

# Morphology, ontogenesis and encystment of a soil ciliate (Ciliophora, Haptorida), *Arcuospathidium cultriforme* (Penard, 1922), with models for the formation of the oral bulge, the ciliary patterns, and the evolution of the spathidiids

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## Summary

We studied and reviewed the morphology, ontogenesis and encystment of two closely related spathidiids, *Arcuospathidium cultriforme* (Penard, 1922) Foissner, 1984 and *A. scalpriforme* (Kahl, 1930) Foissner, 2003, using live observation, protargol impregnation, morphometry, scanning electron microscopy, populations from different geographic regions, and literature data. Both species are biogeographic flagships. They are 200-300 µm long and have a long, steep oral bulge. Our investigations show that they are very similar, differing mainly in the length of the oral bulge and the arrangement of the oral bulge extrusomes. Thus, we classify them as subspecies within the *Arcuospathidium cultriforme* complex, which includes the following taxa: *A. cultriforme cultriforme* (Penard, 1922), *A. cultriforme scalpriforme* (Kahl, 1930), *A. cultriforme megastoma* Foissner et al., 2002, and *A. lorjeae* Foissner et al., 2002. The latter taxa are likely to have a restricted geographic distribution, and there is evidence that the South American and Rwandan populations of *A. cultriforme cultriforme* could represent further distinct (sub)species. During encystment of *A. cultriforme*, the macronucleus becomes strongly shortened and the infraciliature appears to be resorbed. The mature cyst is unique in having a very thick, faceted wall. A detailed scenario of the ontogenetic processes is provided, showing that the ontogenesis of *A. cultriforme* matches those of haptorids in general and other spathidiids in particular. However, there are several characteristic features, for some of which we could find reasonable explanations which, in turn, provided models for the spathidiid ontogenesis and evolution: (i) the “outgrowth model” suggests that the slope of the long spathidiid oral bulge is obtained mainly by faster growth of the dorsal than the ventral side; (ii) the “row detachment model” proposes that the *Arcuospathidium* and *Epispathidium* ciliary patterns are variations of the *Spathidium* pattern which evolved from the *Protospathidium* pattern; and (iii) a model is proposed for a *Dileptus*-like ancestor of the spathidiids.

**Key words:** Hawaii, outgrowth model, phylogeny, row detachment model, South Africa, *Spathidium cultriforme*, *Spathidium lionotiforme*, *Spathidium scalpriforme*

## Introduction

Both morphology and ontogenesis appear highly similar in various haptorid ciliates due to their comparatively simple organization supporting homoplasies (Foissner and Foissner, 1988; Xu and Foissner, 2003). Only on detailed investigation the differences become evident. Typical examples are the time-honoured genera *Trachelophyllum* and *Spathidium*. *Trachelophyllum* has been split into six genera based on the structure of the epicortical scales, and there is evidence for many more undescribed genera and species (Foissner, 2005b; Foissner, unpubl. data). The genus *Spathidium*, which consisted of about 200 species when Dragesco and Dragesco-Kernéis (1979) and Foissner (1984) split it into five genera, now comprises about 300 species distributed over 15 genera (Foissner and Xu, 2005). Similar, but usually less extreme examples can be found in all groups of ciliates, for instance, the hypotrichs (Berger, 1999) and colpodids (Foissner, 1993; Foissner et al., 2002).

Such data and a recent monograph on soil ciliates from Namibia (Foissner et al., 2002) suggest that haptorids are heavily undersplit and their diversity is much greater than previously assumed. Obviously, there are thousands of these often inconspicuous species. Thus, Finlay's (2001) estimation of only 3,000–4,000 free-living ciliate morphospecies is flawed by the facts. There is a continuous flow of new families, genera and species from limnetic, marine, and soil habitats when investigations are performed by experienced taxonomists (Foissner et al., 2002; Song et al., 2003). We also disagree with Finlay's (2001) hypotheses that protists are ubiquitous and cosmopolitan. The soil provides strong arguments against: more than a half of the about 800 species has been found only in one biogeographic region (Foissner, 1998) and many samples contain one or several flagship species not found in more than 1,000 other soil samples collected world-wide (Foissner, 1995, 1999a, 2004a, b, 2005a, b; Foissner et al., 2002, 2005).

The present study reports the morphology and ontogenesis of the *Arcuospathidium cultriforme* complex, which contains large and thus conspicuous species serving as biogeographic flagships. The study provides not only an evidence of a restricted distribution of some taxa of the complex, but also new models for various aspects of the spathidiid ontogenesis and evolution.

## Material and Methods

Over the years, many populations of *A. cultriforme* from soils all over the world were routinely identified according to Penard (1922), Kahl (1930b), and Foissner

(1984). The following compilation contains only those sites where notes and/or preparations of the specimens were made.

### *Arcuospathidium cultriforme cultriforme*

Austria, Burgenland: Upper litter and soil layer (0–2 cm) of a coniferous forest (Ebenauerwald) in southern Burgenland, eastern Austria. This population was described by Foissner (1984).

Austria, Linz: Mud and soil from a flat, ephemeral pond at the foot of the Pöstlingberg, in the surroundings of the town of Linz, capital of Upper Austria.

Germany, Kassel: Litter and soil from the upper 0–5 cm layer of a beech forest on the “Kleinen Gudenberg” about 30 km NW of the town of Kassel. Sample kindly provided by Dr. M. Bonkowski (Göttingen University).

Dominican Republic, site 22: Litter and soil from the upper 0–3 cm layer of a mangrove forest near the village of Maimon, about 10 km west of the town of Puerto Plata. Soil almost black and spongy, contains a lot of grass and mangrove roots, slightly acidic (pH 6.2 in water) and saline (5‰).

Costa Rica, site 11: Tree mosses from the evergreen rain forest in the Braulio-Carillo National Park, Mirador area; pH 4.7 in water.

South America, Venezuelan site 8: Soil from the upper 3 cm of a banana plantation at the farm of Mr. Eisenberg, surroundings of the town of Puerto Ayacucho. The field was founded 15 years ago and has been organically fertilized. The slightly acidic (pH 6 in water) soil is dark and contains many fine roots, but the layer is only about 5 cm thick and followed by yellow sand.

South America, Venezuelan site 20: Tree mosses from a gallery forest in the surroundings of the town of Puerto Ayacucho.

Tropical Africa, Rwanda: Savannah litter and soil from the surroundings of the village of Gabiro, at the foot of the mountains with the Gorilla National Park. Sample kindly provided by Dr. E. Stüber (Salzburg).

Hawaii, Big Island site 12: Highly saline (~ 20‰) mud and soil from dry rockpools in the lower part of a stream between the Kahua Ranch and Kawaihae.

Hawaii, Big Island site 39: Tree bark with mosses and lichens along the Bird Trail in the Volcano National Park; pH 7.2 in water.

Hawaii, Oahu Island site 50: Litter and soil from the upper 0–3 cm layer of a dry swamp overgrown with ferns, Ihi'Ihlanrkea crater area, Koko Head, south of the Hanauma Bay. Sample kindly provided by Mag. Hubert Blatterer (Linz).

### *Arcuospathidium cultriforme scalpriforme*

Austria, Lower Austria: Litter and soil from the upper 0–5 cm layer of a mixed forest (*Asperulo-Fagetum*)

in the surroundings of the village of Baumgarten. Brown-earth soil with many roots and neutral reaction (pH 7 in water). This population was described by Foissner (1984).

South America, Brazil site 30: Litter and soil from the upper 0-5 cm of the floodplain rainforest near the town of Manaus, Janauari region. Soil brown, humic, pH 5.1 in water.

Republic of South Africa, site 44: Litter and soil from the margin of a flat pond (Sirkelsvlei) in the Cape Peninsula National Park. Soil dark, very sandy, with many grass roots, flooded after heavy rain, pH 5.4 in water.

All populations developed in non-flooded Petri dish cultures, as described by Foissner et al. (2002). The stored, air-dried samples were saturated with distilled water and investigated for ciliates weekly. Sufficient specimens for preparations usually developed two weeks after rewetting. No pure cultures were set up, except for the South African population of *A. cultriforme scalpriforme*, where ontogenesis was studied. A semipure culture of this species was obtained by adding some millilitres of percolate from the non-flooded Petri dish culture to a Petri dish filled with Eau de Volvic (French table water) and some crushed wheat grains. A dense culture with many dividers and monsters developed, using as food source the natural ciliate community introduced with the soil percolate.

Specimens were studied *in vivo* using a high power, oil immersion objective and differential interference contrast optics. The ciliary pattern and various cytological structures were revealed by Foissner's or Wilbert's protargol protocol, both described in Foissner (1991). Counts and measurements of stained specimens were performed at a magnification of  $\times 1,000$ . *In vivo* measurements were conducted at magnifications of  $\times 100$ -1,000. Illustrations of live specimens were based on free-hand sketches and video records, while those of cells on preparations were made with a camera lucida. Terminology is mainly according to Corliss (1979) and Foissner (1996).

Scale bars are liked by reviewers and editors. However, in the case of drawings they are usually circumstantial and superfluous, if the size of the specimen is indicated in the figure explanation. The old taxonomists knew that. Imagine Kahl with countless scale bars on the plates! Thus, we adopted the old style and, when appropriate, give the size of the specimen in the figure explanation, not the scale bar in the figure.

The situation is more complex with micrographs. At first glance, scale bars appear indispensable, but when considered critically, they might also be superfluous or even misleading. A good description provides *in vivo* measurements of the main features, such as body length and width (first data set); a good description also provides detailed morphometrics from permanent silver prepa-

rations where, however, the specimens are more or less shrunken (second data set); and when the description contains SEM micrographs, a third data set is generated by scale bars because 20-60% shrinkage is usual in SEM preparations. Now, we have three different values and scale bars for the same structure! Further bias is generated when specimens are more or less flattened by mild coverslip pressure to get detail-rich micrographs. All these problems and the fact that sizes are given in the description and the tabulated morphometrics suggest that scale bars usually can be omitted from micrographs without any loss of important information. Indeed, they are often misleading, indicating higher precision than the actual one. \*)

## Results

### *Arcuospathidium cultriforme* (Penard, 1922) Foissner, 1984

1922 *Spathidium cultriforme* Penard, Infusoires, p. 25.

1984 *Arcuospathidium cultriforme* (Penard, 1922) nov. comb. — Foissner, Stapfia, 12: 78 (designation as type species of the genus *Arcuospathidium*).

2002 *Arcuospathidium cultriforme cultriforme* (Penard, 1922) Foissner, 1984 nov. stat. — Foissner, Agatha and Berger, Denisia, 5: 299 (improved diagnosis and ranking as a subspecies).

**Improved diagnosis:** Size 150-450  $\times$  20-90  $\mu\text{m}$  *in vivo*, usually near 240  $\times$  40  $\mu\text{m}$ . Narrowly to very narrowly knife-shaped with oblique to strongly oblique, very narrowly cuneate to oblong oral bulge occupying 1/3, 1/2 or 2/3 of body length. Macronucleus an indistinctly nodulated, tortuous strand, rarely occur two long pieces, many small nodules or a mixture of nodules and short strands; multimicronucleate. Extrusomes arranged in rows one row in the right and the left half of oral bulge or scattered, basically oblong with a size of 4-8  $\times$  0.7-1.1  $\mu\text{m}$  *in vivo*. On the average 25-37 ciliary rows, 3 anteriorly differentiated to dorsal brush occupying 23-36%, usually about 28% of body length; bristles up to 5  $\mu\text{m}$  long *in vivo*. Resting cyst with thick, faceted wall.

**Etymology:** Not given in the original description. Possibly, *cultriforme* (knife-shaped) refers to both the body (“corps allongé en forme de couteau ...”) and the exploded toxicysts (“... couteau suédois fermé ... mais le couteau s'est ouvert”).

**Remarks:** The diagnosis is based on Foissner et al. (2002) who distinguish three subspecies according to

\*) The editors of the "Protistology" requested to insert the scale bars on the pictures anyway.

the length of the oral bulge; we add the arrangement of the extrusomes: *A. cultriforme cultriforme* (mouth about 1/3 of body length, a row of extrusomes in the right and the left bulge half), *A. cultriforme scalpriforme* (mouth about 1/2 of body length, bulge extrusomes scattered), *A. cultriforme megastoma* (mouth about 2/3 of body length, a row of extrusomes each in right and left bulge half). *Arcuospathidium cultriforme cultriforme* and *A. cultriforme scalpriforme* are the subjects of the present study, while *A. cultriforme megastoma*, for which we do not have any new data, is described in Foissner et al. (2002).

***Arcuospathidium cultriforme cultriforme* (Penard, 1922) Foissner, 1984** (Figs 1-4a, 5-15; Table 1)

1922 *Spathidium cultriforme* Penard, Infusoires, p. 25.

1930 *Spathidium cultriforme* Penard – Kahl, Arch. Protistenk., 70: 383 (brief redescription; likely a misidentification).

1930 *Spathidium cultriforme* Penard, 1922 – Kahl, Tierw. Dtl., 18: 164 (revision).

1975 *Spathidium cultriforme* Penard, 1922 – Fryd-Versavel, Iftode and Dragesco, Protoplastologia, 11: 511-514 (brief redescription from protargol-impregnated, Italian specimens).

1984 *Arcuospathidium cultriforme* (Penard, 1922) nov. comb. – Foissner, Stapfia, 12: 78 (redescription from life and after protargol impregnation of Austrian specimens from Burgenland).

2002 *Arcuospathidium cultriforme cultriforme* (Penard, 1922) Foissner, 1984 nov. stat. – Foissner, Agatha and Berger, Denisia, 5: 299 (improved diagnosis and ranking as a subspecies).

**Improved diagnosis:** Mouth (oral bulge) about one third of body length. A row of extrusomes in the right and the left bulge half, rarely only a single row in left half.

**Type locality:** Mosses in Switzerland (Chemin de la Montagne, on an old wall).

**Morphology:** The description includes the above literature data and the populations mentioned in the Material and Methods section. The morphometric data compiled in Table 1 are repeated only if appropriate.

Size considerably variable within and between populations, with average values ranging from  $192 \times 28 \mu\text{m}$  to  $306 \times 45 \mu\text{m}$  and variation coefficients of up to 18% (Table 1). Taking into account the *in vivo* measurements and 10-20% preparation shrinkage, *in vivo* ranges of  $150\text{--}450 \times 20\text{--}90 \mu\text{m}$  have been calculated, with an overall average of  $240 \times 40 \mu\text{m}$ , which is close to Penard's values ( $200\text{--}260 \times 40\text{--}50 \mu\text{m}$ ). Length:width ratio highly variable within populations (CV 21-37%), but rather constant between populations, ranging from

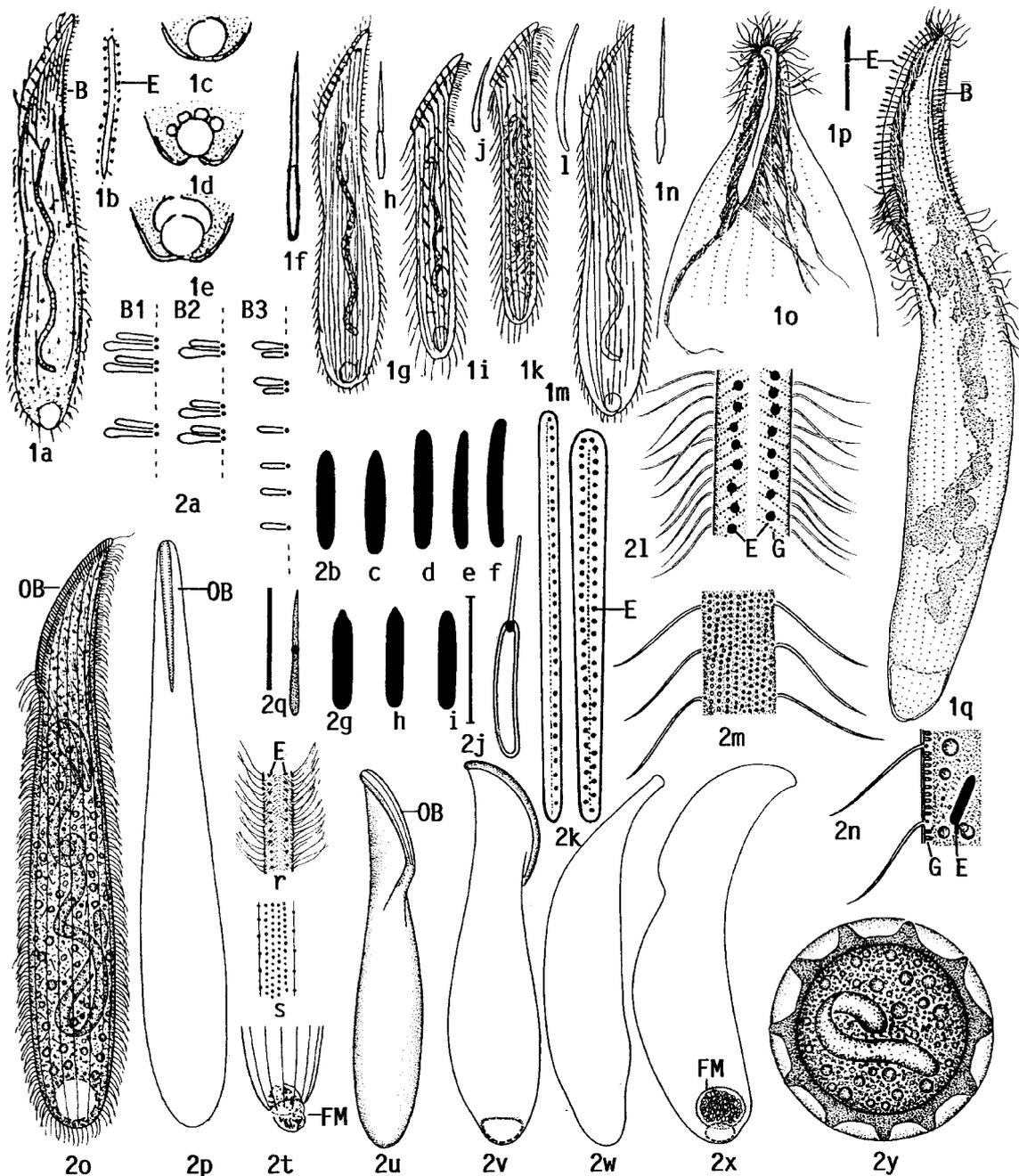
5.7:1 to 7.2:1, with the average of 6.4:1 matching the *in vivo* ratio of about 6:1. Contractile by up to one third of body length, especially in oral region; contracts and extends slowly, size changes thus difficult to recognize.

Usually narrowly to very narrowly knife-shaped or lanceolate (3:1-12:1, on the average about 6:1) due to the long and strongly slanted oral bulge ( $40^\circ\text{--}80^\circ$ , on the average  $65^\circ$ ). Oral portion more or less distinctly curved dorsally and with bluntly pointed anterior end; neck of ordinary distinctness; trunk elongate barrel-shaped, strongly inflated after ingestion of rotifers or large ciliates; posterior end rounded or bluntly pointed after systole of contractile vacuole; laterally flattened up to 2:1 in hyaline oral region, while only slightly flattened or unflattened postorally; sometimes twisted about main body axis, especially when just collected from soil habitat (Figs 1a, g, m, q, 2o, p, u-x, 3a, c, h, 5a-d, 6a, 9a, c, 10a, b).

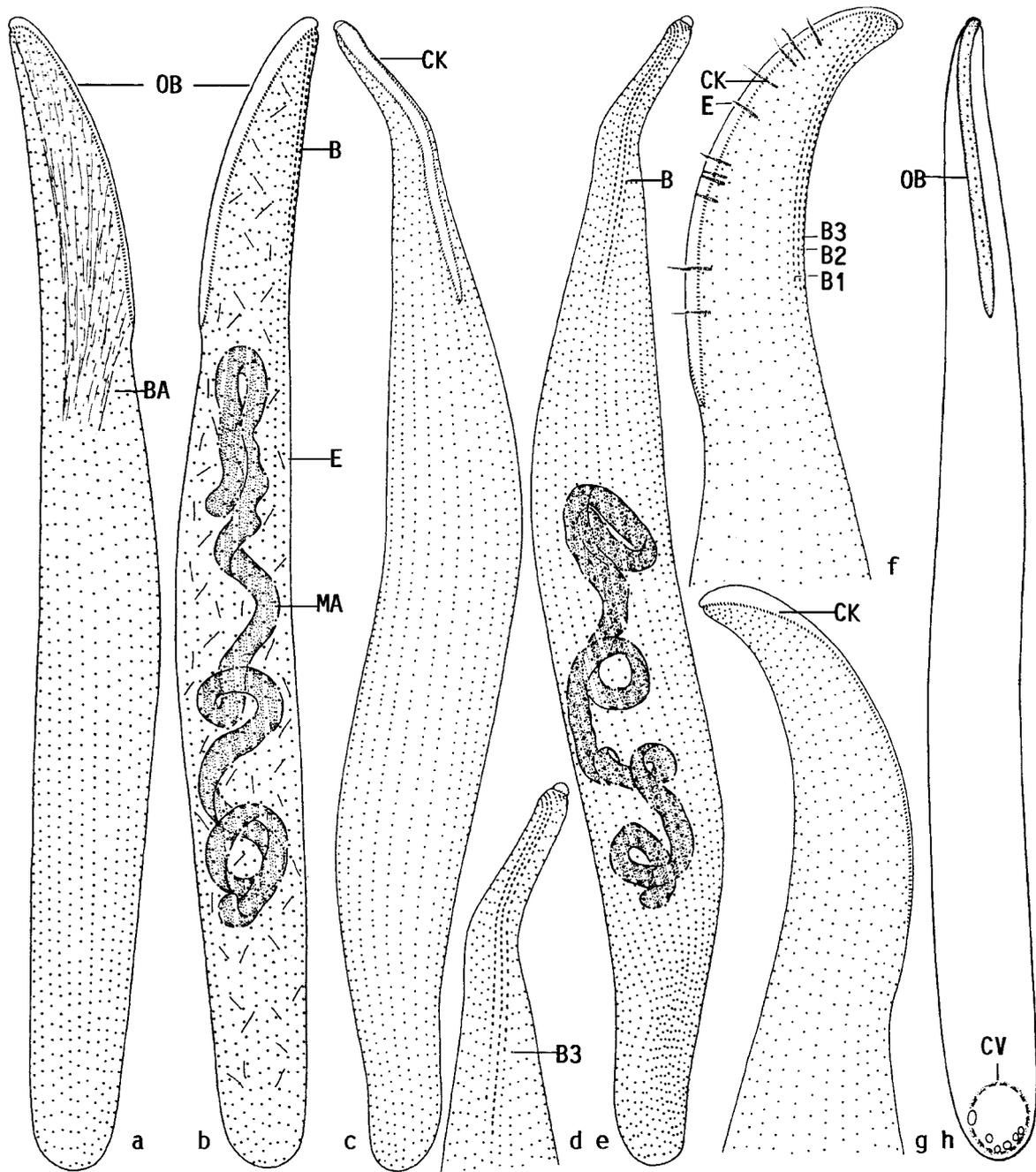
Nuclear apparatus in middle quarters of cell (Figs 1a, g, q, 3b, e, 7a, g, 8a, 9a). Macronucleus a long, tortuous, more or less nodulated, 4-8  $\mu\text{m}$  thick strand in over 30 populations routinely identified over the years, rarely in two or three long, tortuous pieces dividing individually (see *A. cultriforme scalpriforme*); nucleoli granular, rod-like, or lobate, up to 6  $\mu\text{m}$  in size. Macronucleus pattern very variable in one of the three Hawaiian populations: a highly tortuous strand (occasionally in two pieces) in 58% out of 75 specimens analysed, a mixture of short strands and small nodules in 23%, and up to 100 nodules in 19% of specimens (Figs 5a-d, 9a, c-e; Table 1). In a population from Rwanda, all specimens have many nodules (Fig. 6c). Both populations match ordinary cells in all other features, though the Rwandan specimens are the longest on the average (Table 1). Micronuclei scattered along macronucleus strand, 2-4  $\mu\text{m}$  across in protargol preparations, often difficult to distinguish from similarly sized and impregnated cytoplasmic inclusions; number thus difficult to determine, likely between 10 and 20.

Contractile vacuole in rear body end, forms several adventive vacuoles merging to a large vacuole during diastole (Figs 1a, c-e). About 10 excretory pores in pole area (Figs 3h, 6a, b). During defecation, the fecal mass migrates through the contractile vacuole and leaves the cell in the pole centre (Figs 2t, x).

One type of extrusomes in oral bulge. Extrusome shape and size rather similar in the populations investigated, differences recognisable only on careful observation; conspicuous both *in vivo* and in protargol preparations, though small as compared to body size, because forming a row in the right and the left half of oral bulge, as already described and illustrated by Penard (Fig. 1b), producing a conspicuously bright (*in vivo*) or black (protargol preparations) fringe in ventral anterior third of body; often less narrowly spaced, rarely



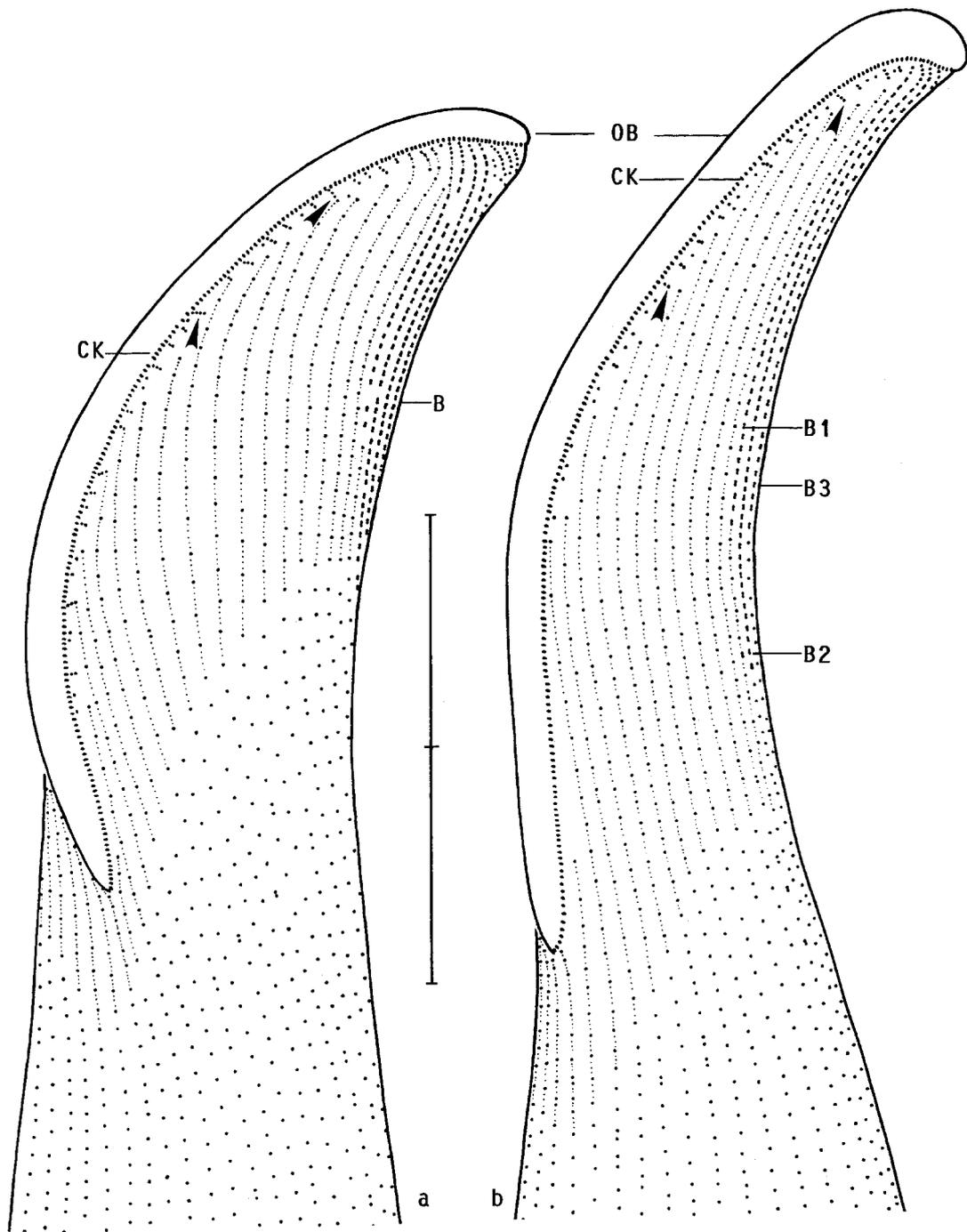
**Figs 1a-q.** *Arcuospathidium cultriforme cultriforme*, alive (1a-n) and after protargol impregnation (1o-q), according to Penard, 1922 (1a-f); Kahl, 1930a (1k-n); Kahl, 1930b (1g-j); and Fryd-Versavel et al., 1975 (1o-q). **1a:** Swiss specimen, 200-260  $\mu$ m. **1b:** Oral bulge view. **1c-e:** Cycle of contractile vacuole. **1f:** Exploded toxicyst. **1g, h, m, n:** Kahl's redrawings of Penard's figures. **1i-l:** A specimen of doubtful identity from the Zillertal, length 180  $\mu$ m; resting extrusome, 8  $\mu$ m (j, l). **1o-q:** Ventral and left side view of Italian specimens, 140  $\mu$ m. **Figs 2a-y.** *Arcuospathidium cultriforme cultriforme*, alive, according to Foissner, 1984 (2o-u) and new observations (2a-n, v-y). **2a:** Dorsal brush, bristles up to 4  $\mu$ m. **2b-i:** Extrusomes from specimens of Austria and Germany (b), Crete (c), Costa Rica (d), Venezuelan site 20 (e, f), and Hawaiian sites 39 (g), 12 (h) and 50 (i). Drawn to scale, bar 5  $\mu$ m. **2j:** Exploded toxicyst, 11  $\mu$ m. **2k:** Variability in shape of oral bulge and arrangement of extrusomes. **2l:** Surface view of oral bulge. **2m, n:** Surface view and optical section showing cortical granulation. **2o, p, u:** Left side, ventral, and ventrolateral view of Austrian (Burgenland) specimens, length 250  $\mu$ m. **2q-t:** Resting (6  $\mu$ m) and exploded toxicyst, surface view of oral bulge and somatic cortex, and defecation. **2v-x:** Various views of specimens from Hawaiian site 12 (video records). **2y:** Resting cyst from an Austrian (Linz) specimen, 70  $\mu$ m. B (1-3) – dorsal brush (rows), E – extrusomes, FM – fecal mass, G – cortical granules, MA – macronucleus, OB – oral bulge.



