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ORIGINAL PAPER

W. Foissner

Two new terricolous spathidiids (Protozoa, Ciliophora) from tropical Africa: *Arcuospathidium vlassaki* and *Arcuospathidium bulli*

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Abstract Soil protozoa of the tropics are poorly known. However, the few data available indicate that tropical soils are inhabited by a highly diverse protozoan community. In the present paper, some of the many new ciliate species (about 300) that I discovered in African soils are described. Descriptions are based on standard methods, i.e. living and silver-impregnated specimens. *Arcuospathidium vlassaki* was discovered in a highly saline soil from the margin of the Etosha Pan in Namibia, Southwest Africa. It differs from congeneric species mainly by its rod-shaped macronucleus, fusiform micronuclei, and oblanceolate extrusomes. *Arcuospathidium bulli* was discovered in a savanna soil of Rwanda, East Africa. It is similar to *Arcuospathidium cultriforme* Penard in most features, but has a second contractile vacuole in the anterior body half.

Key words Etosha Pan · Namibia · New species · Soil ciliates · Spathidiidae · Rwanda

Introduction

Biodiversity studies have become topical since the 1992 Rio Convention, where a global programme was initiated for the "discovery, description, and classification of species living in the biosphere". Unfortunately, most biodiversity studies performed since that time have addressed the Rio Declaration only marginally, i.e. have not described and classified species. Indeed, there is now a tendency to abandon the study of organisms at the species level in biodiversity studies altogether, a contradiction in terms, because of the problems associated with the definition of species (Bachmann 1998). Such radical measures totally neglect practical needs and the fact that the majority of species described are considered bona fide by most taxonomists. There is no reason to abandon this whole field of study because of problems in identifying a minority of species. Species, very likely, will remain the keystones of biodiversity, and cannot be substituted by, for instance, functional groups and family diversities in conservation procedures and many scientific studies [see Cotterill (1995) for a review].

As concerns protozoa, most of their diversity is possibly undescribed, especially for the tropics (Foissner 1987, 1997a; Bamforth and Lousier 1995). In the present paper, I describe two of about 300 new ciliate species found in African soils. They are dedicated to colleagues who have an open mind on alpha taxonomy.

Dedicated to Prof. Dr. K. Vlassak on the occasion of his 65th birthday

W. Foissner (✉)
Universität Salzburg, Institut für Zoologie,
Hellbrunnerstrasse 34, A-5020 Salzburg, Austria
Tel.: +43-662-80445615
Fax: +43-662-80445698

Materials and methods

Arcuospathidium vlassaki was discovered in soil from the margin of the Etosha Pan in Namibia, Southwest Africa (about 16°E, 19°S), taken on 1 March 1994. The sample was collected in the salt bush zone (*Suaeda articulata* zone), where the upper 20 cm of the soil is spongy due to the accumulation of brownish, insufficiently decayed plant residues. The loamy soil, which has pH 9.0 (in water), is highly saline, i.e. a salt crust forms when a drop of the soil solution is evaporated on a slide.

Arcuospathidium bulli was discovered in a soil sample from the Virunga National Park in Rwanda, East Africa (about 30°E, 2°S), taken by Prof. E. Stüber (Haus der Natur, Salzburg) on 27 January 1987. The sample was collected under *Acacia* trees and various bushes. It consisted of plant litter and brownish and yellowish soil crumbs from the upper 0–5 cm.

Both samples were air-dried for 4 weeks in the laboratory of the Zoology Institute (University of Salzburg). The ciliates were reactivated in July 1988 (Rwandan sample) and May 1994 (Namibian sample) from the resting cysts by the non-flooded Petri dish method, as described in Foissner (1987). Briefly, this simple method involves placing 10–50 g soil in a Petri dish (10–15 cm wide,

2–3 cm high) and saturating, but not flooding it, with distilled water. These cultures were analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, and 28. The descriptions of the new species are based on material obtained from such cultures, i.e. no clones were set up. However, *A. vlassaki* was cultivated in a cleaner medium, i.e. part of the soil solution was poured into a Petri dish containing some cracked, sterilised wheat grains to stimulate the growth of bacterivorous ciliates (mainly *Pseudocohnilembus* sp.), which served as food for *A. vlassaki*. *Arcuospathidium vlassaki* and the food ciliates co-existed and divided readily in these cultures.

