ACTA PROTOZOOLOGICA

Four New Fuscheriid Soil Ciliates (Ciliophora: Haptorida) from Four Biogeographic Regions

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Summary. Fuscheriid haptorids are characterized by meridionally extending ciliary rows clearly separated from the dikinetidal circumoral kinety; a two- or three-rowed dorsal brush; and oral basket rods (nematodesmata) originating from both, the circumoral dikinetids and from some oralized monokinetids in the anterior portion of the ciliary rows. Using standard morphological methods, we describe four new fuscheriid taxa, each discovered in a specific biogeographic region. *Fuscheriides tibetensis* nov. gen., nov. spec. has rod-shaped extrusomes and two dorsal brush rows. It is a small (~ 80 × 10 μm), slender ciliate with only seven ciliary rows, and was discovered in grassland soil of southern Tibet, about 4600 m above sea-level. *Aciculoplites ethiopiensis* nov. gen., nov. spec. has acicular extrusomes and two dorsal brush rows. It is a middle-sized (~ 100 × 30 μm), oblong ciliate with about 22 ciliary rows, and was discovered in floodplain soil from a lake in Ethiopia. *Fuscheria uluruensis* nov. spec., which we discovered in mud and aeolic soil from pools on top of the Ayers Rock in the red centre of Australia, is unique in having the macronucleus split into about 12 oblong nodules. The nodules originate post-divisionally via the branching macronucleus strand, as in multinucleate spathidiids. *Fuscheria nodosa salisburgensis* nov. sspec., which was discovered in mud and soil of a meadow pool in Salzburg (Austria), has a long, tortuous macronucleus and about 45 ciliary rows. The resting cyst has an escape apparatus absent from the cyst of *F. uluruensis*, indicating that *Fuscheria* is non-monophyletic.

Key words: Australia, Austria, biogeography, Ethiopia, resting cysts, Tibet.

INTRODUCTION

The haptorid suborder Acropisthiina Foissner and Foissner, 1988 comprises small to middle-sized ciliates with an apical, discoidal oral bulge and an oral basket constructed of nematodesmata originating from both, haptorid oral dikinetids and oralized somatic monokinetids in the anterior region of the ciliary rows. Presently, the suborder contains three families (Acro-pisthiidae, Fuscheriidae, Pleuroplitidae) and 14 genera listed in Oertel *et al.* (2009). Our study adds two genera discovered in Tibet and Ethiopia, two regions being a *tabula rasa* as concerns soil ciliates. The two other new taxa are from Australia and Central Europe. Thus, the four new taxa, as yet found only at their type localities, provide some support for the concept of a biogeographic structuring of protist communities (Foissner 2006, 2008). The Australian species, *Fuscheria uluruensis*, has been discovered at a scenic site with great religious significance for the aborigines, viz., on top of the Ayers Rock, a large granitic outgrowth in the red centre of the continent visited by ten thousands of tourists yearly.

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About half of the acropisthiid genera differ in only two features: the shape of the extrusomes and the number of dorsal brush rows. Possibly, both features are inconspicuous at first glance. However, acropisthiid haptorids have a comparatively simple organization making it difficult to select characters of phylogenetic significance. We consider the shape of the extrusomes, which are toxicysts as in all haptorids (Corliss 1979), a main feature because the extrusomes are an important survival tool, i.e., kill the prey, usually other ciliates. Many haptorids have three dorsal brush rows (Foissner *et al.* 2002). The brush is a complex organelle possibly of great significance for prey recognition and selection. Any deviation from this pattern might thus represent a distinct evolutionary branch, especially because the number is independent of spatial constraints, i.e., tiny species may have three rows as have large species (Foissner and Xu 2007).

MATERIALS AND METHODS

The origin of the material is provided in the individual species descriptions. Basically, all samples are from terrestrial or semiterrestrial habitats. The samples were air-dried for at least one month and then stored in plastic bags. Later, the samples were investigated with the non-flooded Petri dish method, as described by Foissner *et al.* (2002). Briefly, this simple method involved placing 50–500 g soil in a Petri dish (13–18 cm wide, 2–3 cm high) and saturating, but not flooding it, with distilled water. These cultures were analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28. Except of *Fuscheria nodosa salisburgensis*, the descriptions of the new species were based on material obtained from such cultures, i.e., pure cultures were not established. The subspecies mentioned was studied from both, a non-flooded Petri dish culture and a limnetic culture set up with Eau de Volvic, some ml of soil eluate with about 20 *F. nodosa salisburgensis* specimens, and a few crushed wheat grains to stimulate growth of prey ciliates from the soil eluate.

Morphological and presentation methods followed Foissner (1991) and Foissner *et al.* (2002). Terminology followed Corliss (1979) and the refinements introduced by Foissner and Xu (2007).

RESULTS AND DISCUSSION

Genus *Fuscheriides* Foissner and Gabilondo nov. gen.

Diagnosis: Fuscheriidae with oblong extrusomes, two dorsal brush rows, and some ventro-lateral ciliary rows curved leftwards anteriorly.

Type species: *Fuscheriides tibetensis* nov. spec.

Etymology: Composite of the Latin noun *Fuscheria* (Fusch, a village in the Austrian Central Alps) and the Latin suffix *ides* (similar), meaning a ciliate similar to the genus *Fuscheria*. Masculine gender.

Comparison with related genera: *Fuscheriides* differs from the other genera of the family Fuscheriidae Foissner *et al.*, 2002 in that the oral extrusomes are oblong. In *Fuscheria* Berger *et al.*, 1983, they are nail-shaped; in *Actinorhabdos* Foissner, 1984, they are awl-shaped; in *Diplites* Foissner, 1998, which has rod-shaped somatic extrusomes, they are ellipsoidal or clavate, depending on species (Foissner *et al.* 2002); and *Coriplites* Foissner, 1988 and *Apocoriplites* Oertel *et al.*, 2009 lack extrusomes at all. *Dioplitophrya* Foissner *et al.*, 2002 differs from *Fuscheriides* by the number of brush rows (three vs. two) and two shape types (nail-shaped and clavate) of extrusomes anchored to the oral bulge. A more sophisticated feature of *Fuscheriides* is the anterior curvature of some ventro-lateral ciliary rows not described (recognized?) in any other species of both, the Fuscheriidae and Acropisthiidae.

Fuscheriides tibetensis Foissner and Gabilondo nov. spec. (Figs 1a–p, 2a–f; Table 1)

Diagnosis: Size about 80 × 10 µm *in vivo*. Narrowly oblong to cylindroidal. Macronucleus usually reniform; 1 micronucleus. Extrusomes about 2 × 0.3 µm in size. Seven ciliary rows; dorsal brush isomorphic. Oral basket extends in anterior body quarter.

Type locality: Grassland soil from the surroundings of Lake Paiku-Tso, South Tibet, E85°45′ N28°45′, about 4600 m above sea-level.

Type material: One holotype slide and 4 paratype slides with protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip.

Etymology: Derived from Tibet, i.e., the country where it was discovered.

Remarks: Many exconjugants (35%) were found in the protargol slides. They were not included in the morphometric and morphological analyses. In spite of this, body size has high variability coefficients (Table 1), and the prepared specimens are smaller by 40% than the live ones. We do not know the reason for the conspicuous variability and size difference of live and prepared cells; possibly, fixation was insufficient causing strong shrinkage of most specimens. Post-conjugational nuclear reconstruction might be responsible for some of the high variability found in the macronucleus pattern.

Table 1. Morphometric data on *Fuscheriides tibetensis*. Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a non-flooded Petri dish culture. Measurements in μm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean, x̄– arithmetic mean.

Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length	46.9	47.0	6.8	1.5	14.5	33.0	62.0	21
Body, width	10.4	11.0	3.2	0.7	30.7	5.0	17.0	21
Body length:width, ratio	5.3	5.5	1.7	0.4	32.1	3.0	9.4	21
Oral bulge (circumoral kinety), width	3.0	3.0	0.5	0.1	16.7	2.0	4.0	21
Oral bulge, height	1.3	1.0	–	–	–	1.0	2.0	20
Anterior body end to macronucleus, distance	20.0	20.0	5.1	1.1	25.8	11.0	30.0	21
Macronucleus, length[a]	10.0	9.0	–	–	–	6.0	20.0	21
Macronucleus, width in mid	5.0	5.0	1.3	0.3	26.0	3.0	7.0	21
Macronucleus nodules, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronucleus, length[b]	1.9	2.0	–	–	–	1.0	4.0	14
Micronucleus, width[b]	1.6	2.0	–	–	–	1.0	2.0	13
Micronuclei, number[b]	1.0	1.0	–	–	–	1.0	1.0	13
Somatic ciliary rows, number	7.0	7.0	0.0	0.0	0.0	7.0	7.0	21
Circumoral dikinetids, number	7.0	7.0	0.0	0.0	0.0	7.0	7.0	21
Brush rows, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
Brush row 1, length[c]	5.4	5.0	0.9	0.2	16.7	3.0	7.0	21
Brush row 2, length[c]	5.9	5.5	1.2	0.3	20.3	5.0	9.0	20
Brush row 1, number of dikinetids	3.0	3.0	0.4	0.1	13.3	2.0	4.0	21
Brush row 2, number of dikinetids	5.5	5.0	0.8	0.2	14.5	4.0	7.0	20
Subapical condensation, distance[d]	6.0	6.0	1.0	0.2	16.7	4.0	8.0	21
Kinetids in subapical condensation, number	4.7	5.0	0.6	0.1	12.8	4.0	6.0	21
Kinetids in a ventral kinety, number	19.8	20.0	3.9	0.8	19.7	11.0	27.0	21
Kinetids in the subapical condensation kinety, number	22.6	22.0	3.6	0.8	15.9	15.0	30.0	19
Excretory pores, number	1.2	1.0	–	–	–	1.0	2.0	13

[a] Macronucleus "coiled" in some specimens; values thus approximate.

[b] Uncertain because confusion with similar-sized and -impregnated cytoplasmic inclusions could be not excluded.

[c] Distance between circumoral kinety and last dikinetid of row.

[d] Distance between circumoral kinety and last kinetid of subapical condensation.

