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Two Remarkable Soil Spathidiids (Ciliophora: Haptorida), *Arcuospathidium pachyoplites* sp. n. and *Spathidium faurefremieti* nom. n.

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Summary. This paper continues a series of studies on spathidiids, a group of free-living, rapacious ciliates with a high biodiversity. *Arcuospathidium pachyoplites* sp. n. was discovered in saline coastal soil from the Henry Pittier National Park in Venezuela, South America. *Spathidium faurefremieti*, originally described by Tucolesco (1962) from Rumanian cave water, was rediscovered in savannah soil from the Shimba Hills National Reserve in Kenya (Africa) and in floodplain soils of Brazil (South America) and Australia. The morphology of these species was investigated using live observation and protargol impregnation. The South American *A. pachyoplites* differs from the African *A. vlassaki*, possibly the nearest relative, mainly by the extrusomes and dorsal brush. *Spathidium faurei* Tucolesco, 1962 is an objective homonym of *Spathidium faurei* Kahl, 1930 and thus re-named: *Spathidium faurefremieti* nom. n. This species is remarkable in having a second contractile vacuole in anterior body half. However, conspecificity of the European and Kenyan populations is questionable; likewise, the Kenyan and Brazilian populations differ considerably, suggesting that further research might prove that all are different subspecies or even species. The present study shows that (i) an increased number of contractile vacuoles likely evolved independently three times, viz., in *Spathidium*, *Arcuospathidium*, and *Supraspathidium*, and (ii) the bivacuolate species should be separated from the polyvacuolate species, which can be referred to the genus *Supraspathidium*.

Key words: Australia, biodiversity, Brazil, Kenya, *Supraspathidium*, terrestrial Protozoa, Venezuela.

INTRODUCTION

This paper continues a series of studies on spathidiid and *Spathidium*-like ciliates, whose full diversity is still not known (Buitkamp 1977; Dragesco and Dragesco-Kernéis 1979; Foissner 1984, 2000, 2003a; Foissner *et al*. 2002). The two species described here are remarkable in several ways. *Arcuospathidium pachyoplites* from a saline site in Venezuela is rather similar to *A. vlassaki* Foissner, 2000 and *A. etoschense* Foissner *et al*. 2002 from saline inland habitats of Africa. These species might be examples for post-Gondwanan speciation. The second species, *Spathidium faurefremieti* is outstanding in having two contractile vacuoles, a feature which, however, obviously evolved independently at least twice because it is found in spathidiids with either an *Arcuospathidium* or *Spathidium* ciliary pattern, viz., in *A. bulli* Foissner, 2000 and *S. faurefremieti* redescribed here.

Both species are very slender showing that many soil ciliates have the same main morphological adaptation as many metazoan soil inhabitants, viz., a worm-like body.

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Further, they are able to produce dormant stages (resting cysts) to survive periods of dryness, a main physiological adaptation of soil organisms in general (Foissner 1987).

MATERIALS AND METHODS

See type locations and distribution sections for collectors and detailed site descriptions. The samples were air-dried in the Salzburg laboratory and stored in plastic bags until investigation. The ciliates were reactivated from the resting cysts by the non-flooded Petri dish method, as described in Foissner (1987) and Foissner *et al.* (2002). Briefly, this simple method involves placing soil in a Petri dish (10-20 cm wide, 2-3 cm high) and saturating, but not flooding it, with distilled water. These cultures were analyzed for ciliates by inspecting about 2 ml of the run-off (soil percolate) on days 2, 7, 14, 21, and 28. The descriptions of the species are based on material obtained from such cultures, i.e. no clones were set up.

Morphological methods followed those used in our previous studies (e.g. Foissner 1984, 1991; Foissner *et al*. 2002), and thus need not to be detailed here. Briefly, live specimens were studied in bright field and interference contrast, and permanent preparations were made with protargol (Protocol A in Foissner 1991).

RESULTS

Description of *Arcuospathidium pachyoplites* sp. n. (Figs 1-16, 23-25, 27-34, 37-43; Table 1)

Diagnosis: size about 170 x 20 µm *in vivo*. Knife-shaped with steep, slightly cuneate oral bulge occupying about 22% of body length. Macronucleus tortuous, figure formed about 80 µm long. Extrusomes conspicuous because lanceolate and 7 x 1.4 µm in size, scattered in both sides of oral bulge and attached to bulge cortex with narrowed anterior end. On average 10 ciliary rows; dorsal brush inconspicuous, occupies 17% of body length on average.

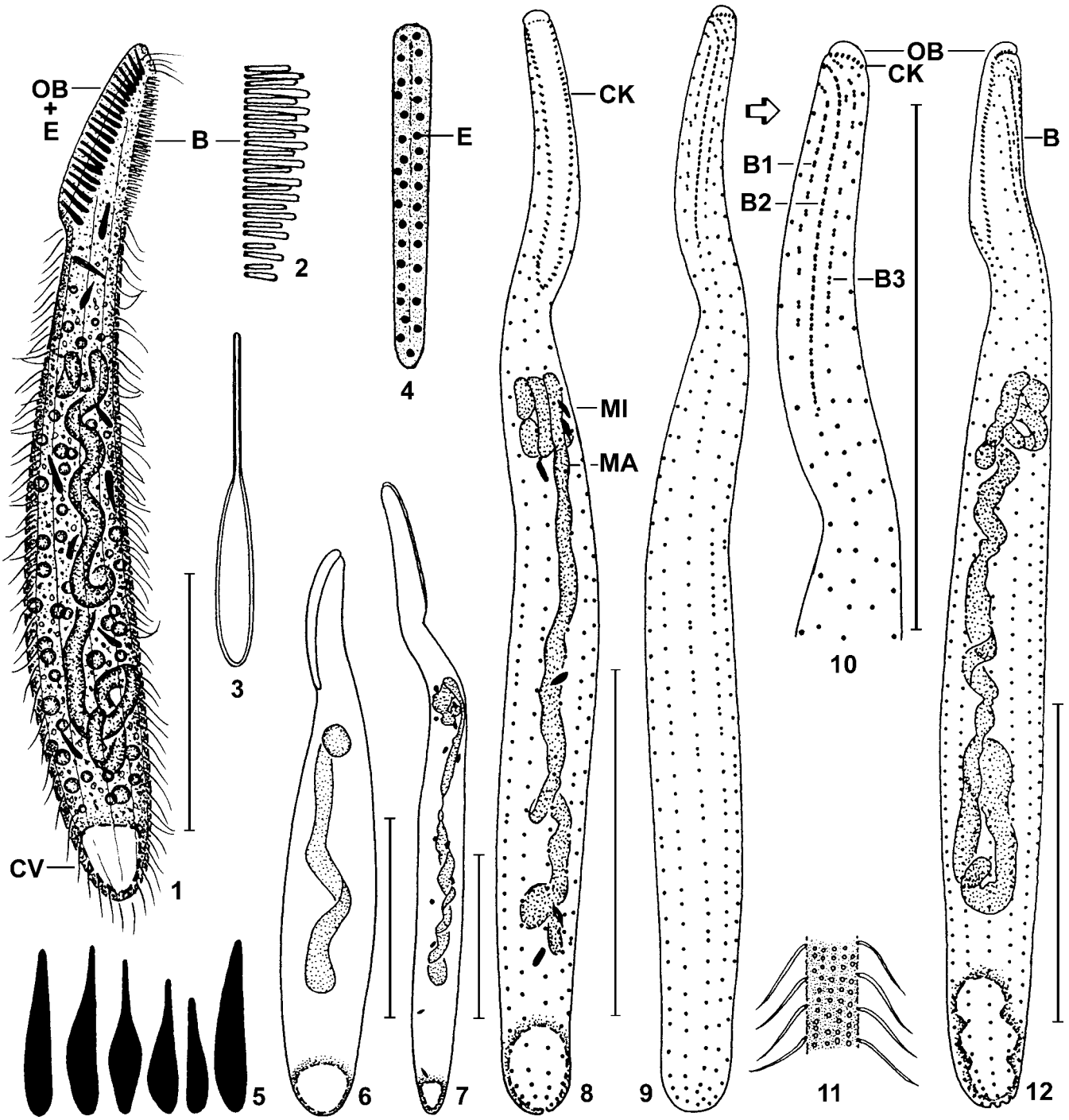
Type location: saline coastal soil in the surroundings of the village of Choroni (67°45'W 10°15'N), Henry Pittier National Park, north coast of Venezuela, South America.

Etymology: apposite noun composed of the Greek words pachy (thick) and (h) oplites (soldier ~ extrusome), referring to the conspicuous extrusomes.