Morphological methods followed those used in my previous studies (e.g. Foissner 1991, 1996a, 1997b) and thus need not to be detailed here. Briefly, live specimens were studied with bright field and interference contrast, and preparations were made with protargol (*protocol A* in Foissner 1991).

Results and discussion

Morphometric data shown in Table 1 are repeated only if needed for clarity

Arcuospathidium vlassaki nov. sp. (Figs. 1–21; Table 1)

Diagnosis

Size in vivo about 200 × 18 μm . Slenderly cylindroidal with inconspicuous, obliquely truncated oral bulge occupying about 13% of body length. Macronucleus rod-shaped, 83 μm long on average. Micronuclei numerous and conspicuously fusiform. Single contractile vacuole in posterior end. Extrusomes oblanceolate, 5 × 1 μm. Eleven somatic kineties on average, three of which differentiated anteriorly to an inconspicuous dorsal brush.

Type location

Highly saline soil from the margin (*Suaeda articulata* zone) of the Etosha Pan, Namibia (about 16°E, 19°S).

Type slides

Two slides (one holotype and one paratype) with many protargol-impregnated, morphostatic and dividing specimens have been deposited in the Oberösterreichische Landesmuseum in Linz , Austria. Relevant cells are marked by a *black circle* on the cover glass.

Dedication

Named in honour of Prof. Dr. K. Vlassak (Katholieke Universiteit Leuven, Belgium), co-editor of Biology and Fertility of Soils, on the occasion of his 65th birthday.

Description