**Figs 3a-h.** *Arcuospathidium cultriforme cultriforme*, Austrian specimens after protargol impregnation. From Foissner, 1984 (a-g) and new observations (h). **a, b:** Right and left side ciliary pattern and macronucleus of a representative specimen from Burgenland site, length 190  $\mu\text{m}$ . **c-e:** Somatic and oral ciliary pattern of ventral and dorsal side of a Burgenland specimen, length 230  $\mu\text{m}$ . **f, g:** Left and right side ciliary pattern in oral body portion of a Burgenland specimen, length 85  $\mu\text{m}$ . **h:** Ventral view of a specimen from Linz, length 300  $\mu\text{m}$ . B (1-3) – dorsal brush (rows), BA – oral basket, CK – circumoral kinety, CV – contractile vacuole with excretory pores, E – extrusomes, MA – macronucleus, OB – oral bulge.

even lacking in right bulge half, for instance, in 3 out of 43 specimens of the Linz population (Figs 1b, g, 2k, 1, r, 3h, 7a, b, d, 8a-e, 9a-c, f, 10g). Size (4-6  $\times$  0.7-1.1  $\mu\text{m}$ ) very similar in the eight populations investigated;

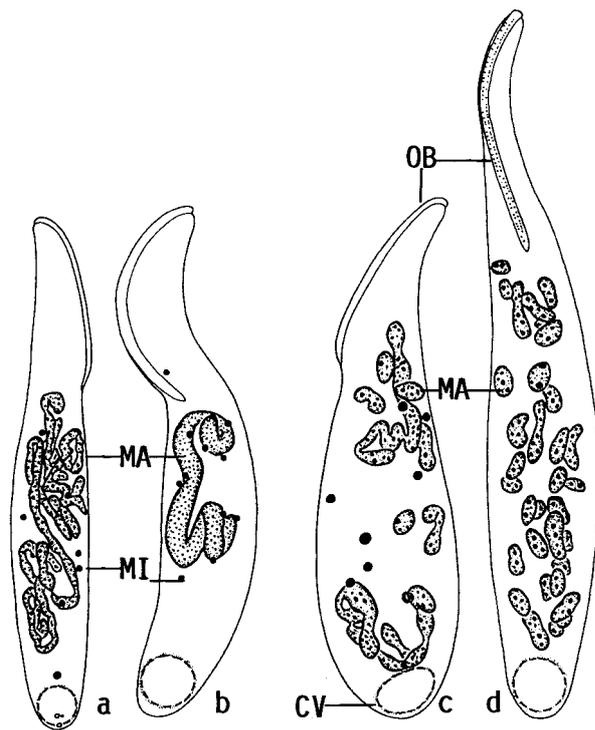
except for the Venezuelan specimens, shape also highly similar, oblong to very narrowly ellipsoidal with rounded ends, anterior end conical in some populations, making extrusomes looking like blunt pencils (Figs 2b-d, g-i,



**Figs 4a, b.** *Arcuospathidium cultriforme cultriforme* (a) and *A. cultriforme scalpriforme* (b), ciliary pattern of left side in oral body portion of an Austrian (Linz, a) and South African (b) specimen after protargol impregnation, length of cord of oral bulge 90  $\mu\text{m}$  and 105  $\mu\text{m}$ , respectively. The specimen of *A. cultriforme cultriforme* has four dorsal brush rows. Arrowheads mark minute, transversely extending kinetofragments (adesmokineties?) left of the circumoral kinety. B (1-3) – dorsal brush (rows), CK – circumoral kinety, OB – oral bulge. Scale bar 50  $\mu\text{m}$ .

9h-k); thinner, slightly acicular, and inconspicuously curved in the two Venezuelan populations (Figs 2e, f, 7c, 10d-f). Fully exploded extrusomes 8-12  $\mu\text{m}$  long and of typical toxicyst structure, that is, with a proximal,

toxin-containing capsule of size and shape similar to that of the resting organelle, and a distal tube about as long as capsule bent at up to 90°; between capsule and tube a refractive granule deeply impregnating with



**Figs 5a-d.** *Arcuospathidium cultriforme cultriforme*, variability of body shape and nuclear apparatus in Hawaiian site (50) specimens after protargol impregnation, length 200  $\mu\text{m}$ , 200  $\mu\text{m}$ , 170  $\mu\text{m}$ , 280  $\mu\text{m}$ . Two thirds of the specimens have an ordinary, more or less tortuous macronucleus strand (**a, b**), while the others have fragmented nucleus in many nodules (**d**). Transitions occur (**c**), showing that they do not represent different species. CV – contractile vacuole, MA – macronucleus (nodules), MI – micronuclei, OB – oral bulge.

protargol and staining red with methyl green-pyronin. Partially exploded toxicysts, usually found in preserved specimens, about 7–10  $\mu\text{m}$  long and often distinctly knife-shaped, as already mentioned and illustrated by Penard (Figs 1f, p, 2q, 3f, 8d, h, 11e, 12e, f). Developing extrusomes studded in cytoplasm, of similar size and shape as mature ones, sometimes form small bundles, impregnating with protargol (Figs 7g, 8e, 9b, g).

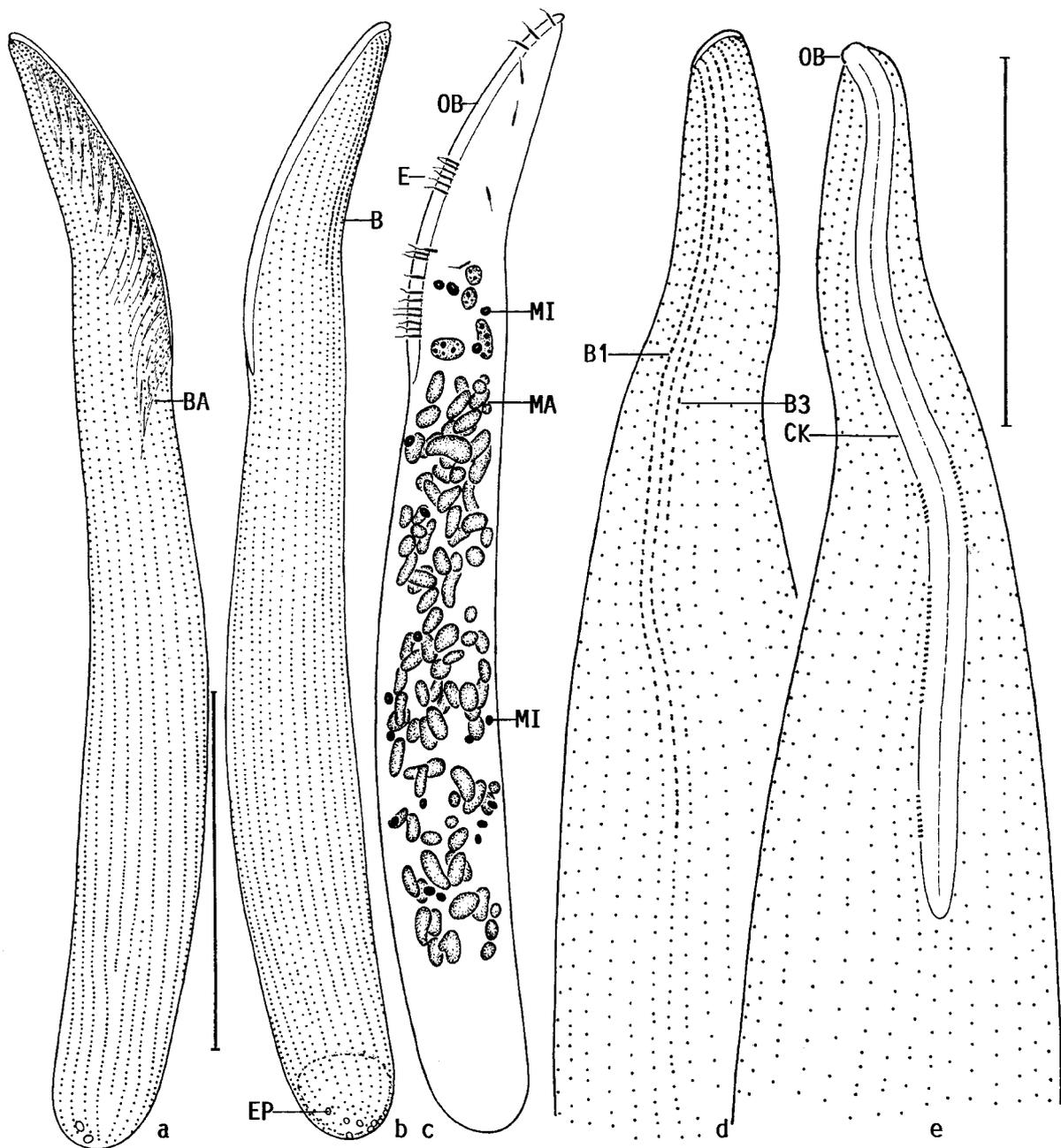
Cortex colourless and very flexible, contains 5–10 granule rows between each two kineties. Individual granules 0.3–0.6  $\times$  0.2–0.3  $\mu\text{m}$  in size, moderately to strongly refractive, depending on population (Figs 1s, 2m, n, 7e, f). Cytoplasm colourless, postorally packed with lipid droplets 1–6  $\mu\text{m}$  across and food vacuoles containing ciliates and rotifers; specimens thus turbid and brownish at low magnification ( $\leq \times 100$ ). Swims and glides moderately rapidly on microscope slides and between soil particles, showing great flexibility, especially in the oral area, which moves to and fro, as already mentioned by Penard (1922). Appears somewhat

flag-like due to the curved oral area and the cylindroidal postoral portion, when rotating about main body axis.

Somatic cilia 8–10  $\mu\text{m}$  long *in vivo*, arranged in 24–44, on the average 25–37 equidistant, densely ciliated rows slightly loosened in oral area. Ciliary rows arranged in typical *Arcuospathidium* pattern, that is, curve dorsally on both sides of oral bulge, except for brush kineties which, as usual, curve ventrally (Fryd-Versavel et al., 1975; Figs 1a, g, q, 2o, 3a–g, 4a, 6a–e, 8a, b, e, 9a, b, 10a, b; Table 1). Short rows, each composed of 1–5 kinetids, extend slightly obliquely from left side circumoral kinety either in line with a certain circumoral dikinetid or separated from kinety by a small space; present in all populations of *A. cultriforme cultriforme* and *A. cultriforme scalpriforme*, but not in all specimens (Figs 4a, b, 8c, h, 9b): for instance, absent in three out of 12 specimens of the Linz population, indistinct (composed of 1–2 kinetids) in four specimens, and distinct (composed of 2–5 kinetids) in five specimens; values similar for Hawaiian site (50) population (n 18): 6/2/10. These short kineties, which suggest a *Dileptus*-like ancestor of the spathidiids (see Discussion), were not mentioned by either Fryd-Versavel et al. (1975) or Foissner (1984), where they are recognisable on a micrograph (Fig. 11c).

Brush exactly on dorsal side, three-rowed, dikinetidal, heterostichad, and slightly shorter than oral bulge, *in vivo* inconspicuous, though occupying 25–30% of body length, because bristles only 2–3  $\mu\text{m}$  long and pairs ordinarily spaced (1–1.5  $\mu\text{m}$  apart). Bristle rows frequently with small irregularities, such as minute breaks and/or some kinetids out of line, rarely occurs a more or less complete fourth row right of row 1; all rows commence with some ordinary cilia anteriorly and continue as somatic kineties posteriorly. Brush row 1 usually slightly shorter than row 2; row 2 by 20–30% longer (heterostichad) than row 3 which, however, has a monokinetidal tail extending to second third to half of body with about 2  $\mu\text{m}$  long bristles. Details of bristle pairs difficult to analyse, even in the scanning electron microscope, likely as shown in figure 2a, that is, anterior bristle of pairs slightly shorter (1.5–2  $\mu\text{m}$ ) than clavate posterior bristle (2–3  $\mu\text{m}$ , rarely up to 5  $\mu\text{m}$ ), while *vice versa* in row 3 (Figs 1a, q, 2o, 3b, d–f, 4a, 6b, d, 7d, e, 8a, c, f, g, 9a, b, 11a–d; Table 1). Bristle pairs obliquely arranged in upper half of rows of Venezuelan site (20 specimens), a conspicuous feature seemingly doubling row number (Figs 12a–d, 14b, c).

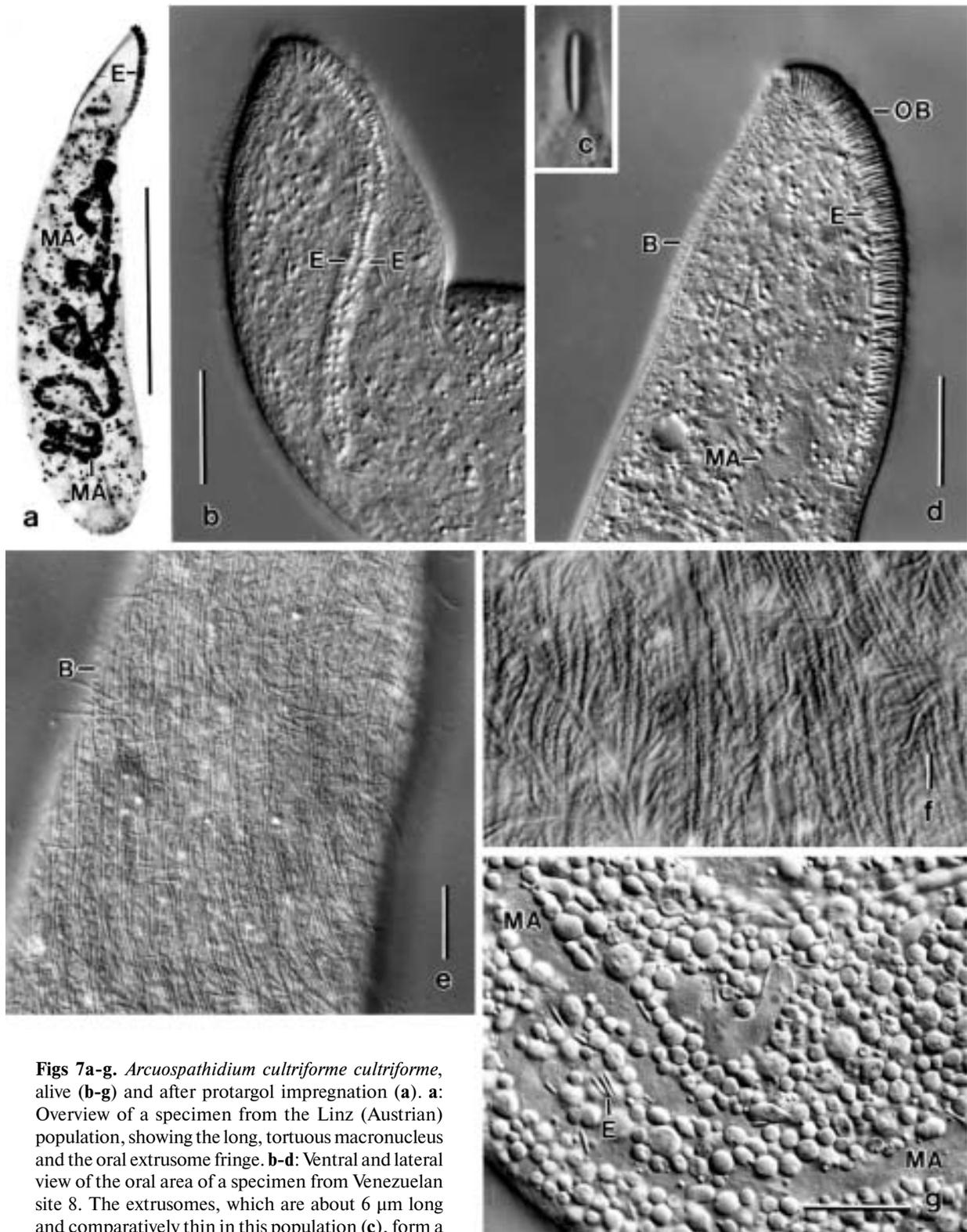
Oral bulge conspicuous due to the highly refractive extrusomes and the strong slope (40°–80°, on the average 65°, n 15) occupying on the average 31% to 35% of body length in five populations; bulge length thus comparatively constant, though ranges (25%–45%) approach *A. cultriforme scalpriforme*; flat to strongly convex, in frontal view very narrowly cuneate to oblong with



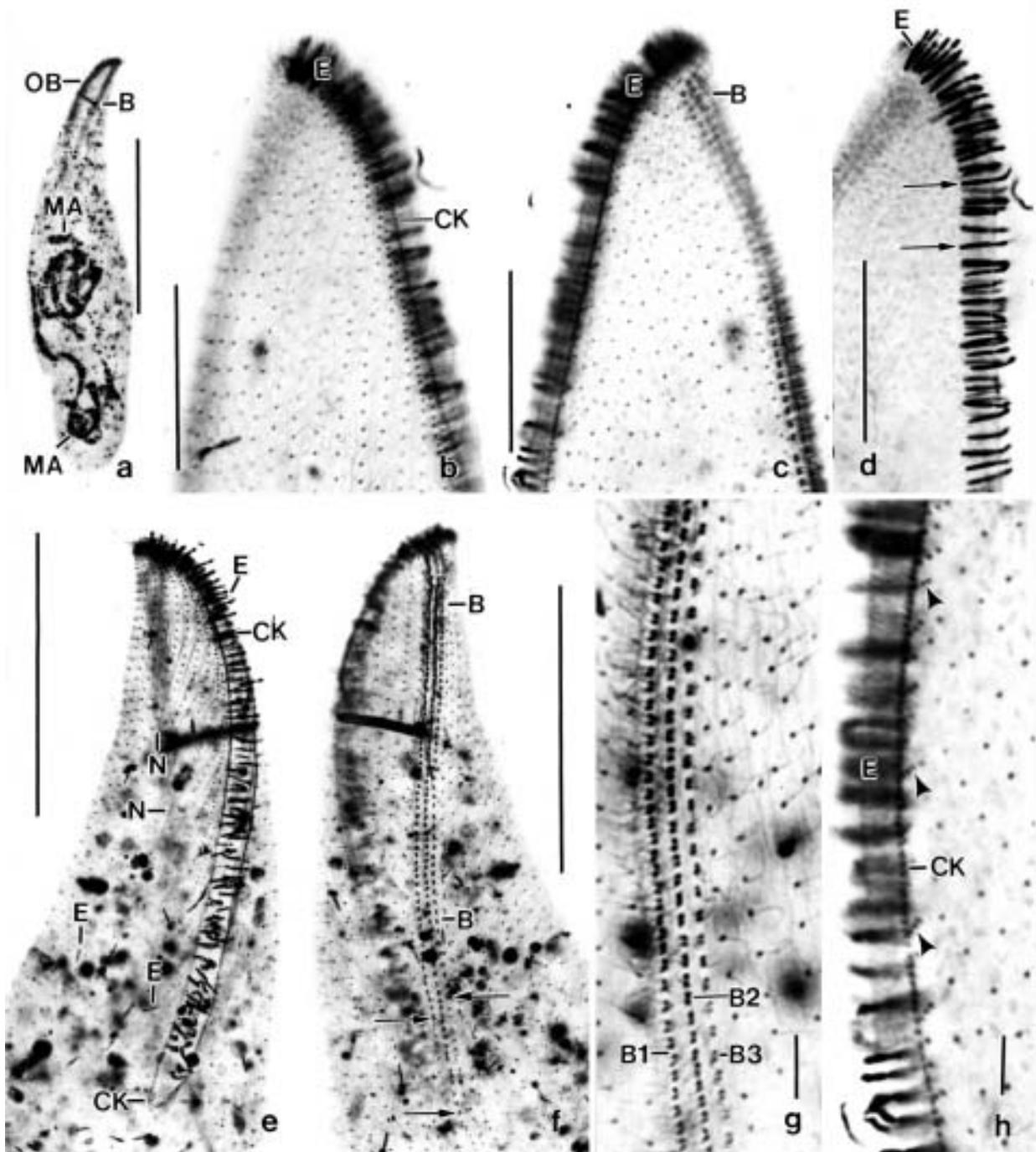
**Figs 6a-e.** *Arcuospathidium cultriforme cultriforme*, Rwandan specimens after protargol impregnation. About 20 specimens are found in the protargol slides, all have many macronucleus nodules (c), similar to some Hawaiian cells (Fig. 5d). Thus, this population might represent a distinct species. **a-c:** Right and left side ciliary pattern and nuclear apparatus of a representative specimen, length 315  $\mu\text{m}$ . **d, e:** Ciliary pattern of dorsal and ventral side in oral body portion, length of circumoral kinety (oral bulge) 97  $\mu\text{m}$ . B (1, 3) – dorsal brush (rows), BA – oral basket, CK – circumoral kinety, E – extrusomes, EP – excretory pores of contractile vacuole, MA – macronucleus, MI – micronuclei, OB – oral bulge. Scale bars 100  $\mu\text{m}$  (a-c) and 40  $\mu\text{m}$  (d, e).

proximal end often bluntly pointed, indistinctly apart from body proper because gradually merging into ventral surface and *in vivo* only about 3  $\mu\text{m}$  high and 5  $\mu\text{m}$  wide. Bulge cortex with arrowhead-like pattern of cortical granules. Circumoral kinety of same shape as

oral bulge, continuous, composed of narrowly spaced dikinetids each associated with a cilium and a basket rod (nematodesma). Oral basket hardly recognisable *in vivo*, prominent after French protargol impregnation (Figs 1o, q), while rather inconspicuous with Foissner's



**Figs 7a-g.** *Arcuospathidium cultriforme cultriforme*, alive (**b-g**) and after protargol impregnation (**a**). **a**: Overview of a specimen from the Linz (Austrian) population, showing the long, tortuous macronucleus and the oral extrusome fringe. **b-d**: Ventral and lateral view of the oral area of a specimen from Venezuelan site 8. The extrusomes, which are about 6  $\mu\text{m}$  long and comparatively thin in this population (**c**), form a row in the right and a row in the left bulge half (**b, d**). Note the minute dorsal brush bristles about 3  $\mu\text{m}$  long. **e, f**: Surface view showing cortical granulation in a specimen from Venezuelan site 8 (**e**) and Costa Rica (**f**). The cortical granules, which have a diameter of only 0.2-0.4  $\mu\text{m}$ , are highly refractive and form long, slightly oblique rows, which are paired in the Costa Rican specimen (**f**). **g**: The cytoplasm of a Hawaiian site (39) specimen is studded with lipid droplets, extrusomes, and the long macronucleus. B - dorsal brush, E - extrusomes, MA - macronucleus, OB - oral bulge. Scale bars 100  $\mu\text{m}$  (**a**) and 20  $\mu\text{m}$  (**b-g**).



**Figs 8a-h.** *Arcuospithidium cultriforme cultriforme*, Linz (Austrian) specimens after protargol impregnation. All flattened by coverslip pressure to reveal as many details as possible. Compare with figure 4a which shows the ciliary pattern of an ordinarily prepared specimen. **a, e, f:** Overview and details of specimen 1, showing the long, tortuous macronucleus (**a**); the conspicuous circumoral kinety (**e**); and the long, three-rowed dorsal brush (**f**) with the posterior row ends marked by arrows. The right side ciliary rows are distinctly curved dorsally along the circumoral kinety (**e**). **b-d, h:** Details of right and left oral area of specimen 2. On the left side, there are curious, short kinetofragments (**c, h**; arrowheads) attached to the circumoral kinety, which is composed of very narrowly spaced dikinetids (**h**). The oral bulge extrusomes, which impregnate intensely forming a conspicuous fringe, are knife-shaped when partially extruded (**d**, arrows). **g:** Details of dorsal brush of specimen 3. The brush is composed of dikinetids spaced wider in row 3 than in rows 1 and 2. B (1-3) - dorsal brush (rows), CK - circumoral kinety, E - extrusomes, MA - macronucleus, N - nematodesmata forming the oral basket, OB - oral bulge. Scale bars 100  $\mu$ m (**a**), 50  $\mu$ m (**e, f**), 20  $\mu$ m (**b-d**), and 5  $\mu$ m (**g, h**).

method (Figs 3a, 6a, 8e, 11c), composed of cuneate bundles of nematodesmata extending into second third of body. Cytopharyngeal entrance recognisable neither *in vivo* nor at preparations (Figs 1a, q, 2k, l, o, p, u-x, 3a-c, f-h, 4a, 5a-d, 6a, e, 7a-d, 8a, e, 9a-c, f, 10a-c, g, 13a; Table 1).

**Resting cyst** (Figs 2y, 15a-i): Resting cysts were studied in the populations from Austria (Linz) and the Dominican Republic. They match well, but the ridges are thinner (Figs 15g, h) and the cysts considerably smaller ( $\bar{x}$  68.8  $\mu\text{m}$ , n 22 vs. 45.2  $\mu\text{m}$ , n 6) in the Dominican specimens. As concerns the ciliary pattern, see *A. cultriforme scalpriforme*.

Only the Linz specimens were studied in detail. For encystment, cells were transferred to Eau de Volvic, where they produced a distinct, but not yet faceted wall within six hours; the facets developed slowly over a week. Thus, observations and measurements were performed on two weeks old cysts.

All components of the cyst are colourless at a magnification of  $\geq \times 400$ , and brown to yellow-brown at low magnifications ( $\leq \times 100$ ) due to the strong light refraction. The cysts are perfectly spherical and have an outer diameter of 55–95  $\mu\text{m}$  ( $\bar{x}$  68.8  $\mu\text{m}$ , M 65, SD 9.6, CV 13.9, n 22), while the inner diameter (cyst proper) is considerably smaller ( $\bar{x}$  48.9  $\mu\text{m}$ , M 48, SD 8.8, CV 18.1, n 22). Thus, the wall has an average thickness of 10  $\mu\text{m}$  (!) and its volume is almost thrice (twice when the facet concavities are detracted) that of the cyst proper (170,430  $\mu\text{m}^3$  vs. 61,570  $\mu\text{m}^3$ ). This is an outstanding feature found, for instance, also in desert strains of *Exocolpoda augustini* (Foissner et al., 2002). The most conspicuous part of the cyst is the deeply and roughly faceted middle layer (mesocyst?) which is compact, flexible, and highly refractive. A 0.5–1  $\mu\text{m}$  thick membrane each is found on the facets (ectocyst?) and the cyst proper; both are also recognisable in squashed, split cysts, showing that the inner membrane is not the cell cortex, but rather the endocyst. The cyst contents consist of colourless lipid droplets 1–5  $\mu\text{m}$  across and the macronucleus shortened to a semicircular strand, as described in *A. cultriforme scalpriforme*. The contractile vacuole and all extrusomes disappear.

**Ontogenesis** (Figs 13a-c, 14a-c, e, f): The SEM micrographs of dividers from the Venezuelan site (20) population will be included in the detailed description of the ontogenesis of *A. cultriforme scalpriforme*. No differences are recognisable in the two subspecies.

**Occurrence and ecology:** There are surprisingly few records in the literature, although *A. cultriforme cultriforme* is rather common in terrestrial habitats, especially in Europe (Foissner et al., 2005). Penard (1922) discovered *A. cultriforme cultriforme* in wall mosses from Switzerland. Kahl (1930a, b) observed a single specimen in moss from the Zillertal in Tyrol,

Austria. However, the shape of the extrusomes suggests that it was a different species (Figs 1i-l). It was only 50 years later, that Fryd-Versavel et al. (1975) reported *A. cultriforme cultriforme* from Italian moss and provided a brief redescription based on protargol impregnation (Figs 1o-q). Later, Foissner (1984) redescribed *A. cultriforme cultriforme* from the soil of a coniferous forest in Austria (Figs 2o-t, 3a-g; Table 1). Since then, it was reported from several terrestrial habitats of Austria (Foissner, 1987a; Aeschl and Foissner, 1993; Foissner et al., 2005) and Germany (Goralczyk and Verhoeven, 1999; Foissner, 2000b). In 1998, Foissner reported the species from all main biogeographic regions, except Antarctica. This is substantiated by the records given in the Materials and Methods section as well as by Blatterer and Foissner (1988) and Foissner (1999a). However, *A. cultriforme cultriforme* seems to be rare in Africa because it was not found in 73 samples from Namibia, where *A. cultriforme megastoma* was discovered (Foissner et al., 2002).

These data show that *A. cultriforme cultriforme* and *A. cultriforme cultriforme*-like taxa are cosmopolites, except Antarctica. In Rwanda and Venezuela, other (sub)species may occur, but more detailed investigations are required. The ecological range is obviously wide and includes terrestrial, semiterrestrial, and saline habitats (see Materials and Methods section). However, true limnetic and extreme habitats, such as alpine soil above the timberline (Foissner, 1987b), Antarctica (Foissner, 1998), and hot deserts (Foissner et al., 2002) are avoided.

***Arcuospathidium cultriforme scalpriforme* (Kahl, 1930) Foissner, 2003** (Figs 4b, 16–29; Tables 2, 3)

1930 *Spathidium scalpriforme* Kahl, Arch. Protistenk., 70: 381.

1930 *Lionotus scalpriforme* Kahl, 1930 – Kahl, Tierw. Dtl., 18: 165 (The figure explanation and the index indicate that Kahl put it to *Lionotus* by mistake).