Description: Size 55–90 × 8–12 μm *in vivo*, usually about 80 × 10 μm. Narrowly oblong to cylindroidal, in preparations frequently slightly clavate and thus widest in third body quarter, slightly to distinctly curved to left side; anterior body portion more or less narrowed, posterior rounded; slightly, rarely distinctly flattened laterally (Figs 1a–g, l, m, 2a, b). Macronucleus in second to third quarter of body; usually reniform (39% out of 100 specimens investigated) but also ellipsoidal (10%), semicircular, and oblong or spiralized (51%); nucleoli numerous and scattered. Possibly one globular to ellipsoidal micronucleus attached to concave or anterior area of macronucleus, difficult to recognize among lipid droplets after protargol impregnation (Figs 1a, d, g, k, l, 2a, b, e, f). Contractile vacuole terminal, usually one excretory pore in or near pole centre. Cytopyge near excretory pore of contractile vacuole, faecal mass spongy (Fig. 1a, d, g, l). Extrusomes in centre of oral bulge and scattered in cytoplasm, oblong, about 2 × 0.3 μm in size, highly refractive and thus easily recognizable *in vivo* (Fig. 1a–c, i, j). Somatic cortex very flexible and distinctly furrowed by ciliary rows, cortical granules not recognizable in three specimens investigated. Cytoplasm colourless, in some speci-
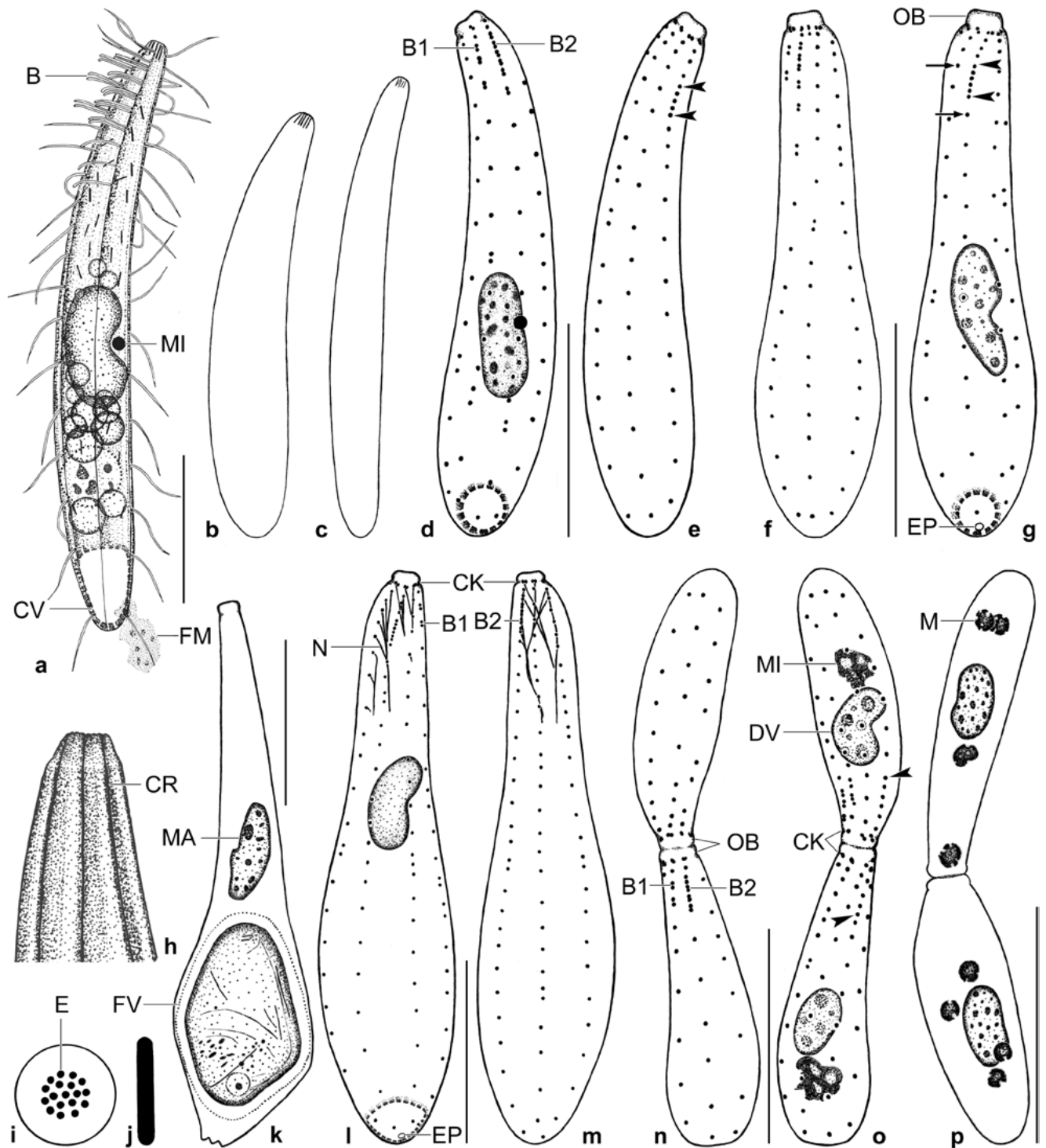
Figs 1a–p. *Fuscheriides tibetensis* from life (a–c, h–j) and after protargol impregnation (d–g, k–p). **a** – dorsolateral view of a representative specimen, length 80 µm; **b**, **c** – shape variants, length 60 µm, 90 µm; **d**, **e** – ciliary pattern of holotype, showing, *inter alia*, the subapical cilia condensation (arrowheads), length 50 µm; **f**, **g** – specimen with two ventral ciliary rows anteriorly curved leftwards (arrows). The arrowheads mark the subapical cilia condensation; **h** – the oral bulge is rugged by the cortex crests; **i** – extrusomes in centre of oral bulge; **j** – extrusome, length 2 µm; **k** – specimen with a big food vacuole containing a naked amoeba; **l**, **m** – dorsolateral and ventrolateral view showing, *inter alia*, the nematodesmata; **n**, **o** – ciliary and nuclear pattern during begin of first maturation. The arrowheads mark the subapical cilia condensation; **p** – conjugants with four maturation derivatives each. B(1–2) – dorsal brush (rows), CK – circumoral kinety, CR – cortex crests, CV – contractile vacuole, DV – degenerating vegetative macronucleus, E – extrusomes, EP – excretory pore, FM – faecal mass, FV – food vacuole, M – maturation derivatives, MA – macronucleus, MI – micronucleus, N – nematodesmata, OB – oral bulge. Scale bars: 20 µm.
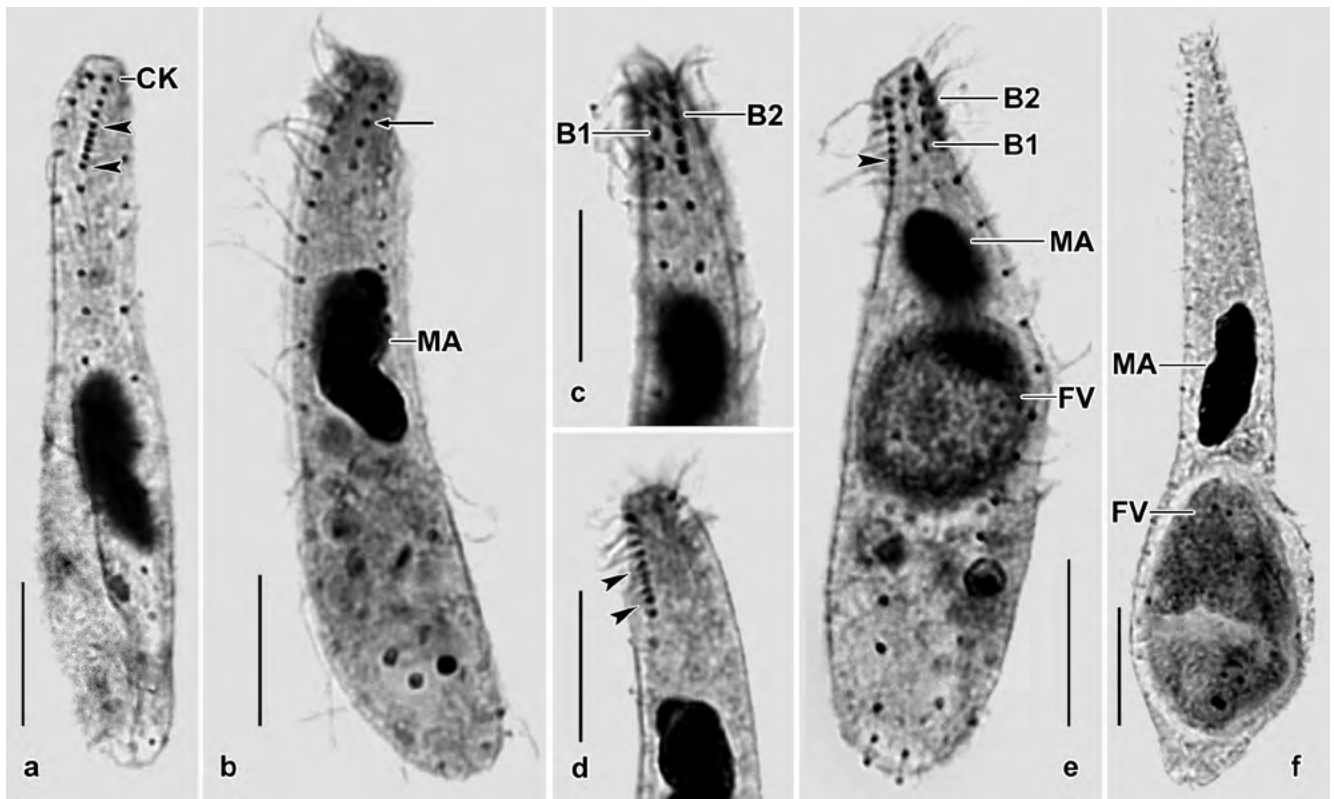
Figs 2a–f. *Fuscheriides tibetensis* after protargol impregnation. **a** – ventrolateral view of a specimen showing the subapical ciliary condensation (arrowheads), which is in one of the two kineties curved leftwards anteriorly, length 50 µm; **b**, **c**, **d** – the same specimen (length 47 µm) at three focal planes to show two ventral ciliary rows curved leftwards anteriorly (arrow), the brush, and the subapical ciliary condensation (arrowheads); **e**, **f** – specimens with food vacuoles containing almost undigested naked amoebae. The arrowhead marks the subapical ciliary condensation. Length of specimens 43 µm and 64 µm. B 1, 2 – dorsal brush rows, CK – circumoral kinety, FV – food vacuole, MA – macronucleus. Scale bars: 10 µm.

mens packed with many lipid droplets 1–4 µm across and food vacuoles containing almost undigested flagellates and/or naked amoebae mainly in mid-body and rear portion (Figs 1a, k, 2e, f). Moves rather slowly by rotation about main body axis.

Cilia about 8 µm long *in vivo*, densely spaced in oral body quarter, while ordinarily beyond; invariably arranged in seven ordinarily spaced, longitudinal rows commencing underneath circumoral kinety; two or three ventral rows anteriorly curved leftwards, a curious feature recognizable only in properly (ventrally or dorsally) oriented specimens. About 3 µm posterior of circumoral kinety a distinct condensation of about five cilia in second row left of brush row 1; condensation caused by two to three extra kinetids, as shown by the morphometric data (Figs 1e, g, l, 2a, d, e; Table 1). Dorsal brush isomorphic, consists of two rows each

usually beginning with a monokinetid possibly bearing an ordinary cilium. Row 1 composed of three widely spaced dikinetids on average; row 2 composed of five ordinarily spaced dikinetids possibly followed by some tail monokinetids (Figs 1a, d, f, l, m, 2c; Table 1). Brush bristles rod-shaped, 4–5 µm long *in vivo*.

Oral bulge discoidal, more distinct in preparations than *in vivo*, in some specimens as distinct as in *Enchelyodon*, centre indistinctly concave, surface rugged by crests between somatic ciliary rows (Fig. 1h). Circumoral kinety at base of oral bulge, invariably composed of seven obliquely to almost horizontally arranged dikinetids one each on top of ciliary rows. Nematodesmata faintly impregnated, originate from circumoral dikinetids and four to eight ciliated basal bodies in anterior region of all somatic kineties, form an indistinct oral basket about 10 µm long (Fig. 1l, m; Table 1).