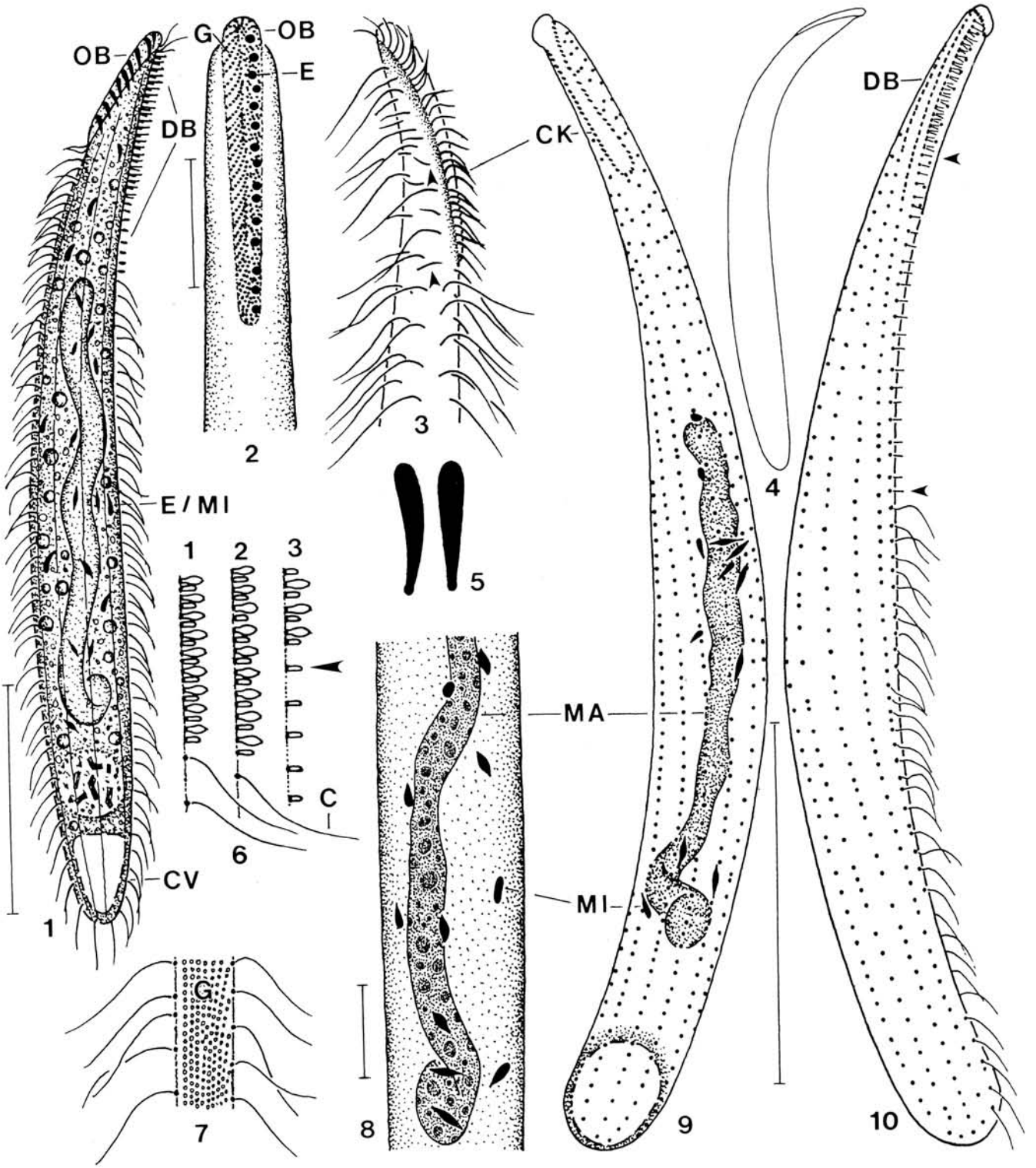
Size highly variable, in vivo 100–260 × 15–25 μm, usually about 200 × 18 μm; length:width ratio also highly variable, i.e. 1:5–1:18, on average 1:11. Cylindroidal, flattened only in obliquely truncated oral region, usually widest in mid-body, largest specimens often distinctly curved (Figs. 1, 4, 9, 16, 17). Macronucleus in middle third of cell, rod-shaped, occasionally tortuous; contains many small and large nucleoli. Micronuclei near macronucleus and scattered in cytoplasm, conspicuously fusiform (Figs. 1, 8, 9), as also evident in middle dividers (Figs. 13–15), and thus easily confused with extrusomes. Single contractile vacuole in posterior body end. Extrusomes attached with broad end to left half of oral bulge (Figs. 1, 2) and scattered in cytoplasm, in vivo oblanceolate, slightly curved, compact, and about 5 × 1 μm in size (Figs. 5, 18–21), usually do not stain with protargol. Cortex flexible, colourless, contains narrowly spaced rows of granules about 0.2 μm across (Fig. 7); granule rows occur also in oral bulge cortex (Fig. 2). Cytoplasm colourless, without crystals, well-fed specimens dark at low magnification ($\leq \times 100$) due to many fat globules 1–5 μm across and 15-μm wide vacuoles with prey remnants, i.e. mainly crystals from hypotrichs (Fig. 1). Feeds on various ciliates, such as *Vorticella astyliformis*, *Pseudocohnilembus* sp., and hypotrichs; overfed specimens heavily deformed. Movement serpentine and slow.