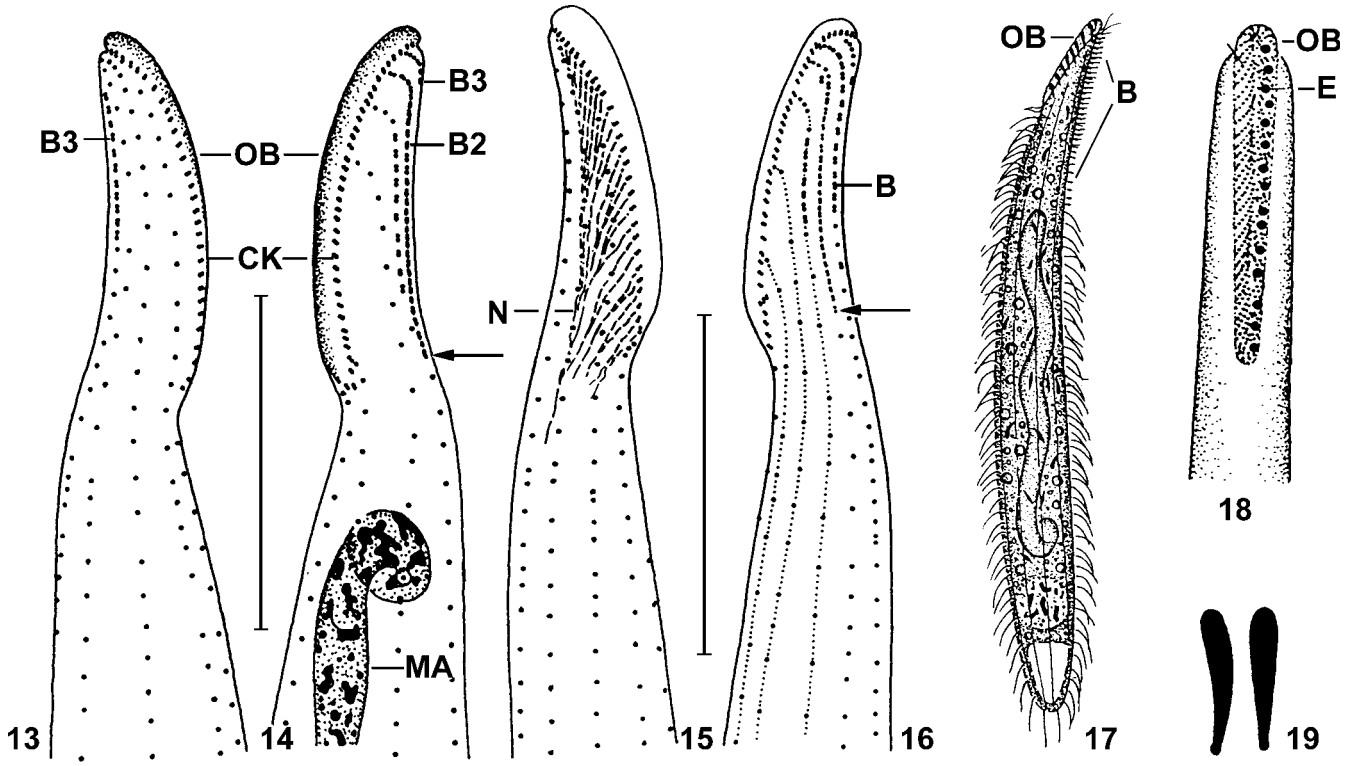
Table 1. Morphometric data on *Arcuospathidium pachyoplites.*

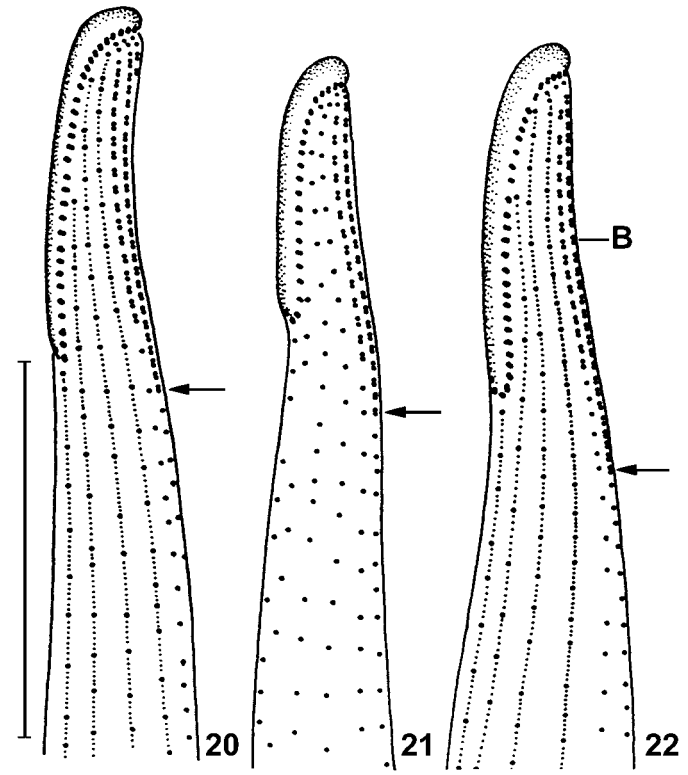
Characteristics[a]	x	M	SD	SE	CV	Min	Max	n
Body, length	158.9	160.0	25.5	5.6	16.1	115.0	210.0	21
Body, width	19.3	18.0	6.1	1.3	31.6	12.0	38.0	21
Body length:width, ratio	9.0	8.2	3.3	0.7	36.7	4.8	17.5	21
Oral bulge, length	34.4	35.0	6.2	1.4	18.0	21.0	44.0	21
Body length:oral bulge length, ratio	4.7	4.6	0.7	0.2	15.4	3.2	6.2	21
Oral bulge, width	4.6	4.5	0.7	0.1	14.6	3.0	5.5	21
Oral bulge, height at anterior end	2.0	2.0	-	-	-	1.5	3.0	21
Circumoral kinety to end of brush row 1, distance	17.2	18.0	3.2	0.7	18.5	11.0	23.0	21
Circumoral kinety to end of brush row 2, distance	27.2	27.0	4.8	1.0	17.6	18.0	38.0	21
Circumoral kinety to end of brush row 3, distance	17.0	18.0	3.8	0.8	21.2	12.0	27.0	21
Anterior body end to macronucleus, distance	57.1	56.0	10.6	2.3	18.5	43.0	84.0	21
Macronucleus figure, length	72.4	72.0	14.0	3.0	19.3	52.0	98.0	21
Macronucleus, length (spread)[b]	111.3	110.0	-	-	-	80.0	150.0	21
Macronucleus, width in middle	4.2	4.0	0.8	0.2	18.3	3.0	5.0	21
Somatic kineties, number (including brush)	10.0	10.0	0.9	0.2	8.7	9.0	12.0	21
Ciliated kinetids in a lateral kinety, number	56.2	56.0	10.8	2.4	19.2	36.0	78.0	21
Dorsal brush rows, number[c]	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
Dikinetids in brush row 1, number	11.4	11.0	2.2	0.5	18.9	7.0	15.0	21
Dikinetids in brush row 2, number	28.0	28.0	5.7	1.2	20.4	18.0	38.0	21
Dikinetids in brush row 3, number	13.3	13.0	2.0	0.4	14.7	11.9	19.0	21

[a]Data based on mounted, protargol-impregnated, selected (see description of species) specimens from a non-flooded Petri dish culture. [b]Very approximate values. [c]Only full rows counted. 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.