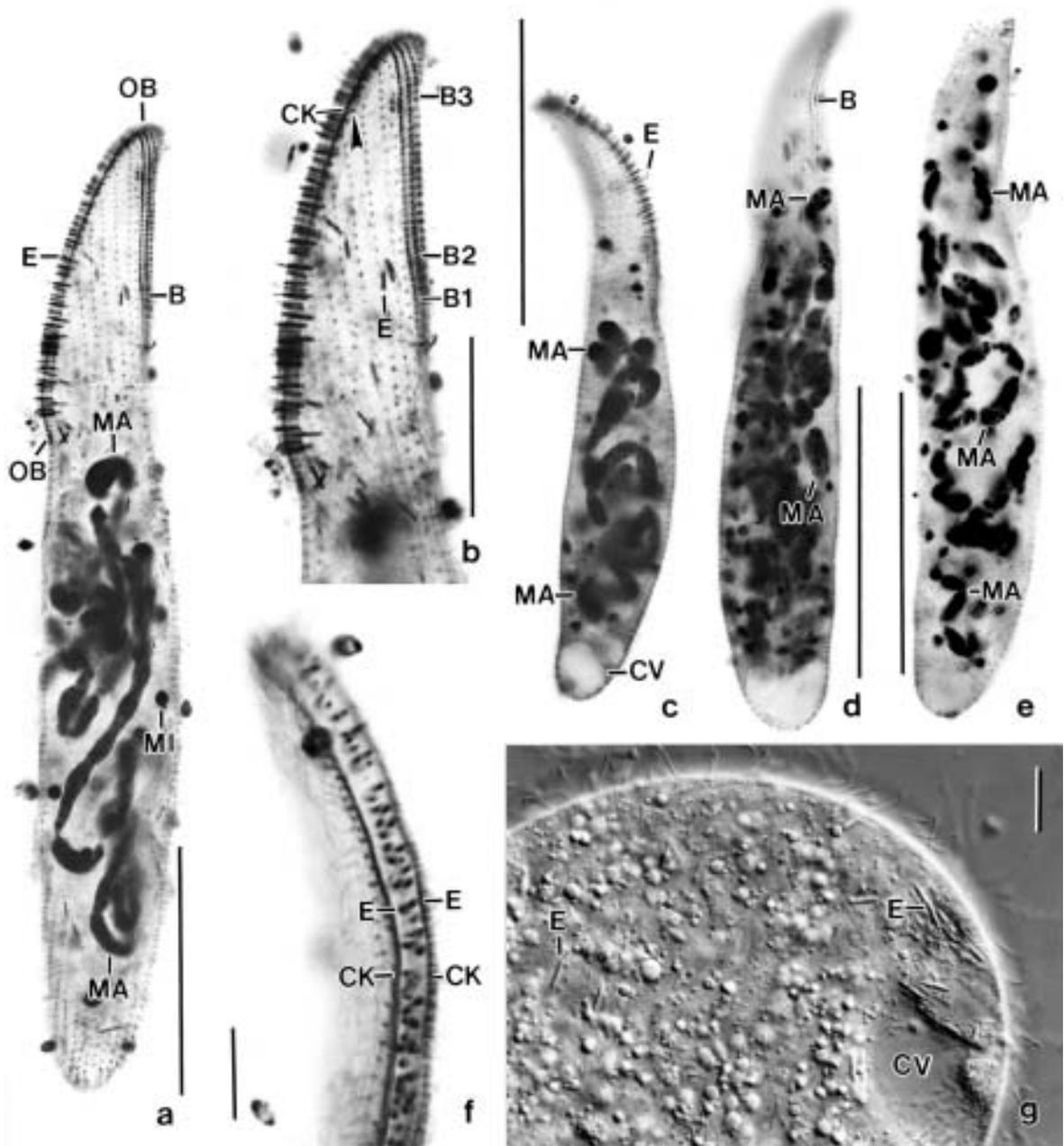
1984 *Arcuospathidium lionotiforme* (Kahl, 1930) nov. comb. – Foissner, Stapfia, 12: 78 (misidentification in that and all subsequent papers!)

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

2003 *Arcuospathidium cultriforme scalpriforme* (Kahl, 1930) comb. n., stat. n. – Foissner, Acta Protozool., 42: 54 (rectification of misidentification and rank lowering).

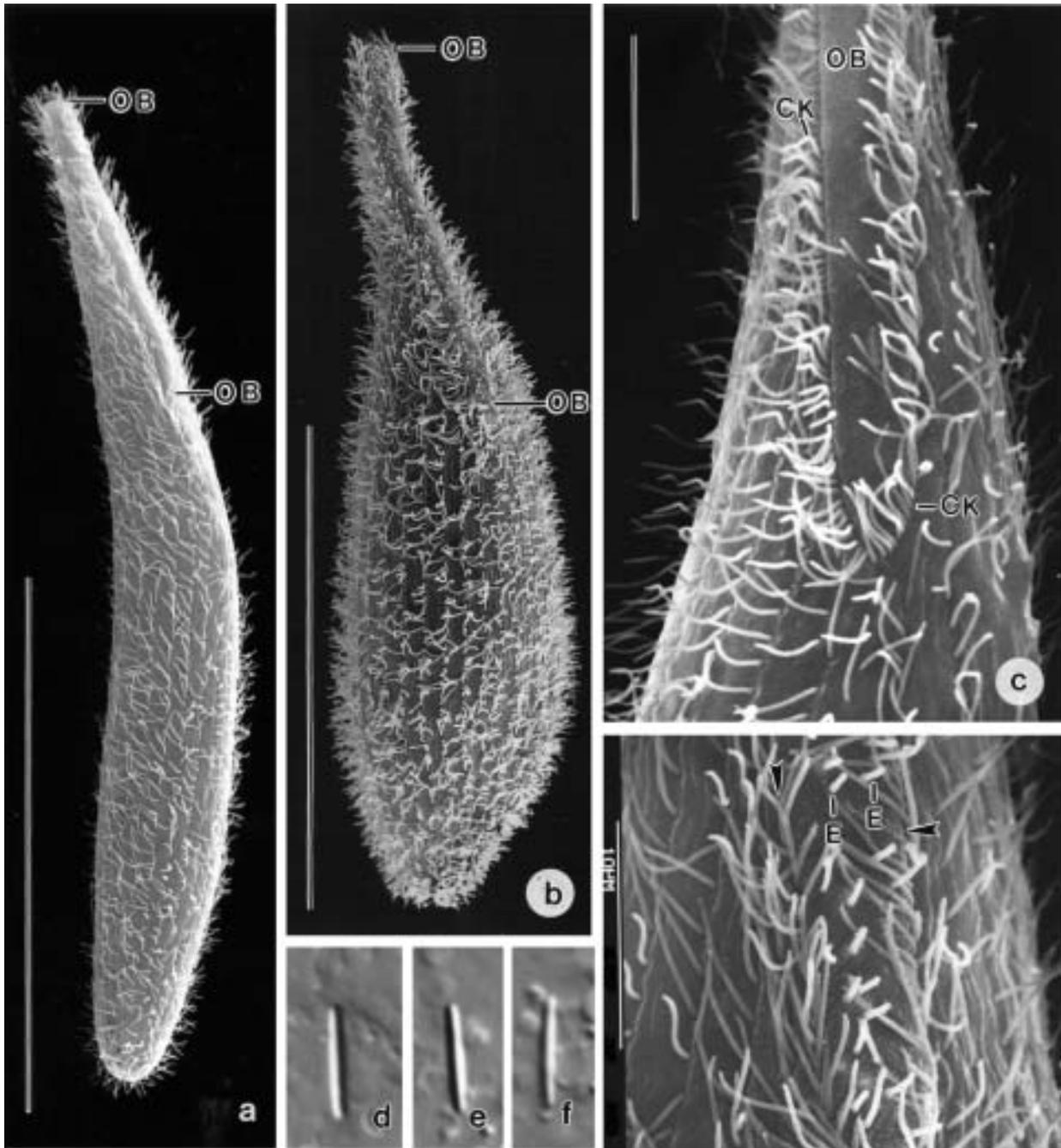
**Improved diagnosis:** Mouth (oral bulge) about half of body length. Extrusomes scattered in right and left bulge half.

**Type locality:** Mosses from the Zillertal in Tyrol, Austria. Kahl (1930a) first observed the species in

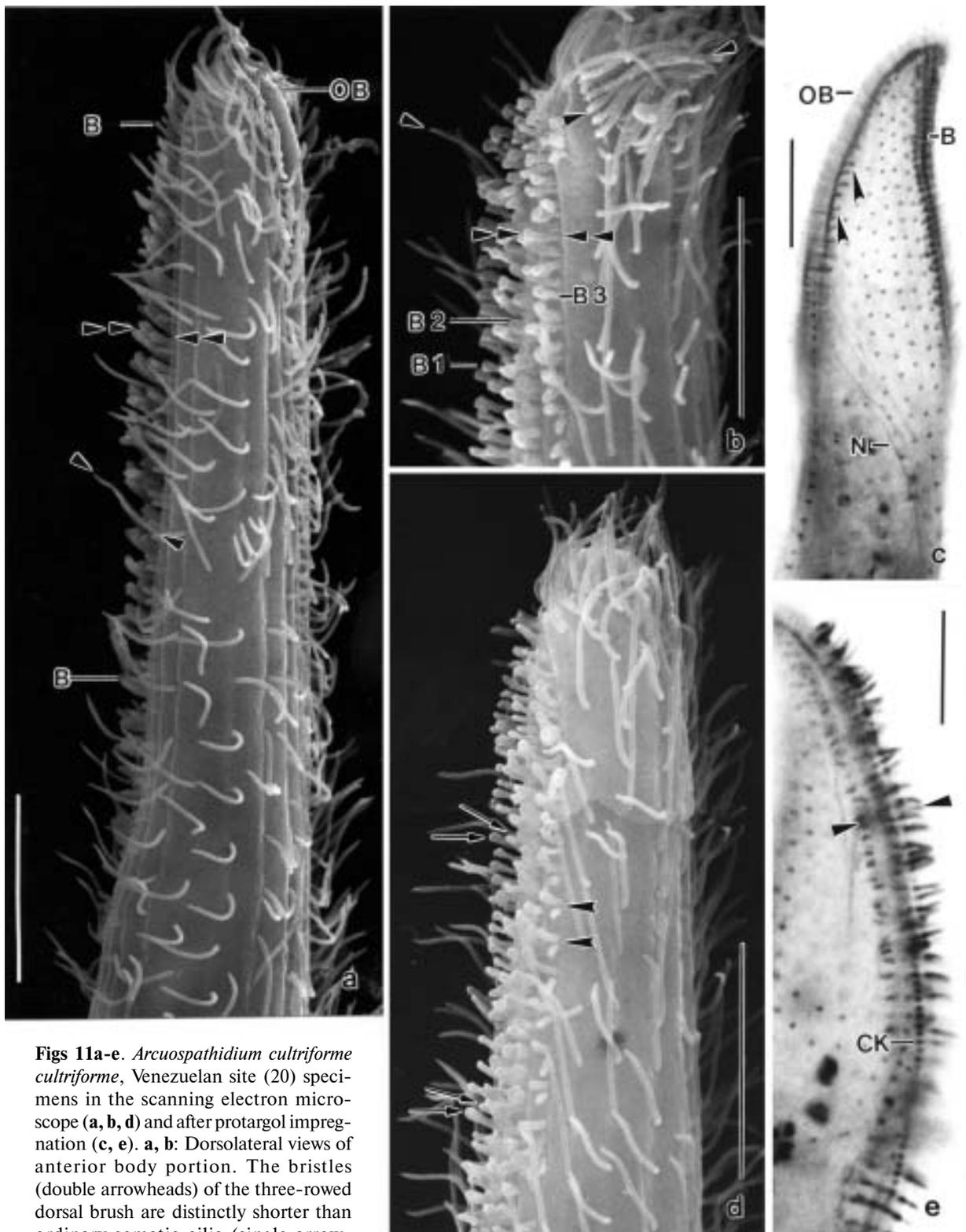


**Figs 9a-k.** *Arcuospathidium cultriforme cultriforme*, Hawaiian sites 50 (a-h) and 39 (i-k) specimens from life (g-k) and after protargol impregnation (a-f). a-e: Two thirds of the Hawaiian site (50) specimens have an ordinary macronucleus, that is, a long, tortuous strand (a, c), while one third have many macronucleus nodules (d). Transitions occur (e), suggesting that specimens with an ordinary macronucleus and those with many nodules/belong to the same species. The oral bulge occupies 30% (a) to 37% (c) of body length, as typical of this subspecies. Short kinetofragments (b, arrowhead) are attached to the left side circumoral kinety, as in the European populations (Figs. 8h, 11c). The anterior end of the dorsal brush kineties is slightly curved ventrally (a, b). f: The extrusomes form a row in the right and a row in the left half of the oral bulge, as typical of this subspecies. g: Posterior body portion studded with minute and moderately-sized lipid droplets and extrusomes. h-k: The oral bulge extrusomes of the Hawaiian site (39) specimens are blunt, 4-5  $\mu\text{m}$  long rods with rounded or bluntly pointed distal end (i-k), just as those occurring in the Hawaiian site (50) specimens (h). B (1-3) - dorsal brush (rows), CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, MA - macronucleus (nodules), MI - micronuclei, OB - oral bulge. Scale bars 100  $\mu\text{m}$  (c-e), 50  $\mu\text{m}$  (a), 25  $\mu\text{m}$  (b), and 10  $\mu\text{m}$  (f, g).

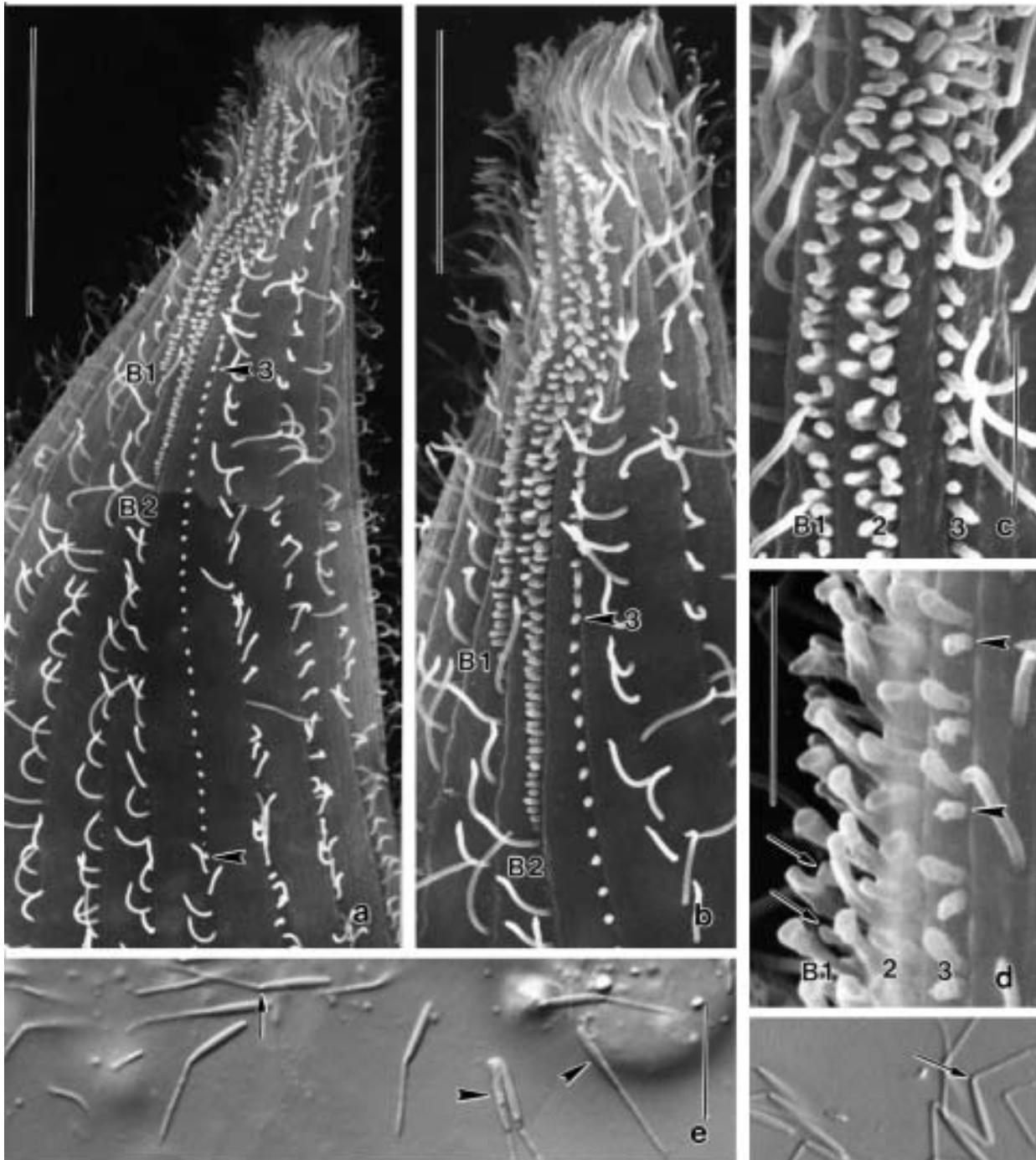
(a) to 37% (c) of body length, as typical of this subspecies. Short kinetofragments (b, arrowhead) are attached to the left side circumoral kinety, as in the European populations (Figs. 8h, 11c). The anterior end of the dorsal brush kineties is slightly curved ventrally (a, b). f: The extrusomes form a row in the right and a row in the left half of the oral bulge, as typical of this subspecies. g: Posterior body portion studded with minute and moderately-sized lipid droplets and extrusomes. h-k: The oral bulge extrusomes of the Hawaiian site (39) specimens are blunt, 4-5  $\mu\text{m}$  long rods with rounded or bluntly pointed distal end (i-k), just as those occurring in the Hawaiian site (50) specimens (h). B (1-3) - dorsal brush (rows), CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, MA - macronucleus (nodules), MI - micronuclei, OB - oral bulge. Scale bars 100  $\mu\text{m}$  (c-e), 50  $\mu\text{m}$  (a), 25  $\mu\text{m}$  (b), and 10  $\mu\text{m}$  (f, g).



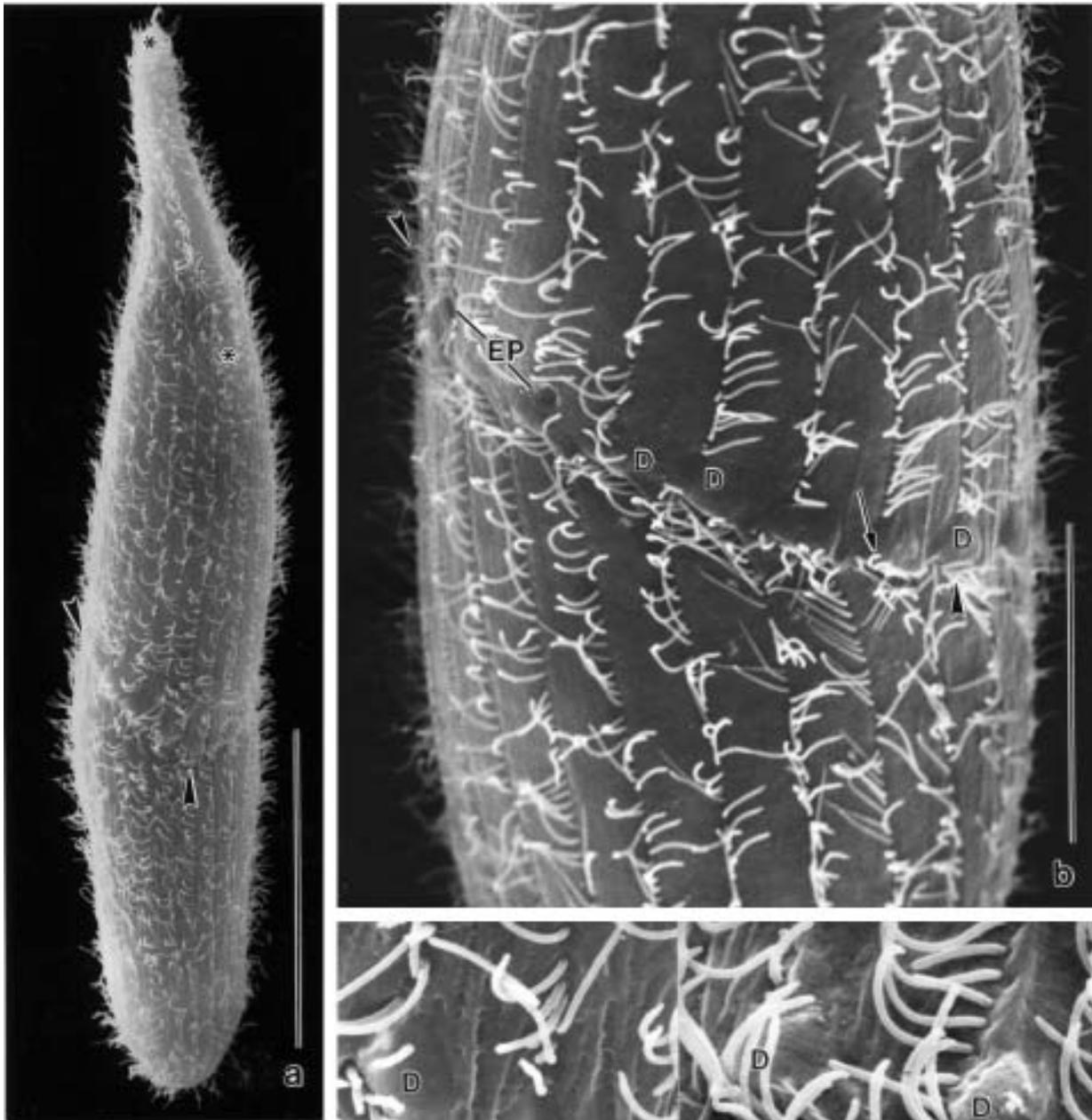
**Figs 10a-g.** *Arcuospathidium cultriforme cultriforme*, Venezuelan site (20) specimens in the scanning electron microscope (**a-c, g**) and alive (**d-f**). **a, b:** Ventrolateral views of a slender and a stout specimen with the oral bulge occupying 33% and 42% of body length; the latter value approaches that in *A. cultriforme scalpriforme*. **c, g:** Ventral views showing posterior portion of oral bulge, which is surrounded by the circumoral kinety (CK and arrowheads), whose cilia have the same length as those on the body. The oral bulge is low, has a flat surface and contains a row of extrusomes in the right and a row in the left half (**g**). **d-f:** The extrusomes are about 6  $\mu\text{m}$  long and much more slender than those of the European and Hawaiian populations (Figs. 1b, c, 9h-k); they are also slender in another Venezuelan population (Fig. 7c). Thus, the Venezuelan populations might represent a distinct subspecies. CK - circumoral kinety, E - extrusomes, OB - oral bulge. Scale bars 100  $\mu\text{m}$  (a, b) and 10  $\mu\text{m}$  (c, g).



**Figs 11a-e.** *Arcuospathidium cultriforme* cultriforme, Venezuelan site (20) specimens in the scanning electron microscope (**a, b, d**) and after protargol impregnation (**c, e**). **a, b:** Dorsolateral views of anterior body portion. The bristles (double arrowheads) of the three-rowed dorsal brush are distinctly shorter than ordinary somatic cilia (single arrowheads). **c:** Left side view of the oral bulge portion of the Burgenland population investigated by Foissner (1984). Arrowheads mark kinetofragments not mentioned by Foissner (Figs 3b, f). **d:** Dorsolateral view of anterior body portion. The posterior, clavate bristle of the dikinetids is slightly longer than the anterior (arrow pairs) in rows 1 and (probably) 2, while vice versa in row 3 (arrowheads). **e:** When partially exploded, the extrusomes become knife-shaped (arrowheads). B (1-3) - dorsal brush (rows), CK - circumoral kinety, N - nematodesmata, OB - oral bulge. Scale bars 10  $\mu$ m.



**Figs 12a-f.** *Arcuospithidium cultriforme cultriforme*, Venezuelan site (20) specimens in the scanning electron microscope (**a-d**) and alive (**e, f**). **a-d**: Dorsal views showing the dorsal brush in overview (**a**) and details at higher magnifications (**b-d**). The dorsal brush is three-rowed (numerals) and composed of dikinetids with up to 3  $\mu\text{m}$  long (*in vivo*), claviform bristles, whose length gradually decreases from anterior to posterior end (**b-d**). The posterior bristle of the dikinetids is slightly longer than the anterior one (**d**, arrows) in rows 1 and 2, while *vice versa* in row 3 (**d**, arrowheads). The dikinetids are obliquely arranged in the anterior two thirds of the rows, producing a distinct zigzag pattern of the bristles (**b, c**). Brush rows 1 and 3 are shorter than row 2, but row 3 has a monokinetidal bristle tail marked by arrowheads in (**a, b**). All brush kineties continue posteriorly as ordinary ciliary rows (**a, b**). **e, f**: Exploded toxicysts are 10-15  $\mu\text{m}$  long and knife-shaped, as mentioned and illustrated by Penard (Fig. 1f). Arrows mark a minute globule between capsule and shaft. Arrowheads denote empty toxicysts (with content extruded). B (1-3) - dorsal brush (rows). Scale bars 20  $\mu\text{m}$  (**a**), 10  $\mu\text{m}$  (**b, e, f**), and 4  $\mu\text{m}$  (**c, d**).



**Figs 13a-d.** *Arcuospathidium cultriforme cultriforme* (a-c) from Venezuelan site (20) and *Spathidium stammeri* (d) in the scanning electron microscope. a-c: Early dividers showing the strongly oblique division axis (arrowheads) and the developing division blebs (D) and excretory pores (EP). Asterisks in a mark the oral bulge. Note growing cilia in the forming oral kinetofragments (arrows).

d: Division blebs in a early mid-divider of *S. stammeri*. The forming oral kinetofragments (KF) curve around the right margin of the division blebs (D) assuming a concave shape. D - division blebs, EP - excretory pores for the proter, KF - forming oral kinetofragments. Scale bars 50  $\mu\text{m}$  (a), 20  $\mu\text{m}$  (b), and 5  $\mu\text{m}$  (c, d).

mosses from Mittenwald, a Bavarian village on the north border of Tyrol. However, the figure he provided seems to be that of the single specimen he found in Zillertal moss (reproduced here as figures 16a-c). Because of this and also because Kahl did not fix a type locality, we choose the Zillertal.

**Synonymy:** Foissner (2003b) explains his misidentification as follows: “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, 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*”.

We adopt this explanation, and the senior author emphasises that all his previous *A. lionotiforme* identifications are wrong (for a review, see Foissner, 1998) and belong to *A. cultriforme scalpriforme*. This is quite reasonable because the “true” *Spathidium lionotiforme* has a rather different organisation making misidentification unlikely (for a review, see Foissner, 2003b); further, *S. lionotiforme* must be a very rare species because it has not been mentioned in the literature since the original description.

**Morphology:** The following description incorporates the data from Kahl (1930a, b) and Foissner (1984) as well as new observations on populations from Brazil and the Cape Peninsula, Republic of South Africa. Kahl (1930a, b) and Gellért (1956) mention small varieties which, in our opinion, belong to other species. Kahl’s “local form” from roof moss in northern Germany is 160–180 µm long and has only about half (15) the number of ciliary rows typical of this species (Fig. 2d). Gellért’s variety from moss humus in Hungary is only 90 µm long, performs a contractile vacuole cycle within 35–40 sec, and feeds on minute zooflagellates.

*Arcuospathidium cultriforme scalpriforme* is very similar to *A. cultriforme cultriforme*, except for the characteristics mentioned in the diagnosis and some other minor features, which will be emphasised in the following description. Some of these might be more important than presently recognised.

Size and length:width ratio highly similar to *A. cultriforme cultriforme*, that is, about 230 × 40 µm *in vivo*, matching data of Kahl, 1930a, b (250–330 µm, length:width ratio 5:1) and Wenzel, 1953 (length about 260 µm); highly dependent on culture conditions, as shown by the South African population: on the average 207 × 39 µm in protargol-impregnated specimens from a non-flooded Petri dish (raw) culture, while 314 × 66 µm in specimens from a flourishing semipure culture. Number of ciliary rows, in contrast, increases only

slightly from 33 to 35 (Tables 2, 3).

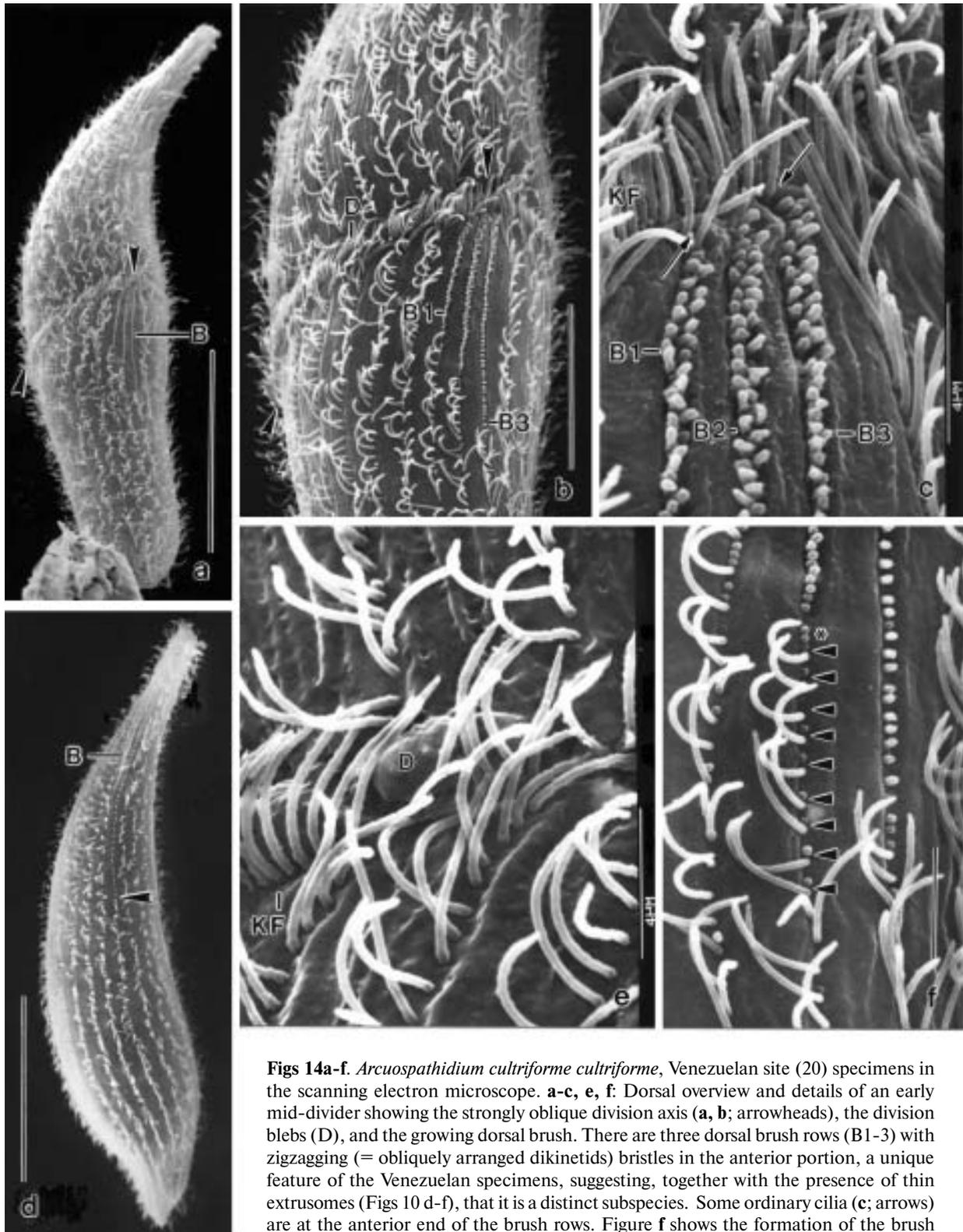
Body usually more distinctly knife-shaped than in *A. cultriforme cultriforme* due to the longer oral area, widest at proximal end of oral bulge and in mid of trunk (Figs 16a-c, f, g, p-t, 17a, b, 18a, b, d, 22a; Table 2); often similar to certain *Dileptus* species (e.g. *Dileptus conspicuus*, redescribed in Foissner, 1989) or various large, limnetic pleurostomatids, for instance, *Amphileptus carchesii* and *Litonotus varsaviensis* (for a review, see Foissner et al., 1995). Ratio of body and oral bulge length 0.42 in Austrian and 0.45 in South African populations, and thus distinctly higher than in the five populations of *A. cultriforme cultriforme* (0.31–0.35; Tables 1, 2).