Somatic cilia rather widely and irregularly spaced, about 10 μm long, except for oral area where 3 to 5-μm-long bristles alternate with ordinary cilia (Fig. 3). Cilia form longitudinal rows anteriorly curved to dorsal side and distinctly separate from circumoral kinety (Figs. 9–12). Dorsal brush at anterior end of three dorsolateral kineties, rows one and two about as long as oral bulge, row three shortened, but continues with monokinetidal 2-μm-long bristles to second quarter of cell; brush dikinetids narrowly spaced, anterior cilium 2.5–3 μm long and slightly inflated distally, posterior cilium about 1.5 μm long and rod-shaped (Figs. 1, 6, 10, 12).

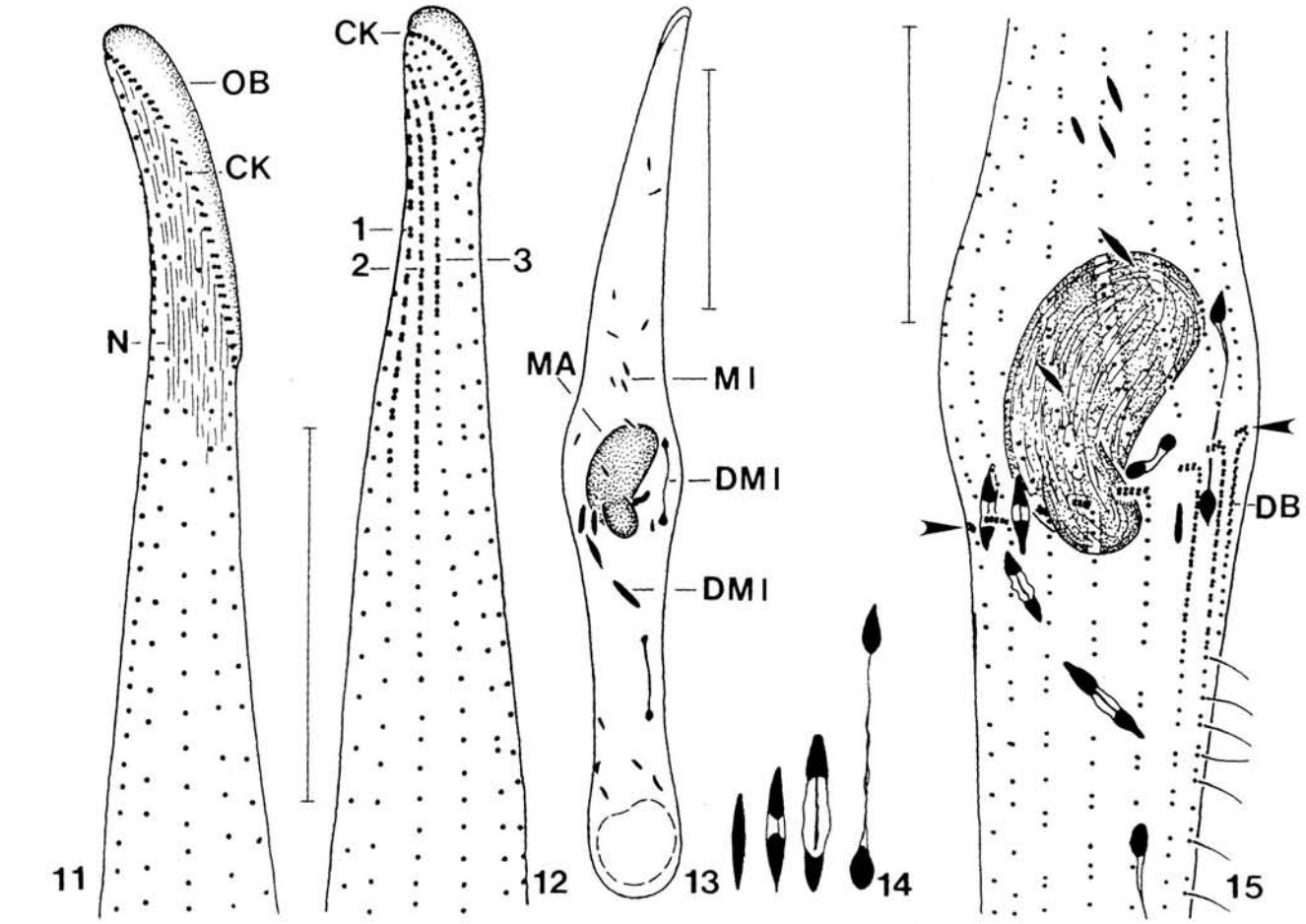
Oral bulge distinctly oblique and slenderly cuneate, hyaline, inconspicuous because only about 25 μm long and 3 μm high, left bulge half filled with extrusomes, as described above, appearing as conspicuous, bright dots when bulge is viewed frontally (Fig. 2). Circumoral kinety at base of oral bulge, slenderly cuneate, composed of rather widely spaced dikinetids each bearing a cilium only about 4 μm long (Figs. 1–3, 9, 11, 12, 16, 17). Nematodesmata (oral basket rods) fine, only very lightly impregnated with protargol (Fig. 11).

Occurrence and distribution

As yet found only at type location, a highly saline soil.



Figs. 1–10 *Arcuospathidium vlassaki* from live specimens (**1–7**) and after protargol impregnation (**8–10**). **1** Left lateral view of a representative specimen. Extrusomes and micronuclei (*E/MI*) have a similar size and shape and are thus difficult to distinguish in vivo. **2** Frontal view of oral bulge, which contains extrusomes only in the left half. **3** Short bristles (*arrowheads*) irregularly alternate with ordinary cilia in the oral portion. **4** Slender shape variant. **5** Two views of the same extrusome, length 5 μm. **6** Posterior brush portion; row three continues posteriorly with single bristles (**10**). **7** Surface view showing cortical granulation. **8** Nuclear apparatus. Note fusiform (more or less lanceolate when viewed obliquely) to elongate ellipsoidal micronuclei, a specific feature of *A. vlassaki*. **9, 10** Ciliary pattern of ventral and dorsal side of same specimen. *Arrowheads* mark monokinetidal tail of brush row three. *Scale bars* 10 μm (**2, 8**) and 50 μm (**1, 9, 10**). *C* ordinary somatic cilium, *CV* contractile vacuole, *CK* circumoral kinety, *DB* dorsal brush, *G* cortical granules, *MA* macronucleus, *OB* oral bulge, (**1–3**) dorsal brush rows