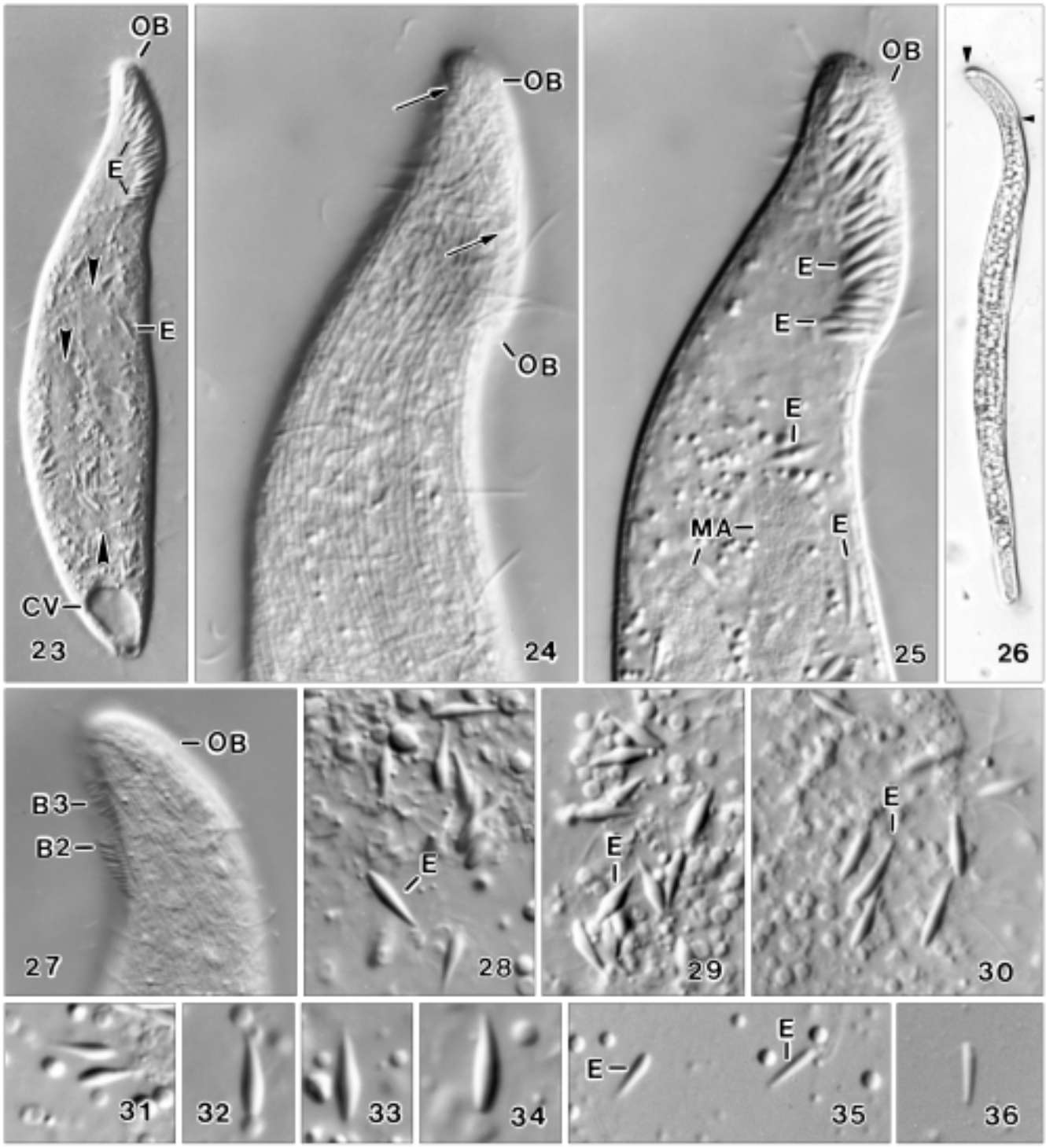


Figs 1-12. *Arcuospathidium pachyoplites* from life (1-5, 11) and after protargol impregnation (6-10, 12). **1 -** left side view of a representative specimen, i.e., "constructed" from live observations and morphometric data shown in table 1. The oral bulge is very conspicuous due to the massive extrusomes contained; **2 -** posterior half of brush row 2, longest bristles 4 µm; **3 -** exploded toxicyst, length 20 µm; **4 -** frontal view of the indistinctly cuneate oral bulge studded with extrusomes; **5 -** oral bulge extrusomes, length 6-8 µm; **6, 7 -** a broad and a slender specimen; note variability of macronucleus; **8-10 -** ciliary pattern of ventral and dorsal side and nuclear apparatus of holotype specimen. Note the oblong circumoral kinety and the dorsal brush dikinetids, which are much more closely spaced in middle row 2 than in rows 1 and 3; **11 -** surface view showing cortical granulation; **12 -** left side view of another specimen with rather distorted dorsal brush and typical macronucleus with coiled and inflated ends. B - dorsal brush, B1-3 - dorsal brush rows, CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, MA - macronucleus, MI - micronucleus, OB - oral bulge. Scale bars - 50 µm.

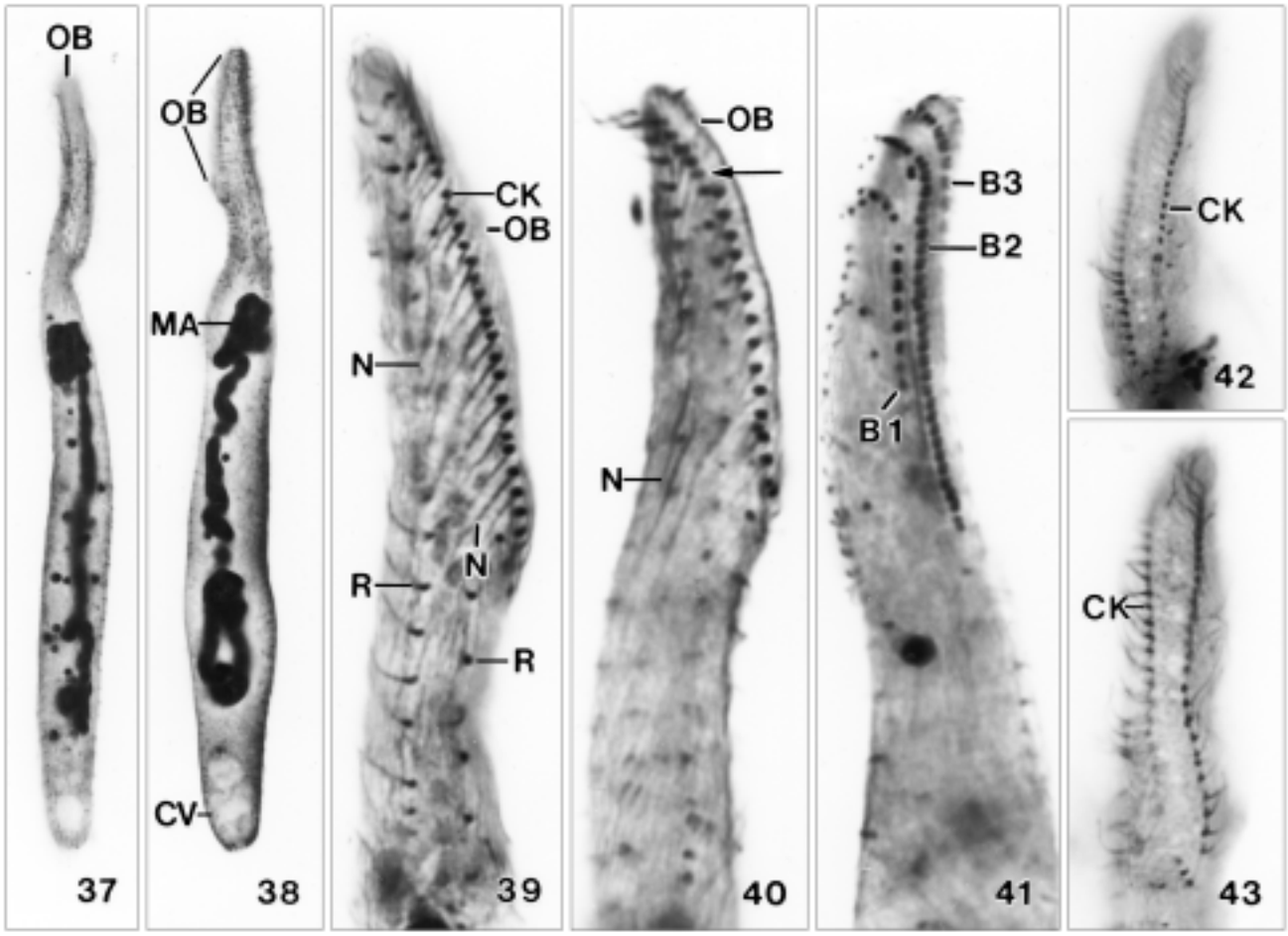




Figs 13-16. *Arcuospathidium pachyoplites*, right and left side view of oral body portion of two specimens after protargol impregnation. Dotted lines connect individual basal bodies of ciliary rows. These figures show: the rather long and very steep oral bulge; the dorsal brush dikinetids much more closely spaced in middle row 2 than in rows 1 and 3; that brush row 2 (arrows) is of about same length as the oral bulge; and, finally, the indistinct *Spathidium* pattern produced by the left side kineties, whose first basal body is very near to the circumoral kinety. B - dorsal brush, B2, 3 - dorsal brush rows, CK - circumoral kinety, MA - macronucleus, N - nematodesmata, OB - oral bulge. Scale bars 30 µm.
Figs 17-22. *Arcuospathidium vlassaki* from life (17-19; from Foissner 2000) and after protargol impregnation (20-22; new drawings from type population). **17** - left side view of a representative specimen (length 190 µm), showing that the oral bulge is much less conspicuous than in *A. pachyoplites*, where it is longer and studded with thick extrusomes (Fig. 1); **18** - frontal view showing extrusomes restricted to the left half of the oral bulge, while they are scattered throughout the bulge in *A. pachyoplites* (Fig. 4); **19** - extrusomes are 5 x 1 µm in size and attached by the broad end to the oral bulge; in *A. pachyoplites*, they are attached by the narrow end and considerably larger (6-8x1-1.7 µm), making the oral bulge very conspicuous (Figs 1, 5, 23, 25); **20-22** - the *Arcuospathidium* ciliary pattern is more distinct in *A. vlassaki* than in *A. pachyoplites* (Figs 14, 16) because the anterior end of the left side kineties is more distinctly directed dorsally (basal bodies of individual ciliary rows connected by dotted lines). Arrows mark end of dorsal brush row 2, which is distinctly longer than the oral bulge, a main difference to *A. pachyoplites*, where it is shorter or of same length as the oral bulge (Figs 14, 16). B - dorsal brush, B1-3 - dorsal brush rows, CK - circumoral kinety, E - extrusomes, MA - macronucleus, N - nematodesmata (oral basket rods), OB - oral bulge. Scale bar 30 µm (20-22).