Ontogenesis: Morphogenesis proceeds as described by Berger *et al.* (1983) in *F. terricola*.

Conjugation: Only three pairs were found, showing the beginning of the first maturation division and the four maturation derivatives (Fig. 1n–p). Conjugants unite with the oral bulge and form a long, slightly curved rod. The ciliary pattern does not change, but cell size and number of basal bodies per ciliary row are about half of those in vegetative specimens, indicating pre-conjugation division(s). During the initial stage, the micronucleus becomes large and reticular (Fig. 10), and then divides twice to generate four globular maturation derivatives containing faintly impregnated granules (Fig. 1p). Many exconjugants occur in the slides. They are easily distinguished from vegetative cells by the deviating nuclear apparatus: two (in 30 out of 53 exconjugants) or four (in 23 out of 53 exconjugants) globular nodules.

Occurrence and ecology: As yet found only at type locality, where it was numerous in the non-flooded Petri dish culture. The sample, kindly provided by Dr. Norbert Winding (Haus der Natur, Salzburg), was taken in a high mountain area covered with vegetation by only about 50%, and consisted of humic surface soil (0–7 cm) with pH 7.9 (in water) and a salinity of about 10‰.

Comparison with similar species: There are several fuscheriids which are highly similar to *Fuscheriides tibetensis* in body size and shape as well as the macronucleus pattern, such as *Diplites arenicola*, *D. telmatobius*, and *Fuscheria terricola* (Berger *et al.* 1983; Foissner 1983, 1984; Foissner *et al.* 2002; Vďačný 2007). *Fuscheriides tibetensis* differs from these and other acropisthiids in having minute, oblong oral bulge extrusomes and a lower number of ciliary rows (invariably seven vs. more than nine). Interestingly, *Fuscheriides tibetensis* shares with *Fuscheria terricola* a subapical condensation of kinetids (Berger *et al.* 1983, Foissner *et al.* 2002).

Genus *Aciculoplites* Foissner and Gabilondo nov. gen.

Diagnosis: Fuscheriidae with acicular extrusomes and two dorsal brush rows.

Type species: *Aciculoplites ethiopiensis* nov. spec.

Etymology: Composite of the Latin nouns *ăcĭcŭla* (diminutive of *ăcus*, needle) and *hoplitês* (soldier or a heavy armed man), referring to the acicular extrusomes. Masculine gender.

Comparison with related genera: *Aciculoplites* belongs to the suborder Acropisthiina because the nematodesmata originate not only from the dikinetidal cir-

cumoral kinety but also from the anterior kinetids of the somatic ciliary rows (Foissner and Foissner 1988). The body organization is enchelyodonid, and thus it belongs to the family Fuscheriidae Foissner *et al.*, 2002. *Aciculoplites* represents a distinct genus because none of the 14 acropisthidiid genera has acicular toxicysts (see Introduction and Oertel *et al.* 2009). As concerns the shape of the extrusomes in the Fuscheriidae, see the discussion of the new genus *Fuscheriides*. *Aciculoplites* has two dorsal brush rows as *Fuscheria*, *Actinorhabdos*, *Diplites* and *Apocoriplites*; while *Coriplites*, *Balantidion* and *Dioplitophrya* have three (Berger *et al.* 1983; Foissner 1984, 1988, 1998; Foissner *et al.* 1999, 2002; Oertel *et al.* 2009).

Aciculoplites ethiopiensis Foissner and Gabilondo nov. spec. (Figs 3a–p, 4a–h; Table 2)

Diagnosis: Size about 100 × 30 μm *in vivo*; oblong. Macronucleus usually horseshoe-shaped; 1 micronucleus. Extrusomes form a bundle in centre of oral bulge, about 7 μm long. 22 ciliary rows on average; dorsal brush isomorphic. Oral basket extends in anterior quarter.

Type locality: Saline (16‰) pasture soil in the surroundings of Lake Ziway, Ethiopia, E38°4′ N7°5′, 1636 m above sea level.

Type material: One holotype slide and 5 paratype slides with protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip.

Etymology: Derived from the Greek noun *Æthiopia* (Αἰθιοπία), which refers to the type locality.

Remarks: About 70 specimens, all mediocre or poorly impregnated, were found in eight protargol slides. With few exceptions, they were distinctly inflated (on average by 48%, Table 2), showing a variety of shapes (Figs 3b–d, j, 4c, e, f). Thus, the measurements shown in Table 2 must be handled with care. Part of this massive variability is, however, caused by a natural reason, viz., the formation of distinct theronts and trophonts (Fig. 3m–o; Table 2).

Description: Size and continuous features rather variable because distinct theronts and trophonts occur: 80–120 × 20–40 μm *in vivo*, ordinary specimens usually near 100 × 30 μm; distinctly smaller in protargol preparations, viz., on average 88 × 43 μm, indicating some shrinkage and strong inflation by the preparation procedures (Table 2). Length:width ratio of both, trophonts and theronts, *in vivo* on average 3.4:1, while

Table 2. Morphometric data on *Aciculoplites ethiopiensis*. Data based, if not mentioned otherwise, on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens (theronts and trophonts) from a non-flooded Petri dish culture. Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean, x̄ – arithmetic mean.

Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length *in vivo* (rough values)	98.3	95.0	–	–	–	80.0	120.0	6
Body, length	88.5	85.0	11.0	2.4	12.4	72.0	106.0	21
Body, width *in vivo* (rough values)	29.2	32.5	–	–	–	20.0	35.0	6
Body, width	43.1	44.0	8.6	1.9	19.9	27.0	65.0	21
Body length:width, ratio *in vivo* (rough values)	3.4	3.2	–	–	–	2.3	6.0	6
Body length:width, ratio	2.1	2.0	0.3	0.1	15.9	1.6	2.8	21
Oral bulge (circumoral kinety), width	4.2	4.0	1.0	0.2	24.6	2.0	5.0	21
Oral bulge, height	1.4	1.0	–	–	–	1.0	2.0	21
Anterior body end to macronucleus, distance	26.0	27.0	11.0	2.4	42.5	6.0	46.0	21
Macronucleus, length	29.0	26.0	7.4	1.6	25.5	22.0	45.0	21
Macronucleus, width	6.0	6.0	1.2	0.3	19.5	4.0	9.0	21
Macronucleus nodules, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	21
Micronuclei, length[a]	2.0	2.0	–	–	–	2.0	3.0	21
Micronuclei, width[a]	2.0	2.0	–	–	–	2.0	2.0	21
Micronuclei, number[a]	1.1	1.0	–	–	–	1.0	2.0	21
Somatic ciliary rows, number[b]	21.8	22.0	–	–	–	18.0	26.0	21
Ciliated kinetids in a ventral kinety, number[b]	36.6	36.0	–	–	–	27.0	60.0	19
Oral bulge extrusomes, length	6.5	6.0	0.5	0.1	7.9	6.0	7.0	21
Oral basket, length (rough values)	22.0	20.0	–	–	–	15.0	37.0	21

[a] Uncertain because confusion with similar-sized and -impregnated cytoplasmic inclusions could be not excluded.

[b] Rough estimation because partially covered by the cortical granules.

2.1:1 in protargol preparations. Theronts *in vivo* oblong to cylindroidal (6:1), slightly more convex dorsally than ventrally, flattened up to 2:1 and occasionally slightly narrowed posteriorly; in protargol preparations rather frequently lageniform. Trophonts ellipsoidal to broadly ellipsoidal, unflattened. Anterior end transverse truncate, posterior narrowly to broadly rounded (Figs 3a–d, j, m–o, 4c, e, f; Table 2). Nuclear apparatus usually in second and third quarter of body, frequently displaced by large food vacuoles. Shape of macronucleus highly variable both *in vivo* and in protargol preparations, i.e., almost circular, oblong, cylindroidal, or tortuous, usually more or less horseshoe-like; nucleoli granular, scattered, and numerous (Figs 3a–d, j, n, o, 4c, e, f; Table 2). Micronucleus globular, usually attached to concave region of macronucleus, while near to the end when oblong or cylindroidal, about 2 µm across in protargol preparations (Fig. 3a, c, d; Table 2). Contractile vacuole