Figs. 11–21 *Arcuospathidium vlassaki* from live specimens (**16–21**) and after protargol impregnation (**11–15**). **11, 12** Ciliary pattern of anterior right and dorsal portion. Note that brush row three is shorter than rows one and two because the bristle tail (**1, 6, 10**) is monokinetidal and thus not recognisable. **13, 15** Middle divider, overview and detail of fission area. The rod-shaped macronucleus (**9**) fused to a globular mass and the micronuclei commence to divide. Small, perpendicularly orientated kinetofragments, which will fuse to form the circumoral kinety, are recognisable at the anterior end of all divided ciliary rows (*arrowheads*; holotelokinetal stomatogenesis). New, not yet ciliated basal bodies are generated within the ciliary rows. **14** Micronucleus division. The micronuclei of *A. vlassaki* have a unique, fusiform shape. **16, 17** Right lateral view of a slender specimen and ventral view of a very slender and long (260 μm) cell. *Arrowheads* mark oral bulge **18–21** The extrusomes (*arrowheads*) are 5 μm long and conspicuously lanceolate. *CK* circumoral kinety, *CV* contractile vacuole, *DB* newly formed dorsal brush, *DMI* dividing micronuclei, *MA* macronucleus, *MI* micronuclei, *N* nematodesmata, *OB* oral bulge, *1, 2, 3* dorsal brush rows. *Scale bars* 25 μm (Figs. **11, 12, 15**) and 50 μm (Fig. **13**)

Generic classification and comparison with related species

Arcuospathidium vlassaki is a typical member of the genus, as defined by Foissner (1984), i.e. has a slenderly cuneate oral bulge and ciliary rows, which are distinctly separate from the circumoral kinety and directed dorsally on both sides of the oral area.

Size, shape, ciliary pattern and terrestrial habitat of *A. vlassaki* highly resemble that of *Arcuospathidium vermiforme* Foissner 1984 and *Arcuospathidium cooperi* Foissner 1996. However, *A. vlassaki* can be easily distinguished from these two species by two "strong" features: *A. vermiforme* has two ellipsoidal macronuclear nodules and lacks extrusomes; *A. cooperi* has an elongate reniform macronucleus with a single micronucleus and lacks, like *A. vermiforme*, extrusomes. Other congeneric species also have a different nuclear apparatus or body shape and/or number of ciliary rows (for literature, see Foissner 1998). No *Spathidium* species was found in the older literature which was identical to *A. vlassaki*.

Arcuospathidium bulli nov. sp. (Figs. 22–43; Table 1)

Diagnosis

Size in vivo about 260 × 25 μm. Slenderly cylindroidal with conspicuous, obliquely truncated oral bulge oc-

Table 1 Morphometric data from *Arcuospathidium vlassaki* (*upper line*) and *Arcuospathidium bulli* (*lower line*), based on protargol-impregnated and mounted specimens. Measurements in micrometres. $\bar{x}$ mean, *M* median, *SD* standard deviation, *SE* standard error, *CV* coefficient of variation in %, *Max* maximum, *Min* minimum, *n* number of individuals investigated