Figs 23-36. *Arcuospathidium pachyoplites* (23-25, 27-34) and *A. vlassaki* (26, 35,36; from Foissner 2000) from life. **23-25 -** overview and oral details of a slightly squashed and thus broadened specimen. *Arcuospathidium pachyoplites* is conspicuous due to the thick and numerous extrusomes contained in the oral bulge. Figure 24 is a surface view showing the closely spaced rows of cortical granules and the right margin of the oral bulge (arrows). Arrowheads in figure 23 mark the long, tortuous macronucleus; **26 -** *Arcuospathidium vlassaki* is usually more slender than *A. pachyoplites* and the oral bulge, marked by arrowheads, is less conspicuous because it contains fewer and smaller extrusomes (cp. figures 32-36); **27 -** right side view showing dorsal brush rows 2 and 3 with bristles up to 4 µm long; **28-31 -** oral bulge extrusomes are 6-8 x 1-1.7 µm in size and rather variable in shape, usually, however, they are lanceolate; **32-36 -** same scale comparison of extrusomes of *A. pachyoplites* (32-34) and *A. vlassaki* (35, 36). The extrusomes of *A. pachyoplites* are considerably larger (7 x 1.4 µm) than those of *A. vlassaki* (5 x 1 µm). B1, 2 - dorsal brush rows, CV - contractile vacuole, E - extrusomes, MA - macronucleus, OB - oral bulge.



Figs 37-43. *Arcuospathidium pachyoplites* after protargol impregnation. **37, 38 -** ventral and left side overviews showing slender shape, steep oral bulge, and the long macronucleus with coiled ends; **39, 40 -** right side views of oral region showing the circumoral kinety composed of comparatively widely spaced dikinetids (arrow) associated with fine rods forming the oral basket; **41 -** left side view of oral body portion showing that dorsal brush dikinetids are much more closely spaced in row 2 than in rows 1 and 3. Note that dorsal brush row (2) is shorter than the oral bulge, a main difference to *A. vlassaki*, where it is longer (Figs 20-22); **42, 43 -** frontal views of circumoral kinety and oral bulge. The white dots within the circumoral kinety (oral bulge) are optical transverse sections of the large, unstained extrusomes. B1-3 - dorsal brush rows, CK - circumoral kinety, CV - contractile vacuole, MA - macronucleus, N - nematodesmata (oral basket rods), OB - oral bulge, R - ciliary rows.

Type material: 1 holotype slide and 2 paratype slides with protargol-impregnated specimens (Foissner's method) have been deposited in the Oberösterreichische Landesmuseum in Linz (LI). All specimens illustrated and some other well-impregnated cells are individually marked by a black ink circle on the cover glass. For comparison, we add a slide of protargol-impregnated *Arcuospathidium vlassaki* from type population, showing the cells illustrated in figures 20-22.

Description: this species does not fix well, as is often the case with saline material. Some specimens look rather distorted and/or inflated by large food inclusions and/or insufficient preservation. Furthermore, the slides contain some very small specimens (postdividers?) and cells with a distinctly shorter oral bulge, possibly belonging to another species. All these poorly preserved and unusual specimens, roughly 10% of the population, are excluded from the description and morphometry.

Size 120-230 x 15-40 µm *in vivo*, usually near 170 x 20 µm, as calculated from some *in vivo* measurements and the morphometric data (Table 1). Knife-shaped with an average length:width ratio of 9:1, but "handle" much longer than "blade", that is 4.7:1, flattened only in oral region; oral bulge bluntly pointed anteriorly and rather distinctly set off from narrowed neck, strongly oblique, that is, almost parallel with main body axis; posterior end

narrowly rounded, in preparations occasionally almost globular when the contractile vacuole is filled (Figs 1, 6, 7, 12, 37, 38). Macronucleus extending in posterior two thirds of body, basically a long, slightly tortuous rod with ends frequently coiled, spiralized and/or inflated; occasionally in two long pieces. Nucleoli small, globular and numerous, rarely reticulate (Figs 1, 6-8, 12, 23, 37, 38). Probably, 5-10 micronuclei not unequivocally distinguishable from extrusomes *in vivo* and protargol preparations; usually fusiform and about 3 x 1-1.5 µm in impregnated specimens (Figs 7, 8). Contractile vacuole in rear body end, several excretory pores in pole area; definitely no second contractile vacuole in anterior body half. Extrusomes accumulated in both sides of oral bulge and scattered in cytoplasm, attached to bulge cortex with pointed anterior end; basically lanceolate, but with several modifications within and between specimens, as shown in figure 5; 6-8 x 1-1.7 µm in size and compact, that is, rather long, thick, and highly refractive, making them very conspicuous at even low magnification (x100; Figs 23, 25) and in silver preparations, where they appear as strongly refractive inclusions (Figs 42, 43); mature (bulge) extrusomes never impregnate with the protargol method used, while a certain cytoplasmic developmental stage impregnates brownish, like the micronuclei (see above). Exploded extrusomes about 20 µm long and of typical toxicyst structure (Figs 1, 3, 5, 23, 25, 28-34, 42, 43). Cortex very flexible, contains about five rows of minute granules approximately 0.2 µm across between each two ciliary rows. Cytoplasm colourless, usually contains many lipid droplets 0.5-5 µm across; rarely specimens with a large food vacuole containing massive prey, likely a ciliate, were observed. Movement conspicuously slow and worm-like, but glides rather rapidly on microscope slide and between soil particles, showing great flexibility.

Cilia about 8 µm long *in vivo*, arranged in an average of 10 equidistant, straight, rather loosely ciliated rows abutting on circumoral kinety in acute angles more distinct at right than left side, where basal bodies are rather widely spaced, producing an intermediate *Arcuospathidium-Spathidium* pattern (Figs 13-16, 39-41, and Discussion). Dorsal brush not as stable as in many congeners, but often with small irregularities, such as minute breaks within rows, supernumerary dikinetids outside rows, or even some extra bristles forming a short fourth row; basically, however, three-rowed and inconspicuous because shorter than oral bulge, occupying only 17% of body length and bristles merely up to 4 µm long, decreasing to 2 µm at end of rows; all rows have one or few ordinary cilia anteriorly and continue as somatic kineties posteriorly; *in vivo*, bristles slightly inflated distally, and anterior bristles of dikinetids shorter than posterior. Brush rows 1 and 3 of similar length and with rather widely spaced dikinetids; row 2 distinctly longer than rows 1 and 3 and with dikinetids so narrowly spaced (~ 1 µm) that they are difficult to illustrate; row 3 with some minute, about 1 µm long, monokinetidal bristles forming short tail extending to second body third (Figs 1, 8-10, 12-16, 27, 39, 41; Table 1).

Oral bulge very conspicuous due to the large and highly refractive extrusomes contained and the strongly oblique orientation almost in parallel with main body axis (Figs 1, 6, 7, 12, 13, 15, 23-25, 38, 39, 41; Table 1); basically, however, of ordinary size and shape, that is, about twice as long as widest trunk region, moderately convex, and dorsally slightly higher than ventrally; oblong to narrowly cuneate and studded with extrusomes in frontal view (Fig. 4). Circumoral kinety of same shape as oral bulge, composed of comparatively widely spaced and frequently slightly irregularly arranged dikinetids, each associated with a cilium and a fine basket rod recognizable only in over-impregnated specimens (Figs 8, 12-16, 24, 39, 40, 42, 43).

Distribution and ecology: as yet found only at type location, that is, 10-20 m inshore the beach of Choroni (67°45'W 10°15'N), Henry Pittier National Park, north coast of Venezuela. The sample consisted of very sandy coastal soil up to 10 cm depth and the mouldy top leave litter from shrubs, Cactaceae, and grasses. The rewetted mixture had 10‰ salinity and pH 6.7. The species become abundant one week after rewetting the sample. Prey is obviously digested rapidly because only few specimens with food vacuoles containing prey remnants were found in the protargol slides.

Redescription of *Spathidium faurefremieti* nom. n. (Figs 44-59, 65-70; Table 2)

Nomenclature: Tucolesco (1962) named a new species *Spathidium faurei* in honour of the great French protozoologist Fauré-Fremiet (1883-1971). Unfortunately, this name is preoccupied by *Spathidium faurëi* Kahl, 1930a. Thus, a new name is required: *Spathidium faurefremieti* nom. n. Accordingly, in future, Tucolesco's species must be referenced as: *Spathidium faurefremieti* Foissner, 2003.

Material: from 3 sites, as described in the distribution and ecology section, but only the Kenyan population was fully investigated. The Brazilian specimens were also studied rather carefully, while the Australian popu-

lation was routinely identified *in vivo* by the main characteristics of the species, viz., the two contractile vacuoles, body and extrusome size and shape, and the nuclear pattern.

Voucher slides: 5 slides with protargol-impregnated specimens (Foissner's method) from Kenya have been deposited in the Oberösterreichische Landesmuseum in Linz (LI). All specimens illustrated and some other well-impregnated cells are individually marked by a black ink circle on the cover glass. The Brazilian specimens, which are only mediocre impregnated, are contained in the type slides of *Cultellothrix velhoi* Foissner, 2003a and *Cephalospatula brasiliensis* Foissner, 2003b.