in rear end, possibly only one excretory pore (Fig. 3a). Extrusomes form a dense, conical bundle in central area of oral bulge, many scattered in cytoplasm (Figs 3a, b, e–g, j, l, 4a–d, f–h); acicular with an about 0.5 µm wide globule at proximal end, 6–8 µm long *in vivo*; anterior half very hyaline, posterior filled with toxin; become rod-shaped when only partially exploded or disturbed by, e.g., coverslip pressure (Figs 3a, e, l, 4a, b). Acicular shape of mature extrusomes basically preserved in protargol preparations; developing cytoplasmic extrusomes very narrowly ovate and only about half as long as mature ones, toxin containing posterior half rather deeply impregnated (Figs 3g, j, 4c, d, g). Cortex very flexible, with broad ridges between ciliary rows; cortical granules inconspicuous because colourless, rather loosely spaced, and only about 0.2 µm across; usually impregnate more or less deeply with the protargol method used, disturbing the analysis of the ciliary pat-
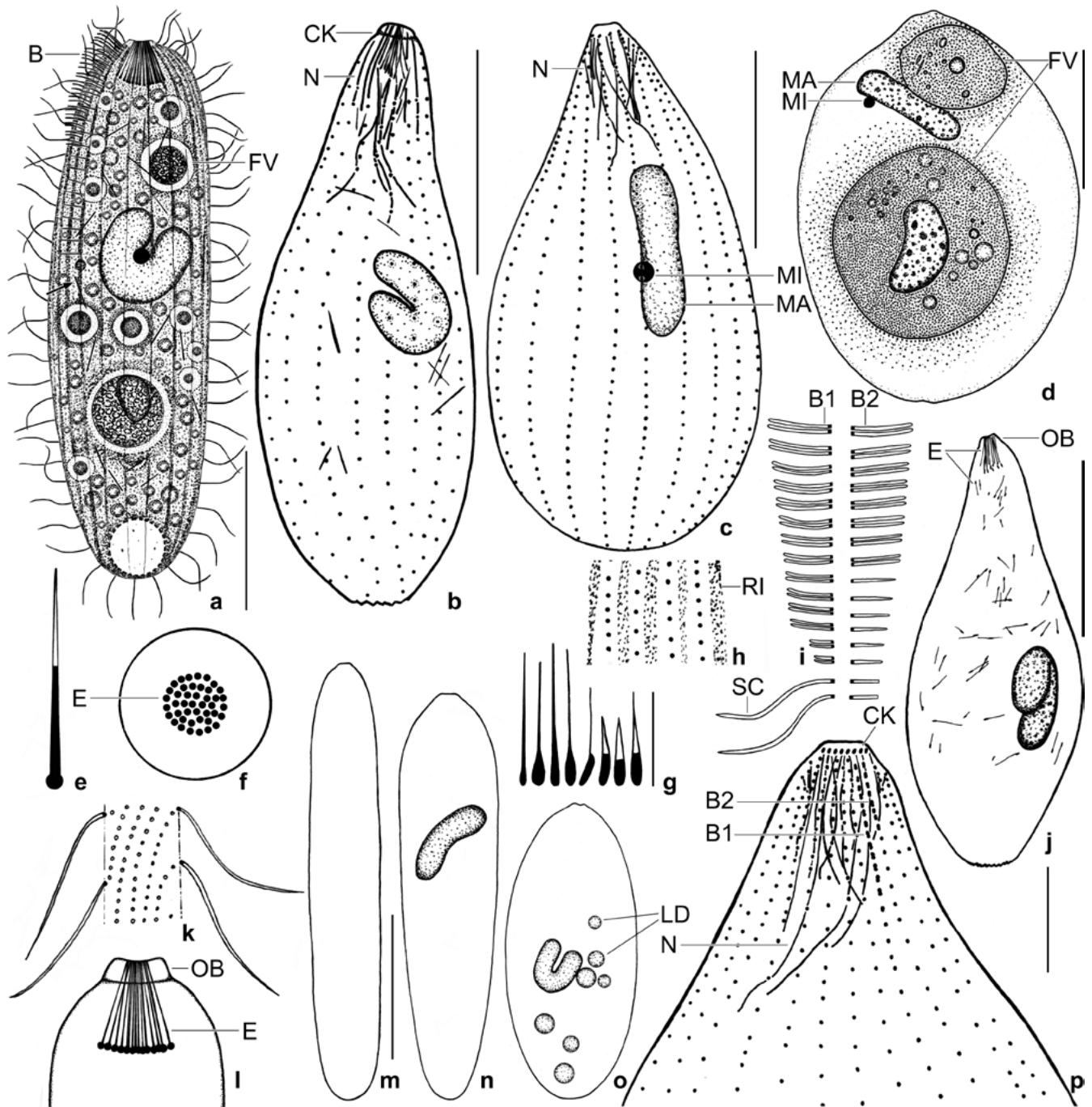
Figs 3a–p. *Aciculoplites ethiopiensis* from life (a, e, f, i, k–o) and after protargol impregnation (b–d, g, h, j, p). **a** – dorsolateral view of a representative specimen, length 100 μm; **b**, **c** – ventral views showing ciliary pattern, nuclear apparatus, and oral basket rods; **d** – a specimen with large food vacuoles displacing the nuclear apparatus; **e** – resting extrusome, length 7 μm; **f** – frontal view showing arrangement of extrusomes in oral bulge; **g** – the acicular shape of the oral bulge extrusomes is recognizable also in protargol-impregnated specimens (left four), while those in the cytoplasm are smaller and usually very narrowly ovate (right four). Drawn to scale, 5 μm; **h** – broad cortical ridges between ciliary rows; **i** – dorsal brush, longest bristles 3–4 μm (slightly schematized); **j** – specimen with extrusomes in oral bulge and cytoplasm; **k** – surface view showing rows of rather loosely spaced cortical granules about 0.2 μm across; **l** – the extrusomes form a dense bundle in the central area of the oral bulge; **m**, **n**, **o** – length:width variants caused by the formation of theronts (m, n) and trophonts (o). Drawn to scale, 40 μm; **p** – dorsal view of holotype, showing important oral structures and the two-rowed dorsal brush. B(1–2) – dorsal brush (rows), CK – circumoral kinety, E – extrusomes, FV – food vacuoles, LD – lipid droplets, MA – macronucleus, MI – micronucleus, N – nematodesmata, OB – oral bulge, RI – cortical ridge, SC – somatic cilium. Scale bars: 5 μm (g), 10 μm (p), 30 μm (a–d, j), and 40 μm (m–o).
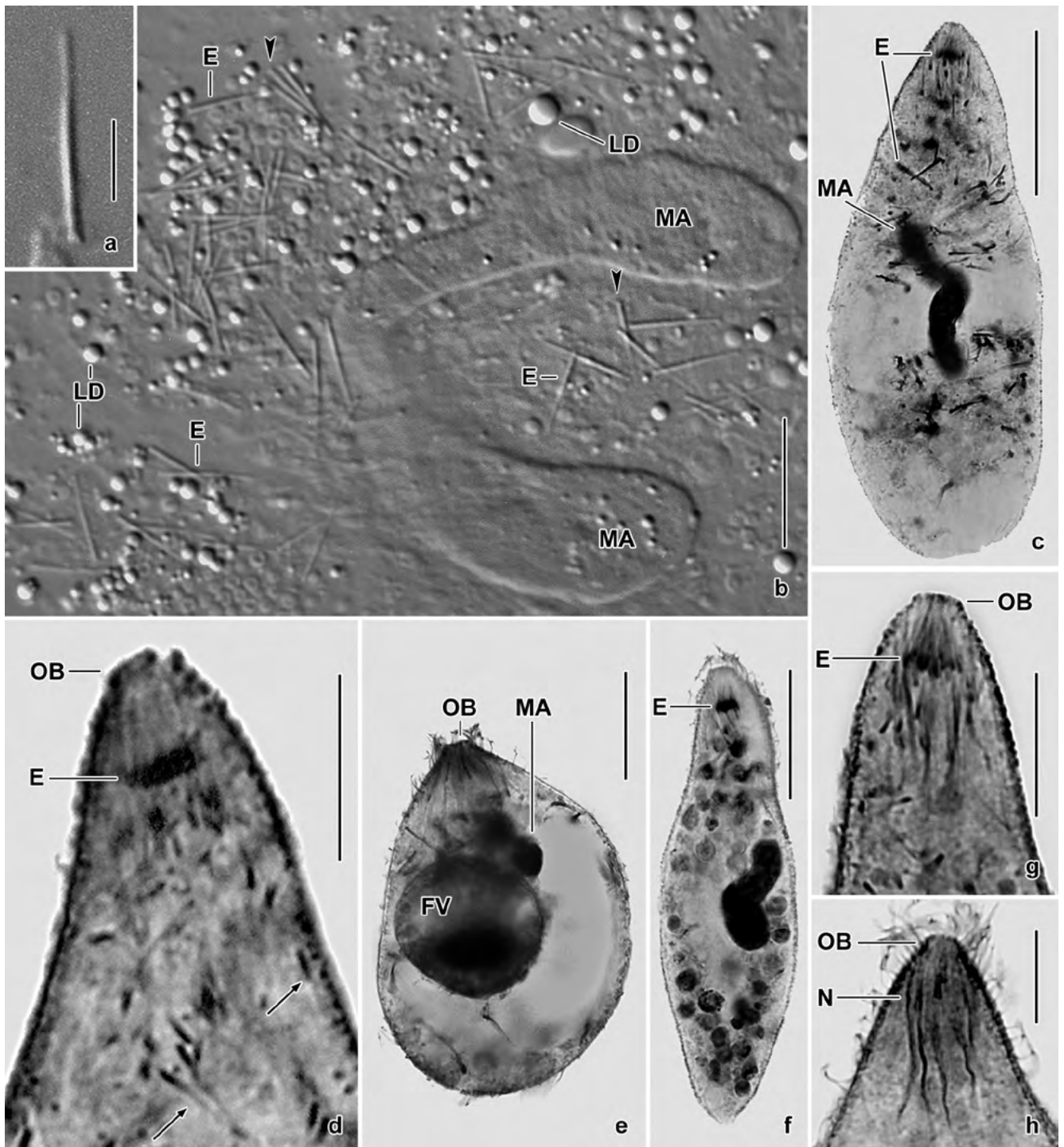
Figs 4a–h. *Aciculoplites ethiopiensis* from life (a, b) and after protargol impregnation (c–h). **a**, **b** – the about 7 μm long extrusomes are acicular and have a minute globule proximally (arrowheads). When disturbed, they become almost rod-shaped and the toxin containing portion becomes more distinct (a); **c**, **f** – an elongate ovoid and a lageniform specimen showing the macronucleus and the extrusomes in the oral bulge and the cytoplasm; **d** – anterior portion showing the dense extrusome bundle in the oral bulge. Arrows mark cytoplasmic extrusomes having strongly impregnated the posterior, toxin-containing portion; **e** – specimen with a large food vacuole; **g**, **h** – anterior portion showing the extrusome bundle and the nematodesmata, which form a wrinkled oral basket. Basket rods originate from the circumoral dikinetids and from about 5 ciliated basal bodies in the anterior region of all somatic kineties. E – extrusomes, FV – food vacuole, LD – lipid droplets, MA – macronucleus, N – nematodesmata, OB – oral bulge. Scale bars: 3 μm (a), 10 μm (b, d, g, h), 20 μm (e, f), and 30 μm (c).

tern (Fig. 3a, h, k). Cytoplasm hyaline in theronts, while packed with lipid droplets up to 5 μm across and one or two large and several small food vacuoles in trophonts; larger lipid droplets in distinct vacuoles. Feeds on small to medium sized ciliates, viz., the haptorid *Pleuroplitoides smithi* and the peritrich *Vorticella* (Figs 3a, d, o, 4b, e). Swims moderately rapid by rotation about main body axis, showing great flexibility under coverslip pressure.

Cilia about 8 μm long *in vivo*, ordinarily spaced, distance gradually increasing from anterior to posterior in some specimens; arranged in an average of 22 ordinarily spaced, longitudinal rows commencing underneath circumoral kinety (Fig. 3a–c, p; Table 2). Two rows anteriorly modified to an isomorphic dorsal brush occupying about 20% of body length. Brush row 1 composed of an average of 13 dikinetids; row 2 distinctly shorter than row 1 and thus composed of only 8 dikinetids, followed by a monokinetidal bristle tail extending to second body third. Bristles rod-shaped, decreasing in length from 3–4 μm anteriorly to 1–2 μm posteriorly; anterior tail bristles acicular and about 3 μm long, followed by 1–2 μm long stumps (Fig. 3a, i, p; Table 2).

Oral bulge about 4 μm wide and 2 μm high *in vivo*, central area slightly concave and filled with extrusomes (Figs 3a–d, f, j, l–p, 4c–h; Table 2). Circumoral kinety at base of oral bulge, composed of dikinetids, usually one each on top of ciliary rows. Nematodesmata originate from circumoral dikinetids and about 5 ciliated basal bodies in the anterior region of all somatic kineties, form a distinct but wrinkled oral basket about 22 μm long in protargol preparations (Figs 3b, c, p, 4h; Table 2).

Occurrence and ecology: This species was found in a soil sample from the flooded margin (if there is much rain) of Lake Ziway in Ethiopia. When the sample was taken, the area was used as a pasture for goats, horses and cows. The sample, kindly provided by Dr. Christian Jersabeck (Salzburg University), had 16‰ salinity and pH 8.4. *Aciculoplites ethiopiensis* reached moderate numbers in the non-flooded Petri dish culture five days after rewetting the sample.

Comparison with similar species: This species, preliminarily identified as *Fuscheria terricola*, attracted our closer attention by some comparatively slender specimens (Fig. 3m). As the genus is still monotypic, the acicular extrusomes are the main identification means (Figs 3a, e, g, j, l, 4a–d, f). Both *in vivo* and in protargol preparations, *A. ethiopiensis* is easily confused with the common and highly variable *Fuscheria terricola*, dif-

fering from *A. ethiopiensis* mainly by the nail-shaped extrusomes (Berger *et al*. 1983, Foissner *et al*. 2002). Fortunately, the shape of the extrusomes is well preserved in protargol preparations, confirming not only the *in vivo* observations but providing also a tool for separating both in protargol slides (Figs 3g, j, 4c, d, f, g).