Nuclear apparatus as in *A. cultriforme cultriforme*, but specimens/populations with numerous macronuclear nodules were not observed (Figs 16a, c, f, 17b, d, 18d, 22a). Macronucleus strand “broken” into two pieces dividing individually in about 5% of specimens (Figs 27a-g). Contractile vacuole and cortex also as in *A. cultriforme cultriforme*. Cortical granules very distinct and in about 8 rows between each two kineties in South African specimens (Figs 20a, b).

Extrusome shape and size very similar in the populations investigated and thus rather dissimilar to those of *A. cultriforme cultriforme*, where they are shorter and thus comparatively thicker, as already mentioned by Kahl (1930a); data of Foissner (1984) do not meet the modern standard, but basically match the new observations. Extrusomes conspicuous both *in vivo* and at protargol preparations, though small as compared to body size, because they form a bright stripe in the right and the left half of oral bulge, especially in broader dorsal half (Figs 16a, c, f-h, o, 18c-f, 20a-e). Size about 7 µm in specimens studied by Kahl (1930a), 5 µm in Austrian cells (Foissner, 1984), and 6 × 0.8 µm in Brazilian and 7–8 × 0.7–0.9 µm in South African specimens. Shape rather similar in Brazilian and South African specimens, that is, indistinctly acicular to very narrowly ellipsoidal and somewhat curved and asymmetric (Figs 16v, w). Fully exploded extrusomes 15–20 × 1 µm in size and of typical toxicyst structure (Fig. 16u); partially exploded toxicysts less distinctly knife-shaped than in *A. cultriforme cultriforme*, usually more or less distinctly clavate (Figs 16h, 18f). Developing extrusomes in cytoplasm as shown in figures 16i, k.

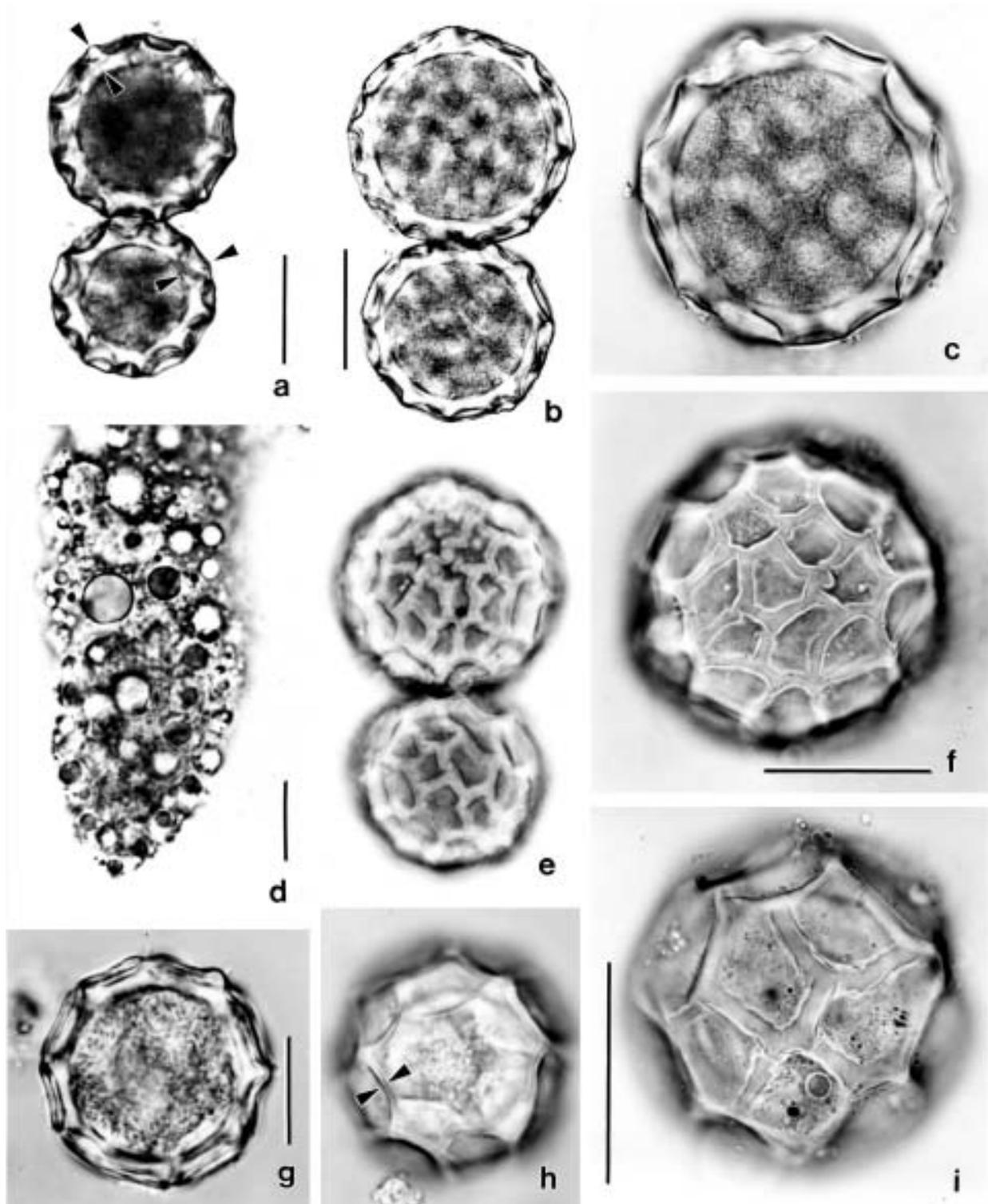
Cytoplasm and movement as described in *A. cultriforme cultriforme*. Feeds also on rotifers and middle-sized hypotrichs, such as *Gastrostyla steinii*, *Gonostomum affine* and a large *Urosoma* species. Appears elegant and conspicuous when gliding on microscope slide due to the nicely curved dorsal outline and the large size.

Somatic ciliature highly similar to that of *A. cultriforme cultriforme*, except dorsal brush (Figs 4b,



**Figs 14a-f.** *Arcuospathidium cultriforme cultriforme*, Venezuelan site (20) specimens in the scanning electron microscope. **a-c, e, f.** Dorsal overview and details of an early mid-divider showing the strongly oblique division axis (**a, b**; arrowheads), the division blebs (D), and the growing dorsal brush. There are three dorsal brush rows (B1-3) with zigzagging (= obliquely arranged dikinetids) bristles in the anterior portion, a unique feature of the Venezuelan specimens, suggesting, together with the presence of thin extrusomes (Figs 10 d-f), that it is a distinct subspecies. Some ordinary cilia (**c**; arrows) are at the anterior end of the brush rows. Figure **f** shows the formation of the brush dikinetids (asterisk): a new bristle is formed anteriorly of the parental somatic cilia,

which become gradually shortened to brush bristles (triangle series). The division blebs (D) are now very conspicuous (**b, e**), and the cilia of the new oral kinetofragments have reached full length (**b, c, e**; KF). **d:** Dorsal view of a morphostatic specimen showing the monokinetidal bristle tail of brush row 3 extending to mid-body (arrowhead); then, the row continues as ordinary somatic kinety to rear body end. The length of the tail of brush row 3 is an important feature for distinguishing *Spathidium* s. l. species. B (1-3) - dorsal brush (rows), D - division blebs, KF - kinetofragments. Scale bars 50  $\mu\text{m}$  (**a, d**), 20  $\mu\text{m}$  (**b**), and 4  $\mu\text{m}$  (**c, e, f**).



**Figs 15a-i.** *Arcuospathidium cultriforme cultriforme*, resting cysts (diameter 42-95  $\mu\text{m}$ ) of specimens from Linz, Austria (**a-f, i**) and the Dominican Republic (**g, h**). **a**: Optical section of two cysts showing the extremely thick wall delimited by arrowheads, scale bar 40  $\mu\text{m}$ . **b, e**: Optical section and surface view of two cysts showing the thick wall and the polygonally faceted surface, scale bar 40  $\mu\text{m}$ . **c, f**: Optical section and surface view of same specimen, showing the broad ridges (**f**) causing the spotted appearance of the cyst content in optical section (**c**), scale bar 40  $\mu\text{m}$ . **d**: Cyst contents mainly consisting of highly refractive lipid droplets 1-5  $\mu\text{m}$  across, scale bar 10  $\mu\text{m}$ . **g, h**: Optical section and surface view of a cyst from the Dominican specimen, scale bar 20  $\mu\text{m}$ . Obviously, it is very similar to the Austrian specimens, except for the thinner ridges (arrowheads) and the smaller diameter (45.2 vs. 68.8  $\mu\text{m}$  on average). **i**: Surface view of a cyst with facets distinctly larger than those recognisable in (**e, f**), scale bar 40  $\mu\text{m}$ .

16a, f, l, m, 17a-d, 18a, b, d, f; Tables 1, 2). Cilia 8-10  $\mu\text{m}$  long, more loosely spaced ( $\sim 3 \mu\text{m}$ ) in oral than postoral area ( $\sim 1.5 \mu\text{m}$ ), arranged in about 30 rows according to Kahl (1930a, b), matching averages (32-33) of Austrian and South African populations (Tables 1, 2). Row number conspicuously constant, that is, 33 rows in South African specimens from the non-flooded Petri dish (raw) culture and 35 in the 100  $\mu\text{m}$  longer specimens from the semipure culture (Table 2). At right anterior end of dorsal brush of most Austrian specimens an accumulation of scattered basal bodies (Fig. 16m), likely adesmokineties as in the South African specimens (Fig. 4b). Dorsal brush basically as in *A. cultriforme cultriforme*, that is, almost heterostichad (row 3 shorter than row 2 by about 20%) occupying 31-35% of body length; rarely occur specimens with one or two more or less distorted bristle rows right of row 1; tail of row 3 extends into second body third (Figs 4b, 16a, f, m, 17c, d, f, 18d, f, i, 19d; Table 2). Details of bristle pairs studied only in South African specimens, likely as shown in figure 16n, that is, slightly clavate anterior bristle longer (3-5  $\mu\text{m}$ ) than posterior (2-3  $\mu\text{m}$ ) in rows 1 and 2, while vice versa in row 3 (Figs 16n, 19a-f).

Oral bulge conspicuous, though only about 3  $\mu\text{m}$  high near dorsal end, because almost half as long as body (42-45%; Table 2), strongly slanted ( $50^\circ$ - $70^\circ$ , on the average  $63^\circ$ , n 10), rather distinctly projecting from body proper at proximal end (Figs 16p-s, 17a), and studded with refractive extrusomes (Figs 20a, b); in lateral view slightly to distinctly convex, in frontal view about 5  $\mu\text{m}$  wide and very narrowly cuneate to oblong with proximal end bluntly pointed. Circumoral kinety and oral basket as described in *A. cultriforme cultriforme* (Figs 16a, c, f, o-t, 17a, 18a, b, d-h, 20a-e, 22a-c, 27b; Table 2).

**Encystment** (Figs 21a-g): The protargol preparations from the non-flooded Petri dish culture and the semipure culture contain encysting and encysted specimens, but only those from the former impregnated well, and were used for reconstructing the encystment process. However, few encysting specimens were found, and the description is not very detailed, though definitely more complete than the data available in the literature.

When encystment commences, cells become smaller and stouter (Figs 21a, b). This diminution is associated with the gradual resorption of somatic and oral kinetids, especially those of the dorsal brush and the circumoral kinety, both having reduced about half of their length and kinetids. The macronucleus appears more tortuous, but is basically unchanged, while the micronuclei move apart from the macronucleus and distribute throughout the body, as in early dividers. The contractile vacuole is still recognisable.

In the next stage, cells become almost globular and the number of somatic and oral kinetids is greatly

reduced (Fig. 21c). The remaining, still ciliated kinetids are somewhat irregularly arranged within the rows which lose the *Arcuospathidium* pattern, and become more and more meridionally arranged. The dorsal brush kinetids still have bristles, but lose the dikinetid appearance. The postciliary microtubule ribbons are very distinct at this stage. Then a really remarkable process commences, a strong shortening of the macronucleus, which is eventually reduced by about 80% of its length and becomes semicircular in the encysted specimens (Fig. 21g). This length reduction is not achieved by thickening of the strand or chromatin extrusion, but by resorption of nuclear mass. It is evident from simple volume calculations: the nuclear volume decreases from about 5,000  $\mu\text{m}^3$  to 1,000  $\mu\text{m}^3$  in the mature cyst. The micronuclei are still scattered, and the contractile vacuole is still recognisable.

The next stage is characterised by the appearance of the cyst wall, which is about 1  $\mu\text{m}$  thick and does not impregnate with the protargol method used (Fig. 21e). The specimens are now globular and lose the oral apparatus, while some somatic basal bodies are still recognisable, becoming more and more scattered. The shortening of the macronucleus is now distinct, and the micronuclei arrange around the macronucleus strand. The contractile vacuole is still recognisable.

During the last stages, micronuclei resorption commences and the faceted ectocyst develops, as described in *A. cultriforme cultriforme*. The infraciliature probably disappears completely, though this is difficult to prove because the maturing cysts are rather poorly impregnated (Fig. 21f). The mature cyst wall, although appearing quite stable *in vivo*, is usually distorted and the cell is distinctly shrunken at the preparations, leaving a more or less wide space between wall and cortex. The macronucleus is shortened to a semicircular strand, and about half of the micronuclei have been resorbed. The nucleoli are very small. The contractile vacuole disappears (Fig. 21g).

**Occurrence and ecology:** Kahl (1930a) discovered *A. cultriforme scalpriforme* in mosses from Mittenwald (a village in southern Bavaria near the Tyrolean border) and the Zillertal in Austria (a valley in the Tyrol Alps). The "local form" from roof moss in northern Germany appears to belong to another species (see discussion of synonymy). The same is supposed for Gellért's (1956) variety, which is only 90  $\mu\text{m}$  long. The next reliable record is from the surroundings of Erlangen in Bavaria, where Wenzel (1953) observed *A. cultriforme scalpriforme* in only two of over 100 moss samples, that is, in dry moss at pH 5.1 and leaf litter. In 1984, Foissner redescribed the species from soil of a deciduous forest in Burgenland, Austria. More recently, some unsubstantiated records were added from Bulgaria (Detcheva, 1972b) and from Germany (Foissner, 2000b). Note also

**Table 1.** Morphometric data on *Arcuospathidium cultriforme cultriforme* populations (Pop) from Hawaii (HA50, original; HA12, original), Austria (AUL from Linz, original; AUB from Burgenland, according to Foissner, 1984 and his original notes), and Rwanda (RW, original).

Characteristics <sup>1</sup>	Pop	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	HA50	222.5	220.0	28.0	8.4	12.6	193.0	280.0	11
	HA12	270.3	270.0	30.9	9.3	11.4	233.0	348.0	11
	AUL	275.7	282.0	43.5	8.7	15.8	145.0	382.0	25
	AUB	191.6	180.0	33.7	8.7	17.6	145.0	245.0	15
	RW	305.8	300.0	48.5	16.2	15.9	250.0	400.0	9
Body, width	HA50	36.3	35.0	8.4	2.5	23.2	25.0	56.0	11
	HA12	51.5	53.0	14.5	4.4	28.2	34.0	76.0	11
	AUL	50.3	49.0	12.0	2.4	23.8	26.0	85.0	25
	AUB	27.5	28.0	4.5	1.2	16.6	20.0	34.0	15
	RW	44.9	47.0	11.0	3.7	24.6	27.0	62.0	9
Body length:width, ratio	HA50	6.4	6.4	1.5	0.4	23.2	4.4	9.2	11
	HA12	5.7	5.1	2.1	0.6	36.7	3.6	10.2	11
	AUL	5.8	5.4	2.1	0.4	35.8	3.2	11.9	25
	AUB	7.2	6.6	1.9	0.5	26.7	4.5	11.4	15
	RW	7.1	6.5	1.5	0.5	21.3	5.2	9.7	9
Oral bulge, length (cord)	HA50	78.7	75.0	14.6	4.4	18.6	58.0	105.0	11
	HA12	84.6	80.0	10.3	3.1	12.2	70.0	107.0	11
	AUL	93.9	95.0	17.4	3.5	18.5	56.0	138.0	25
	AUB	58.1	60.0	8.3	2.1	14.3	37.0	70.0	15
	RW	102.8	100.0	26.6	8.9	25.9	65.0	145.0	9
Oral bulge (circumoral kinety), width	HA50	4.0	4.0	0.8	0.4	19.8	3.0	5.0	5
	AUL	3.9	4.0	0.2	0.1	5.7	3.5	4.0	5
Oral bulge, height	HA50	2.4	2.5	0.4	0.1	15.6	2.0	3.0	11
	AUL	2.3	2.5	0.3	0.1	11.5	2.0	2.5	11
Oral bulge length:body length, ratio	HA50	0.35	0.35	-	-	10.4	0.29	0.43	11
	HA12	0.32	0.3	-	-	13.9	0.26	0.38	11
	AUL	0.34	0.34	-	-	11.3	0.28	0.43	25
	AUB	0.31	0.3	-	-	15.7	0.22	0.39	15
	RW	0.34	0.31	0.1	-	19.3	0.25	0.45	9
Circumoral kinety to last dikinetid of brush row 1, distance	HA50	52.2	52.0	6.5	2.0	12.4	44.0	62.0	11
	RW	66.3	67.0	20.3	6.8	30.6	45.0	100.0	9
Circumoral kinety to last dikinetid of (the longest) brush row 2, distance	HA50	58.6	58.0	5.4	1.6	9.3	50.0	67.0	11
	AUL	68.5	68.0	9.2	2.6	13.4	51.0	86.0	13
	AUB	50.9	49.0	7.9	2.0	15.6	39.0	70.0	15
	RW	71.4	73.0	20.6	6.9	28.8	45.0	100.0	9
Circumoral kinety to last dikinetid of brush row 3, distance	HA50	47.7	49.0	4.2	1.3	8.8	40.0	54.0	11
	RW	51.8	52.0	13.4	4.5	25.9	35.0	70.0	9
Anterior body end to macronucleus, distance	HA50	72.8	72.0	10.1	3.0	13.9	58.0	90.0	11
Macronucleus figure, length	HA50	113.9	110.0	28.8	8.7	25.3	60.0	155.0	11
	AUL	158.7	173.0	39.9	9.1	25.1	47.0	220.0	19
	AUB	100.6	94.0	27.5	7.1	27.3	69.0	160.0	15
	RW	10.0	9.0	2.7	0.9	26.9	7.0	14.0	9
Macronucleus nodules, length	HA50	5.6	5.0	1.8	0.5	32.0	4.0	9.0	11
Macronucleus, width in mid	AUB	4.9	5.0	0.6	0.2	12.3	4.2	5.6	15
	RW	4.2	4.0	0.9	0.3	22.2	3.0	6.0	9
Macronucleus, number	HA50					see footnote <sup>2</sup>			
	HA12	1.0	1.0	0.0	0.0	0.0	1.0	1.0	11
	AUL	1.0	1.0	0.0	0.0	0.0	1.0	1.0	25
	AUB	1.0	1.0	0.0	0.0	0.0	1.0	1.0	15
	RW <sup>3</sup>	96.0	100.0	15.2	6.8	15.8	70.0	110.0	5
Macronucleus nodules, number	HA50	2.6	2.5	0.2	0.1	6.9	2.5	3.0	8
	AUL	2.8	3.0	0.4	0.1	15.0	2.0	3.5	19
	AUB	2.7	2.7	0.2	0.1	5.6	2.5	2.8	4
	RW	2.9	3.0	0.5	0.2	18.9	2.0	4.0	9
	HA50	15.0	13.0	-	-	-	12.0	20.0	3
Micronuclei, across	AUL	13.3	12.0	3.3	0.7	24.5	9.0	23.0	19
	HA50	31.4	31.0	1.6	0.5	5.0	30.0	35.0	11
Somatic kineties, number	AUL	36.7	36.0	3.6	0.8	9.8	30.0	44.0	23
	AUB	28.1	29.0	1.3	0.3	4.6	26.0	30.0	15
	RW	28.4	28.0	2.2	0.7	7.7	25.0	31.0	9

Table 1. (Continuation).

Characteristics <sup>1</sup>	Pop	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Basal bodies in a right side kinety, number	HA50	116.8	113.0	14.5	4.4	12.4	100.0	147.0	11
	RW	161.0	165.0	32.5	14.5	20.2	110.0	200.0	5
Dorsal brush rows, number	HA50	3.0	3.0	0.0	0.0	0.0	3.0	3.0	11
	HA12	3.0	3.0	0.0	0.0	0.0	3.0	3.0	11
	AUL	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
	AUB	3.0	3.0	0.0	0.0	0.0	3.0	3.0	15
	RW	3.0	3.0	0.0	0.0	0.0	3.0	3.0	9
Dikinetids in brush row 1, number	HA50	48.7	46.0	11.4	3.4	23.4	35.0	76.0	11
	RW	64.6	61.0	15.7	5.9	24.3	44.0	91.0	7
Dikinetids in brush row 2, number	HA50	55.3	54.0	8.8	2.6	15.9	48.0	78.0	11
	AUL	52.4	50.0	7.8	3.0	15.0	47.0	70.0	7
	RW	59.6	58.0	9.9	3.7	16.6	47.0	72.0	7
Dikinetids in brush row 3, number	HA50	40.2	39.0	7.8	2.3	19.3	28.0	51.0	11
	RW	35.6	38.0	6.1	2.3	17.2	24.0	41.0	7

<sup>1</sup> Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from non-flooded Petri dish cultures. Measurements in  $\mu\text{m}$ . 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,  $\bar{x}$  - arithmetic mean.

<sup>2</sup> Four types are recognizable: Of 75 specimens analysed, 58% have the usual macronucleus pattern, while 19% have many (up to 100) scattered nodules, and 23% have a mixture of nodules and one to several short strands.

<sup>3</sup> See text for explanations.

the records from South Africa and Brazil described in the Materials and Methods section.

All other records are from limnetic habitats, viz. the mud and Aufwuchs of various polluted rivers in Bulgaria (Detcheva, 1972a, c, 1975, 1979a, b, 1986, 1993; Russev et al., 1976, 1994) and some natural freshwaters and a sewage plant in China (Wang, 1977; Ning et al., 1993; Wang and Ma, 1994). We consider all these unsubstantiated reports as highly questionable because the senior author never found this species in over 1,000 river samples investigated over the years. The above mentioned authors confused *A. cultriforme scalpriforme* with other ciliates, especially pleurostomatids, some of which have a similar size and shape, as mentioned in the descriptive section. Therefore, we do not document the many abiotic environmental data provided by Detcheva and her students. On the other hand, our South African habitat, the soil from the margin of a flat pond, indicates that this subspecies may occur as a guest in the true limnetic habitats.

Reliable records of *A. cultriforme scalpriforme* are available from Germany, Austria, Brazil, and the Republic of South Africa. Foissner (1998) also lists a record from Australia. Basically, the reliable records indicate that *A. cultriforme scalpriforme* is a terrestrial cosmopolitan rarer than *A. cultriforme cultriforme*.

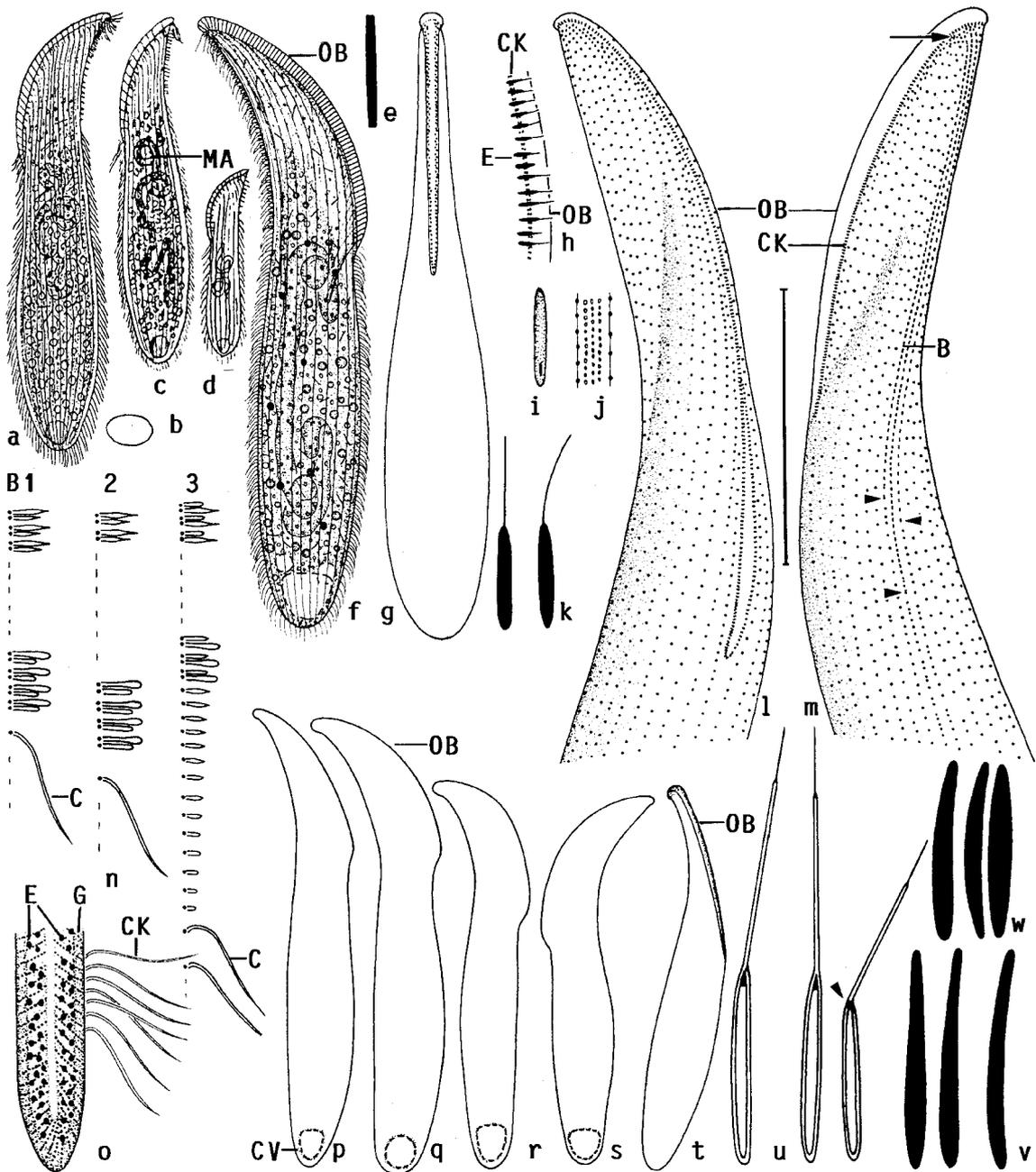
**Ontogenesis:** Although ontogenesis is a continuous process, we distinguish seven stages, each characterised by specific events, for the sake of clarity. Most stages depicted were seen in at least two, usually three or four

specimens. This is also evident from the second series showing division in specimens with two macronucleus pieces (Figs 27a-g). We could not follow the origin of the oral basket because the nematodesmata impregnated too faintly. The parental oral apparatus and dorsal brush do not show any changes.