Description of Kenyan population: size 170-330 x 13-22 µm *in vivo*, usually near 240 x 17 µm, as calculated from some *in vivo* measurements and the morphometric data (Table 2). Very slenderly spatulate or vase-shaped with an average length:width ratio of about 14:1, widest in or slightly underneath mid-body, rather distinctly narrowed subapically producing a slender neck bearing the slightly widened and flattened oral area; posterior end narrowly rounded, in preparations sometimes bulbous due to the contractile vacuole (Figs 44, 48; Table 2). Macronucleus extending in central body quarters, basically a long, irregularly nodulated rod with more or less tortuous, coiled ends; nucleoli small, globular, and numerous. On average 8.5 globular, spongious micronuclei near or attached to macronucleus (Figs 44, 48, 51, 69; Table 2). Invariably two contractile vacuoles without collecting canals: one, as usual, in rear end with scattered excretory pores in pole are; and a second slightly above mid-body with 2-7 serially arranged excretory pores attached to the kinety bearing the middle row of the dorsal brush (Figs 44, 48, 52, 70; Table 2). Extrusomes inconspicuous, accumulated in both sides of oral bulge and scattered in cytoplasm, where a certain, fusiform developmental stage impregnates with protargol; mature oral bulge extrusomes *in vivo* rod-shaped with rounded ends and slightly curved, about 6 µm long, do not impregnate with the protargol method used (Figs 44, 46, 52). Cortex very flexible, contains rows of inconspicuous granules less than 1 µm across. Cytoplasm colourless, contains few to many lipid globules, depending on state of nutrition, mainly in middle body third. Movement conspicuously slow and worm-like.

Cilia about 8 µm long *in vivo*, arranged in an average of 12 equidistant, straight, moderately densely ciliated rows abutting on circumoral kinety in typical *Spathidium* pattern (Foissner 1984), that is, in acute angles at right side and nearly at right angles at left with rows still attached to circumoral kinety fragments (Figs 44, 47-50, 65-67; Table 2). Dorsal brush of usual location and structure, inconspicuous because occupying only 20% of body length on average and bristles merely 3 µm long *in vivo*; all rows of similar length, an unusual feature; dikinetids narrowly spaced in rows 1 and 2, while widely spaced in row 3 which has, as usual, a monokinetidal bristle tail extending to second body third; specimens with a short, fourth row of bristles rarely occur (Figs 44, 49, 50, 52, 68; Table 2).

Oral bulge inconspicuous because only slightly longer than widest trunk region and ordinarily slanted (~ 45°); slightly convex and higher dorsally than ventrally; obovate and studded with extrusomes in frontal view (Figs 44, 45, 47-52, 65-68; Table 2). Circumoral kinety not obovate as oral bulge, but distinctly cuneate, composed of more or less perfectly aligned, dikinetidal kinetofragments frequently still attached to the somatic ciliary rows from which they were produced, especially at left side; dikinetids narrowly spaced, each associated with a cilium and a fine basket rod hardly recognizable *in vivo*; oral basket, however, fairly distinct in protargol-impregnated specimens (Figs 48-52, 65-68).

Observations on Brazilian and Australian specimens (Figs 53, 55-58; Table 2)

The Brazilian population matches the Kenyan specimens in many main features, for instance, the two contractile vacuoles and the dorsal brush, while the number of ciliary rows (17 *vs.* 11) and body width (27 µm *vs.* 15 µm) are conspicuously different (Table 2). Furthermore, the extrusomes are longer, viz. 9-12 µm and thus extend into the somatic cytoplasm, while the short (6 µm) extrusomes of the Kenyan specimens just fill the oral bulge; the macronucleus is more distinctly nodulated and thicker; body shape is cylindroidal rather than spatulate (Figs 56-58); and the oral bulge is elliptical, not obovate in frontal view (Fig. 55). These are rather distinct differences causing doubt on conspecificity (see Discussion).

The Australian specimens were identified *in vivo*, where they showed the same features as those from Kenya. Extrusomes, however, were 9-10 µm long and thus in between those of specimens from Kenya (6 µm) and Brazil (9-12 µm).

Distribution and ecology: Tucolesco (1962) discovered *S. faurefremieti* in subterranean cave water in Rumania. I found it, as yet, only in soils from Gondwanan areas, viz., Africa, South America, and Australia. These data indicate a cosmopolitan distribution of

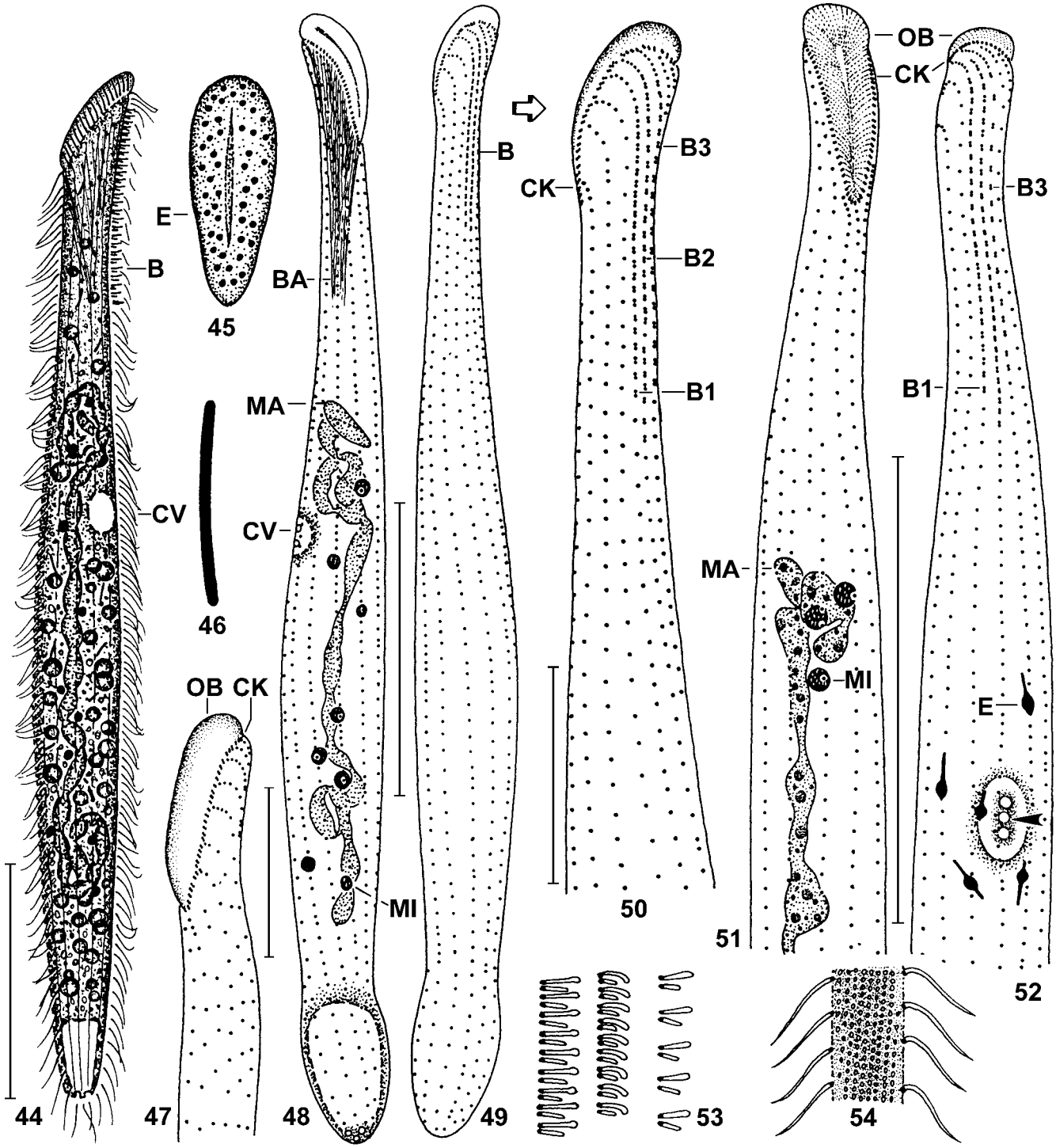
Table 2. Morphometric data on *Spathidium faurefremieti* from Kenya (upper line) and Brazil (lower line).