Genus *Fuscheria* Foissner, 1983

Fuscheria uluruensis Foissner and Gabilondo nov. spec. (Figs 5a–u, 6a–j; Table 3)

Diagnosis: Size about 100 × 60 μm *in vivo*; ellipsoidal. On average 12 oblong macronucleus nodules and several micronuclei. Extrusomes nail-shaped, about 12 × 0.2 μm in size, form a torus in oral bulge. 46 ciliary rows on average; dorsal brush isomorphic. Oral basket extends two thirds of body length.

Type locality: Mud and aeolic soil from pools on top of Ayers Rock, Uluru-Kata Tjuta National Park, Australia, 863 m above sea level, E131°02´10´´ S25°20´42´´.

Type material: One holotype slide and 10 paratype slides with protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip.

Etymology: The Aboriginal noun *Uluru* refers to the type locality.

Remarks: This species was rather difficult to impregnate because of the considerable size and the large food inclusions. Thus, several preparations with different fixatives (Stieve, Stieve and Bouin alcohol, and pure alcohol) were made over a period of two months. The best specimens were selected for observations and morphometry.

Description: Size 80–120 × 60 μm *in vivo*, usually about 100 × 60 μm, considerably smaller in protargol preparations, either due to strong shrinkage or due to some size decrease over the observation period (Table 3). Broadly to ordinarily ellipsoidal and slightly flattened, even if containing many food vacuoles; anterior end flat to slightly concave, in prepared specimens sometimes convex; posterior end broadly rounded (Figs 5a, 6a–c). Nuclear apparatus mainly in second and third quarter of cell (Figs 5a, b, 6e, i, j; Table 3). Number (8–28) and length of macronucleus nodules highly variable, possibly due to late post-dividers having not yet fully segregated the macronuclear reticulum (see ontogenesis), on average elongate ellipsoidal with width varying much less than length (3–6 μm, CV 15.4% vs. 5–60 μm, CV 66.7%; Table 3); nucleoli globular and scattered. Several micronuclei according to dividing cells (Fig. 5q);

Table 3. Morphometric data on *Fuscheria uluruensis*. Data based on mounted, protargol-impregnated (Foissner's method), and selected specimens (see Result section) from a non-flooded Petri dish culture. Specimens were collected several times within two months and fixed either in Stieve's solution, Bouin's solution with ethanol, or in pure ethanol. For measurements (µm) only specimens fixed in Stieve's solution were used. 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, x̄ – arithmetic mean.

Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length	74.3	73.7	6.8	1.5	9.1	65.0	87.0	21
Body, width	53.1	53.7	5.0	1.1	9.4	45.0	62.0	21
Body length: width, ratio	1.4	1.4	0.1	0.0	7.1	1.2	1.6	21
Oral bulge (circumoral kinety), width	8.1	8.0	1.5	0.3	17.9	6.0	11.0	21
Oral bulge, height	1.8	2.0	–	–	–	1.0	2.0	21
Anterior end to first macronucleus nodule, distance	13.7	14.0	3.5	0.8	25.3	10.0	21.0	21
Macronucleus, length of largest nodule	23.5	21.0	9.7	2.1	41.1	16.0	60.0	21
Macronucleus, length of smallest nodule	7.9	8.0	2.0	0.4	25.0	5.0	12.0	21
Macronucleus nodules, average length[b]	15.7	14.0	10.5	1.6	66.7	5.0	60.0	42
Macronucleus, width of largest nodule in cell	4.6	5.0	0.8	0.2	17.4	3.0	6.0	21
Macronucleus, width of smallest nodule in cell	5.0	5.0	0.6	0.1	12.6	4.0	6.0	21
Macronucleus nodules, average width[b]	4.8	5.0	0.7	0.1	15.4	3.0	6.0	42
Macronucleus nodules, number	12.4	12.0	4.8	1.1	38.8	8.0	28.0	21
Somatic ciliary rows, number	46.1	46.0	2.0	0.4	4.3	42.0	50.0	21
Ciliated kinetids in a ventral kinety, number	63.3	64.0	12.8	2.8	20.2	41.0	88.0	21
Brush rows, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	21
Brush row 1, length[a]	24.0	20.0	6.7	2.2	28.1	19.0	37.0	9
Brush row 2, length[a]	12.6	12.0	4.0	1.3	31.5	9.0	19.0	10
Brush row 1, number of dikinetids	23.6	24.0	3.9	0.9	16.7	12.0	30.0	21
Brush row 2, number of dikinetids	13.9	14.0	1.8	0.4	13.2	12.0	18.0	21
Oral bulge extrusomes, length	11.4	11.0	1.8	0.5	16.0	9.0	15.0	13
Oral basket, length (rough values)	55.0	55.0	–	–	–	44.0	66.0	21

[a] Distance between circumoral kinety and last dikinetid of row.

[b] Calculated from the largest and the smallest nodule in the individual cells.

difficult to recognize in morphostatic specimens because of similar-sized cytoplasmic inclusions. Contractile vacuole in posterior body end, likely several excretory pores in pole area (Figs 5a, j, 6a, c, e). Extrusomes numerous and nail-shaped, about 12 × 0.2 µm *in vivo* and in protargol preparations; scattered in cytoplasm, while forming a torus-like pattern in oral bulge leaving free bulge centre; oral extrusome bundle prominent *in vivo*, while individual extrusomes are very fine (0.2 µm) and, thus, difficult to recognize; impregnate lightly with the protargol method used, especially the globular proximal end (Figs 5a, f, g, j, 6a, b, d, e; Table 3). Cortex flexible and about 1 µm thick, i.e., rather prominent *in vivo* and distinctly furrowed by ciliary rows, forming a serrate outline in transverse view (Fig. 5a, h). Cyto-

plasm colourless and turbid due to many granules, lipid droplets, small food vacuoles, and macronucleus nodules; usually contains one or several large food vacuoles with ciliates, such as *Urosoma*, *Urosomoida* and *Vorticella*, pharyngeal baskets of *Colpodidium caudatum*, and possibly remnants also small naked amoebae (Figs 5a, 6a–e). An attack on *Cyrtohymena primicirrata*, an oxytrichid hypotrich, was unsuccessful because *F. uluruensis* could not break the cortex during a period of five minutes, meanwhile becoming almost globular and producing a large, clear (food) vacuole in cell centre (Fig. 5m–o).

Cilia densely spaced, arranged in an average of 46 narrowly spaced, longitudinal rows most commencing underneath circumoral kinety and loosely ciliated and/

or slightly shortened in posterior pole area (Fig. 5a–c, i, p, q; Table 3); rarely some anterior kinetids lacking in ciliary rows neighbouring dorsal brush. Ciliary condensation left of dorsal brush, as found in *F. terricola*, definitely absent from *F. uluruensis*. Dorsal brush occupies about one third of body length, consists of two rows with ordinarily spaced dikinetids. Brush row 1 composed of an average of 24 dikinetids; row 2 distinctly shorter than row 1 because composed of only 14 dikinetids followed, however, by a monokinetidal bristle tail extending beyond anterior body half. Bristles of some anterior dikinetids *in vivo* 8–10 μm long with distal half thread-like narrowed, other bristles 4–5 μm long and rod-shaped; bristles of monokinetidal tail of row 2 rod-shaped and decreasing in length from 4 μm anteriorly to 3 μm posteriorly (Fig. 5a, b, e, i, l, p, q; Table 3).

Oral bulge in centre of anterior pole area, discoidal and about 2 μm high *in vivo*; contains the conspicuous extrusome bundle described above. Circumoral kinety at base of oral bulge, composed of obliquely arranged dikinetids, usually one each on top of ciliary rows (Fig. 5b–e, i, p, q). Nematodesmata originate from circumoral dikinetids and 5 to 11 ciliated basal bodies in anterior region of all somatic kineties ("oralized somatic monokinetids"); inconspicuous *in vivo*, while comparatively thick and long in protargol preparations, thus forming a conspicuous basket extending two thirds of body length (Figs 5c, q, 6i, j; Table 3). Internal basket inconspicuous, conical or obconical (Fig. 5c).

Ontogenesis: Some dividers (Figs 5p–u, 6h, i) show that somatic ontogenesis and cell fission proceed as described by Berger *et al*. (1983) in *F. terricola*. In contrast, the division of the macronucleus is quite different because of the different patterns (a single strand vs. many nodules). The macronucleus nodules of *F. uluruensis* fuse to an irregular mass in early mid-dividers (Fig. 5p, q); this mass develops to a long strand extending into proter and opisthe in late dividers (Fig. 5r, s). Fragmentation of the strand occurs in post-dividers via a reticulate stage (Figs 5t, u, 6h), as described in *Spathidium turgitorum* by Foissner *et al*. (2002).

Resting cyst: Resting cysts were obtained from 12 specimens isolated from the non-flooded Petri dish culture (Figs 5k, 6f, g). The cysts were mature after three days, having an average diameter of 50.7 μm (M = 51 μm, SD = 5.8 μm, SE = 1.7 μm, CV = 11.5%, Min = 42 μm, Max = 60 μm, n = 12). They are colourless and covered by an about 7 μm thick wall, consisting of two distinct layers (Figs 5k, 6f, g). The outer layer, which

is about 5 μm thick, appears slimy and very hyaline, becoming recognizable mainly due to adhering bacteria and debris; the inner layer is 1–1.5 μm thick and rather compact, thus being distinct not only in interference contrast but also under bright field illumination. The cyst content is also bipartite (Figs 5k, 6f, g): the peripheral third is finely granular and thus hyaline, while the central area is rather turbid due to autophagous vacuoles, lipid droplets, and the macronucleus nodules, which do not fuse (Fig. 6f, g). There is no escape apparatus.

Occurrence and ecology: As yet found only at type locality, where it was rather sparse in the non-flooded Petri dish culture. However, the species persisted for two months, making possible several collections for preparations. This rather conspicuous species might be an Australian endemic because we did not find it in about 1500 soil and mud samples collected globally.

Generic assignment and comparison with related species: This population belongs to the genus *Fuscheria* because it has nail-shaped extrusomes and two dorsal brush rows (Oertel *et al*. 2009). However, it could be also the representative of a new genus because the resting cyst lacks the escape apparatus present in *F. terricola* (Foissner, unpubl.) and *F. nodosa salisburgensis* nov. subspec. (see below).

Fuscheria uluruensis is unique in having the macronucleus split into several nodules, while it is globular or strand-like in the congeners (Berger *et al*. 1983; Foissner 1983, 1984; Foissner and Foissner 1988; Foissner and O'Donoghue 1990; Foissner *et al*. 2002; Petz *et al*. 1995; Song and Wilbert 1989; Vďačný 2007). Fortunately, dispersed macronucleus nodules occur in only two out of the 14 related genera, viz., in *Chaenea* spp. (all very slender) and *Papillorhabdos multinucleatus* Foissner, 1984 (similar in size and shape, but with three or four dorsal brush rows and simple, rod-shaped extrusomes). Thus, *Fuscheria uluruensis* is easily identified. Further, *Fuscheria uluruensis* has a very long oral basket, the significance of which will be discussed in connection with *F. nodosa salisburgensis* described below.