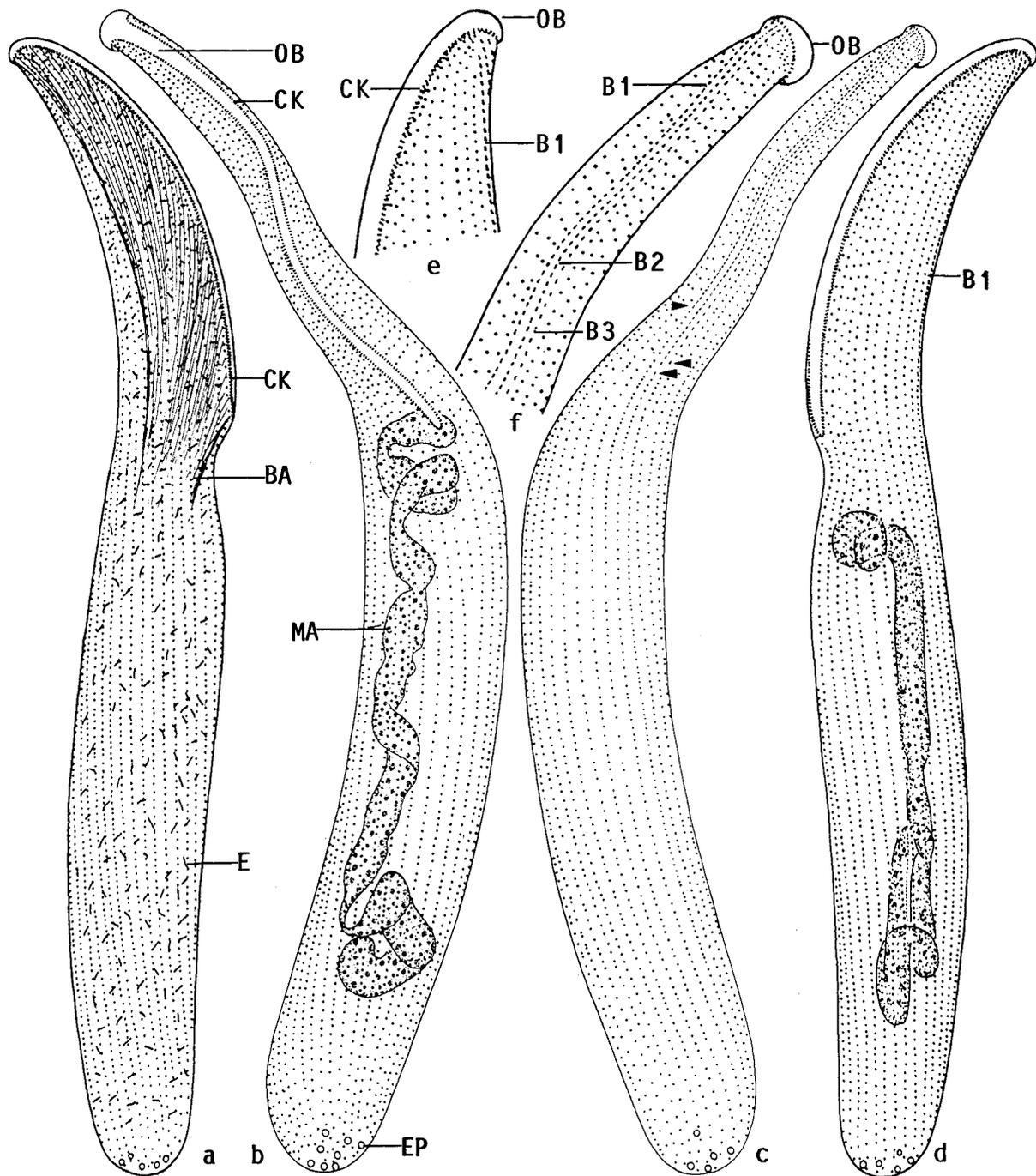
**Ordinary specimens** (Figs 13, 14, 22-26; Table 3).

**Stage 1** (very early dividers commencing production of oral kinetofragments and dorsal brush row 2; Figs 22a-c, 23a, b, d, e; Table 3). When ontogenesis begins, the cells are on the average slightly larger than morphostatic specimens (Table 3). Basal bodies are produced distinctly underneath mid-body, as evident from middle and late dividers which show that the proter is considerably longer than the opisthe (Table 3). The division plane is not transverse but slightly oblique from dorsal to ventral. Soon, some of the new basal bodies form minute kinetofragments, each probably consisting of monokinetids, except of a dikinetid at anterior end. There is also a basal body proliferation in the opisthe's ciliary rows, as recognisable by the narrow and uneven spacing of the kinetids.

When entering stage 2, a remarkable change occurs in body shape - a slight indentation develops in the prospective fission area (Figs 22b, c), as observed also in *Protospathidium serpens* and *Arcuospathidium coemeterii* in which, however, the indentation develops slightly later and is transient. Basal body proliferation continues in all opisthe ciliary rows; the oral kinetofragments become dikinetidal and associated with minute



**Figs 16a-w.** *Arcuospathidium cultriforme scalpriforme*, alive (**a-g, j, n-w**) and after protargol impregnation (**h, i, k-m**), according to Kahl, 1930a (**a, b**); Kahl, 1930b (**c, d**); Foissner, 1984 (**e-m**); and new observations of populations from South Africa (**n-v**) and Brazil (**w**). **a-c**: Left side and transverse (**b**) view of a specimen probably from the Zillertal in Austria, length 260  $\mu\text{m}$ . **d**: Left side view of another species from northern Germany, length 160  $\mu\text{m}$ . **e**: Resting extrusome, length 6  $\mu\text{m}$ . **f, g**: Right side and ventral view of a specimen from Austria, length 270  $\mu\text{m}$ . **h**: Part of oral bulge of specimen shown in figure 17a. **i, k**: Cytoplasmic extrusomes, capsule 4  $\mu\text{m}$ . **j**: Cortical granulation. **l, m**: Ventrolateral and dorsolateral view of oral area. Arrow marks an accumulation of basal bodies. Arrowheads denote proximal end of brush rows. Scale bar 50  $\mu\text{m}$ . **n**: Dorsal brush, bristles (up to 5  $\mu\text{m}$ ) and somatic cilia (10  $\mu\text{m}$ ) drawn to scale. **o**: Proximal portion of oral bulge (width 5  $\mu\text{m}$ ), showing the scattered extrusomes and the cortical granules, which form an arrowhead-like pattern. **p-t**: Variability of body shape and length of oral bulge (37-51% of body length) of specimens redrawn from video records. **u**: Exploded toxicysts, length 15-20  $\mu\text{m}$ . Arrowhead marks refractive granule. **v, w**: Resting oral bulge extrusomes from populations of South Africa (**v**) and Brazil (**w**), length 8  $\mu\text{m}$  and 6  $\mu\text{m}$ . B (1-3) - dorsal brush (rows), C - somatic cilia, CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, G - cortical granules, MA - macronucleus, OB - oral bulge.



**Figs 17a-f.** *Arcuospathidium cultriforme scalpriforme*, protargol-impregnated ciliary pattern and macronucleus of the Austrian population studied by Foissner (1984). **a, d, e:** Right and left side view, length 295  $\mu$ m. The dorsal brush is exactly on the dorsal side of the cell. Therefore, the specimen cannot be *Spathidium lionotiforme*, as proposed by Foissner (1984), which has the dorsal brush on the left side of the oral area (Kahl, 1930b; Foissner, 2003b). The oral bulge and the cytoplasm are studded with extrusomes shown at higher magnification in figures 16 h, k. **b, c, f:** Ventral and dorsal view, length 240  $\mu$ m. Note the long, slightly cuneate oral bulge and the dorsal brush, which is composed of three rows of dikinetids with short, bristle-like cilia; arrowheads denote proximal end of brush rows. The brush is isotrichad (all rows of similar length) and almost as long as the oral bulge. B (1-3) - dorsal brush (rows), BA - oral basket made up of nematodesmata originating from the circumoral dikinetids, CK - circumoral kinety, E - extrusomes, EP - excretory pores of the contractile vacuole, MA - macronucleus, OB - oral bulge.

**Table 2.** Morphometric data on *Arcuospathidium cultriforme scalpriforme* populations (Pop) from South Africa (SA, original) and Austria (AUS, from Foissner, 1984 and his original notes).

Characteristics <sup>1</sup>	Pop	$\bar{X}$	M	SD	SE	CV	Min	Max	n
Body, length	SA	206.8	200.0	25.6	5.6	12.4	167.0	275.0	21
	AUS	251.3	260.0	30.8	8.9	12.3	205.0	290.0	12
Body, width	SA	39.3	42.0	7.7	1.7	19.5	25.0	51.0	21
	AUS	35.3	34.5	3.9	1.1	11.0	30.0	43.0	12
Body length:width, ratio	SA	5.4	5.3	1.0	0.2	18.0	4.0	7.8	21
	AUS	7.2	7.0	1.2	0.4	16.9	5.2	9.7	12
Oral bulge, length (cord)	SA	92.1	94.0	12.8	2.8	13.9	61.0	113.0	21
	AUS	105.8	106.0	7.8	2.2	7.4	92.0	120.0	12
Oral bulge, height	SA	2.6	2.5	0.3	0.1	11.1	2.0	3.0	21
	AUS	0.42	0.43	-	-	8.7	0.37	0.49	12
Oral bulge length:body length, ratio	SA	0.45	0.46	0.1	-	11.8	0.35	0.52	21
	AUS	0.42	0.43	-	-	8.7	0.37	0.49	12
Circumoral kinety to last dikinetid of brush row 1, distance	SA	53.6	53.0	7.9	1.7	14.7	41.0	65.0	21
	AUS	64.0	63.0	8.1	1.8	12.7	50.0	80.0	12
Circumoral kinety to last dikinetid of brush row 2, distance	SA	88.4	88.0	8.3	2.4	9.4	70.0	100.0	12
	AUS	51.6	49.0	9.1	2.0	17.7	35.0	72.0	21
Circumoral kinety to last dikinetid of brush row 3, distance	SA	51.6	49.0	9.1	2.0	17.7	35.0	72.0	21
	AUS	51.6	49.0	9.1	2.0	17.7	35.0	72.0	21
Anterior body end to macronucleus, distance	SA	78.8	78.0	16.8	3.7	21.3	47.0	113.0	21
Macronucleus figure, length	SA	87.5	90.0	21.5	4.7	24.6	46.0	134.0	21
	AUS	106.3	108.0	25.9	7.5	24.4	42.0	142.0	12
Macronucleus, length (spread, thus approximate)	SA	187.4	200.0	-	-	-	130.0	230.0	21
Macronucleus, width in central third	SA	6.1	6.0	1.4	0.3	22.6	4.0	9.0	21
	AUS	7.2	7.0	0.8	0.2	10.7	6.0	8.5	12
Macronucleus, number	SA	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
	AUS	1.0	1.0	0.0	0.0	0.0	1.0	1.0	12
Micronuclei, across	SA	3.0	3.0	0.3	0.1	10.6	2.5	4.0	21
	AUS	2.9	2.8	0.1	0.0	4.0	2.8	3.1	12
Micronuclei, number	SA	11.5	12.0	2.6	0.6	22.2	7.0	18.0	21
	AUS	32.7	32.0	3.0	0.7	9.1	27.0	40.0	21
Somatic kineties, number <sup>2</sup>	SA	32.7	32.0	3.0	0.7	9.1	27.0	40.0	21
	AUS	32.5	32.0	1.2	0.4	3.8	31.0	35.0	12
Basal bodies in a right side kinety, number	SA	124.4	130.0	25.0	5.5	20.1	85.0	180.0	21
Basal bodies in each somatic kineties of 10 $\mu$ m long, number	AUS	6.1	6.0	0.9	0.3	14.8	5.0	8.0	12
Dorsal brush rows, number	SA	3.0	3.0	0.0	0.0	0.0	3.0	3.0	21
	AUS	3.0	3.0	0.0	0.0	0.0	3.0	3.0	12
Dikinetids in brush row 1, number	SA	40.6	39.0	7.3	1.6	18.0	30.0	56.0	21
Dikinetids in brush row 2, number	SA	55.0	55.0	7.0	1.5	12.8	40.0	67.0	21
Dikinetids in brush row 3, number	SA	34.9	35.0	6.1	1.3	17.4	23.0	48.0	21

<sup>1</sup> Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from non-flooded Petri dish cultures. Measurements in  $\mu$ m. 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,  $\bar{x}$  - arithmetic mean.

<sup>2</sup> Row number in specimens from semipure culture, which are much larger (Table 3):  $\bar{x}$  35.0, SD 3.6, CV 10.3, Min 30, Max 41, n 21.

blebs described below; and some somatic dikinetids appear to form a short dorsal brush row 2 (Figs 23b, e). The macronucleus appears smoothed and the micronuclei are distributed throughout the cell.

**Stage 2** (early dividers separating kinetofragments from proter kineties and producing brush rows 3 and 1 and the excretory pores; Figs 13a-d, 14a-c, 22d, 23c, f, 24a, b; Table 3). Early dividers are distinctly longer and narrower than morphostatic specimens (Table 3). Body indentation and division blebs become more distinct in the prospective fission area (Figs 22d, 23c).

The division blebs are about 5  $\mu$ m-sized convexities left and slightly above of the kinetofragments (Figs 13b-d). They disappear in late dividers. The kinetofragment belt is now clearly recognisable and distinctly oblique (30°-45° in three appropriate specimens), which is likely to be due to faster growth of the dorsal than the ventral side (Figs 13a, 14a, 22d). The newly formed oral kinetofragments, which are longer on the ventral than the dorsal side and detach from the proter's ciliary rows in a dorsoventral gradient, commence to curve rightwards on the dorsal surface and later also on the

ventral one (Figs 13b, 22d, 23c, f). Slightly later, when three brush rows are recognisable, the fragments are arranged transversely and become concave on the left side, while the right side fragments remain almost straight, but become more distinctly slanted (Figs 24a, b). At this stage, the newly formed kinetofragments consist of six to eleven, on the average eight dikinetids each, that is, they are almost complete; only one dikinetid is added in middle and late dividers (Table 3). Taking the averages of the fragment dikinetids (9) and ciliary rows (35), 315 dikinetids are available for the new circumoral kinety, which is close to the number found in the proter of the dividers (Tables 2, 3). Thus, there is no second round of dikinetid proliferation, not even during post-divisional growth.

Many new somatic basal bodies have been produced in two regions of the ciliary rows, the posterior area of the proter and the anterior area of the opisthe. However, some basal body production is likely to occur throughout the division process and even in morphostatic specimens, as suggested by barren kinetids found throughout the ciliary rows of morphostatic cells. Dorsal brush row 2 is still growing and has now about one third of the dikinetids found in morphostatic specimens (Table 3). Next, brush row 3 is generated, and finally row 1. This curious sequence was observed in several specimens. All brush rows have an anterior tail of ordinary monokinetids (Figs 14a-c). Now, the nuclear apparatus shows some changes (Fig. 22d): the macronucleus becomes less tortuous, that is, commences to contract, and the micronuclei appear more or less distinctly inflated. Further, a new contractile vacuole and new excretory pores are generated at the proter's posterior end. Usually, the new pores are located dorsally or dorso-laterally, but in some specimens they are near the ventral side.

**Stage 3** (early mid-dividers with condensing macronucleus and dividing micronuclei; Figs 14a-f, 22e, f; Table 3). Early mid-dividers are smaller and stouter than early dividers, but larger than morphostatic cells (Table 3). The indentation underneath the prospective fission area is still recognisable, but becomes indistinct, especially in cells prepared for scanning electron microscopy (Figs 14a, 22e, f). The macronucleus commences to shorten towards the centre, as indicated by the swollen ends; the nucleoli begin to disappear. The micronuclei grow up to 6  $\mu\text{m}$ , that is, double size. In the post-brush area, one can observe the origin of dikinetids within the parental ciliary rows: a bristle is generated ahead of each parental cilium which gradually decreases in length eventually becoming a bristle (Fig. 14f).

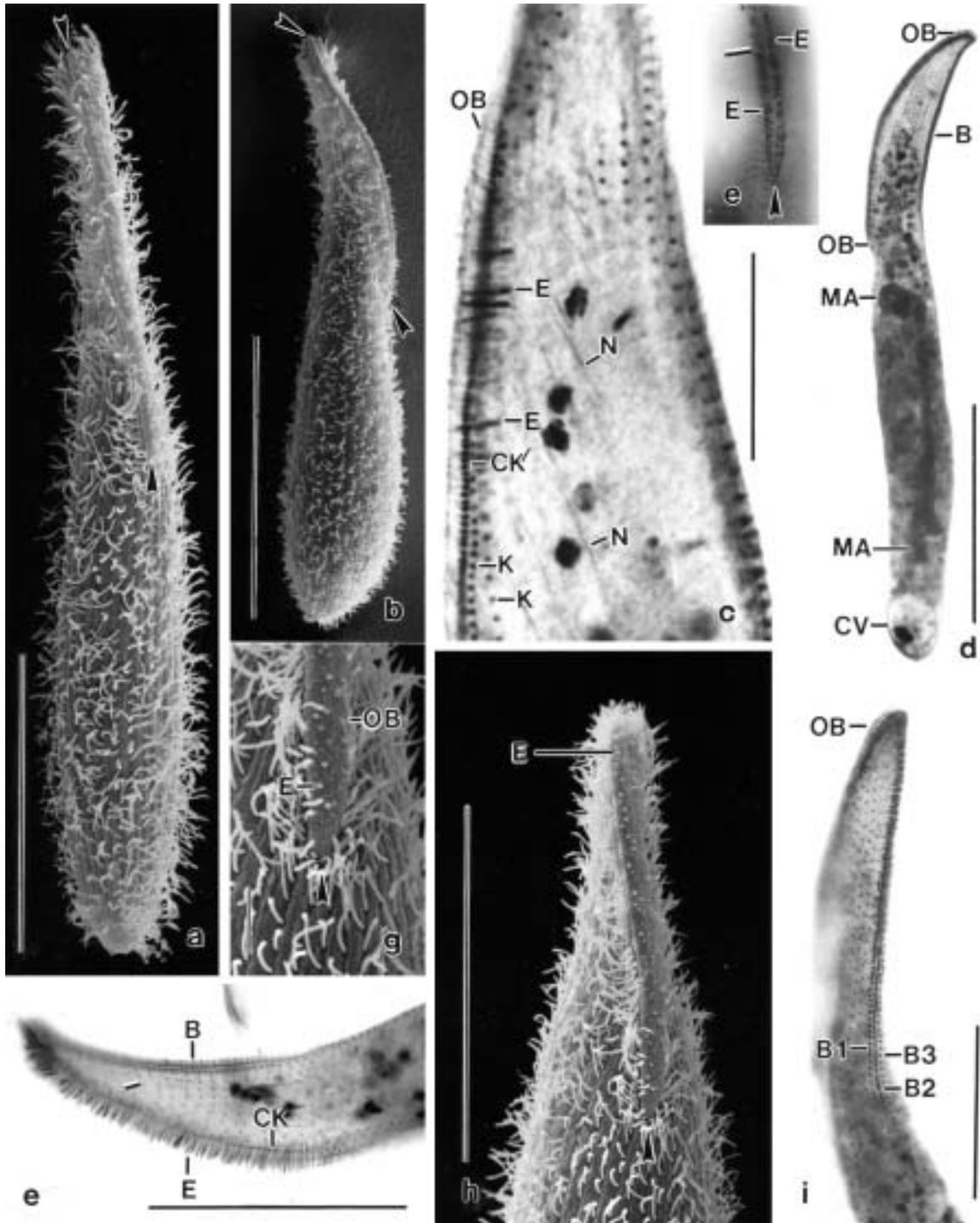
**Stage 4** (mid-dividers with transversely oriented, concave kinetofragments and condensed, globular macronucleus; Figs 14a-f, 22g, h, 24c, d; Table 3).

These cells are the smallest and stoutest dividers, approaching the length of morphostatic specimens (Table 3). The subequatorial indentation is indistinct, while the division blebs are still distinct (Figs 14a, b, e, 22g, h). All oral kinetofragments are now transversely arranged and more or less distinctly concave. The new brush rows have about two thirds of their dikinetids (Table 3). No further increase of brush dikinetids occurs in late dividers and early post-dividers, suggesting that the final number is obtained only in late post-dividers or morphostatic cells. The macronucleus condenses to a globular, fibrous mass with lightly impregnated karyoplasm. The dividing micronuclei move apart, still being connected by a bundle of fibres.

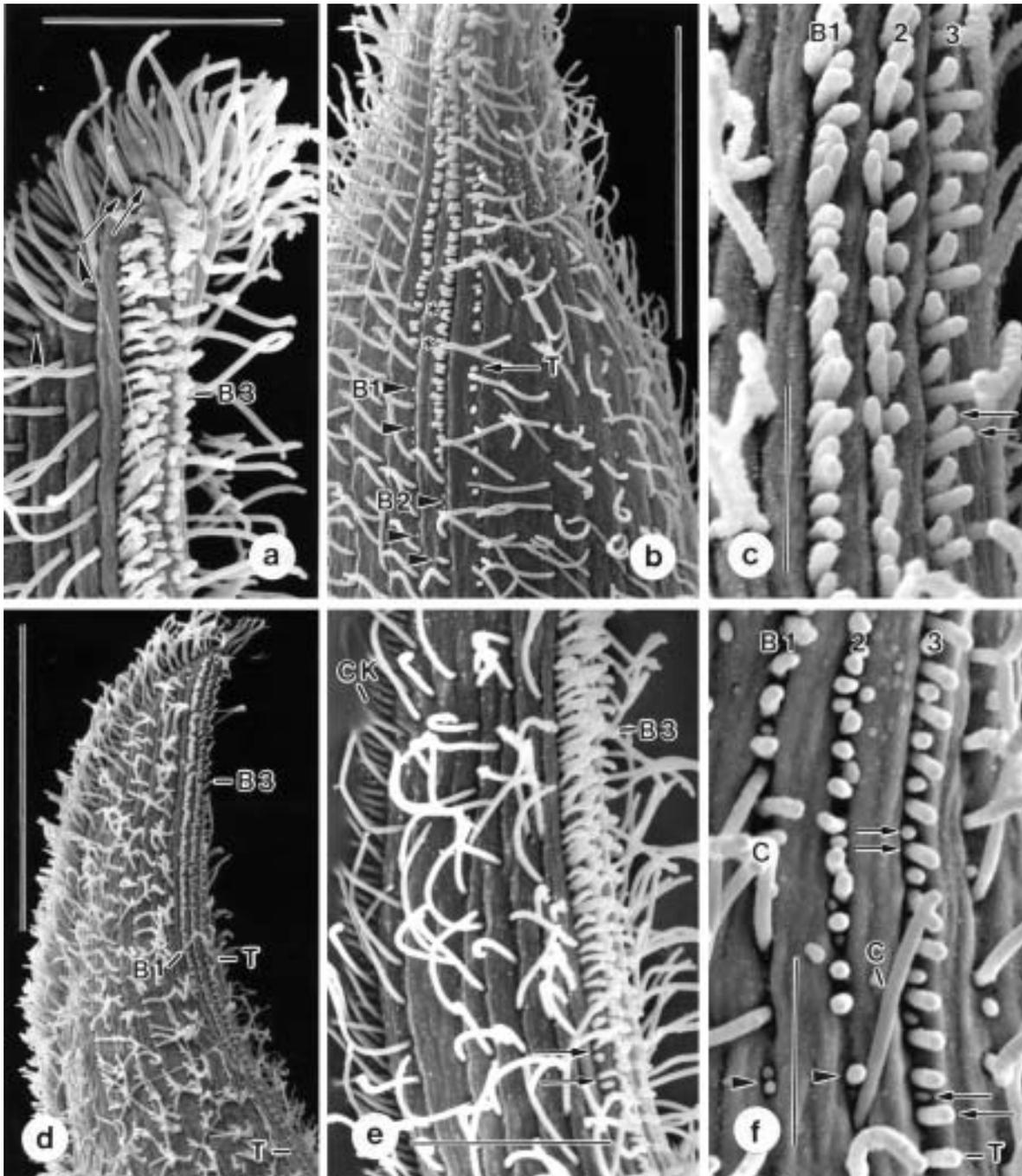
**Stage 5** (late dividers with forming division furrow and extending macronucleus; Figs 22i, j; Table 3). Late dividers grow in length, the division blebs disappear, and the division furrow becomes recognisable, dividing the cell into an ellipsoidal opisthe shorter by about one third than the conspicuously conical proter (Table 3). The body constriction causes the onset of kinetofragment alignment, that is, the distances between the individual kinetofragments become smaller. The globular macronuclear mass of the mid-dividers extends and grows to a more or less curved rod with fibrous contents. Most micronuclei have completed fission and commence to condense.

**Stage 6** (very late dividers with aligning kinetofragments, forming oral bulge, and separating daughter cells; Figs 22k, l, 25a, b, 26a; Table 3). Very late dividers may grow to a length of 565  $\mu\text{m}$  and are distinctly furrowed. The developing opisthe oral area is conspicuously clavate and the oral bulge, which develops at its margin, becomes recognisable as a bare protuberance. The oral kinetofragments align to the new opisthe circumoral kinety. This process is obviously complex because many fragments overlap more or less and some become strongly curved, as in *A. muscorum* (Berger et al., 1983). Now, the ciliary rows are distinctly separate from the kinetofragments but still straight, showing that the genus-specific pattern is obtained only in post-dividers. The macronucleus becomes a long, tortuous strand and shows fibrous contents with many minute globules, probably developing nucleoli (Fig. 22l). Finally, the daughters separate. The anterior daughter cell is longer than the posterior one by about one third; the latter is more or less distinctly pointed in the split area. The micronuclei are still scattered through the cell.

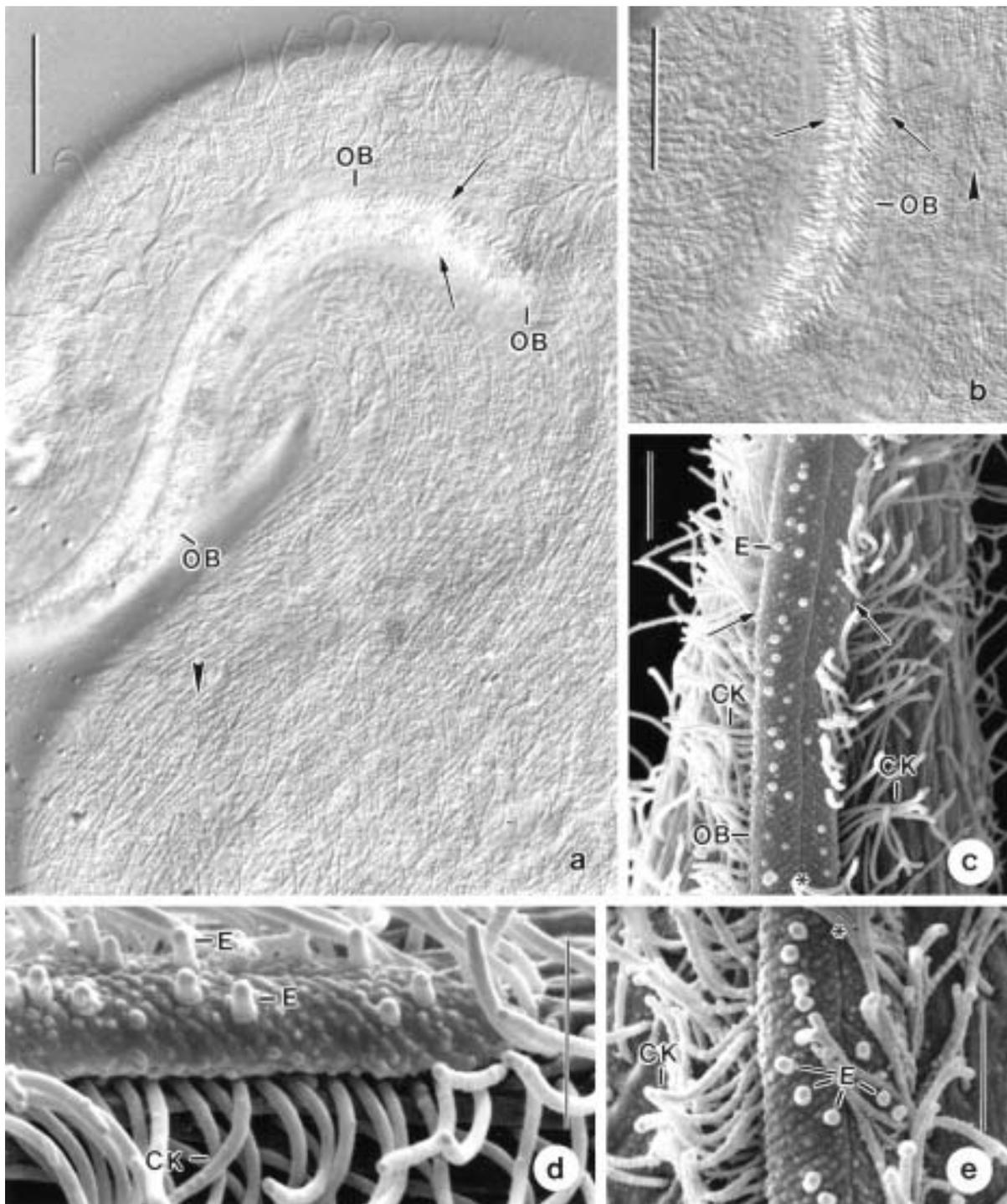
**Stage 7** (early opisthe post-dividers with small size, pointed anterior macronucleus end, and short dorsal brush and circumoral kinety/oral bulge with more or less conspicuous irregularities; Figs 26b-g). Very early opisthe post-dividers are bursiform and still have a rather distorted circumoral kinety and almost meridionally arranged ciliary rows (Figs 26d, e). The oral



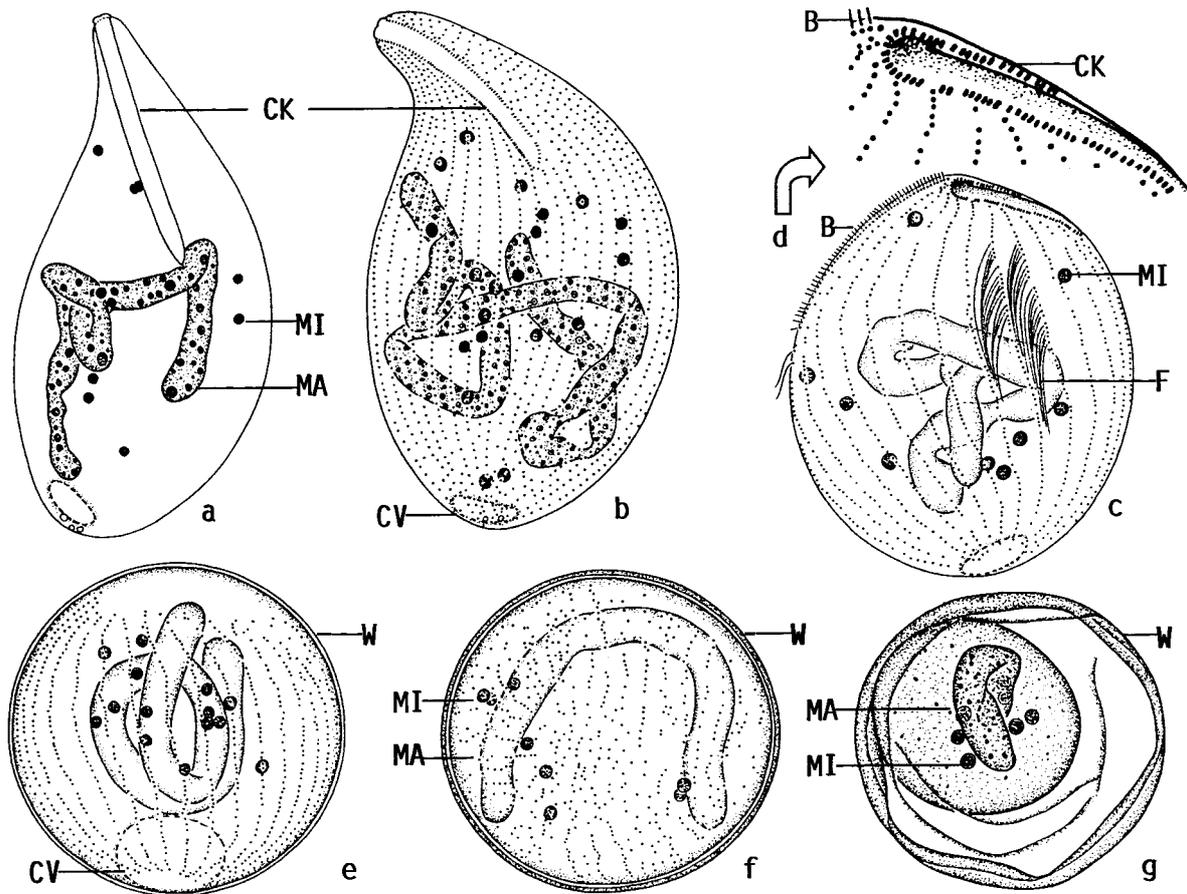
**Figs 18a-i.** *Arcuospathidium cultriforme scalpriforme*, South African (**a, b, e-h**) and Austrian (**c, d, i**; from Foissner, 1984) specimens in the scanning electron microscope (**a, b, e, g, h**) and after protargol impregnation (**c, d, f, i**). **a, b:** Ventrolateral overviews showing the oral bulge (ends marked by arrowheads) extending to almost mid-body. **c:** Left side oral area showing the comparatively thin, resting extrusomes and the circumoral kinety composed of very narrowly spaced dikinetids. **d:** Left side overview showing the dorsally curved ciliary rows in the oral area. **e, g, h:** Ventral views showing the bluntly pointed proximal end of the oral bulge and circumoral kinety (arrowheads). Note the scattered extrusomes in right and left bulge half. **f:** Left side view of oral area showing the dorsal brush and the partially extruded, thin extrusomes. **i:** Dorsolateral view showing the long dorsal brush. B (1-3) - dorsal brush (rows), CK - circumoral kinety, CV - contractile vacuole, E - extrusomes, K - somatic kineties, MA - macronucleus, N - nematodesmata, OB - oral bulge. Scale bars 100 µm (b, d), 50 µm (a, f, h, i), 20 µm (c), and 5 µm (g).