Characteristics[a]	x	M	SD	SE	CV	Min	Max	n
Body, length	213.8	202.5	41.6	12.0	19.4	160.0	305.0	12
	255.9	240.5	43.4	14.5	16.9	186.0	330.0	9
Body, width	15.6	15.0	2.2	0.7	14.3	13.0	20.0	12
	28.3	27.0	8.6	2.9	30.2	21.0	47.0	9
Body length:width, ratio	13.9	12.9	2.9	0.8	20.6	10.4	20.3	12
	9.6	10.9	2.8	0.9	28.9	4.9	12.3	9
Oral bulge, length	22.7	22.0	2.0	0.6	9.2	19.0	25.0	12
	25.6	26.0	3.0	1.0	11.6	20.0	30.0	9
Body length:oral bulge length, ratio	9.6	9.5	1.1	0.3	11.8	8.0	12.2	12
	10.0	9.4	1.1	0.4	11.2	8.6	11.8	9
Circumoral kinety to end of brush row 1, distance	38.4	36.5	5.8	1.7	15.0	30.0	48.0	12
	39.2	40.0	8.9	3.0	22.7	22.0	52.0	9
Circumoral kinety to end of brush row 2, distance	43.3	40.0	8.5	2.5	19.6	30.0	58.0	12
	47.8	50.0	9.7	3.2	20.3	30.0	62.0	9
Circumoral kinety to end of brush row 3, distance	37.7	37.5	7.2	2.1	19.1	30.0	50.0	12
	35.6	36.0	9.2	3.1	25.8	20.0	47.0	9
Anterior body end to first excretory pore, distance	91.7	90.0	16.5	4.8	18.0	66.0	122.0	12
	91.3	90.0	14.2	4.7	15.6	60.0	110.0	9
Macronucleus figure, length	105.6	96.5	28.8	8.3	27.3	66.0	165.0	12
	115.0	105.0	26.0	8.7	22.6	82.0	150.0	9
Macronucleus, width in middle	3.6	4.0	-	-	-	3.0	4.0	12
	6.0	6.0	1.0	0.3	16.7	5.0	8.0	9
Micronuclei, largest diameter	3.1	3.0	-	-	-	3.0	3.5	12
	4.9	5.0	0.9	0.3	18.4	4.0	6.0	7
Micronuclei, number	8.5	8.5	1.0	0.3	11.8	7.0	10.0	12
	9.5	9.5	1.5	0.6	15.8	8.0	12.0	6
Somatic kineties, number (including brush)	11.7	11.0	0.9	0.3	7.6	11.0	13.0	12
	17.1	17.0	1.3	0.4	7.4	15.0	19.0	9
Ciliated kinetids in a lateral kinety, number	90.4	82.5	24.6	7.1	27.2	64.0	152.0	12
	62.1	63.0	7.2	2.7	11.6	50.0	70.0	9
Dorsal brush rows, number[b]	3.0	3.0	0	0	0	3.0	3.0	12
	3.0	3.0	0	0	0	3.0	3.0	9
Dikinetids in brush row 1, number	23.3	23.0	5.5	1.6	23.6	15.0	32.0	12
	24.0	25.0	3.6	1.2	15.0	15.0	27.0	9
Dikinetids in brush row 2, number	29.5	29.5	6.6	1.9	22.4	20.0	40.0	12
	34.8	34.0	4.7	1.6	13.6	25.0	42.0	9
Dikinetids in brush row 3, number	19.2	18.5	3.1	0.9	16.0	14.0	23.0	12
	23.1	23.0	3.8	1.3	16.4	17.0	27.0	9
Excretory pores of anterior contractile vacuole, number	3.3	3.0	1.5	0.4	45.7	2.0	7.0	12
	similar as above, but exact number difficult to count							

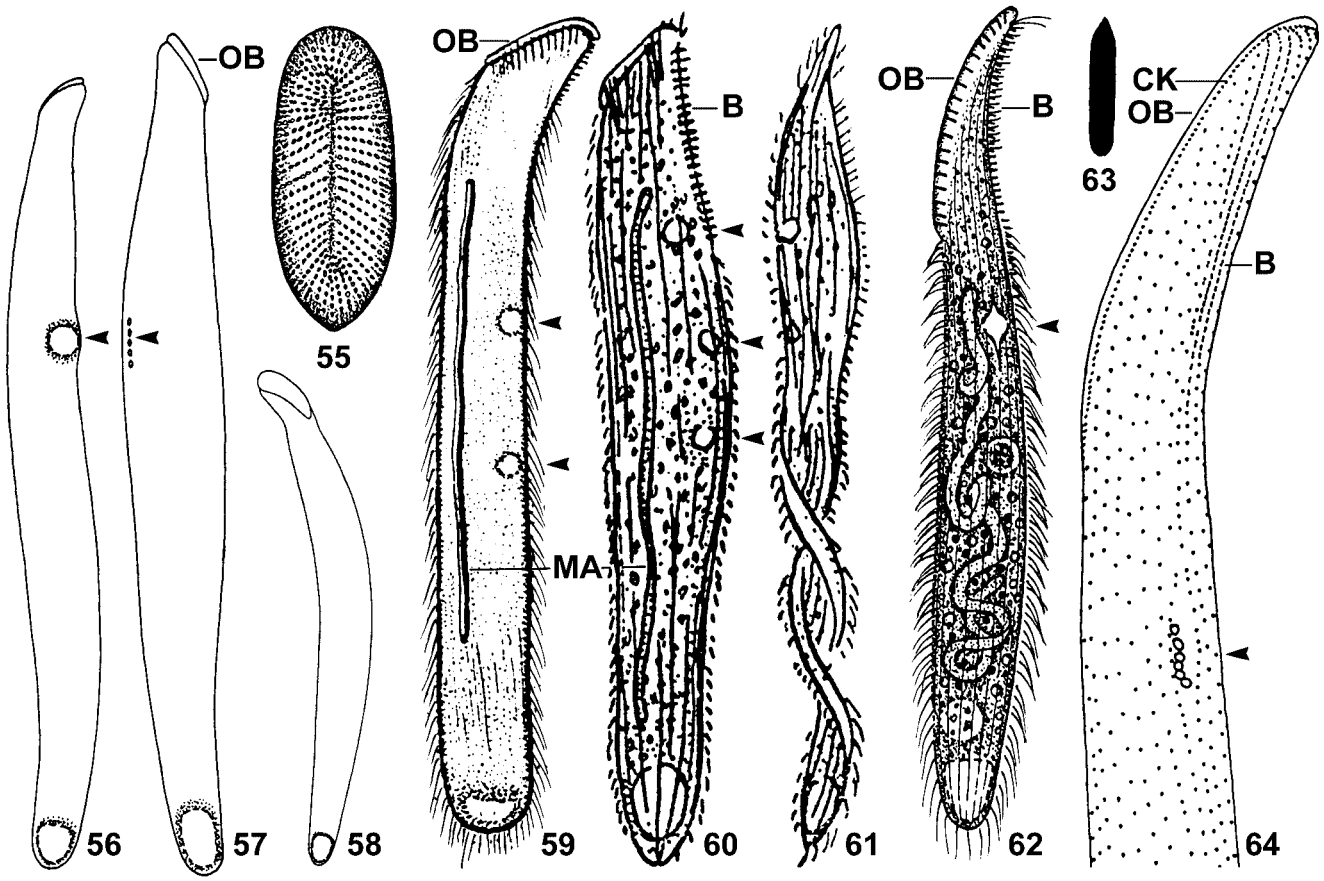
[a]Data based on mounted, protargol-impregnated, randomly selected specimens from non-flooded Petri dish cultures. [b]Only full rows counted. Measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SE - standard error of arithmetic mean, x - arithmetic mean.

S. faurefremieti, but this is not definite because conspecificity of all populations is doubtful (see Discussion).

In Kenya (Africa), *S. faurefremieti* occurred in a sample collected by Emmerich Petz (Upper Austria) in the surroundings of the Taita Hills Lodge in the Shimba Hills Nature Reserve (39°25'E 5°S), about 40 km south of the town of Mombassa. This sample was composed of red soil mixed with some grass and shrub litter. The Brazilian (South America) population occurred in a soil sample, kindly provided by Dr. L. F. Machado Velho, from the floodplain of the Paraná River near the town of Maringá (53°15'W 22°40'S). The dark, humic soil was mixed with much partially decomposed leaf litter and had pH 5.1 (in water). The Australian population of *S. faurefremieti* was found in soil from the Murray



Figs 44-54. *Spathidium faurefremieti*, Kenyan (44-52, 54) and Brazilian (53) specimens from life (44-46, 53, 54) and after protargol impregnation (47-52). **44 -** left side view of a representative specimen, i.e., "constructed" from live observations and morphometric data shown in table 2. Note the slender shape and the second contractile vacuole above mid-body, a main feature of this species; **45 -** frontal view of oral bulge studded with extrusomes; **46 -** oral bulge extrusome, length 6 µm; **47 -** left side view of anterior body portion, showing circumoral kinety and somatic ciliary rows arranged in typical *Spathidium* pattern; **48-50 -** ciliary and contractile vacuole pattern and nuclear apparatus of main voucher specimen. Note the second contractile vacuole above mid-body and the dorsal brush rows, which terminate at almost same level, another unusual feature. The circumoral kinety and the ciliary rows are arranged in the typical *Spathidium* pattern (50); **51, 52 -** ventral and dorsal view of anterior body half of another specimen. Note the cuneate circumoral kinety, the widely spaced dikinetids of dorsal brush row 3, and the three excretory pores (arrowhead) of the anterior contractile vacuole; **53 -** middle portion of dorsal brush, longest bristles 5 µm; **54 -** surface view showing cortical granulation. B - dorsal brush, B1-3 - dorsal brush rows, BA - oral basket, CK - circumoral kinety, CV - contractile vacuoles, E - extrusomes, MA - macronucleus, MI - micronucleus, OB - oral bulge. Scale bars 20 µm (47, 50); 50 µm (44, 48, 49, 51, 52).



