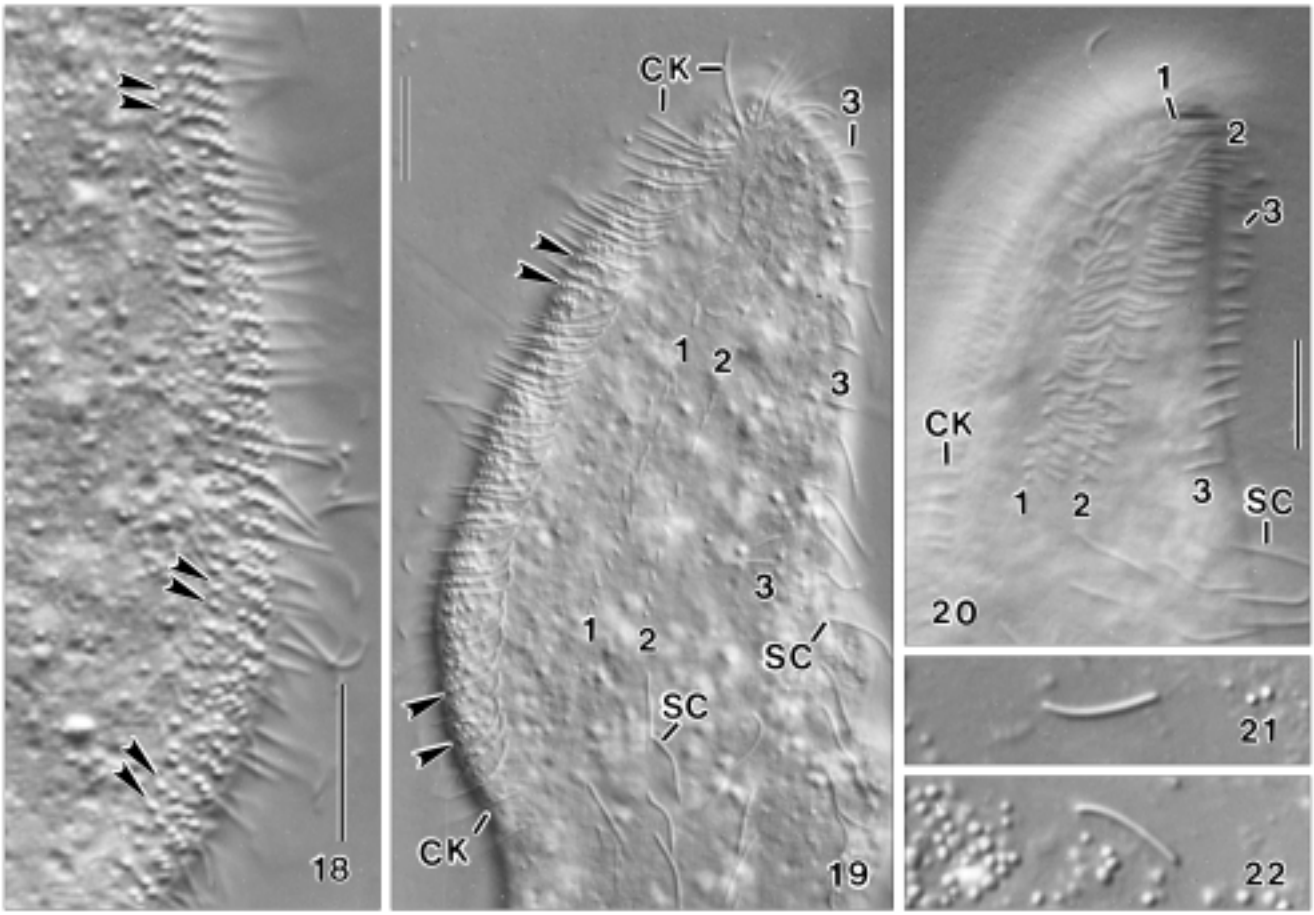
Description: size 80-150 x 20-40 µm *in vivo*, laterally flattened up to 2:1, fragile and thus frequently more or less inflated in protargol preparations (Table 1). Slenderly to broadly knife-shaped with blade (oral portion), however, often indistinctly separated from cylindroidal handle; dorsal outline more or less distinctly sigmoidal, ventral slightly convex; anterior end bluntly pointed, posterior more or less broadly rounded (Figs 1, 2, 7, 9, 15). Nuclear apparatus slightly underneath midbody in postoral portion of cell. Macronucleus about 40 µm long, basically rod-shaped, usually, however, more or less tortuous and coiled; contains many small and