**Figs 19a-f.** *Arcuospathidium cultriforme scalpriforme*, dorsal brush area of South African specimens in the SEM. **a:** Bluntly pointed anterior end, where (brush) ciliary rows curve ventrally. The brush rows have some ordinary cilia at anterior end (arrows). Arrowheads mark short kinetofragments (adesmokineties?) left of circumoral kinety. The brush bristles are three times shorter than the somatic cilia, that is, about 3  $\mu\text{m}$  long *in vivo*. **b:** Posterior half of brush. The rows end at similar level, but row 3 has a monokinetidal bristle tail (arrow) extending into second third of body. Arrowheads mark heteromorphic tail of brush rows 1 and 2, where minute bristles irregularly alternate with ordinary cilia. Asterisks denote row 1 dikinetids with a very short anterior bristle and an ordinary posterior cilium. **c, f:** Mid-portion and end of dorsal brush showing that, in row 3, the anterior bristle of the dikinetids is shorter than the posterior one (arrows). Arrowheads mark strongly shortened bristles. **d:** Oral portion showing that brush bristle length decreases at both ends of rows. **e:** Posterior third of brush showing that, in row 1, the posterior bristle of the dikinetids is shorter than the anterior (arrows). B (1-3) - dorsal brush (rows), C - somatic cilia, CK - circumoral kinety, T - tail of brush row 3. Scale bars 40  $\mu\text{m}$  (d), 20  $\mu\text{m}$  (b), 10  $\mu\text{m}$  (a, e), and 4  $\mu\text{m}$  (c, f).



**Figs 20a-e.** *Arcuospathidium cultriforme scalpriforme*, South African specimens alive (**a, b**) and in the scanning electron microscope (**c-e**). **a, b:** Surface views of oral area. The oral bulge appears as a bright band due to the refractive extrusomes which are scattered in the bulge halves, while forming a row in the right and a row in the left bulge half of *A. cultriforme cultriforme* (Fig. 7b). The bulge cortex contains oblique rows of cortical granules arranged in an arrowhead-like pattern (arrows). The same granules occur in the somatic cortex, where they form long rows (arrowheads). **c-e:** In the scanning electron microscope, the oral bulge is much more distinct than *in vivo*. The scattered bulge extrusomes are just exploding and appear as distinct granules in surface view (**c, e**) and as small domes in side view (**d**). Note the bulge midline (asterisks), the oblique striation (arrows) caused by the cortical granules, and the narrowly spaced cilia of the circumoral kinety (CK). E - extrusomes, OB - oral bulge. Scale bars 10  $\mu\text{m}$  (a, b) and 4  $\mu\text{m}$  (c, d, e).



**Figs 21a-g.** *Arcuospathidium cultriforme scalpriforme*, encystment of South African specimens, protargol impregnation. **a, b:** Early stages showing reduction of body size and diminution of circummoral kinety, length 120  $\mu\text{m}$  and 108  $\mu\text{m}$ . **c, d:** Next, the cell rounds up and the resorption of the somatic and oral ciliary pattern becomes distinct, length 71  $\mu\text{m}$ . **e, f:** Formation of the cyst wall and shortening of the macronucleus, diameter 67  $\mu\text{m}$  and 65  $\mu\text{m}$ . Resorption of the ciliary structures proceeds. **g:** Young, completed cyst. The macronucleus is now semicircular, most micronuclei and the infraciliature have been resorbed, and a faceted cyst wall has developed, diameter 58  $\mu\text{m}$ . Both wall and cell are distinctly shrunken due to the preparation procedures. B - dorsal brush, CK - circummoral kinety, CV - contractile vacuole, F - (postciliary?) fibres, MA - macronucleus, MI - micronuclei, W - cyst wall.

dikinetics are more narrowly spaced than in morphostatic specimens: there are about 17 vs. 11 in an area of 10  $\mu\text{m}$ . This explains why the bulge can grow (become longer) without a second round of basal body production.

Early (to late) post-dividers are already spatulate, have a distinct but short oral bulge, and the circummoral kinety still shows some small irregularities. The ciliary rows commence to assume the typical *Arcuospathidium* pattern, that is, curve dorsally in the anterior portion. The dorsal brush is still incomplete, as explained in stage 4. The macronucleus still has a pointed or even filiform anterior end and grows to a long, tortuous strand with distinct nucleoli. The micronuclei commence to arrange near the macronucleus (Figs 26b, e, f).

**Specimens with two macronucleus strands** (Figs 26a, 27a-g). Specimens with two, comparatively short macronucleus strands occur in both the non-flooded Petri

dish culture (5%, Figs 27a, b) and the semipure culture, from which we could reconstruct a full division series (Figs 27a-g) showing the following: (i) the two macronucleus pieces divide individually, that is, do not fuse to a single, globular mass as in ordinary specimens (Figs 22g, h) and multimacronucleate species (Foissner et al., 2002); (ii) the binucleate state is stable and heritable, although figure 27g indicates that the opisthe might become mononucleate; (iii) binucleate specimens are a natural phenomenon in *A. cultriforme*, that is, are not caused by chance or injury; and (iv) ontogenesis of the oral and somatic ciliary pattern as well as the contractile vacuole is as in ordinary specimens.

**Monsters** (Figs 28a-i, 29a-e). Monsters occurred in both the non-flooded Petri dish (raw) culture, though very rarely (< 1%), and in the semipure culture, where they were frequent (~14%). We could not clarify the

**Table 3.** Morphometric data on *Arcuospathidium cultriforme scalpriforme* in cultivated morphostatic and dividing specimens from South Africa.

Characteristics <sup>1</sup>	Stages	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	Morphostatic	313.9	300.0	45.4	10.4	14.5	240.0	410.0	19
	Very early	353.9	350.0	50.2	19.0	14.2	270.0	420.0	7
	Early	420.0	440.0	-	-	-	380.0	440.0	3
	Early middle	367.1	370.0	44.8	16.9	12.2	295.0	435.0	7
	Middle-early late	316.4	330.0	42.2	16.0	13.3	255.0	370.0	7
Body, width (maximum)	Morphostatic	65.8	67.0	15.0	3.4	22.7	44.0	95.0	19
	Very early	76.1	80.0	12.7	4.8	16.7	55.0	90.0	7
	Early	79.3	80.0	-	-	-	78.0	80.0	3
	Early middle	79.6	75.0	17.8	6.7	22.4	58.0	110.0	7
	Middle-early late	78.3	78.0	14.5	5.5	18.5	61.0	97.0	7
Body length:width, ratio	Morphostatic	4.9	4.9	0.9	0.2	17.4	3.2	6.4	19
	Very early	4.7	4.7	0.6	0.2	13.4	3.9	5.7	7
	Early	5.3	5.5	-	-	-	4.8	5.6	3
	Early middle	4.8	4.8	1.1	0.4	23.2	3.4	6.5	7
	Middle-early late	4.1	4.1	0.4	0.1	9.4	3.5	4.8	7
Proter, length	Middle	217.6	220.0	41.0	15.5	18.8	145.0	265.0	7
	Late	221.7	205.0	45.9	17.3	20.7	170.0	292.0	7
Proter, width	Middle	80.6	76.0	17.1	6.5	21.2	61.0	110.0	7
	Late	81.6	80.0	13.2	5.0	16.1	62.0	97.0	7
Proter length:width, ratio	Middle	2.8	2.5	0.6	0.2	23.1	2.1	4.0	7
	Late	2.7	2.7	0.6	0.2	20.0	2.1	3.5	7
Opisthe, length	Middle	145.7	150.0	20.4	7.7	14.0	108.0	165.0	7
	Late	143.6	150.0	28.5	10.8	19.9	95.0	180.0	7
Opisthe, width	Middle	79.1	75.0	19.3	7.3	24.3	53.0	110.0	7
	Late	78.7	80.0	13.2	5.0	16.7	57.0	92.0	7
Opisthe length:width, ratio	Middle	1.9	1.9	0.4	0.1	19.9	1.4	2.5	7
	Late	1.8	1.8	0.4	0.1	19.3	1.4	2.4	7
Proter:opisthe length, ratio	Middle	1.5	1.5	0.1	-	6.1	1.3	1.6	7
	Late	1.6	1.5	0.2	0.1	11.7	1.4	1.9	7
Macronucleus, length (spread, thus approximate)	Morphostatic	351.4	350.0	-	-	-	230.0	430.0	7
	Very early	368.6	360.0	-	-	-	320.0	430.0	7
	Early	370.0	390.0	-	-	-	320.0	400.0	3
Micronuclei, largest diameter	Morphostatic					about 3 $\mu\text{m}$			
	Very early	3.4	3.0	0.8	0.3	22.9	3.0	5.0	7
	Early-middle					up to 6 $\mu\text{m}$			
Circumoral dikinetids of proter, number	Middle-late	332.8	330.0	24.9	10.2	7.5	305.0	370.0	6
Dikinetids in corresponding opisthe's kinetofragments, number	Middle	275.0	285.0	-	-	-	240.0	300.0	3
	Late	330.0	320.0	-	-	-	320.0	350.0	3
Dikinetids in opisthe's individual kinetofragments, number	Early	8.6 <sup>2</sup>	8.0	-	-	-	6.0	11.0	6
	Middle	8.8 <sup>2</sup>	9.0	-	-	-	6.0	13.0	9
	Late	9.3 <sup>2</sup>	9.0	-	-	-	7.0	12.0	9

**Table 3.** (Continuation).

Characteristics <sup>1</sup>	Stages	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Dikinetids in proter's brush row 2, number	Early to late stages	62.2	62.0	4.9	1.1	7.9	54.0	75.0	21
Dikinetids in opisthe's brush row 2, number	Early	21.0	20.0	9.9	3.8	47.4	10.0	41.0	7
	Middle	40.8	40.0	3.9	1.3	9.6	36.0	49.0	9
	Late	40.6	41.0	3.0	1.1	7.4	35.0	45.0	7

<sup>1</sup> Data based on mounted and protargol-impregnated (Wilbert's method) specimens from a semipure culture. Measurements in  $\mu\text{m}$ . 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,  $\bar{x}$  - arithmetic mean.

<sup>2</sup> Based on four kinetofragments each from 6 or 9 specimens.

reason(s) of formation of monster, but noted that they were most common during a "bloom" of the culture. The following observations were made from the protargol slides: (i) monsters occur in specimens with a single macronucleus strand (Figs 28a, f, 29a, b, e) and in cells with two strands (Fig. 28b), as described above; (ii) monsters can divide and grow to ordinary size, as evidenced, for instance, by post-dividers (Figs 29a, b, e) and the mirror-image doublet shown in figure (28a); (iii) the new oral apparatus can develop from underneath the parental oral bulge to near mid-body on the ventral or dorsal, but never lateral surface (Figs 28b, d, g-i); (iv) there is indication that specimens can produce an ordinary opisthe and a monster proter by generating a third oral apparatus above the fission area (Fig. 28b); (v) the new circumoral kinety, probably invariably composed of dikinetids, develops not from somatic kinetofragments but interkinetally, that is, *de novo* and grows by proliferation of dikinetids within the forming kinety (Figs 28c, e); and (vi) the new oral apparatus, even if small, always has associated extrusomes, while ordinary dividers show extrusomes only at late stages.

## Discussion

### GENERIC ASSIGNMENT

*Arcuospathidium cultriforme* is the type of the genus *Arcuospathidium* Foissner, 1984. *Arcuospathidium* is characterised by the arrangement of the ciliary rows which are separated from the circumoral kinety and curve dorsally on both sides of the oral bulge. Further, the dorsal brush is located dorsally, which distinguishes *Arcuospathidium* from *Cultellothrix* Foissner, 2003, the latter having the brush located laterally.

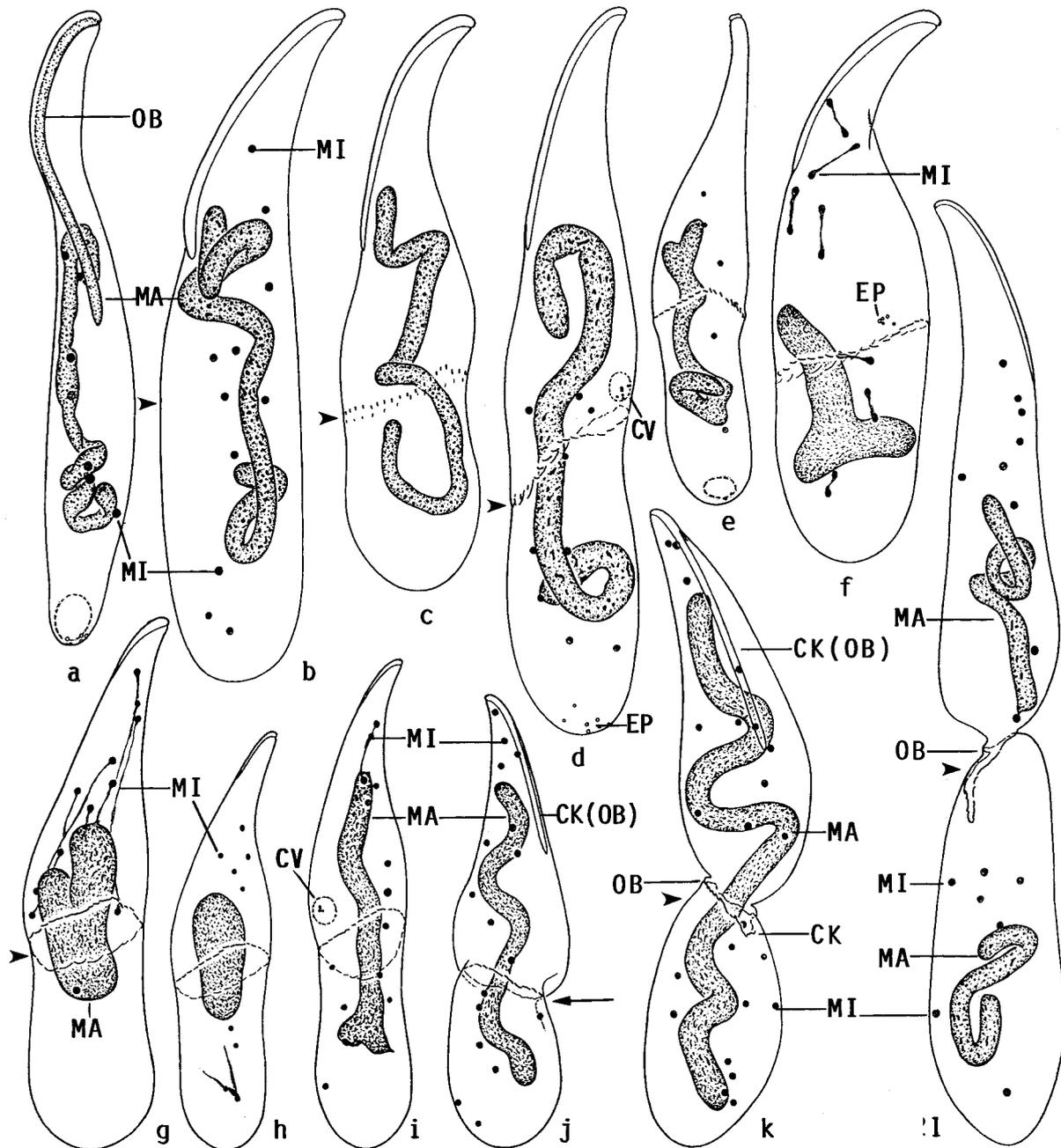
### NEOTYPIFICATION

We consider neotypification of *A. cultriforme cultriforme* and *A. cultriforme scalpriforme* as inappropriate because the original descriptions are sufficiently detailed for recognition and their status has not yet settled. None the less, we deposit voucher slides of all populations shown in Tables 1-3 at the Linz (LI) museum in Austria, where Foissner (1984) stored the protargol slides of his populations. If there ever should be a combined morphological and gene sequence study of *A. cultriforme* and *A. scalpriforme*, these populations should be fixed as neotypes because, basically, their identity is threatened by *A. cultriforme megastoma* and populations with more or less different macronucleus pattern.

### SEPARATION OF (SUB)SPECIES IN THE *ARCUOSPATHIDIUM CULTRIFORME* COMPLEX

Foissner et al. (2002) and Foissner (2003b) introduced the "*Arcuospathidium cultriforme* complex" to draw attention to some closely related species, viz., *A. cultriforme* (Penard, 1922) Foissner, 1984; *A. scalpriforme* (Kahl, 1930) Foissner, 2003; *A. cultriforme megastoma* Foissner et al., 2002; and *A. lorjeae* Foissner et al., 2002. As concerns the general organisation, *A. bulli* Foissner, 2000 also belongs to the *A. cultriforme* complex, but has two contractile vacuoles indicating a special lineage within this or another complex.

*Arcuospathidium cultriforme megastoma*, a Namibian species, differs from *A. cultriforme cultriforme* in the length of the oral bulge (60% vs. 33% of body length); it differs from *A. cultriforme scalpriforme* in the length of the oral bulge (60% vs. 45% of body length) and the arrangement of the extrusomes (in a single row in the right and the left bulge half vs. several more or less scattered rows). *Arcuospathidium lorjeae*, which has a



**Figs 22a-l.** *Arcuospathidium cultriforme scalpriforme*, body and nuclear changes of South African specimens during ontogenesis. All drawn to scale, except figure (a), from protargol preparations (Wilbert's method). Arrowheads mark regions shown in detail on the following plates. **a:** Ventral view of a morphostatic specimen, length 325  $\mu\text{m}$ . **b, c:** Left side views of very early dividers, which soon become indented in the prospective fission area (**c**), length 410  $\mu\text{m}$  and 352  $\mu\text{m}$ . The macronucleus appears smoothed and the micronuclei distribute throughout the cell. **d:** Left side view of an early divider, length 440  $\mu\text{m}$ . Early dividers are conspicuously larger than morphostatic cells (Table 3), have a distinctly oblique division axis, and generate a contractile vacuole in the proter. The macronucleus is smoothed and less tortuous. **e, f:** Dorsal and left side view of early mid-dividers, length 295  $\mu\text{m}$  and 335  $\mu\text{m}$ . The macronucleus commences condensation and the micronuclei begin to divide. **g, h:** Dorsolateral views of mid-dividers, length 325  $\mu\text{m}$  and 255  $\mu\text{m}$ . Mid-dividers are the smallest and stoutest dividers, approaching the length of morphostatic specimens. The macronucleus condensed to a globular mass in mid-body. **i, j:** Late dividers with extending macronucleus and developing division furrow (arrow), length 295  $\mu\text{m}$  and 275  $\mu\text{m}$ . **k, l:** Very late dividers with splitting macronucleus and developing oral structures, length 400  $\mu\text{m}$  and 565  $\mu\text{m}$ . The opisthe is considerably smaller than the proter. CK - circumoral kinety, CV - contractile vacuole, EP - excretory pores, MA - macronucleus, MI - micronuclei, OB - oral bulge.

similar bulge length as *A. cultriforme cultriforme*, differs from the other members of the complex in the body's length:width ratio (11.4:1 vs. 5.4-7.2:1), the number of ciliary rows (17 vs. about 30) and, especially, the length of the dorsal brush bristles (up to 10 µm vs. up to 5 µm); it is the last feature that makes *A. lorjeae* so conspicuous.

Literature data and our new observations show that *Spathidium cultriforme* Penard and *S. scalpriforme* Kahl are very similar in all main features, such as body size and shape, oral bulge shape, nuclear and contractile vacuole pattern, number of ciliary rows, and the length and gross shape of the extrusomes. The distinguishing characteristics given in the diagnoses are recognisable only on detailed observation and one (bulge length) is purely statistical. Thus, we persist in our opinion that *S. scalpriforme* is a subspecies of *cultriforme* (Foissner et al., 2002; Foissner, 2003b). As concerns recognition of subspecies, see Foissner et al. (2002) and Xu and Foissner (2005). Contrary to the geographical isolation concept, we distinguish ciliate subspecies by significant morphometrics and minor morphological features.

Foissner et al. (2002) distinguish *A. cultriforme cultriforme* and *A. cultriforme scalpriforme* only by the length of the oral bulge. Our comparison of several populations suggests two additional features: the arrangement (in rows vs. scattered) of the extrusomes and the brush bristles of row 3 (anterior bristle shorter than posterior one in *A. cultriforme scalpriforme*, while vice versa in *A. cultriforme cultriforme*, *A. lorjeae*, and *A. bulli*; Figs 11a, d, 12d, 19c, f). However, accurate data are available only from a few populations of each species. Thus, we cannot yet be entirely confident in these features, but they are good starters for falsification. The first feature is included in the diagnoses because it was seen in at least three populations each, while brush bristle details are reliably documented (by SEM) in only one population each.

Further studies should painstakingly document the following features: ratio of body and oral bulge length; the macronucleus pattern; the arrangement of the extrusomes (in rows or scattered); shape and size of the extrusomes; the arrangement of the brush dikinetids (straight or oblique); the number of ciliary rows; and brush row 3 (anterior or posterior bristle of pairs shortened). Certainly, gene sequence data would be very helpful.

#### THE MACRONUCLEUS PATTERN AS A TAXONOMIC FEATURE

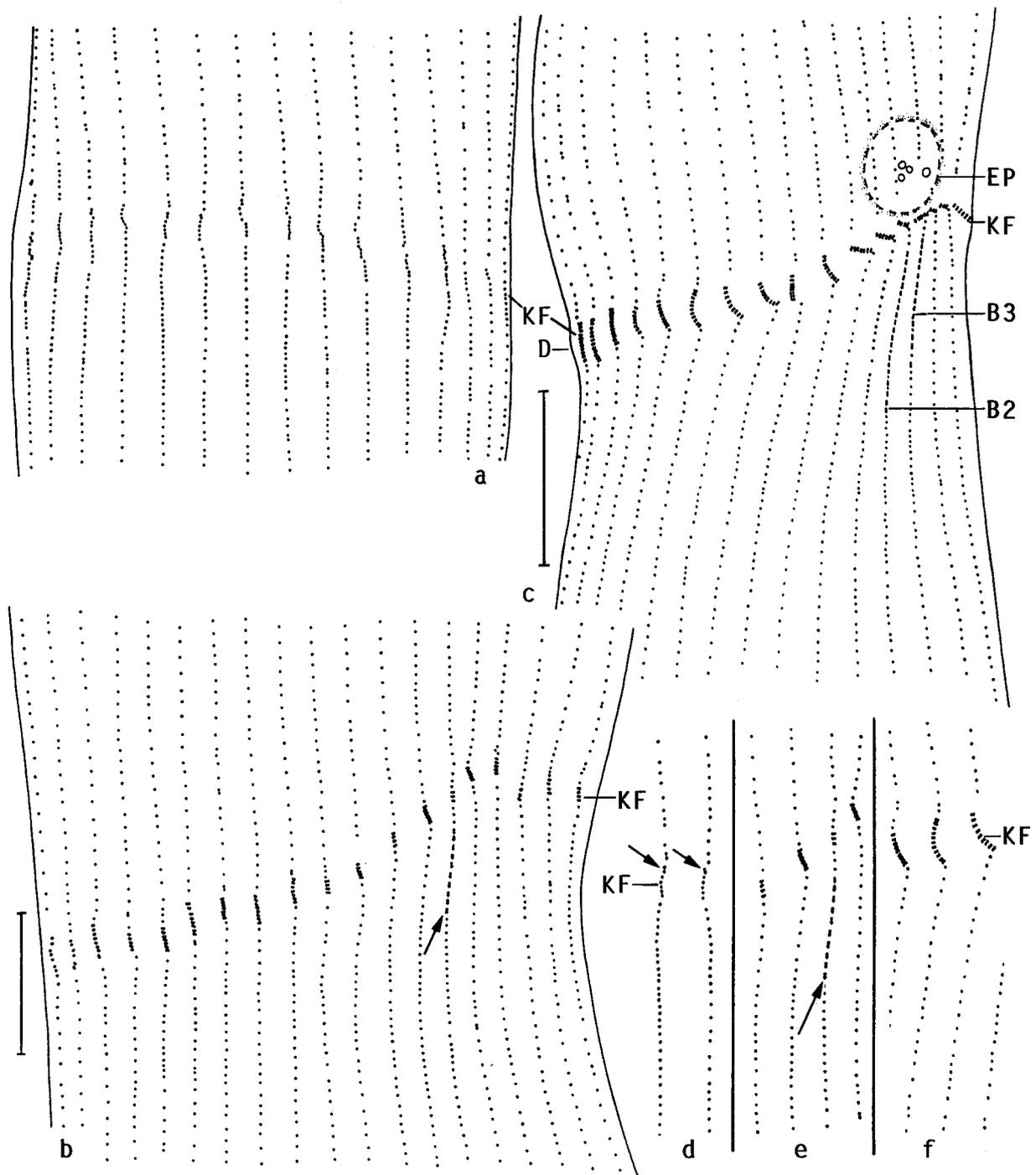
The macronucleus pattern is widely used for separating species and genera in spathidiids and ciliates in general because it is considered as an important, stable feature (Kahl, 1930b; Hirshfield et al., 1973; Foissner, 1993; Berger, 1999; Foissner et al., 2002; Foissner and Xu, 2005). Therefore, it was an unpleasant surprise to

find an *A. cultriforme* in Hawaii, where one third of the specimens had many macronucleus nodules, instead of the usual, tortuous strand. The situation is even worse in the population from Rwanda, where all specimens have macronucleus nodules, although we cannot exclude the possibility that this population represents a distinct species. Further, intergradation between different macronucleus patterns occurs, for example, also in *Epispathidium papilliferum* (Foissner and Xu, 2005) and in *Blepharisma* spp. (Hirshfield et al., 1973). This is emphasised by specimens with two macronucleus pieces. Our observations show that this pattern occurs in all populations, though with very low frequency, and is hereditary. Accordingly, four macronucleus patterns occur in *A. cultriforme*: a long, tortuous strand (usual), two long pieces (very rare), a mixture of pieces and nodules (in one of many populations), and many nodules (in one of many populations; possibly a distinct species). All this variation is not part of the life cycle; it is not caused by reorganisation processes during or after division (this paper) and conjugation (Xu and Foissner, 2004b).