Figs 55-64. *Spathidium faurefremieti* (55-59) and related species, viz., *Spathidium vermiforme* (60, 61) and *Arcuospathidium bulli* (62-64) from life (55, 59-63) and after protargol impregnation (56-58, 64). Arrowheads mark the anterior contractile vacuole(s), a main feature of these species. **55 -** frontal view of oral bulge of a Brazilian specimen. The bulge contains distinct rows of cortical granules; **56-58 -** outline drawings of Brazilian specimens of *S. faurefremieti*, length 300 µm, 200 µm, 200 µm; **59 -** original figure of *S. faurefremieti*, length 400 µm (from Tucolesco 1962). Tucolesco did not mention the contractile vacuoles in the description, and thus it is unknown whether both or only one of the anterior contractile vacuoles has excretory pores; **60, 61 -** *Spathidium vermiforme* (from Penard 1922) is 200-400 µm long, strongly flattened, and has "une grande vésicule contractile postérieure, puis une autre en avant, dans laquelle viennent éclater des vacuoles plus petites"; **62-64 -** *Arcuospathidium bulli* (from Foissner 2000) is about 260 x 25 µm in size; has a long, steep oral bulge containing thick, 4 µm long extrusomes (63); and shows a typical *Arcuospathidium* ciliary pattern (64; somatic ciliary rows separated from circumoral kinety and directed dorsally), while *S. faurefremieti* has a *Spathidium* pattern (ciliary rows attached to circumoral kinety and curved ventrally to form ~ 90° angles with circumoral kinety; Figs 47, 50). B - dorsal brush, CK - circumoral kinety, MA - macronucleus, OB - oral bulge.

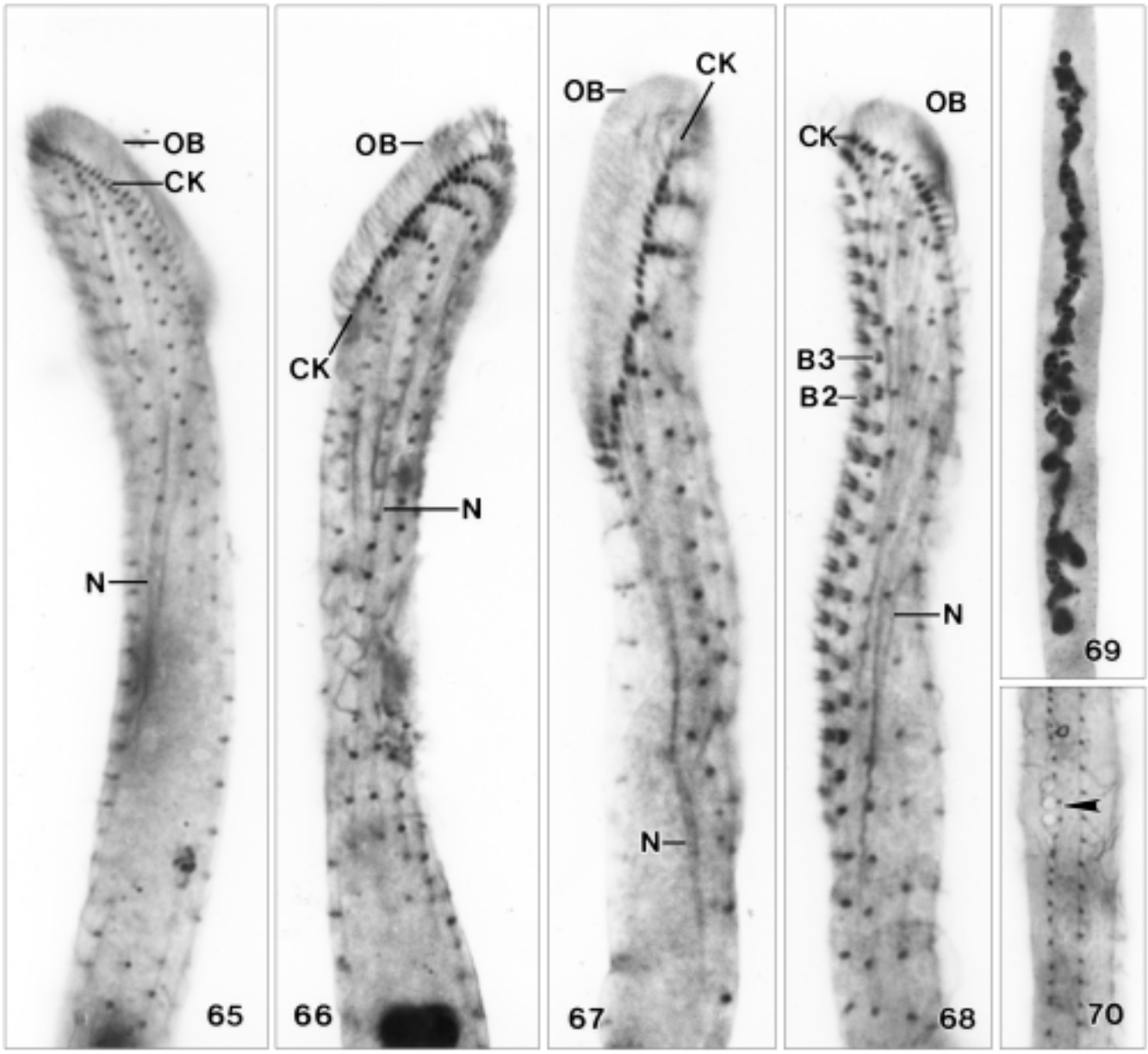
River floodplain near the town of Albury (37°S 147°E). The sample, kindly provided by Dipl.-Biol. Hubert Blatterer, was a mixture of leaf litter and light brown soil with pH 5.2 (in water).

The records from cave water (Tucolesco 1962) and two floodplain soils indicate that *S. faurefremieti* occurs in both soil and freshwater, while the slender shape suggests a preference for soil or muddy environments (Foissner 1987). Abundances were low at all sites, and Tucolesco (1962) possibly observed only a single specimen, as indicated by the range-less size value. None of the prepared cells contained definite food inclusions, indicating that prey is digested rapidly and/or dissolves during ingestion, as observed in several members of the group (Foissner, unpubl.).

DISCUSSION

Arcuospathidium pachyoplites

The boundaries between the spathidiid genera distinguished by Foissner (1984) and Foissner *et al*. (2002) are not very sharp, but greatly aid in distinguishing



Figs 65-70. *Spathidium faurefremieti*, Kenyan specimens after protargol impregnation. **65, 66 -** ciliary pattern of right and left side in anterior body portion of same specimen. This species has a typical *Spathidium* ciliary pattern, that is, the somatic ciliary rows are attached to the circumoral kinety and curved dorsally at right side and ventrally at left, forming ~ 90° angles with the circumoral kinety; **67 -** left side view of another specimen showing the right angles formed by the circumoral kinety and the curved anterior end of the somatic ciliary rows; **68 -** dorsolateral view showing the widely spaced dikinetids of dorsal brush row 3; **69 -** nodulated and tortuous macronucleus; **70 -** the anterior contractile vacuole has three excretory pores (arrowhead). B2, 3 - dorsal brush rows, CK - circumoral kinety, N - nematodesmata, OB - oral bulge.

species groups within this highly diverse assemblage (for detailed discussion, see Foissner 1984 and Foissner *et al*. 2002). The present species, at first glance, appears as a typical *Arcuospathidium* because the anterior ends of the kineties seemingly are curved dorsally at both sides of the oral bulge, a diagnostic character for the genus. However, closer investigation shows that most left side kineties have a basal body very near to the circumoral dikinetids, giving the kineties a *Spathidium*-like pattern (Figs 14, 16). On the other hand, the knife-shaped body and the steep, oblong oral bulge argue for a classification in *Arcuospathidium*, as does a compari-

son with the supposed nearest relative, *A. vlassaki*, where the *Arcuospathidium* ciliary pattern is more distinct (Figs 20-22).

There are few species with a close resemblance to *A. pachyoplites*, viz., *A. vlassaki* Foissner, 2000; *A. etoschense* Foissner *et al*., 2002; and *Spathidium etoschense* Foissner *et al*., 2002. At first glance, *A. pachyoplites* is indistinguishable from *A. vlassaki* because all obvious features are rather similar (Figs 17-22). However, a more detailed comparison reveals differences significant at species level, most related to the extrusomes emphasizing the need of thorough *in vivo* data: lanceolate with narrow end attached to the oral bulge *vs.* oblanceolate attached with broad end (Figs 1, 5, 17, 19); scattered in entire oral bulge *vs.* forming a single row in left bulge half (Figs 4, 18); 7 x 1.4 µm and thus highly conspicuous *vs.* 5 x 1 µm and of ordinary appearance (Figs 32-36). Although there is some variation in these features, the differences are obvious and conspicuous. In this context, it should be mentioned that I found a second population of *A. vlassaki* in Saudi Arabia having the same extrusome characteristics as the type population from Namibia, especially the unusual broad end-attachment. The second main distinguishing feature concerns the relative lengths of the oral bulge and dorsal brush: in *A. pachyoplites* the longest brush row (2) is of same length or shorter than the oral bulge (Figs 8, 9, 12, 14, 16, 41), while in *A. vlassaki* the longest brush row (2) is of same length or longer than the oral bulge (Foissner 2000 and Figs 20-22); the average values are 34.4 µm and 27.2 µm *vs.* 21.8 µm and 25 µm. Further, minor differences in sum supporting separation of the South American from the African population: dikinetids very narrowly spaced (~ 1 µm) only in brush row 2 *vs.* rows 2 and 3; body width:oral bulge length ratio 0.56 *vs.* 0.75, that is, bulge relatively longer in *A. pachyoplites* than in *A. vlassaki*; *Arcuospathidium* ciliary pattern less distinct in the former than the latter, as proved by the reinvestigation of the African type population (Figs 20-22). Observations on other populations are needed to prove whether these minor differences are stable or within the range of natural variability of the taxa concerned.