Characteristics	$\bar{x}$	M	SD	SE	CV	Max	Min	n
Body, length	162.3	160	43.0	9.4	26.5	94	260	21
	223.0	200	47.8	14.4	21.4	156	295	11
Body, maximum postoral width	16.4	16	2.6	2.7	16.2	13	25	21
	21.6	21	3.2	1.0	15.0	17	28	11
Circumoral kinety, length[a]	21.8	21	5.2	1.1	24.1	13	37	21
	62.0	60	9.9	3.0	16.0	50	80	11
Macronucleus, length[b]	82.9	80	32.0	7.0	38.6	40	160	21
	121.4	103	40.1	12.1	33.0	75	200	11
Macronucleus, width	5.1	5	0.6	0.1	11.2	4	6	21
	4.0	4	0.9	0.3	22.4	3	5	11
Micronucleus, length	3.6	4	0.6	0.1	16.7	3	5	21
	3.2	3	–	–	–	3	4	11
Micronucleus, width	1.0	1	–	–	–	~1		21
	3.1	3	–	–	–	~3		11
Brush row one, length[c]	20.8	20	6.3	1.4	30.4	13	35	21
	47.9	49	9.5	2.9	19.9	35	60	11
Brush row two, length[c]	25.0	25	5.4	1.2	21.7	16	40	21
	55.7	54	11.2	3.4	20.1	42	80	11
Brush row three, length[c,d]	17.7	17	3.7	0.8	20.9	12	27	21
	40.6	35	10.0	3.0	24.7	30	60	11
Somatic kineties, no.	11.6	11	1.5	0.3	12.9	9	15	21
	22.7	22	1.7	0.5	6.8	21	26	11
Basal bodies, no. in a right lateral kinety[e]	64.7	65	16.2	3.5	25.1	38	100	21
	101.7	95	21.9	6.6	21.5	75	145	11
Brush rows, no.	3.0	3	0.0	0.0	0.0	3	3	21
	3.0	3	0.0	0.0	0.0	3	3	11
Brush row one, no. of dikinetids	15.7	15	4.1	0.9	26.0	12	30	21
	48.0	50	8.1	2.4	16.8	29	60	11
Brush row two, no. of dikinetids	24.4	24	4.6	1.0	18.9	19	35	21
	49.6	50	7.2	2.2	14.6	36	60	11
Brush row three, no. of dikinetids	16.8	17	3.5	0.8	21.0	10	27	21
	26.4	27	3.6	1.1	13.8	20	32	11
Macronuclei, no.	1.0	1	0.0	0.0	0.0	1	1	21
	1.0	1	0.0	0.0	0.0	1	1	11
Micronuclei, no.	11.6	12	2.7	0.6	22.1	7	17	21
	21.8	20	10.9	3.3	50.2	7	40	11
Anterior contractile vacuole, distance to anterior cell end	–	–	–	–	–	–	–	–
	81.8	71	19.3	5.8	23.6	65	130	11
Anterior contractile vacuole, no. of pores	–	–	–	–	–	–	–	–
	5.8	5	1.7	0.5	28.6	3	8	11

[a]Measured as length of cord from distal to proximal end of kinety
[b]*A. vlassaki*: optically "uncoiled" if tortuous, measurements thus inexact. *A. bulli*: measured as length of nuclear figure, i.e. in coiled condition
[c]Measured as distance from circumoral kinety
[d]Excluding monokinetidal tail
[e]Including unciliated granules in line with ciliated ones

cupying about 27% of body length. Macronucleus very long and tortuous. Micronuclei numerous and globular. Two contractile vacuoles, one in anterior body half, the other in rear end. Extrusomes pencil shaped, 4×1 μm. 22 somatic kineties on average, three of which differentiated anteriorly to inconspicuous dorsal brush.

Type location

Savanna soil near the village of Gabiro, Virunga National Park, Rwanda (about 30°E, 2°S).

Type slides

Two slides (one holotype and one paratype) with protargol-impregnated specimens have been deposited in the Oberösterreichische Landesmuseum in Linz, Austria. Relevant cells are marked by a *black circle* on the cover glass.

Dedication

Named in honour of Prof. Alan T. Bull (University of Kent, UK), chief editor of *Biodiversity and Conservation*, who has an open mind on alpha taxonomy.