Figs 15-17. *Cultellothrix velhoi* sp. n., right side (15) and left side (16) surface view, and optical section (17) of a slightly squeezed and flattened (by cover glass pressure), live specimen under interference contrast optics. Note the loose ciliation and the dorsal brush rows (arrows) on the left side of the cell; row 3 (B3) is short, but has a monokinetidal bristle tail. The extrusomes are fine and thus inconspicuous in cells viewed laterally (17); the postoral extrusome row is out of focus. B2, 3 - dorsal brush rows; CK - circumoral kinety; CV - contractile vacuole; E - extrusomes; MA - macronucleus. Scale bar 50 µm.

medium-sized nucleoli. Micronucleus attached to middle third of macronucleus, broadly ellipsoidal, 3-4 µm *in vivo*. Contractile vacuole in posterior body end; excretory pore(s) not impregnated. Cortex thin, bright and very flexible; cortical granules and/or extrusomes not recognizable, not even with interference contrast optics. Extrusomes 6-7 x 0.3 µm and slightly curved *in vivo*, do not impregnate with the protargol method used, form many short, oblique rows in oral bulge and a single postoral row extending across posterior body end to dorsal side of cell; some scattered in cytoplasm (Figs 1, 2, 4, 5, 17-19, 21, 22). Oral area flat and hyaline, postoral portion, depending on state of nutrition, also flattened and hyaline or packed with lipid droplets 1-5 µm across and some large food vacuoles containing rotifers in various stages of digestion. Slowly glides on and between soil particles and the microscope slide showing great flexibility.

Somatic cilia 7-8 µm long *in vivo*, widely spaced, arranged in an average of 12 meridional rows commencing at and near curved anterior end of body and thus more or less distinctly curved dorsally at both sides of circumoral kinety (oral bulge), as in *Arcuospathidium* (Foissner 1984), except of a single or two, rather irregular postoral rows, which, interestingly, lack cilia; all kineties equidistantly spaced, except of more narrowly arranged postoral and dorsal brush rows 1 and 2 (Figs 1, 7, 9, 10, 15, 16; Table 1). Dorsal brush occupies left surface of anterior body third, produced by paired bristles at anterior end of first three ciliary rows left of circu-



Figs 18-22. *Cultellothrix velhoi* sp. n. from life, interference contrast optics. **18 -** a heavily flattened (by cover glass pressure) specimen showing the oblique extrusome rows, some marked by arrowheads, in the oral bulge; **19 -** same specimen as depicted in figure 18, showing the up to 3 µm long dorsal bristles forming three rows (numbers 1-3) in the anterior portion of the cell's left side; brush row 3 mainly consists of 3 µm long, monokinetidal bristles. Arrowheads mark oblique extrusome rows in oral bulge, shown at higher magnification in figure 18. The cilia of the circumoral kinety (CK) are slightly longer (10 µm) than the ordinary somatic cilia (7 µm; SC); **20 -** left anterior portion of a specimen with about 5 µm long dorsal bristles, which are arranged in three rows (numbers 1-3); the posterior portion of row 3 consists of monokinetidal, 4 µm long bristles; **21, 22 -** extrusomes, length 6-7 µm. CK - circumoral kinety; SC - somatic cilia. Scale bars 10 µm.

moral kinety; posterior bristle of dikinetids 3-5 µm long and slightly inflated distally, anterior granule-like because only 1-2 µm long; bristle length gradually decreases in posterior third of rows 1 and 2 and is rather variable in individual cells; one or few monokinetids may occur at anterior end of one or all brush rows. Brush row 1 composed of an average of 29 narrowly spaced dikinetids, of similar length as row 2 consisting of only 23 dikinetids; row 3 extends along dorsal margin of cell, strongly shortened posteriorly, composed of only 7 dikinetids on average, has , however, a monokinetidal tail of 3-4 µm long bristles extending to second body third (Figs 1, 3, 6-8, 10, 16, 19, 20; Table 1).