Arcuospathidium pachyoplites differs from *A. etoschense* mainly by the macronucleus, which is tortuous in the former and nodular in the latter. Furthermore, *A. pachyoplites* is considerably stouter than *A. etoschense* (9.0:1 *vs.* 13.7:1), and its extrusomes are much longer and thicker (7 x 1.4 µm *vs.* 3-4 x < 1 µm). *Arcuospathidium pachyoplites* differs from *Spathidium etoschense* by the ciliary pattern, the much longer and tortuous macronucleus, and the thicker (1.4 µm *vs.* 1 µm), and thus highly conspicuous extrusomes.

Spathidium faurefremieti

This species poses the opportunity to discuss some basic problems, especially of recognizing the genus *Supraspathidium* and of species in general. I shall discuss these and other questions in a loose sequence showing my views and ways to solve them.

(1) The genus *Supraspathidium* and generic assignment of *Spathidium faurefremieti*: Foissner and Didier (1981) diagnosed *Supraspathidium* as follows: "Spathidiidae with several to many, serially arranged contractile vacuoles and the oral bulge indistinctly set off from body proper". They assigned to *Supraspathidium* all spathidiids with more than the ordinary posterior contractile vacuole and, unfortunately, fixed as a type species *Spathidium teres* Stokes, 1886, a poorly known species that awaits redescription. Nonetheless, three "typical" *Supraspathidium* species have been described meanwhile: *S. multistriatum* Foissner and Didier, 1981; *S. etoschense* Foissner *et al*., 2002; and *S. armatum* Foissner *et al*., 2002. All have a clear identity and are massive, densely ciliated organisms with one or two rows of contractile vacuoles, each comprising five or more individual vacuoles with several excretory pores. The ciliary pattern is *Epispathidium*-like, with ciliary rows and cilia within rows even more densely spaced, especially at the anterior end of the kineties, where a kinetofragment-like polymerization occurs. Clearly, such spathidiids represent a type of its own and can be classified in the genus *Supraspathidium*, for which I suggest the following refined diagnosis: Massive, densely ciliated Spathidiidae with *Epispathidium*-like ciliary pattern and many (> 5) contractile vacuoles, each having several excretory pores, in one or two rows.

Another group of spathidiids has the ordinary posterior contractile vacuole and a second one in the mid-body region. The general organization and the ciliary pattern of these bivacuolate species look like those of classical spathidiids, as shown by *Arcuospathidium bulli* Foissner, 2000 (Figs 62-64) and *Spathidium faurefremieti* redescribed here. Thus, generic separation would appear premature given the present state of knowledge, though the second contractile vacuole is a distinct feature. On the other hand, it is now obvious that an increased number of contractile vacuoles evolved independently in

several evolutionary lines of the Spathidiidae, viz., in *Spathidium*, *Arcuospathidium* and *Supraspathidium*. Each of these lines can be considered as a distinct evolutionary branch, requiring generic or subgeneric separation, especially when further such species are discovered differing also in other features, for instance, an *Arcuospathidium* with two contractile vacuoles (like *A. bulli*), but scattered macronuclear nodules (unlike *A. bulli*, which has a single, long strand).

(2) Conspecificity of the African and Brazilian population: Several main features, especially the two contractile vacuoles and the shape of the extrusomes agree well, while two other main characteristics do not even overlap, viz., the number of ciliary rows and body width (Table 2). While the latter might be considered as less important because both populations are still slender (body length:width ratio 13.9:1 and 9.6:1, respectively) and body width is known to depend on nutritional state, the former is an important difference because kinety number is rather stable within and between populations in many haptorids (Foissner 1984, Foissner *et al*. 2002). Furthermore, extrusome length also does not overlap (6 µm *vs.* 12 µm). This, however, is probably a minor difference because extrusome length is rather variable in general and the Australian specimens are intermediate (9-10 µm).

Basically, the differences argue to consider the Brazilian population as a distinct subspecies because two main morphometric features do not overlap, of which one is very important. Furthermore, geographic distance would support such a separation (Foissner *et al*. 2002). On the other hand, only one population each has been studied in detail so that the "true" variability of the species is insufficiently known. Furthermore, if such differences are rated too high at the present state of knowledge, it would hardly be possible to identify the populations with the European *S. faurefremieti*, whose description is much more incomplete and thus requires a broad species concept.

In this situation, the most pragmatic solution is to consider all populations as belonging to a single species, but to avoid neotypification, as suggested by Foissner (2002) and Foissner *et al*. (2002), because conspecificity is not beyond reasonable doubt and detailed data on the supposed European population (= *S. faurefremieti*) are lacking. However, if the detailed investigation of an European population shows similar differences as those found between the African and Brazilian populations (Table 2), all should obtain species or subspecies status. Ideally, such data should be supplemented by gene sequences.

(3) Identification: Identification largely depends on the species concept and the treatment of literature data. Our concept is thoroughly discussed in Foissner *et al*. (2002) and thus needs not to be explained here. Briefly, we apply a simple, population-based, morphological concept and identify populations with previously described species, even if data are poor, whenever it is feasible; specifically, at least the general appearance and one main feature must agree.

Tucolesco (1962) provided the following description of *Spathidium faurei* (now *S. faurefremieti*) and a single illustration, reproduced here as figure 59: "Taille 400 µ. Cellule de couleur jaunàtre, de forme allongée et très étroite, à bords parallèles. Pòle postérieur largement arrondi. Partie antérieur de la cellule fortement déviée. Troncanture antérieure presque droite, légèrement inclinée sur l'axe longitudinal. Noyau très long (un peu plus long que la moitié de la cellule) et très mince. Striation serrée. Cils fins, épais et courts. Trouvé dans les eaux souterraines de la grotte Pestera Ialomicioara, en août 1958".

Obviously, my populations agree with Tucolesco's species in several main features, suggesting conspecificity: size, slender shape; short, moderately oblique oral bulge; macronuclear and contractile vacuole pattern. Unfortunately, Tucolesco did not provide information on two other main features: extrusomes and the number of ciliary rows, which is possibly considerably higher than in my populations because Tucolesco's illustration appears to indicate a narrow striation pattern (Fig. 59). Further, details of the shape are different because my specimens are definitely not parallel-sided, especially those from Africa have a distinctly narrowed neck. Possibly, of even greater importance is the habitat difference, viz., soil *vs.* cave water. However, the Australian and the Brazilian populations are from floodplain soils, indicating that my species might occur also in freshwater.

In summary, uncertainties and differences are too pronounced to be entirely confident about conspecificity. Thus, neither the Kenyan nor Brazilian population can serve as a neotype given the present state of knowledge, as explained above.

(4) Comparison with related species: Unfortunately, Tucolesco (1962) did not compare *Spathidium faurefremieti* with any described species, especially *S. vermiforme* Penard, 1922. It is unclear whether he did not know of *S. vermiforme* or considered his

population as sufficiently different to classify it as a new species. In my opinion, *S. vermiforme* and *S. faurefremieti* agree in most main features, especially size, slender shape, and the nuclear and contractile vacuole pattern (Figs 59-61). However, there is an important difference in the habitat: Penard discovered *S. vermiforme* in a sapropelic environment, according to Kahl (1930b), while Tucolesco found *S. faurefremieti* in clean cave water. Sapropelic spathidiids are poorly known, but most are probably different from those living in ordinary freshwaters and soils (Kahl 1926, 1930b; Foissner 1998, and unpublished). Accordingly, synonymy of Penard's and Tucolesco's species is questionable given the present state of knowledge. This is emphasized by slight shape differences (Penard emphasized a dorsal convexity, while Tucolesco mentioned that his species is parallel-sided) and the remark of Penard that one of his populations has symbiotic algae, indicating that he might have mixed two species.

Both populations of *Spathidium faurefremieti* are very similar to *S. procerum* Kahl, 1930a, as redescribed by Foissner (1984), except of that the latter has only one ordinary, posterior contractile vacuole in over one hundred populations checked. At first glance, *Arcuospathidium bulli* Foissner, 2000 is also similar to *S. faurefremieti*, as already discussed by Foissner (2000), because it has two contractile vacuoles. However, the oral bulge is distinctly longer and much more oblique in *A. bulli*, and the ciliary patterns are conspicuously different (Figs 62-64). Thus, *Spathidium faurefremieti*, as redescribed here, is a very distinct species easily recognizable, even *in vivo*, by the long, slender body; the short oral bulge; the long macronucleus; and, especially, the two contractile vacuoles.

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