Description

Size highly variable, in vivo 190–300×20–40 μm, usually about 260×25 μm, contracts by up to 30% under slight cover glass pressure; length:width ratio also very variable, 7:1–15:1, on average 10:1. Cylindroidal, flattened only in obliquely truncated oral region, usually widest in mid-body region (Figs. 22, 25, 34). Macronucleus in middle third of cell, long and tortuous, occasionally composed of two to three long pieces; contains many small and large nucleoli. Micronuclei near macronucleus, globular, not as compact as is usual (Figs. 22, 34). One to three contractile vacuoles, usually one in anterior body half and a second in the rear end, both with several excretory pores; anterior vacuoles with short collecting canals (Figs. 22, 25, 32, 35). Extrusomes (toxicysts) attached with pointed end to left half of oral bulge and scattered in cytoplasm (Figs. 22, 24, 34, 39–43); in vivo shaped like a blunt pencil and about 4×1 μm in size, very compact and thus appearing as bright dots when viewed frontally (Figs. 24, 27, 37, 38); exploded toxicysts in vivo club shaped and about 8 μm long, hyaline with double-contoured wall (Fig. 28); cytoplasmic toxicysts with darkly impregnated anterior rod and small globule in posterior end of trunk (Figs. 29, 43); partially extruded bulge toxicysts with an intensively impregnated globule, both in proximal half of rod and posterior end of trunk (Figs. 30, 42). Cortex very flexible, colourless, contains narrowly spaced rows of granules about 0.5 μm across (Fig. 26); granule rows also in oral bulge cortex. Cytoplasm colourless, without crystals, well-fed specimens dark at low magnification (≤ ×100) due to many fat globules 1–5 μm wide. Feeds mainly on rotifers and ciliates. Glides slowly on and between soil particles and on slide surface.

Somatic cilia rather widely and irregularly spaced, about 10 μm long, form longitudinal rows anteriorly curved to dorsal side and distinctly separate from circumoral kinety (Figs. 22, 32, 33). Dorsal brush at anterior end of three dorsolateral kineties, rows one and two each about 50 μm long, row three shorter, but continues with monokinetidal 2-μm long bristles to second quarter of cell; brush dikinetids narrowly spaced except in row three, anterior cilium about 4 μm long and lanceolate, posterior cilium 2 μm long and rod-shaped (Figs. 22, 23, 32, 35, 42).

Oral bulge distinctly oblique and slightly convex, slenderly cuneate, conspicuous because about 70 μm long and filled with thick, bright extrusomes (Figs. 22, 24, 25, 34, 36, 37, 39, 42). Circumoral kinety at base of oral bulge, slenderly cuneate, composed of rather widely spaced dikinetids (Figs. 32, 33, 36, 39–41). Nematodesmata (oral basket rods) distinct, form long, obliquely extending bundles (Figs. 34, 39).