Oral area more or less distinctly curved dorsally, bulge thus convex; hyaline because flattened about 2:1 and containing only few, small lipid droplets. Mouth occupies anterior body half on average, very steep because extending parallel to main body axis, conspicuous due to the oblique rows of refractive extrusomes contained. Oral bulge inconspicuous because less than 3 µm high and thus hardly set off from body proper, contains short, anteriorly directed fibres, originating from circumoral dikinetids, forming arrowhead-like pattern; in frontal view elongate cuneate to almost parallel-sided with proximal portion wedge-like narrowed, about 5 µm wide *in vivo*. Circumoral kinety of same shape as oral bulge, composed of comparatively widely spaced dikinetids each associated with a slightly elongated, about 10 µm long cilium lacking in proximal, wedge-shaped portion of kinety. Nematodesmata originate only from right side dikinetids of circumoral kinety, gradually decrease in length from anterior to posterior, the long

anterior rods form bundles extending to mid-body; I could not decide whether the left side basket rods are lacking or present, but not impregnated (Figs 1, 2, 6-11, 15-17, 18, 19; Table 1).

Occurrence and ecology: *Cultellothrix velhoi* sp. n. is a rare species because I did not find it in about 1000 other soil and moss samples, including approximately 100 samples from similar habitats, collected world-wide. Although looking fragile, *C. velhoi* is a rapacious predator feeding mainly on large rotifers, showing that the oral apparatus can open widely and the body is very flexible. The second species, *C. lionotiformis*, is obviously also rare because it has not yet been redescribed (see Discussion).

DISCUSSION

Spathidiid classification and the new genus *Cultellothrix*

Cultellothrix belongs to the gymnostomatous haptorids, as defined by Corliss (1979) and Foissner and Foissner (1988). Both live and protargol-impregnated specimens of *Cultellothrix* highly resemble pleurostomatid ciliates, such as *Litonotus* and *Amphileptus*, many of which have a similar body shape and basic ciliary pattern (Figs 1, 2, 7-11). However, pleurostomatids invariably have the left side ciliature reduced to short bristles, and many have a special nuclear pattern, viz., two globular macronuclear nodules and a micronucleus in between (Kahl 1931, Foissner and Leipe 1995, Foissner *et al.* 1995). *Cultellothrix* has ordinary cilia at both body sides and a tortuous macronucleus and thus does not belong to the Pleurostomatida.

The body plan and ciliary pattern show that *Cultellothrix* belongs to the Spathidiina, as defined by Foissner and Foissner (1988). Within this suborder, it resembles members of the family Spathidiidae. *Cultellothrix* is unique among the spathidiids in having the dorsal brush not dorsally or dorsolaterally located, as the other genera mentioned in the Introduction (for examples, see Foissner *et al.* 2002), but on the left side (Figs 1, 7, 10, 16). Admittedly, this is a rather inconspicuous difference; however, the two species known look quite unique, indicating that further characters, especially the curious body shape and the special arrangement of the extrusomes, can be added to the diagnosis when further species with the same features have been discovered. Generally, most spathidiid genera are separated by rather sophisticated features; nonetheless, their recognition greatly aids in recognizing species within this rich assemblage.

Within the spathidiids, *Arcuospathidium*, *Apertospathula*, and *Cultellothrix* form a special group characterized by the *Arcuospathidium* type somatic ciliature, that is, all lateral ciliary rows form acute angles with the circumoral kinety because they are directed dorsally at both sides of the oral bulge. This is an indication for a common ancestor and a (sub)familial split. The arrangement of the oral bulge extrusomes in short, transverse rows and the single postoral row in *C. velhoi* resemble several *Bryophyllum* species, whose oral bulge and circumoral kinety, however, extend along the whole body length and curve around the posterior body end to terminate on the dorsal side of the cell (for description of representative species, see Foissner *et al.* 2002). The oral bulge and circumoral kinety of *Cultellothrix*, in contrast, extend only to mid-body. Thus, it is more closely related to the spathidiid than bryophyllid evolutionary path.