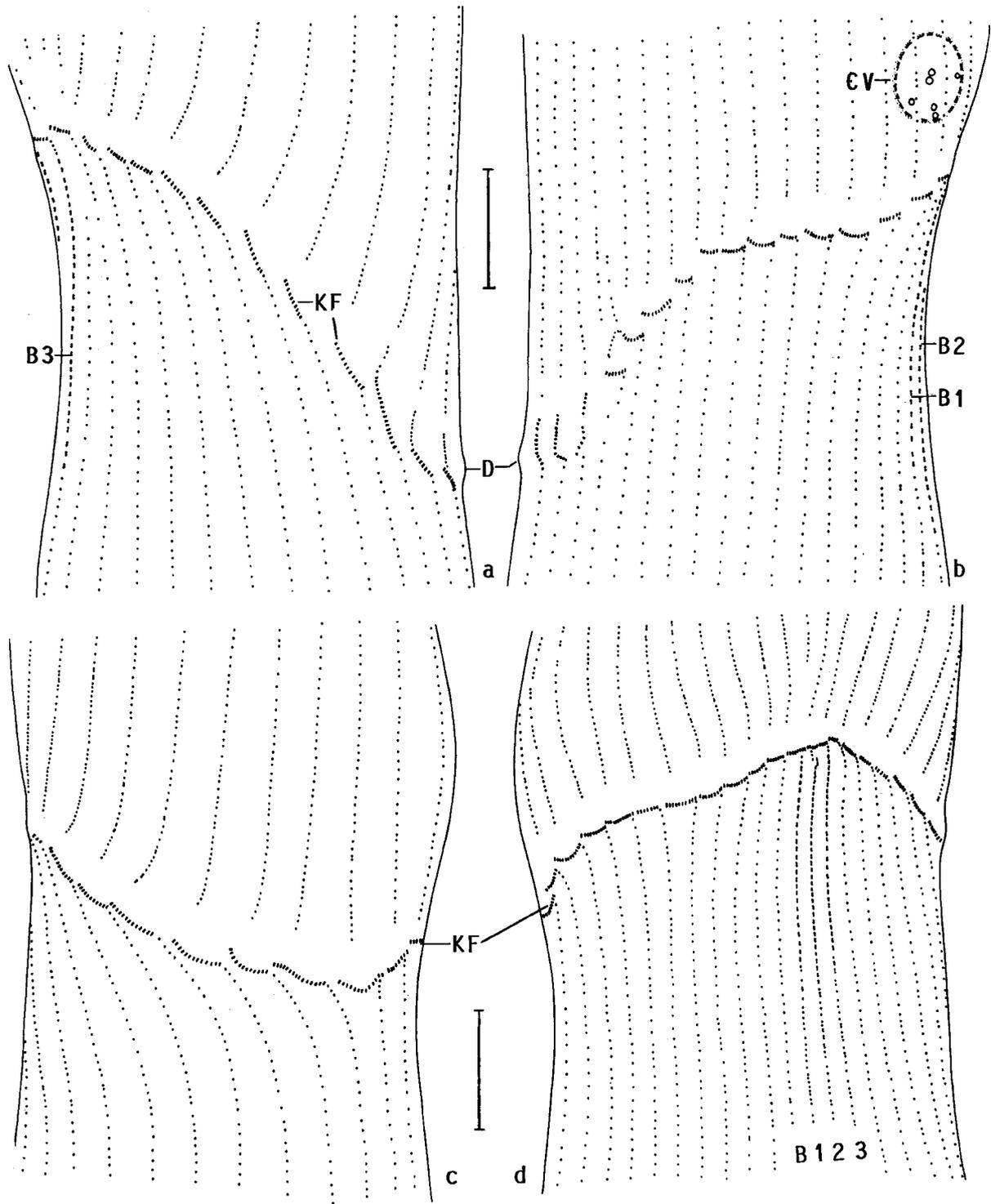
Thus, the macronucleus pattern is as variable as many other features used for defining species and genera. Accordingly, it must be applied cautiously. However, the situation is not as hopeless as it appears at first glance. The populations that contain ciliates with "unusual" macronucleus patterns constitute only a small fraction of all the populations studied. Further, some even might represent distinct species, especially at molecular level. There are cases when the species status is suggested not only by a different macronucleus pattern but also by other characteristics. Representative examples are *Epispathidium ascendens*, as redescribed by Foissner (1987b), and *E. polynucleatum* Foissner et al., 2002. In the descriptions, these species differ only in the macronucleus pattern (a long, tortuous strand vs. many small nodules). However, recent investigations show a further distinct feature: the resting cyst wall is spiny in the former and smooth in the latter (checked in two populations from different biogeographic regions; Foissner and Xu, 2005).

#### BIOGEOGRAPHY

Biogeography of ciliates is strongly impeded by the rarity of reliable faunistic data and other shortcomings discussed in Foissner (1999b, 2004a, 2005a) and Foissner et al. (2002). Therefore, we heavily depend on the so-called "flagship species", that is, taxa with large body size and/or conspicuous morphology (Foissner, 2004a, b, 2005a, b). However, a restricted geographic distribution can probably be established for less conspicuous species as well, if a combination of classic and modern methods is used (Foissner et al., 2003; Fokin et al., 2004).



**Figs 23a-f.** *Arcuospathidium cultriforme scalpriforme*, ciliary pattern of very early and early dividers from South African population after protargol impregnation (Wilbert's method). **a, d** (cp. Fig. 22b): Very early divider commencing basal body proliferation underneath mid-body. Arrows in **(d)** mark a dikinetid at the anterior end of the developing kinetofragments. **b, e** (cp. Fig. 22c): Very early divider developing a body indentation in the prospective fission area and dorsal brush row 2 (arrows). The newly produced opisthe oral kinetofragments become dikinetidal. **c, f**: Early divider with growing dorsal brush rows 2 and 3, row 1 is generated later. The newly produced opisthe oral kinetofragments separate from the parental ciliary rows in a dorsoventral gradient. The kinetofragments are orientated ventrally and become curved due to the developing division blebs (D, cp. Figs 13b-d). A new proter contractile vacuole and excretory pores have developed above the opisthe's dorsal brush rows. B2, 3 - dorsal brush rows, D - division blebs, EP - excretory pores, KF - oral kinetofragments. Scale bars 20  $\mu$ m.



**Figs 24a-d.** *Arcuospathidium cultriforme scalpriforme*, ciliary pattern of an early divider and an early mid-divider from South African population after protargol impregnation (Wilbert's method). **a, b** (cp. Fig. 22d): Right and left side view of an early divider with fully developed oral kinetofragments and growing brush rows. The kinetofragments are longer on the right than on the left side, where they arranged horizontally. **c, d** (cp. Fig. 22g): Ventrolateral and dorsolateral view of a mid-divider. The opisthe ciliature is almost complete, that is, the oral kinetofragments are now transversely arranged and the new brush rows have about two thirds of their dikinetids; the final number is obtained in post-dividers or morphostatic cells. B1-3 - dorsal brush rows, CV - contractile vacuole, D - division blebs, KF - oral kinetofragments. Scale bars 20  $\mu$ m.

As concerns *A. cultriforme cultriforme* and *A. cultriforme scalpriforme*, both appear to be cosmopolites (Foissner, 1998). However, our new data cast doubt on this view because both South American populations of *A. cultriforme cultriforme* have thinner extrusomes and obliquely arranged bristle dikinetids (Figs 2e, f, 7c, d, 10d-f, 12a-c), suggesting that they represent a distinct (sub)species, especially if gene sequence data would be in the same line. The extrusome differences become impressive, if the micrographs are compared (Figs 7c, 9h-k, 10d-f). Interestingly, the South American populations of *A. cultriforme cultriforme* have very similar extrusome shapes as the *A. cultriforme scalpriforme* populations from South Africa and Brazil (compare Figs 2d-f and 16v, w). Likewise, the rather different averages in the number of ciliary rows (Table 1) suggest that the complex consists of more (sub)species that presently recognized.

The two other taxa of the *A. cultriforme* complex, *A. cultriforme megastoma* and *A. lorjeae* have been found as yet only in Namibia (*A. lorjeae* recently also in Brazil). They did not occur in over 1,000 other soil samples collected all over the world (Foissner et al., 2002), suggesting a restricted (Gondwanan) distribution. This applies also to the *A. cultriforme cultriforme* population from Rwanda which probably represents a distinct (sub) species, as indicated by the large body size and the multinucleate macronucleus pattern. The Rwandan sample contains several undescribed ciliates, especially *Arcuospathidium bulli*, a large and very distinct species with two contractile vacuoles (Foissner, 2000a). Samples with one or several “flagships” are found all over the world (Foissner, 1995, 1999b, 2004a, b, 2005a, b; Foissner et al., 2002, 2005), providing the strongest evidence for a restricted distribution of certain ciliate species.

## ONTOGENESIS

*Arcuospathidium cultriforme* and related species show many interesting ontogenetic processes for some of which we could find reasonable explanations (Table 4). Others remain obscure, for instance, the division blebs, the indentation in the prospective fission area in early dividers, and the different length of proter and opisthe; possibly some of the unexplained features are plesiomorphic or species-specific.

### • Why is the division axis oblique in *A. cultriforme*?

The long circumoral kinety (mouth) of *A. cultriforme* needs a lot of dikinetids. As there is no second (late) round of oral kinetid proliferation, the individual oral kinetofragments must be as long as possible to suffice for the new kinety. Basically, this is achieved by making the division axis oblique so that the larger perimeter of the fragment belt becomes larger (Fig. 30; Table 3). Accordingly, blunt spathidiids (e.g., *A. muscorum*) and

species with short mouth (e.g., *Spathidium turgitorum*) have a simple, transverse division axis (Berger et al., 1983; Foissner et al., 2002). A further small space increase for dikinetids is possible by curving the individual kinetofragments. A simple planimetric calculation shows that oblique division axis and fragment curving increase the useable perimeter by about 40%. Basically, however, curving of the oral kinetofragments is caused by the division blebs and is independent of the space available for the growing kinetofragments (see below). Thus, it is unlikely that curving of the kinetofragments is a special adaptation to the restricted space available for the growing kinetofragments of *A. cultriforme* and other haptorids.

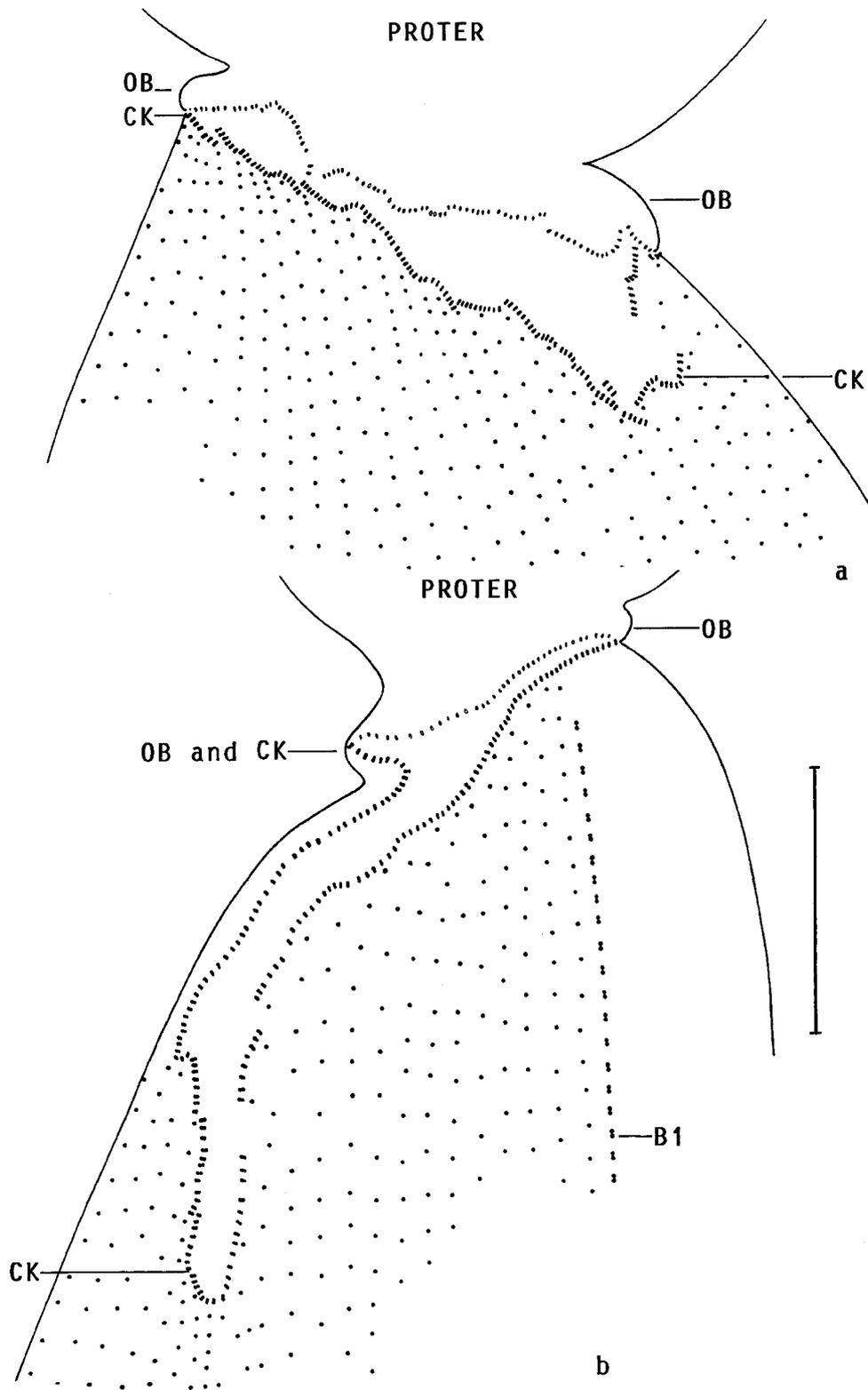
### • Why are the growing oral kinetofragments curved?

The concave shape of the newly produced oral kinetofragments is a conspicuous and widespread feature in haptorids (Figs 23c, 24c, Table 3). In spathidiids (Figs 13b-d) and *Homalozoon* (Leipe et al., 1992), the kinetofragments grow around the posterior and right margin of the division blebs, and thus assume the concave shape observed. There is little doubt that this applies also to other haptorids, for instance, *Dileptus* (Golińska, 1995).

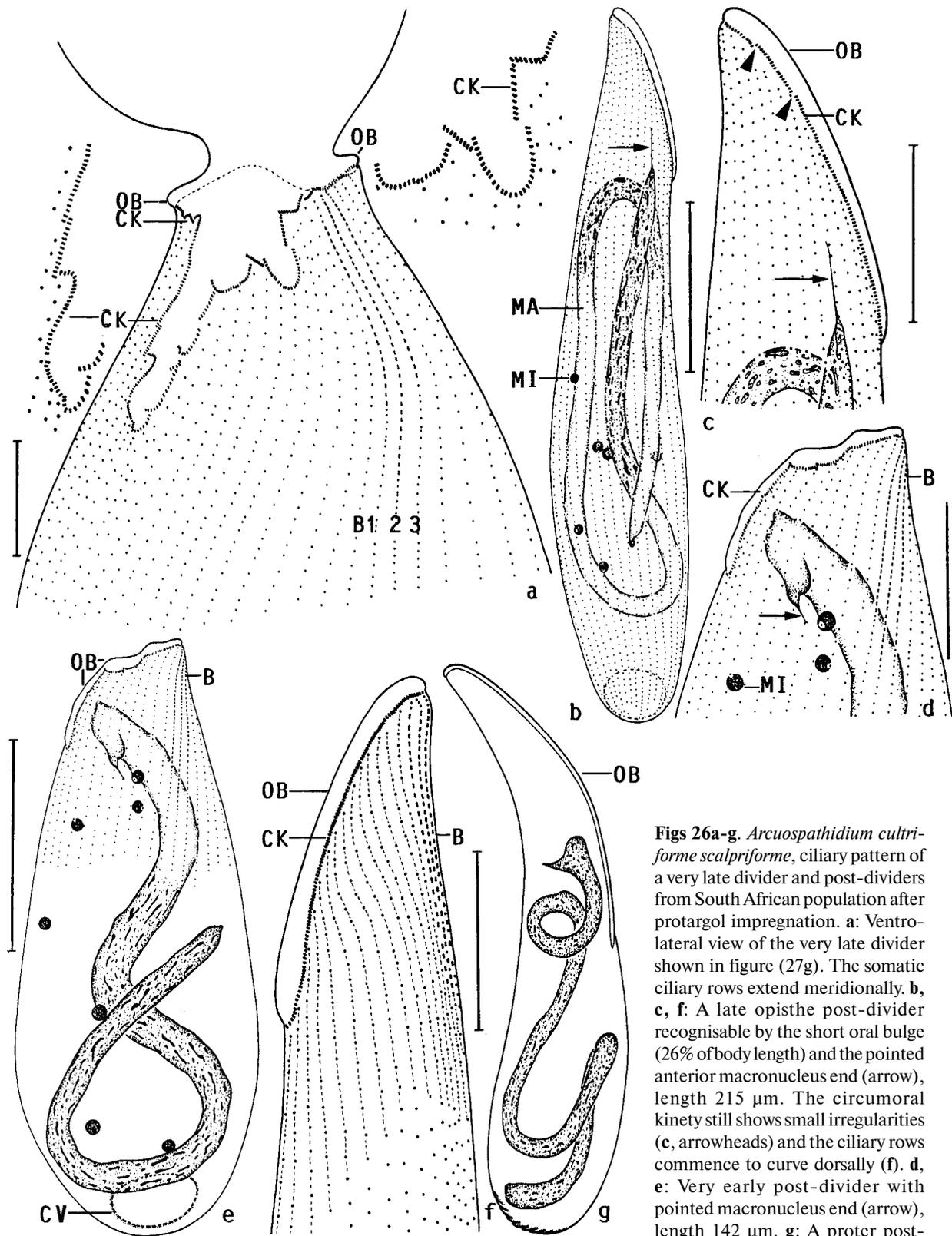
• **Why do kinetofragments become disordered in late dividers?** In late and very late dividers of *A. cultriforme* (Figs 25a, b, 26a) and *A. muscorum* (Berger et al., 1983), the opisthe's oral kinetofragments overlap more or less distinctly, some even becoming arranged one upon the other or side by side. At first glance, this appears to be an effect of the general disorder caused by cell furrowing and the reduced space available for the fragment belt. However, the fragments never overlap in *A. coemeterii*, a small species with a comparatively short oral bulge (Foissner and Lei, 2004). Thus, the overlap must have other reasons in long-mouthed species, probably the lack of a second round of oral dikinetid production, which causes the necessity of generating the oral kinetofragments in full length. This, however, results in spatial constraints and disorder during furrowing.

• **How are the long mouth (oral bulge, circumoral kinety) and its steep slope obtained?** Theoretically and in accordance with most data available, a spathidiid mouth could develop either by ingrowth or outgrowth. The ingrowth model suggests that mouth and slope are obtained by resorption of the anterior portion of the ventral ciliary rows or, in other words, the oral bulge grows into the ventral parental cortex and obtains, almost automatically, the slope. The outgrowth model assumes that mouth and slope are obtained by some ventral growth of the forming oral area (Figs 32a-c) and, in post-dividers, by a faster growth of the dorsal than the ventral area (Figs 32d, e).

None of the models can be rejected *a priori* because the monsters show that a new circumoral kinety can be generated between the ciliary rows, probably involving

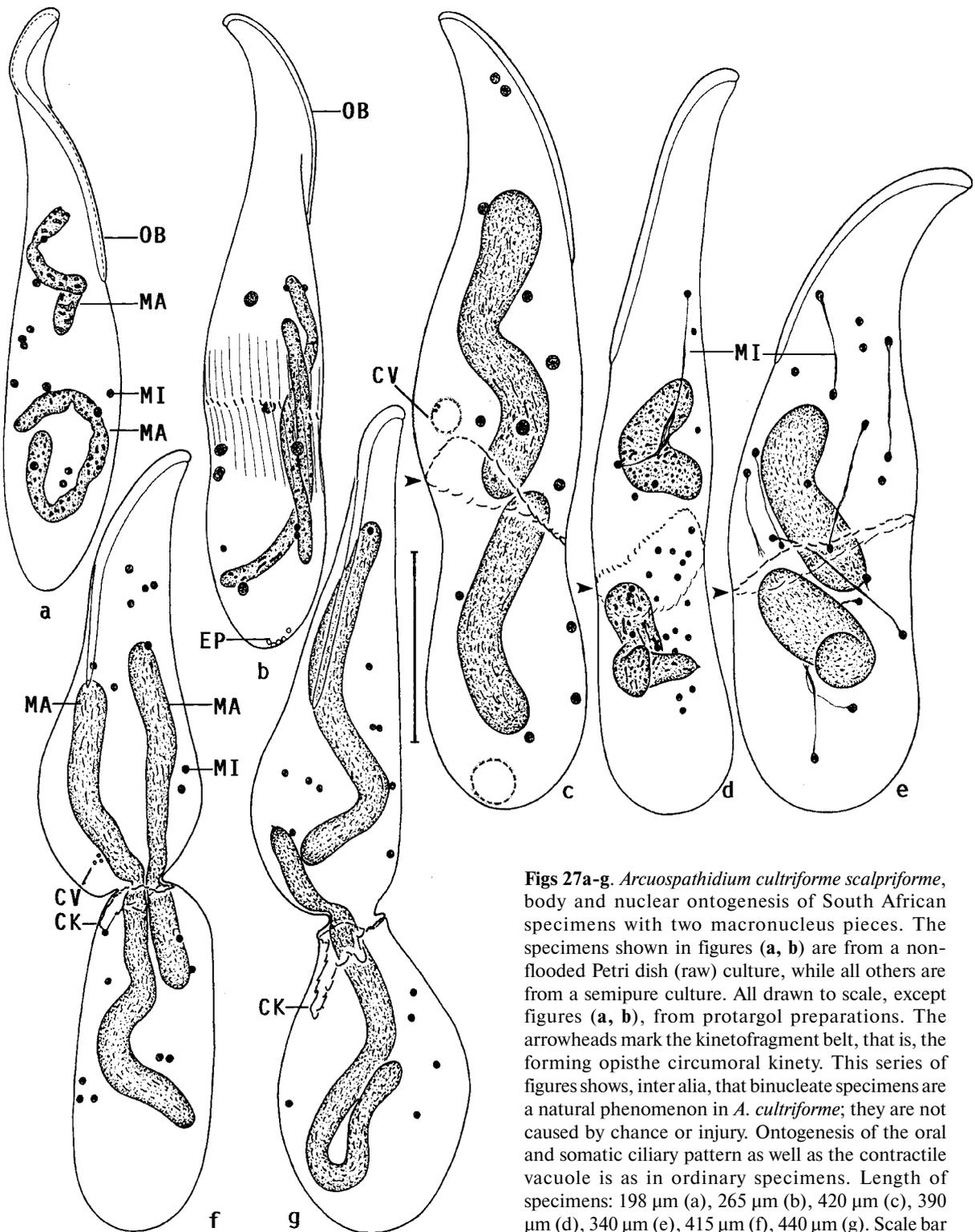


**Figs 25a, b.** *Arcuospathidium cultriforme scalpriforme*, ciliary pattern of very late dividers from South African population after protargol impregnation (Wilbert's method). These are details of the specimens shown in figures 22k, l. They show the complex processes of oral bulge growth and alignment of the kinetofragments to the opisthe's circumoral kinety. B1 - dorsal brush row 1, CK - circumoral kinety, OB - oral bulge. Scale bar 20  $\mu$ m.

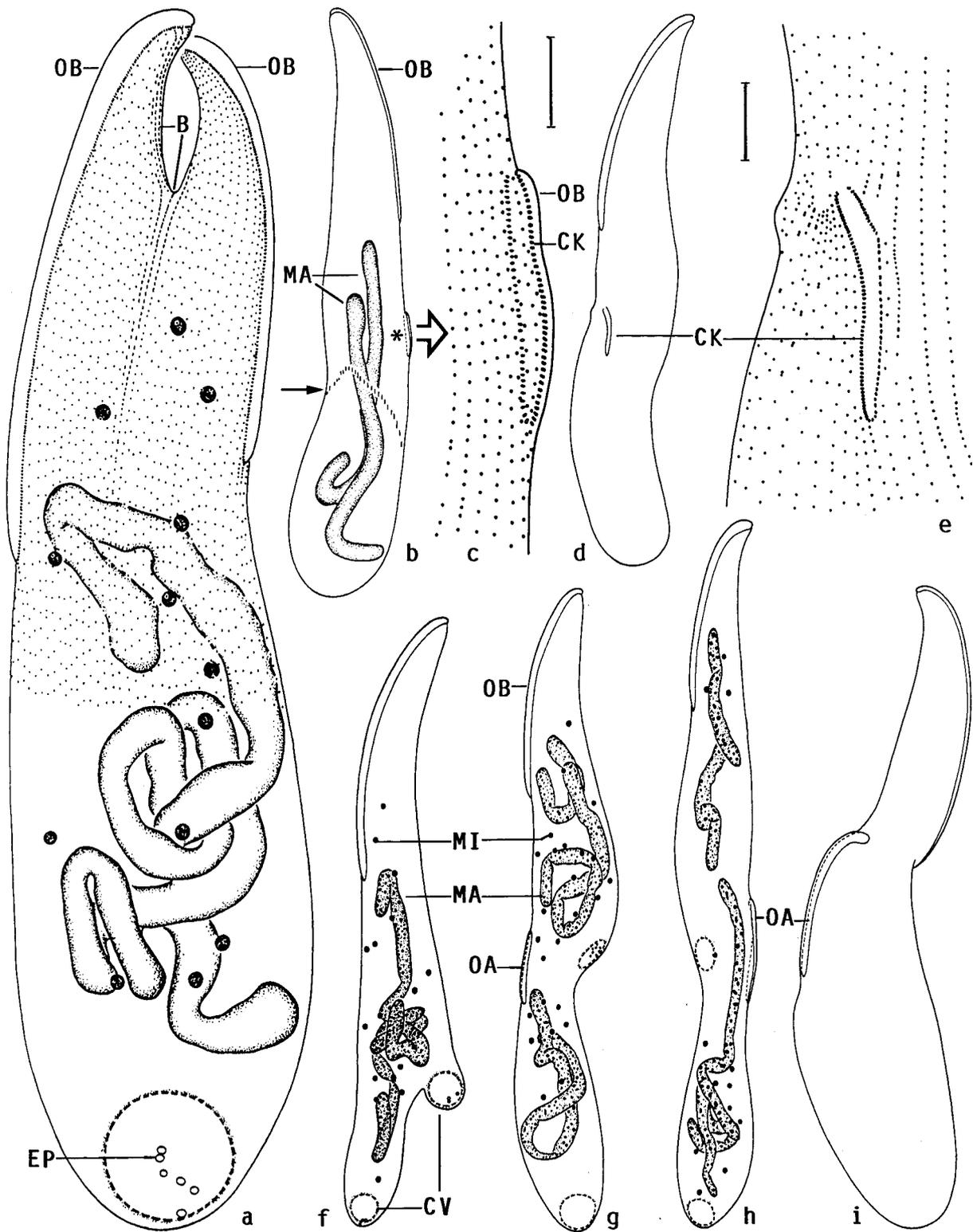


**Figs 26a-g.** *Arcuospathidium cultriforme scalpriforme*, ciliary pattern of a very late divider and post-dividers from South African population after protargol impregnation. **a:** Ventrolateral view of the very late divider shown in figure (27g). The somatic ciliary rows extend meridionally. **b, c, f:** A late opisthe post-divider recognisable by the short oral bulge (26% of body length) and the pointed anterior macronucleus end (arrow), length 215 µm. The circumoral kinety still shows small irregularities (c, arrowheads) and the ciliary rows commence to curve dorsally (f). **d, e:** Very early post-divider with pointed macronucleus end (arrow), length 142 µm. **g:** A proter post-divider recognisable by the long oral

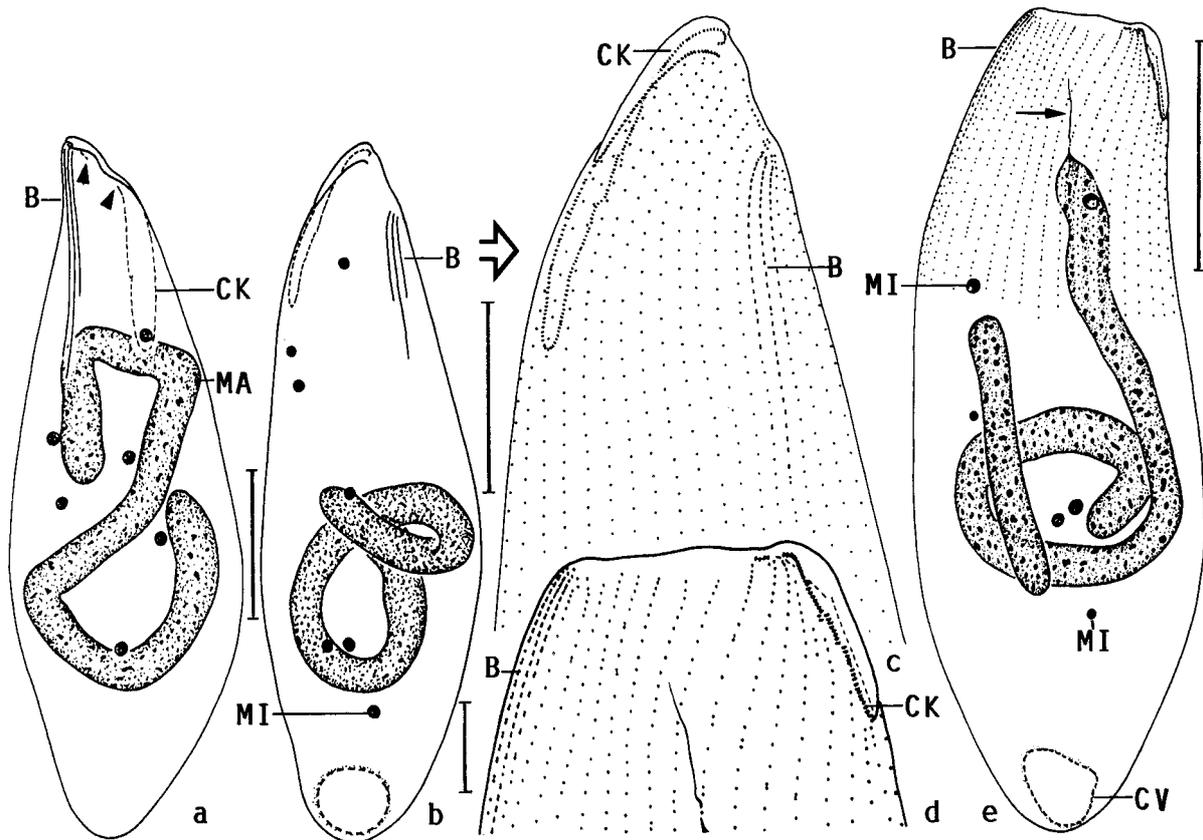
bulge (60% of body length), length 290 µm. B (1-3) - dorsal brush (rows), CK - circumoral kinety, CV - contractile vacuole, MA - macronucleus, MI - micronuclei, OB - oral bulge. Scale bars 50 µm (b, e), 25 µm (c, d, f), and 20 µm (a).