Fuscheria nodosa Foissner, 1983

We split this species into two subspecies according to the criteria suggested by Foissner *et al*. (2002). Very likely, *F. terricola* Berger *et al*., 1983 also consists of several subspecies because some populations are rather different (Vďačný 2007, Foissner, unpubl.). Further, the genus is possibly non-monophyletic because the resting cyst may have (*F. nodosa salisburgensis*; *F. terricola*,
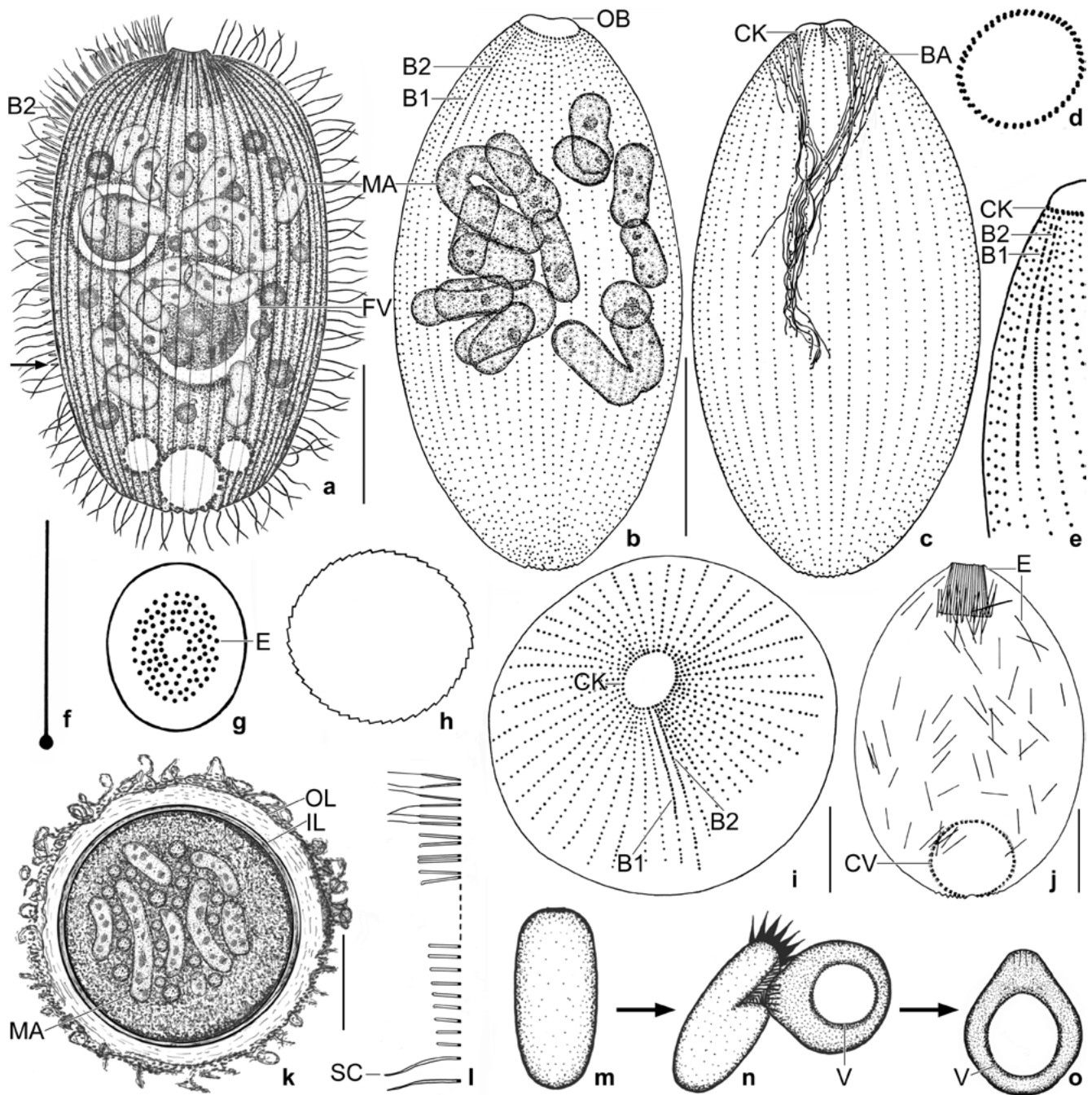
Figs 5a–o. *Fuscheria uluruensis* from life (a, f–h, k–o) and after protargol impregnation (b–e, i, j). **a** – lateral view of a representative specimen, length 100 µm. Note the distinctly furrowed ciliary rows and the monokinetidal end of dorsal brush row 2 (arrow); **b**, **c**, **e** – dorsolateral and ventrolateral view of ciliary pattern of holotype specimen, length 94 µm. Note the conspicuous oral basket, the oralized somatic monokinetids, and the dorsal brush (e). The about 12 macronucleus nodules are the main feature of this species; **d**, **i** – anterior polar view showing the dorsal brush and the circumoral kinety composed of obliquely arranged dikinetids, width 80 µm; **f** – extrusome, length 12 µm; **g** – frontal view of oral bulge with extrusomes forming a torus-like pattern; **h** – serrate outline in transverse view; **j** – a specimen with extrusomes attached to the oral bulge and scattered in the cytoplasm, length 80 µm; **k** – resting cyst, diameter 50 µm; **l** – dorsal brush row 2 consists of an anterior portion with dikinetids having 4–5 µm long bristles and a monokinetidal tail. The anteriormost bristles have a thread-like process; **m**, **n**, **o** – sequence of an unsuccessful attack on *Cyrtohymena primicirrata*. *Fuscheria uluruensis* could not break the cortex but became globular and produced a large, clear (food) vacuole within 5 minutes. B 1, 2 – dorsal brush rows, BA – oral basket, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, FV – food vacuole, IL – inner layer, MA – macronucleus, OB – oral bulge, OL – outer layer, SC – somatic cilium, V – forming food vacuole without contents. Scale bars: 20 µm (i–k) and 30 µm (a–c).
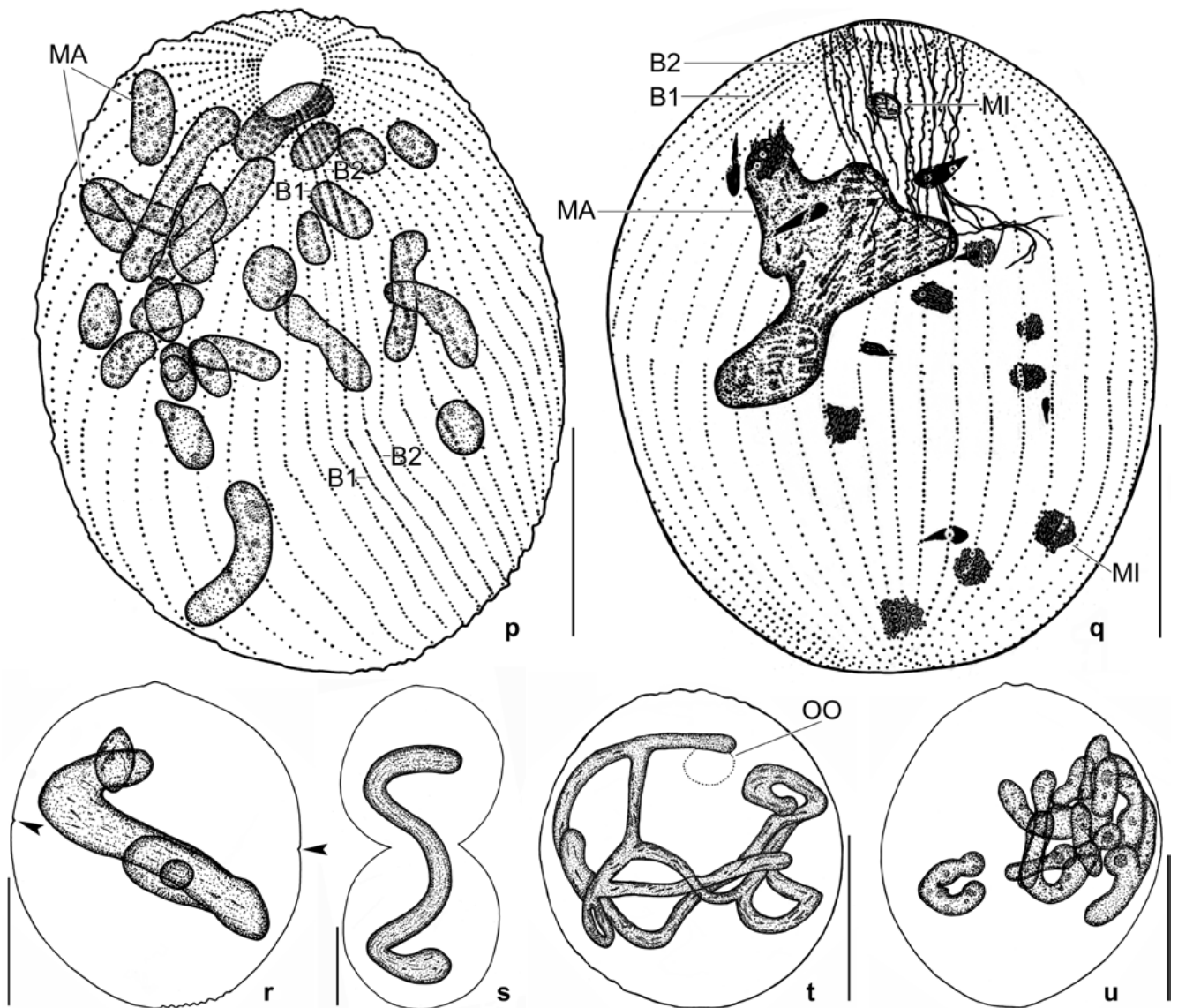
Figs 5p–u. *Fuscheria uluruensis*, ciliary and nuclear pattern of dividers (p–s) and post-dividers (t, u) after protargol impregnation. **p** – dorsal view of an early divider. The opisthe brush is fully developed, while the macronucleus nodules have not yet started to fuse; **q** – dorsolateral view of a middle divider. The macronucleus nodules fused to a roundish mass and the micronuclei commence to divide; **r** – middle-late divider showing the nuclear mass developing to a long strand and an indentation in the fission area (arrowheads); **s** – late divider showing the macronucleus strand extending into proter and opisthe; **t, u** – post-dividers. The macronucleus strand elongates to a three-dimensional reticulum (t) which eventually breaks into several nodules (u), i.e., the species-specific pattern. B 1, 2 – dorsal brush rows, MA – macronucleus (nodules), MI – micronuclei, OO – oral opening. Scale bars: 30 μm.

Foissner, unpubl.) or not have (*F. uluruensis*) an escape apparatus.

**Improved diagnosis:** Size 45–130 × 25-60 μm *in vivo*, usually about 90 × 50 μm; ellipsoidal to ovate or obovate. Macronucleus semicircular to highly tortuous and about half as long or as long as body; several micronuclei. Extrusomes about 10 μm long *in vivo*. 25–50 ciliary rows. Oral basket extends one to two thirds of body length.