Species assignable and comparison of *Cultellothrix velhoi* sp. n. with similar species

Spathidium lionotiforme Kahl, 1930a has the same generic features as *Cultellothrix velhoi* and is thus transferred to this genus: *Cultellothrix lionotiformis* (Kahl, 1930a) comb. n. (Figs 12a, b). This species, which Kahl (1930a, b) discovered in *Sphagnum* moss and in a pond among *Hottonia palustris* in the surroundings of Hamburg, differs from *C. velhoi* by body length (140-200 µm *vs.* 80-150 µm), the lack of a postoral extrusome row, and in having two ordinary ciliary rows between brush row 1 and circumoral kinety (none in *C. velhoi*). Kahl (1930a, b) recognized that *S. lionotiforme* is an "atypical species" showing "transistions to *Amphileptus*". Indeed, this kind of spathidiids is easily confused with pleurostomatids, as discussed above. Further, Kahl (1931) mentioned a single specimen of an *Amphileptus* sp., found among colonies of *Carchesium polypinum*, which might be a further distinct *Cultellothrix* species. *Cranotheridium stilleri* Lepsi, 1959 (Fig. 14a) and *Litonotus dubius* Vuxanovici, 1962 (Fig. 14b) might also belong to *Cultellothrix*. Unfortunately, data are too incomplete for a definite transfer; both need redescription with modern methods.

As concerns the redescription of *S. lionotiforme* Kahl (1930a, b) by Foissner (1984), see following chapter on *Arcuospathidium cultriforme scalpriforme*. Kahl

described *S. lionotiforme* as species nova two times, viz., in 1930a, b. I suggest Kahl (1930a) as correct date because he described also many other new species in this paper later referred to "Kahl, 1930a" by Kahl (1930b).

Arcuospathidium cultriforme scalpriforme (Kahl, 1930) comb. n., stat. n. (Fig. 13)

1930 *Spathidium scalpriforme* Kahl, *Arch. Protistenk*. 70: 381.1930 *Lionotus scalpriforme* Kahl,

1930 - Kahl, *Tierwelt Dtl*. 18: 165 (although seemingly transferred to *Lionotus*, described under the genus *Spathidium*).

1984 *Arcuospathidium lionotiforme* (Kahl, 1930) nov. comb. - Foissner, *Stapfia* 12: 78 (misidentification).

2002 *Arcuospathidium cultriforme lionotiforme* (Kahl, 1930) Foissner, 1984 nov. stat. - Foissner, Agatha and Berger, *Denisia* 5: 300 (ranked as a subspecies).

The present study shows that my former redescription of *S. lionotiforme* Kahl (1930a, b) is based on an unfortunate misidentification caused by insufficient experience and the widespread opinion that *Spathidium* is highly variable. The species I investigated in 1984 obviously belongs to the *Spathidium cultriforme* complex, specifically to *S. scalpriforme* Kahl, 1930a (Fig. 13), a species which I never found in soil and moss (Foissner 1998), although it lives there (Kahl 1930a, b), simply because I continuously misidentified it as *S. lionotiforme*.

Based on the investigations by Foissner *et al.* (2002), I here consider *S. scalpriforme* as a subspecies of *S. cultriforme* Penard, 1922. My published (Foissner 1984, Foissner *et al*. 2002) and unpublished notes on that type of *Spathidium* show that all belong to *Arcuospathidium* and have similar extrusomes, viz., moderately fine and sometimes slightly acicular rods with a size of 4-6 x 0.8-1 µm; when the extrusomes are partially exploded, as in some protargol preparations, they look like small knifes, just as depicted by Penard (1922) in *S. cultriforme*.

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