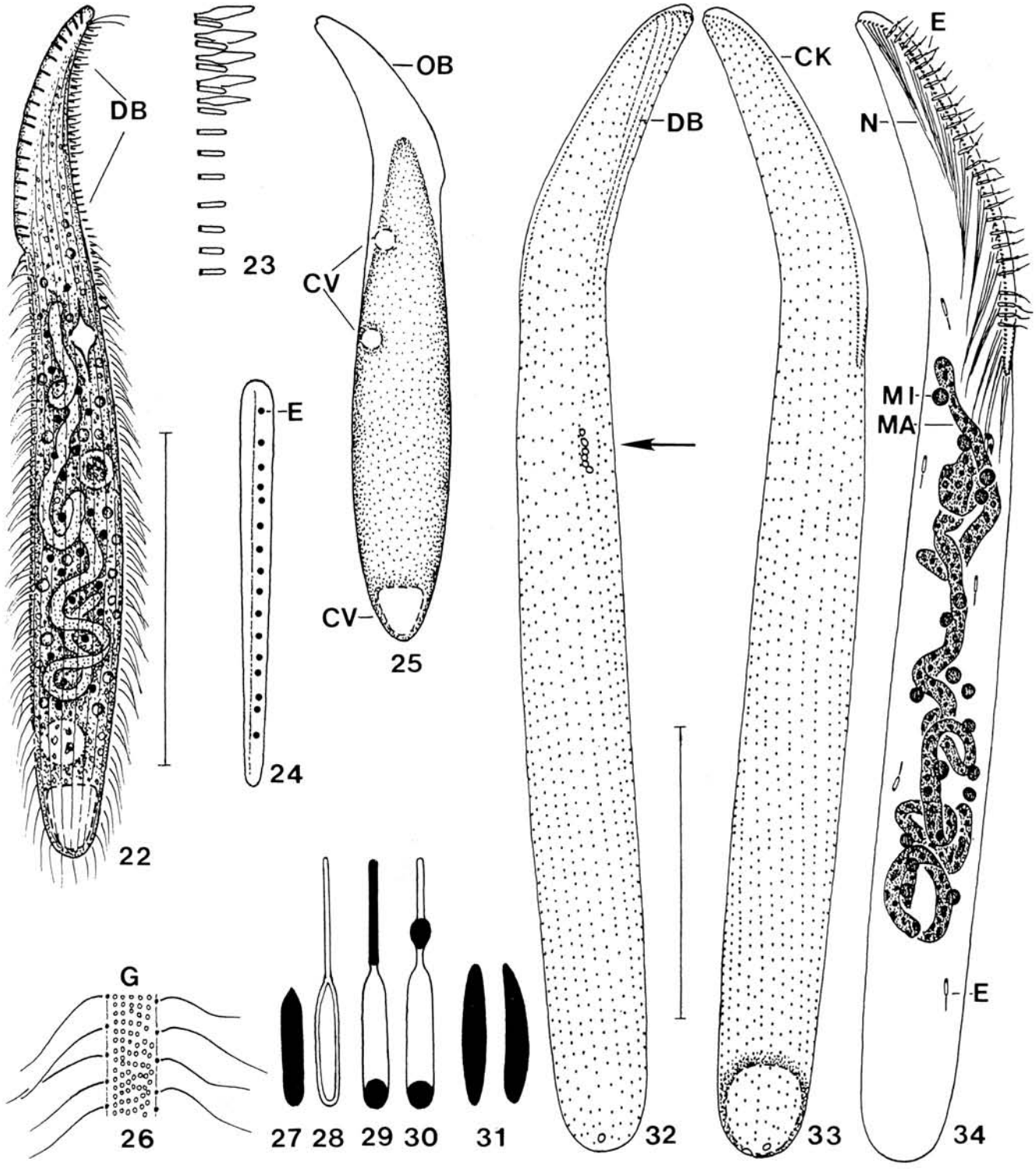
Occurrence and distribution

Found in a savanna soil in Rwanda (tropical Africa, type location) and, with exactly the same characteristics, in a sandy, slightly saline coastal soil (pH 7.5) of Phuket Island, Thailand. A population from soil of the surroundings of Lake Titicaca (Peru) possessed fusiform, slightly curved extrusomes (Fig. 31), indicating that it might be a distinct subspecies. This is supported by the specimens from Thailand, whose extrusomes were exactly like those from the type population. However, at the present state of knowledge, such differences appear insufficient for establishing distinct taxa.

Generic classification and comparison with related species

Aruospathidium bulli has two to four contractile vacuoles and should thus be classified within the genus *Supraspathidium* Foissner and Didier, 1981. However, the general organisation and the ciliary pattern are highly similar to the type species of *Arcuospathidium*, i.e. *A. cultriforme*, as redescribed by Foissner (1984). Thus, as a consequence of this and because the characteristic "several contractile vacuoles" possibly evolved independently in several spathidiids, I assigned the Rwandan population to *Arcuospathidium*.

Generally, the spathidiid genera are distinguished by rather indistinct features, sometimes making it difficult to assign a certain population unequivocally (Foissner



Figs. 22–34 *Arcuospathidium bulli* from live specimens (**22–28, 31**) and after protargol impregnation (**29, 30, 32–34**). **22** Left lateral view of a representative specimen. **23** Posterior portion of dorsal brush row three, which has a monokinetidal bristle tail (not shown in full length) extending to mid-body. **24** Frontal view of oral bulge, which contains extrusomes only in left half. **25** Broad shape variant with three contractile vacuoles. **26** Surface view showing cortical granulation. **27** Bulge extrusome (toxicyst) in vivo, about 4×1 μm. **28** Exploded bulge toxicyst in vivo, about 8×1 μm. **29, 30** Cytoplasmic and partially exploded bulge toxicysts after protargol impregnation. **31** Two views of the same extrusome from a Peruvian population. **32–34** Main cytoplasmic organelles and infraciliature of left and right side of same specimen. *Arrow* marks excretory pores of the anterior contractile vacuole, a main character of *A. bulli*. *CK* circumoral kinety, *CV* contractile vacuoles, *DB* dorsal brush, *E* extrusomes, *G* cortical granules, *MA* macronucleus, *MI* micronucleus, *N* nematodesmata (oral basket rods), *OB* oral bulge. *Scale bars* 100 μm (Fig. **22**) and 50 μm (Figs. **32–34**)