Fuscheria nodosa nodosa Foissner, 1983 nov. stat.

Diagnosis: Macronucleus about half as long as body, usually semicircular. 20–30, usually about 25–30 ciliary rows. Oral basket extends in anterior body third.
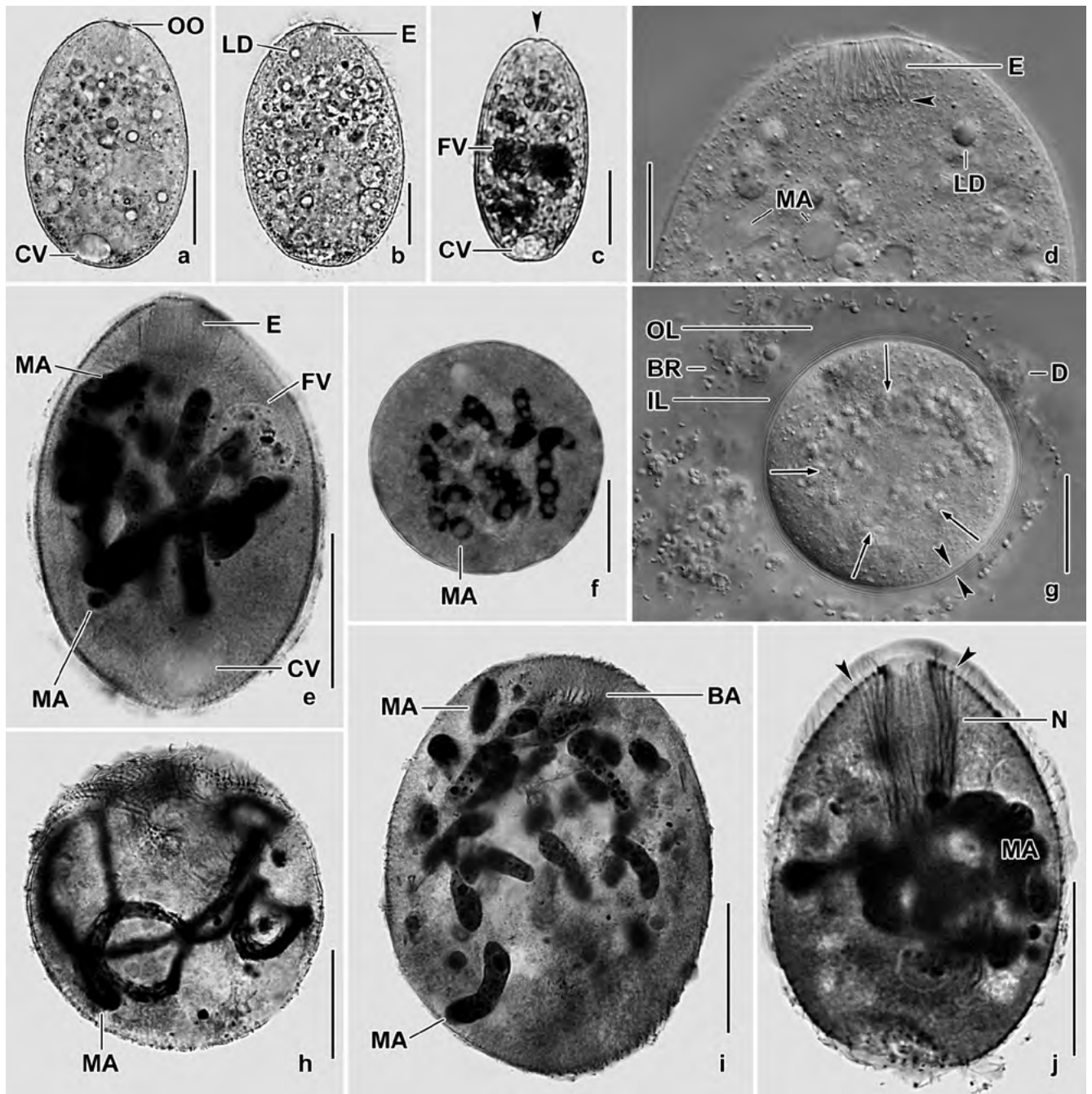
Figs 6a–j. *Fuscheria uluruensis* from life (a–d, g), after protargol impregnation (e, h–j), and methyl green-pyronin stain (f). **a**, **b**, **c** – total views of freely motile specimens showing the ellipsoidal outline and the slightly concave anterior end (arrowhead). The cytoplasm is turbid due to many lipid droplets, macronucleus nodules, and food vacuoles; **d** – the oral extrusome bundle is prominent, while the individual extrusomes are very fine with the posterior globule hardly recognizable (arrowhead); **e** – the vegetative specimen has several oblong macronucleus nodules; **f** – the macronucleus nodules do not fuse in the resting cyst; **g** – the resting cyst is covered with a hyaline outer layer (OL) and a thin but compact inner layer (arrowheads). The arrows denote the turbid central area with macronucleus nodules and autophagous vacuoles; **h** – early postdivider with reticular macronucleus; **i** – very early divider with macronucleus nodules not yet fused; **j** – nematodesmata originate from circumoral dikinetids and 5 to 11 ciliated basal bodies (arrowheads) in the anterior region of all somatic kineties. BA – oral basket, BR – rod-shaped bacteria, CV – contractile vacuole, D – debris, E – extrusomes, FV – food vacuole, IL – inner layer, LD – lipid droplets, MA – macronucleus, N – nematodesmata, OL – outer layer, OO – oral opening. Scale bars: 20 μm (d, f–h) and 30 μm (a–c, e, i, j).

Type locality: Eutrophic pool east of the Fuscher-lacke, Grossglockner-Hochalpenstrasse, Austria, E12°50´ N47°05´, about 2260 m above sea-level.

Remarks: Both, the original description and the redescription by Foissner and O'Donoghue (1990) are rather incomplete, especially as concerns the morphometric data. Few impregnated cells were available to Foissner (1983), and the specimens investigated by Foissner and O'Donoghue (1990) are strongly shrunken. Thus, we suggest to investigate new preparations either from the original or new localities.

Fuscheria nodosa salisburgensis Foissner and Gabilondo nov. subspec. (Figs 7a–q, 8a–w; Tables 4, 5)

Diagnosis: Macronucleus about as long as body and highly tortuous. About 40–50, usually 45 ciliary rows. Oral basket extends in anterior two thirds of body.

Type locality: Upper mud and soil layer of a shallow meadow puddle in the surroundings of the former Henkerhaus (house of the hangman) near the centre of the town of Salzburg, Austria, E13°02´ N47°47´.

Type material: One holotype slide and 10 paratype slides with protargol-impregnated specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip.

Etymology: *Salisburgum* is the Latin name of Salzburg, the type locality.

Remarks: Morphology and morphometry were analysed separately in cells from a non-flooded Petri dish culture ("environmental specimens") and from cells of a semi-pure "limnetic culture" (Table 4). The description is based on protargol preparations and scanning micrographs because the live specimens are highly similar to *F. nodosa nodosa*.

Description: Size of environmental specimens 82–137 × 45–97 μm in protargol preparations, considerably smaller (64–96 × 45–65 μm) in limnetic cultures, length:width ratio, however, 1.6:1 in both culture types (Table 4). Body broadly to ordinarily ellipsoidal or ovate to obovate, posterior end occasionally nearly transverse truncate; laterally flattened up to 2:1 *in vivo* (Figs. 7a, b, m–q, 8a, u–w). Nuclear apparatus usually in second and third quarter of cell, frequently displaced by large food vacuoles (Figs 7a, b, m–q, 8p, u–w). Macronucleus strand about as long as body in both culture types, usually highly tortuous; broken into several globular pieces, possibly due to post-conjugational reorganization, in 1.3% of limnetic specimens. Several

micronuclei, exact number difficult to recognize due to similar-sized and -impregnated cytoplasmic inclusions. Contractile vacuole in posterior body end, with up to six, usually three or four excretory pores in pole area (Fig. 7a). Extrusomes nail-shaped, about 10 x ≤ 0.2 μm *in vivo*, form a dense bundle in central area of oral bulge (Fig. 7a, d, e; Table 4); globular posterior end of individual extrusomes impregnates lightly, producing a dark transverse line in protargol-impregnated specimens (Fig. 8d, u–w); bundle partially extruded in some cells (Fig. 8d). Cortex flexible, prominent *in vivo* and in scanning micrographs due to comparatively broad and high ridges between ciliary rows; ridges more or less deeply impregnated with protargol, become sharp lines when viewed laterally (Figs 7a, h, 8a–c, r); postciliary microtubule ribbons sometimes well impregnated (Fig. 8t). Cytoplasm colourless, contains lipid droplets and, usually, one or two large food vacuoles with prey ciliates, such as *Tetrahymena*, *Dileptus*, *Vorticella*, *Pseudochilodonopsis algivora*, and hypotrichs (Figs 7a, n–q, 8o, w).

Cilia ordinarily or densely spaced on body and in oral area, respectively; arranged in an average of 42 (limnetic cells) to 45 (environmental cells) ordinarily spaced, longitudinal rows commencing underneath circumoral kinety, some slightly shortened in posterior pole area (Figs 7a–c, l, p, 8a–c, m, q–t; Table 4). Some anterior kinetids lacking in ciliary rows neighbouring dorsal brush, forming an indistinct suture in 32% of specimens; of these, 11% have the suture left of the brush, 52% right, and 37% have a shortened row at both sides. Ciliary condensation left of dorsal brush, as found in *F. terricola*, definitely absent from *F. nodosa salisburgensis*. Dorsal brush usually composed of two rows, occupies slightly less than one third of body length, dikinetids ordinarily spaced; three-rowed in 3% environmental specimens and in 31% (!) limnetic cells (for details, see discussion). Brush row 1 composed of an average of 21 dikinetids, bristles of individual dikinetids of similar length in one specimen (Fig. 8b), while anterior bristle distinctly shortened in another specimen (Fig. 8c); row 2 distinctly shorter than row 1, composed of an average of 13 dikinetids followed by a monokinetidal tail with acicular bristles (Figs 7a, c, f, g, p, 8a–c, e, n, q, s, v; Tables 4, 5); extra brush row comprising an average of 10 dikinetids.