**Figs 27a-g.** *Arcuospathidium cultriforme scalpriforme*, body and nuclear ontogenesis of South African specimens with two macronucleus pieces. The specimens shown in figures (a, b) are from a non-flooded Petri dish (raw) culture, while all others are from a semipure culture. All drawn to scale, except figures (a, b), from protargol preparations. The arrowheads mark the kinetofragment belt, that is, the forming opisthe circumoral kinety. This series of figures shows, inter alia, that binucleate specimens are a natural phenomenon in *A. cultriforme*; they are not caused by chance or injury. Ontogenesis of the oral and somatic ciliary pattern as well as the contractile vacuole is as in ordinary specimens. Length of specimens: 198  $\mu\text{m}$  (a), 265  $\mu\text{m}$  (b), 420  $\mu\text{m}$  (c), 390  $\mu\text{m}$  (d), 340  $\mu\text{m}$  (e), 415  $\mu\text{m}$  (f), 440  $\mu\text{m}$  (g). Scale bar 100  $\mu\text{m}$  for c-g. CK - circumoral kinety, CV - contractile vacuole, EP - excretory pores, MA - macronucleus, MI - micronuclei, OB - oral bulge.



**Figs 28a-i.** *Arcuospathidium cultriforme scalpriforme*, protargol-impregnated (Wilbert's method) monsters from South African population. **a:** A doublet with 61 ciliary rows, 270  $\mu\text{m}$ . **b, c:** A binucleate specimen with a de novo (asterisk) and an ordinarily (arrow) developing oral apparatus, 450  $\mu\text{m}$ . Scale bar 10  $\mu\text{m}$ . **d, e:** De novo formation of circumoral kinety, 390  $\mu\text{m}$ . Scale bar 10  $\mu\text{m}$ . **f:** A specimen with doubled rear end, 400  $\mu\text{m}$ . **g:** A specimen with a de novo developing oral apparatus and without opisthe brush rows, 425  $\mu\text{m}$ . **h, i:** Specimens in which the opisthe oral apparatus develops de novo on the opposite of the proter oral apparatus, 470  $\mu\text{m}$  and 435  $\mu\text{m}$ . B - dorsal brush, CK - circumoral kinety, CV - contractile vacuole, EP - excretory pores, MA - macronucleus, MI - micronuclei, OA - oral apparatus, OB - oral bulge.



**Figs 29a-e.** *Arcuospathidium cultriforme scalpriforme*, protargol-impregnated (Wilbert's method) monster opisthe post-dividers from a semipure culture of the South African population. These figures show that monsters successfully divide, giving rise to malformed post-dividers and morphostatic cells. **a:** A slightly distorted specimen with partially lacking circumoral kinety (arrowheads), length 255  $\mu\text{m}$ . **b, c:** Left side view of a monster with malformed dorsal brush, length 248  $\mu\text{m}$ . **d, e:** Right side view of a monster with malformed circumoral kinety, length 180  $\mu\text{m}$ . Arrow marks pointed anterior end of macronucleus, showing that it is an opisthe post-divider. B - dorsal brush, CK - circumoral kinety, CV - contractile vacuole, EP - excretory pores, MA - macronucleus, MI - micronuclei. Scale bars 50  $\mu\text{m}$  (a, b, e), 30  $\mu\text{m}$  (c), and 10  $\mu\text{m}$  (d).

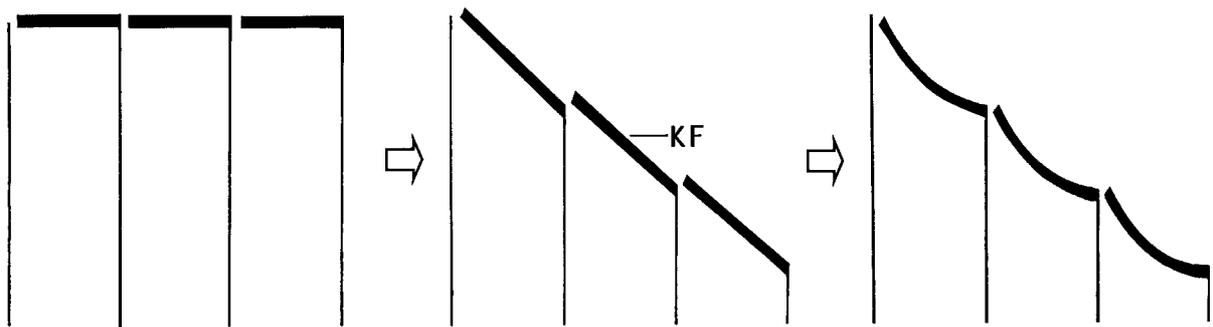
the resorption of parental somatic kinetids (Fig. 28e). However, in *A. cultriforme* and some other spathidiids (Berger et al., 1983; Foissner et al., 2002), the ingrowth model is disproved by the lack of evidence for resorption of parental kinetids in the growing mouth area. Further, only half of the basal bodies found in morphostatic specimens occur in the oral area of early post-dividers, and the distances between the individual basal bodies are larger orally than postorally. To compensate for this deficit, the oral area should not extend into the parental ventral cortex and dissolve existing kinetids, but grow as a whole and concomitantly produce new kinetids. Thus, we favour the outgrowth model, where length and slope of the oral bulge are obtained by unequal growth of the dorsal and the ventral side (Figs 32a-e).

As concerns *A. cultriforme* and other long-mouthed species, the oral area not only grows transversely in the fission region, but extends more or less far ventrally (posteriorly) in late dividers (Figs 25a, b, 26a). We

suggest this as a genus and/or species-specific process producing some space for the long oral kinetofragments because no growth can occur in the dorsal region where the daughters adhere.

The observations and models do not explain the dorsal curve of the ciliary rows in the mouth area of all spathidiids. This curve augments the oral ciliature and therefore is thus probably related to the feeding process. At first glance, the curve seems to be caused by the sometimes distinct dorsal curve of the spathidiid oral area. However, such relationship is disproved by many species which have the oral area hardly curved dorsally, for instance, *Spathidium spathula* and *S. turgitorum* (Foissner, 1984; Foissner et al., 2002). The lack of specific ontogenetic processes suggests that the dorsal curve is genetically fixed and belongs to the basic (plesiomorphic) equipment of the spathidiids.

• **How are the spathidiid ciliary patterns obtained?** Foissner (1984) distinguished four spathidiid ciliary



**Fig. 30.** The long circumoral kinety of *A. cultriforme* needs a lot of dikinetids, so that the individual oral kinetofragments (KF) must be as long as possible to suffice for the new circumoral kinety. This is achieved by making the division axis oblique and the fragments curved. Both provisions increase the useable perimeter by about 40%.

patterns. Based on the outgrowth model discussed above, we suggest the “row detachment model” for the origin of the spathidiid ciliary patterns (Fig. 31). An *Arcuospathidium* pattern is generated if the ciliary rows detach from the oral kinetofragments in very late and post-dividers, while a *Protospathidium* or *Spathidium* pattern is generated if the ciliary rows remain attached to the oral kinetofragments. This explains three of the four basic spathidiid ciliary patterns. The many variations of the patterns found within and between genera are likely produced by (i) some remodelling of the oral and perioral cortex, (ii) by different growth intensities of the dorsal and ventral oral area, and (iii) by an increased basal body proliferation at the anterior end of the ciliary rows. The latter produces the typical condensation of the cilia at the anterior end of the kineties in many *Spathidium* and, especially, *Epispathidium* species. Thus, the *Epispathidium* ciliary pattern is a variation of the *Spathidium* pattern. However, detailed data on *Epispathidium* are lacking and so it is not known whether the *Epispathidium* pattern is generated directly from the *Spathidium* pattern or indirectly via an *Arcuospathidium* pattern.

The row detachment model also explains some other characteristic features of the spathidiid ciliary patterns: (i) that the dorsal brush kineties even in typical *Arcuospathidium* species are often arranged in *Spathidium* pattern (Fig. 3f) and (ii) that the *Arcuospathidium* pattern is frequently indistinct, that is, the left side ciliary rows are straight or only slightly curved dorsally, sometimes even ventrally. This is so because the *Arcuospathidium* ciliary pattern is basically a *Spathidium* pattern masked by the separation of the ciliary rows from the circumoral kinety, as shown in our scheme (Fig. 31).

The ontogenetic data and the row detachment model suggest an evolution of the spathidiid ciliary pattern as shown in Figure 31. Most of these patterns evolved several times not only in the spathidiids (Xu and Foissner, 2003, 2004a) but also in related families, such as the Myriokaryonidae (Foissner, 2003a) and

Enchelyodontidae (Foissner et al., 2002; Xu and Foissner, 2003, 2004a).

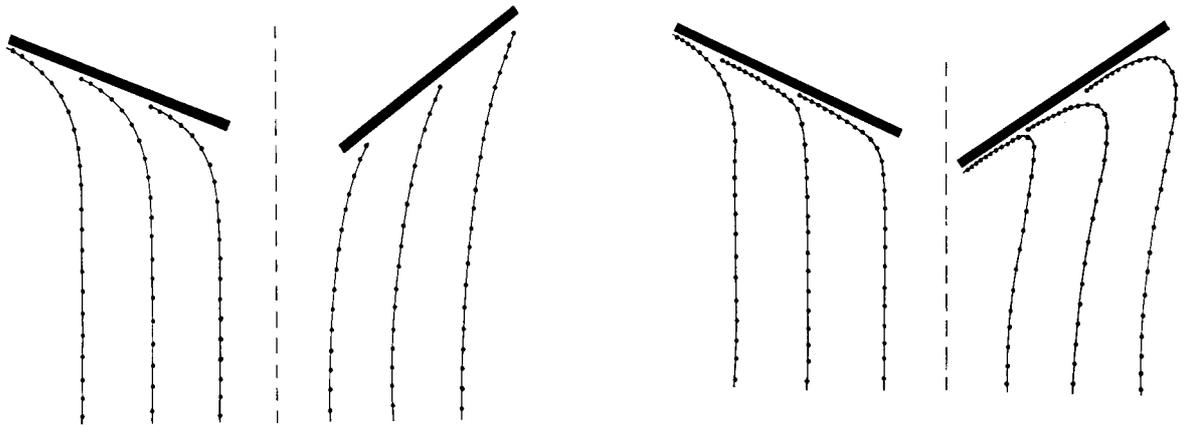
• **How is the shape of the oral bulge obtained?** When viewed ventrally, the spathidiid oral bulge may be obovate, oblong, or cuneate (Foissner, 1984; Foissner et al., 2002). We have not found any indication that these shapes are caused by special ontogenetic constraints, which means that they are genetically determined.

• **Was the ancestral dorsal brush two-rowed or three-rowed?** The dorsal brush of *A. cultriforme* develops in a remarkable way unknown in *A. muscorum* and *A. coemeterii* (Berger et al., 1983; Foissner and Lei, 2004): first, row 2 is generated, followed by rows 3 and 1 (Figs 23b, c, 24a, b). In *Spathidium*, the brush rows develop concomitantly (Table 4), while a certain population of *Protospathidium serpens* generates rows 2 and 3 during ontogenesis and row 1 in post-dividers (Foissner and Xu, 2005). Further, most *Protospathidium* species have brush row 1 strongly shortened, and in some it is even lacking (Foissner et al., 2002; Foissner and Xu, 2005).

These observations and the proposed evolution of the spathidiids (Fig. 31) suggest an ancestor with only two brush rows. We would not like to generalise this hypothesis, that is, to apply it to other groups of haptorids, because ontogenetic data are very sparse, the evolution of the haptorids is obscure, and most species/genera have a three-rowed brush, suggesting this is the plesiomorphic state.

• **What is the function of the division blebs?** Division blebs are hemispherical protrusions underneath the prospective division furrow. They were first described in *Homalozoon vermiculare*, where they contain various microtubules and cortical granules (Leipe et al., 1992). Later, division blebs were described also in *Spathidium turgitorum* (Foissner et al., 2002). We found them in *A. cultriforme* and other *Spathidium* species (Figs 13b-d, 14e), which suggests their widespread occurrence in haptorid ciliates.

At first glance, the blebs appear to be the precursors of the prospective oral bulge. However, they are distinct

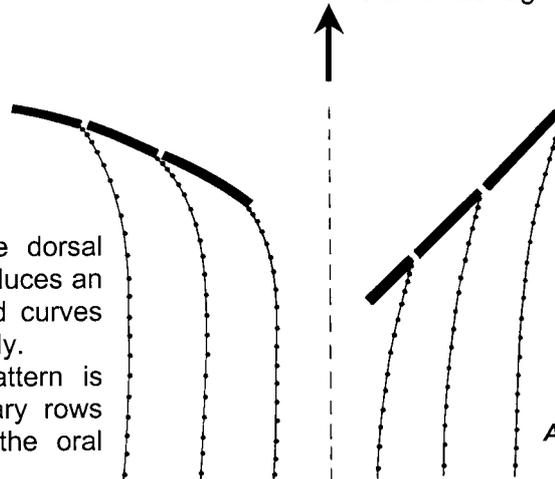


An **Arcuospathidium** pattern is produced if the ciliary rows detach from the oral kinetofragments.

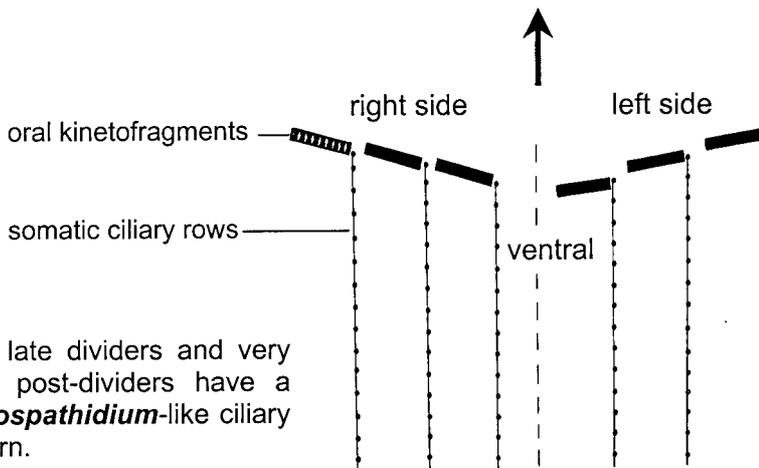
An **Epispathidium** pattern is produced if intense basal body proliferation occurs in the anterior region of the ciliary rows and the rows curve ventrally and detach from the oral kinetofragments.

Faster growth of the dorsal than ventral area produces an oblique oral area and curves the ciliary rows dorsally.

A **Spathidium** pattern is generated if the ciliary rows remain attached to the oral kinetofragments.



**Arcuospathidium pattern**      **Epispathidium pattern**



Very late dividers and very early post-dividers have a **Protospathidium**-like ciliary pattern.

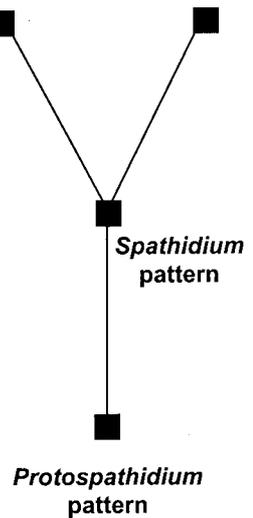
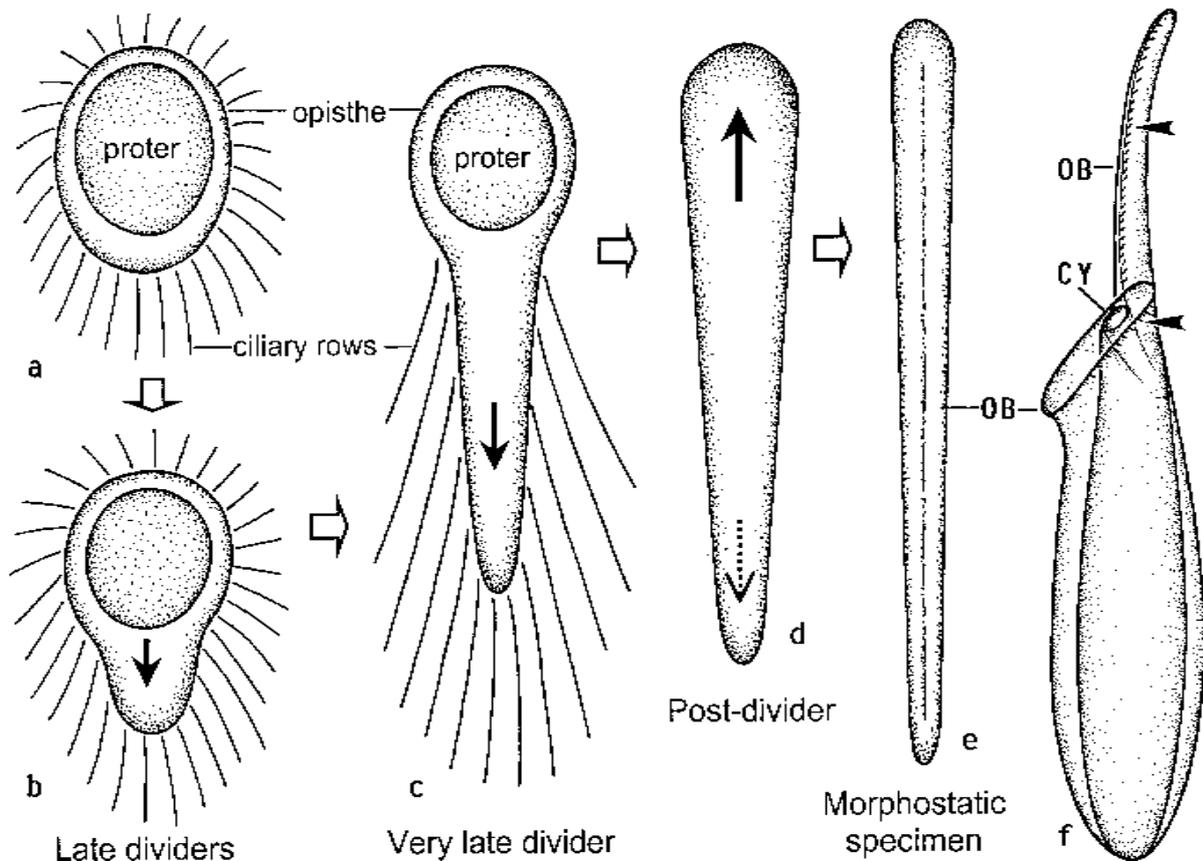


Fig. 31. An evolutionary scheme of the spathidiid ciliary patterns.

Table 4. Ontogenetic comparison of spathidiids.

Characteristics	<i>Arcuospathidium cultriforme scalpiforme</i>	<i>A. muscorum</i>	<i>A. coemeterii</i>	<i>Protospathidium serpens</i>	<i>P. muscicola</i> <sup>2</sup>	<i>Spathidium apospathidiiforme</i>	<i>S. turgitorium</i>	<i>Homalozoon vermiculare</i>
Body becomes longer and more slender in early dividers	yes (distinctly)	?	yes	no (only stouter)	yes	no (likely only stouter)	no	? (contractile)
Body distinctly inflated in fission area during middle stages	no	no	yes	yes	?	yes	yes	no
Proter longer than opisthe	yes (ratio 1.5:1)	yes	yes	yes	yes	?	yes	yes
Early dividers with indentation in fission area	yes	?	yes	yes	?	no?	no?	yes
Blebs recognizable in fission area	yes	?	no	no	no	yes	yes	yes
Macronucleus distinctly elongates in early dividers	no	no	yes	no	no	no	no	no
Micronucleus greatly ( $\geq 3$ times) enlarges in early-middle dividers	no	no	yes	no	no	no	no	no
Dorsal brush row 1 generated in post-dividers	no	no	no	yes	no	no	no	no
Dorsal brush row 2 produced distinctly earlier than rows 1 and 3	yes	?	no	no	?	no	no	no
Left side oral kinetofragments curve rightwards earlier than right ones	yes	yes	yes	no	no	no	no	?
Oral kinetofragments straight or slightly/distinctly curved	distinctly curved	distinctly curved	slightly curved	straight	straight	slightly curved	slightly curved	distinctly curved
Individual oral kinetofragments separate or loosely aligned in very late dividers	aligned	aligned	aligned	separate	separate	separate	separate	separate
Circumoral kinety develops in simple or complex manner <sup>1</sup>	complex	complex	simple	simple	simple	simple	simple	simple
Shaping of oral bulge and circumoral ciliature completed in late dividers or post-dividers	late post-dividers	late post-dividers	late post-dividers	very late dividers	very late dividers or early post-dividers	post-dividers	post-dividers	post-dividers
Division axis distinctly oblique?	yes	no	no	no	no	no	no	no
Anterior portion of opisthe's kineties strongly curved in mid-dividers	yes	yes	no	no	no	no	no	no
Shape of fission area in late dividers	clavate	clavate	roundish	roundish	roundish	roundish	roundish	roundish
References	Berger et al., 1983	Foissner and Lei, 2004	Foissner and Xu, 2005	Foissner and Xu, 2005	Berger et al., 1984	Foissner and Xu, 2005	Foissner et al., 2002	Leipe et al., 1992

<sup>1</sup> simple = by alignment of kinetofragments one after another; complex = by shifting and overlapping individual oral kinetofragments.<sup>2</sup> Misidentified as *Protospathidium serpens*.



**Figs 32a-e.** A model of production of the spathidiid oral bulge. In *A. cultriforme* and several other spathidiids, both the oral bulge and ciliary pattern are generated mainly post-divisionally. Late and very late dividers perform growth mainly in the ventral fission area (a-c). After separation of the daughter cells, faster growth of the dorsal than the ventral area makes the oral bulge more or less distinctly oblique and curves the ciliary rows dorsally (d, e). The shape of the oral bulge is genetically determined. f. A model of a *Dileptus*-like ancestor of the spathidiids. It is based on the adesmokineties (arrowheads) and the acentric location of the cytopharyngeal entrance (CY) in *Spathidium*. The dileptid proboscis becomes reduced. OB - oral bulge.

only in early and middle dividers, disappearing during late ontogenesis, long before oral bulge formation commences (Figs 13a-d, 14b; Foissner et al., 2002). Thus, the function of the blebs remains obscure.

• **Adesmokineties in spathidiids?** The term “adesmokineties” was coined by Jankowski for the preoral kinetofragments of *Dileptus*; later the Americans named them “paratenes”. Recently, adesmokinety-like kinetofragments were found in *Myriokaryon*, a relative of the spathidiids (Foissner, 2003a).

Our observations show adesmokinety-like fragments in a typical spathidiid. The ontogenetic analysis reveals that these fragments are produced during post-divisional growth and shaping of the oral area, possibly by lateral proliferation of the circumoral kinety or by some re-arrangement of the somatic kinetids (Figs 4a, b). This would be different from *Dileptus*, where the adesmokineties are produced by the anterior end of the somatic kineties (Golińska, 1995). However, Golińska’s

evidence is not entirely convincing, since it does not rule out production by the circumoral kinety. Generally, there is a tendency to produce extra kinetofragments around the oral opening in haptorids, especially in the endocommensal Buetschliidae (Wolska, 1965; Grain, 1966). In this group, the fragments are produced interkinetally, probably *de novo*, but origin via migrating basal bodies cannot be excluded, as in the endodinomorphids (Furness and Butler, 1986; Foissner, 1996).

Only a part of the individuals of *A. cultriforme* in a population have adesmokinety-like fragments, suggesting that they are vestiges of an ancestral state. Possibly, spathidiids *sensu stricto* evolved from a *Dileptus*-like ancestor by reduction of the proboscis and its adesmokineties. This hypothesis is supported by two curious features of the spathidiids: (i) adesmokinety-like fragments occur only on the left side, where *Dileptus* has these structures, and (ii) the cytopharyngeal opening is near the dorsal end of the oral bulge. Figure 32f shows

an evolutionary scenario, where this pattern is produced by reduction of the proboscis and unequal (ventral) growth of the oral bulge.

• **Monsters.** A huge literature is available on natural and experimental monsters in ciliates. It was carefully reviewed by Frankel (1989) who concluded from all the observations and experiments: “My own view is that the true mechanisms of ontogenesis are not yet understood, not even in principle, and that answers will be found by scientists who are willing to think and work unconventionally”. None the less, our observations are of some interest because they confirm and extend earlier data on other species, mainly *Tetrahymena*, *Paramecium*, *Stentor*, hypotrichs, and *Dileptus*. For instance, many processes are obviously controlled epicortically, and a new mouth can be generated only on the ventral and dorsal surface, never laterally. Of particular interest is the observation that the spathidiid circumoral kinety can develop apokinetally (*de novo*), that is, without soma-based oral kinetofragments. This provides support for the molecular phylogenies proposing a sister-group relationship between Gymnostomatea ( $\cong$  Litostomatea) and Spirotrichea (Lynn and Small, 1997), the sole ciliate groups with apokinetal stomatogenesis (Foissner, 1996).

Malformed specimens basically divide as ordinary ones. For instance, specimens with two macronucleus pieces divide the pieces individually, so that the offspring also have two pieces. Wenzel (1955) showed that such changes could stabilise in *Spathidium* and produce a new species.

#### COMPARATIVE ONTOGENESIS

Foissner (1996) briefly reviewed the ontogenetic literature on haptorids. Although some studies were added recently (e.g., Foissner et al., 2002; Foissner and Lei, 2004; Table 4), this compilation shows that reliable data are available on only 6 out of about 1,000 species described. None the less, Foissner’s review indicates a great homogeneity of the division process in haptorids: the macronucleus is homomeric and cell division occurs in active (non-encysted) condition; stomatogenesis is holotelokinetal (all ciliary rows produce kinetofragments) except in the pleurostomatids, where it is monotelokinetal (only one kinety produces the new circumoral kinety); and the parental oral apparatus is not reorganised. *Arcuospathidium cultriforme* perfectly matches the general pattern, while details may be different even in the same or related genera (Table 4). However, we cannot exclude that some variation is caused by incomplete or misinterpreted data.

Evolution and classification of the haptorids into orders and families are poorly understood (Foissner and Foissner, 1988; Lipscomb and Riordan, 1990; Foissner,

1996). Ontogenetic data will certainly contribute to a more rigid system, providing they become available in sufficient quality and quantity. For instance, ontogenetic features suggest that the bryophyllids and dileptiids are distinct groups (Fryd-Versavel et al., 1975; Golińska, 1995). Likewise, the nuclear apparatus shows interesting variations. For instance, in multinucleate species the macronucleus nodules fuse to a globular mass in mid-dividers, except for *Dileptus* where the nodules divide individually (Jones, 1951). The same is known for some stichotrichine spirotrichs (Foissner, 1996).

#### ENCYSTMENT AND RESTING CYSTS

Detailed data on encystment of *Spathidium* are very scant and time-honoured. Until recently, the sole study available was that from Moore (1924), who provided a thorough description of body and nuclear changes in encysting and encysted cells of *Spathidium spathula*. While the body changes described by Moore (1924) in *S. spathula* match our observations in *A. cultriforme* and *Protospathidium fraterculum* (Xu and Foissner, 2005), the nuclear processes do not. In cysts of *S. spathula*, the macronucleus strongly fragmentates, while it is maintained, but distinctly shortened in *A. cultriforme*. However, Moore’s data are doubtful because she could stain the nuclear apparatus only after mechanical rupture of the wall. Certainly, such crude method can produce many artifacts.

The strong shortening of the macronucleus by cytoplasmic resorption is an outstanding feature of the resting cyst of *A. cultriforme*. Some reviews (Corliss and Esser, 1974; Gutiurrez et al., 2003) indicate that such process has been not described in other ciliates, although Padnos et al. (1954) observed that 4-year-old cysts expelled, in three phases, a good half of the macronucleus as well as cytoplasm and deposited the material in the cyst wall. In *A. cultriforme*, a strong reduction of the macronucleus from  $\sim 5,000 \mu\text{m}^3$  to  $\sim 1,000 \mu\text{m}^3$  might be associated, at least partially, with the small cyst volume ( $\sim 45,000 \mu\text{m}^3$  without the thick wall) as compared to the large cell volume ( $\sim 300,000 \mu\text{m}^3$ ). In several other spathidiids, the ratio cell:cyst volume is not 6:1, as in *A. cultriforme*, but only about 2:1, for instance, in the *Protospathidium serpens* complex, where the macronucleus is only slightly shortened during encystment (Xu and Foissner, 2005).

As concerns the ciliary pattern ( $\sim$  infraciliature), three types of resting cysts have been distinguished in ciliates (Gutiurrez et al., 2003): kinetosome-resorbing cysts (KR type), non-kinetosome-resorbing cysts (NKR), and partial-kinetosome-resorbing cysts (PKR). *Arcuospathidium cultriforme* very probably produces KR cysts, as do, for instance, oxytrichid hypotrichs (Gutiurrez et al., 2003), while some

colpodids have PKR cysts (Foissner, 1993). However, definite proof requires transmission electron microscopy data not available on any spathidiid. In related taxa, such as *Didinium* and *Dileptus*, some basal bodies and cortical microtubules are maintained (Dippell and Grimes, 1966; Kink, 1973).

Few data are available on resting cyst morphology of spathidiids. Moore (1924) described a fairly thin, wrinkled wall in *Spathidium spathula*. The wall is also thin, but smooth in *S. ascendens*, *S. turgitorum*, and *S.* (now *Arcuospathidium*) *muscorum* (Wenzel, 1955; Berger et al., 1983; Foissner et al., 2002). *Spathidium stammeri*, in contrast, has a thin wall with many conspicuous spines about 4 µm long (Wenzel, 1959 and unpubl. observations of the authors). Last but not least, *Protospathidium serpens* makes smooth or spiny cysts, depending on population. The smooth type has been classified as a new species by Xu and Foissner (2005). The resting cysts of the three populations of *A. cultriforme* are very similar, though there are differences in size and thickness of the ridges. The cysts are unique not only because of the strong shortening of the macronucleus (Figs 2y, 21a-g) but also because of a distinctly faceted cyst wall about 10 µm thick (Figs 15a-i). Obviously, resting cyst morphology is conspicuously different in *Spathidium sensu lato*, providing some support for the pronounced split of the genus (Foissner, 1984; Foissner et al., 2002; Foissner and Xu, 2005).

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