Oral bulge in centre of anterior pole, circular, very inconspicuous because only about 10 μm wide and 1–2 μm high; surface basically flat but with a fairly deep central concavity marking the internal basket, in some specimens with a diaphragm-like pattern possibly

Table 4. Morphometric data on *Fuscheria nodosa salisburgensis*. Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a non-flooded Petri dish culture (upper line) and a semi-pure limnetic culture (lower line). Measurements in μm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of arithmetic mean, x̄ – arithmetic mean.

| Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length	101.8	100.0	16.7	3.7	16.4	82.0	137.0	21
	81.9	82.0	8.8	1.9	10.7	64.0	96.0	21
Body, width	64.0	59.0	13.3	2.9	20.8	45.0	97.0	21
	52.8	52.0	5.4	1.2	10.2	45.0	65.0	21
Body length:width, ratio	1.6	1.6	0.3	0.1	18.3	1.3	2.6	21
	1.6	1.6	0.2	0.0	9.7	1.3	1.8	21
Oral bulge (circumoral kinety), width	9.4	10.0	2.2	0.5	23.9	5.0	14.0	21
	9.0	9.0	1.6	0.3	17.3	7.0	12.0	21
Oral bulge, height	1.6	2.0	–	–	–	1.0	2.0	21
	1.6	2.0	–	–	–	1.0	2.0	21
Anterior body end to macronucleus, distance	25.8	26.0	7.9	1.7	30.7	14.0	42.0	21
	29.8	29.0	10.6	2.3	35.7	15.0	55.0	21
Macronucleus, length (spread and thus approximate)	146.9	144.0	–	–	–	75.0	239.0	21
	81.2	87.0	–	–	–	47.0	116.0	21
Macronucleus, width	8.0	7.0	2.0	0.4	25.6	6.0	12.0	21
	6.9	7.0	1.3	0.3	19.2	5.0	10.0	21
Micronuclei, length[a]	2.0	2.0	–	–	–	2.0	2.0	21
	2.0	2.0	–	–	-	2.0	2.0	21
Micronuclei, width[a]	2.0	2.0	–	–	–	2.0	2.0	21
	2.0	2.0	–	–	–	2.0	2.0	21
Micronuclei, number[a]	9.3	9.0	–	–	–	3.0	18.0	21
	11.5	12.0	–	–	–	3.0	15.0	21
Somatic ciliary rows, number	45.4	46.0	1.9	0.4	4.2	43.0	50.0	21
	42.1	41.0	2.2	0.5	5.3	40.0	47.0	21
Ciliated kinetids in a ventral kinety, number	47.2	46.0	10.7	2.3	22.7	33.0	66.0	21
	43.1	45.0	7.7	1.7	17.9	31.0	54.0	21
Brush rows, number	2.0	2.0	0.2	0.0	8.6	2.0	3.0	96
	2.3	2.0	0.5	0.0	20.1	2.0	3.0	90
Brush row 1, length (two-rowed specimens only)[b]	26.5	27.0	5.1	1.1	19.1	17.0	35.0	21
	24.0	25.0	4.8	1.0	20.0	15.0	34.0	21
Brush row 2, length (two-rowed specimens only)[b]	15.3	15.0	2.7	0.6	17.8	10.0	24.0	21
	15.8	15.0	4.2	0.9	26.7	9.0	26.0	21
Brush row 1, number of dikinetids (two-rowed specimens only)	21.0	21.0	2.1	0.5	10.0	17.0	25.0	21
	20.3	21.0	3.1	0.7	15.3	15.0	25.0	21
Brush row 2, number of dikinetids (two-rowed specimens only)	12.8	13.0	1.3	0.3	10.5	10.0	15.0	21
	14.2	14.0	2.6	0.6	18.6	10.0	19.0	21
Oral bulge extrusomes, length	10.6	11.0	0.8	0.2	7.7	9.0	12.0	21
	10.0	10.0	0.7	0.2	7.4	9.0	11.0	21
Nematodesma of oral basket, length (rough data because proximal end difficult to discern)	49.0	49.0	–	–	–	21.0	86.0	21
	59.0	60.0	–	–	–	50.0	77.0	21

[a] Uncertain because confusion with similar-sized and -impregnated cytoplasmic inclusions could be not excluded.

[b] Distance between circumoral kinety and last dikinetid of row.
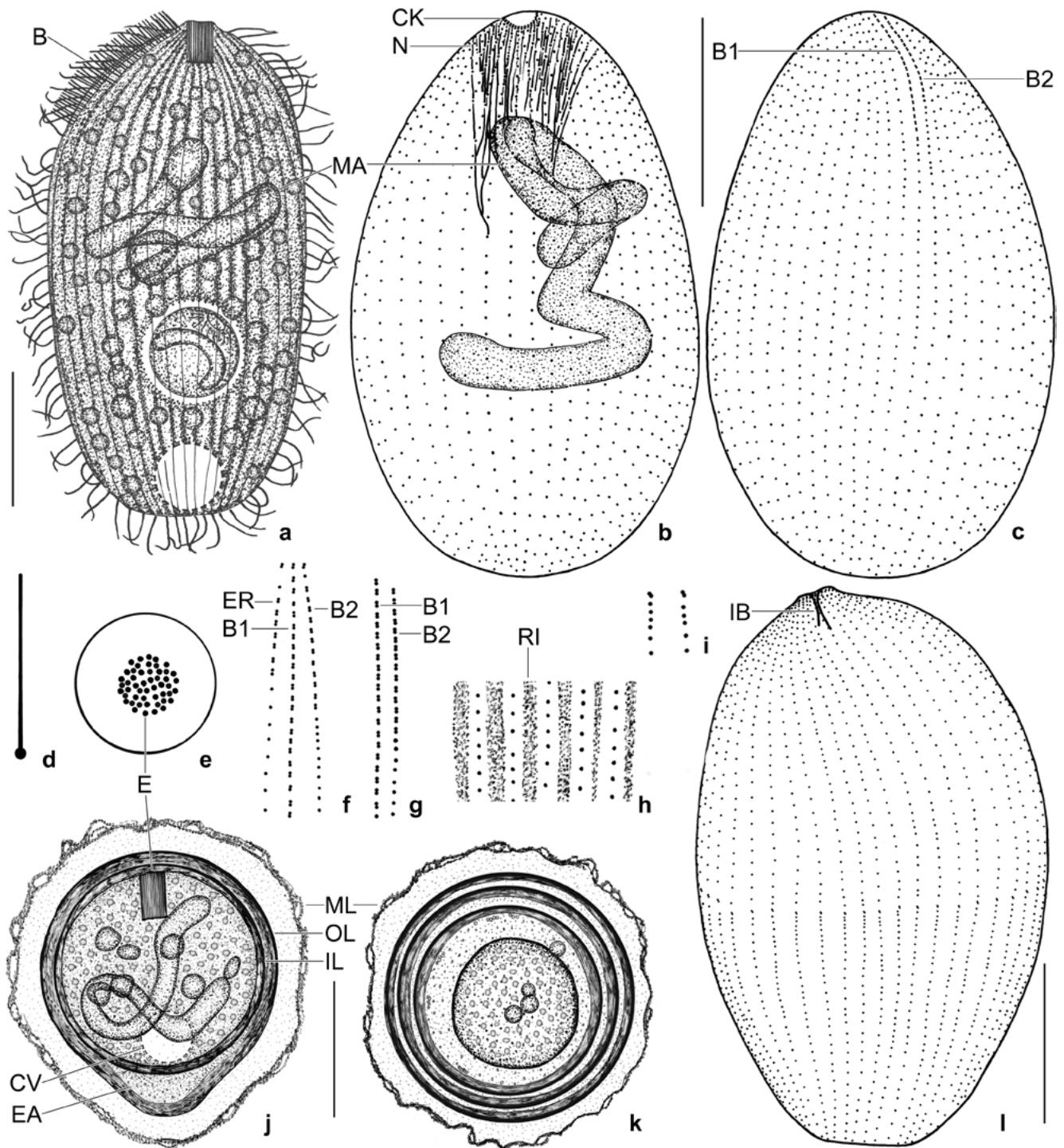
Figs 7a–l. *Fuscheria nodosa salisburgensis* from life (a, d, e, j, k) and after protargol impregnation (b, c, f–i, l). **a** – lateral view of a representative specimen, length 110 μm; **b**, **c** – ciliary pattern of ventral and dorsal side of holotype specimen, length 90 μm. Note the long, tortuous macronucleus; **d** – extrusome, length 10 μm; **e** – oral bulge with extrusomes in the centre; **f**, **g** – three-rowed and two-rowed dorsal brushes occur; **h** – broad cortical ridges between ciliary rows; **i**, **l** – ciliary pattern of an early divider, length 108 μm. Note an obliquely disposed, newly formed dikinetid at the begin of each opisthe ciliary row (i). These dikinetids will form the new circumoral kinety; **j**, **k** – resting cysts after three and ten days, diameter about 50 μm. B(1–2) – dorsal brush (rows), CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, EA – escape apparatus, ER – extra dorsal brush row, IB – internal oral basket, IL – inner layer, MA – macronucleus, ML – mucus layer, N – nematodesmata, OL – outer layer, RI – cortical ridge. Scale bars: 30 μm.
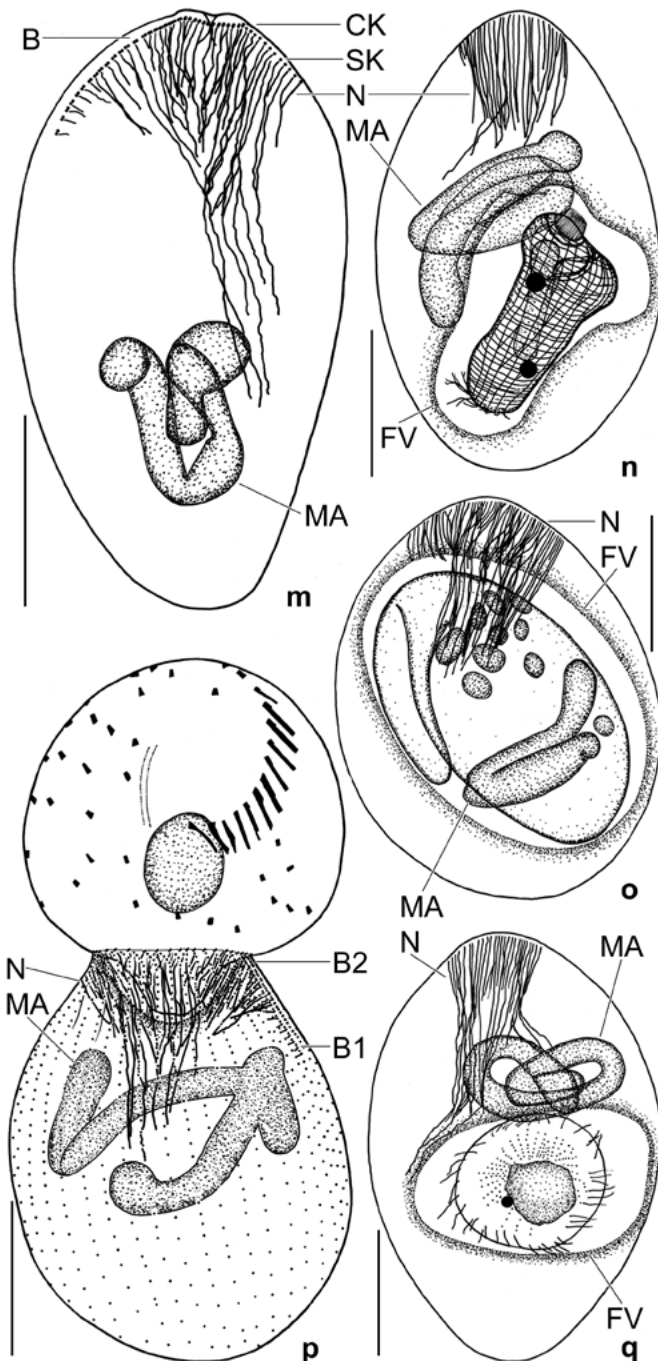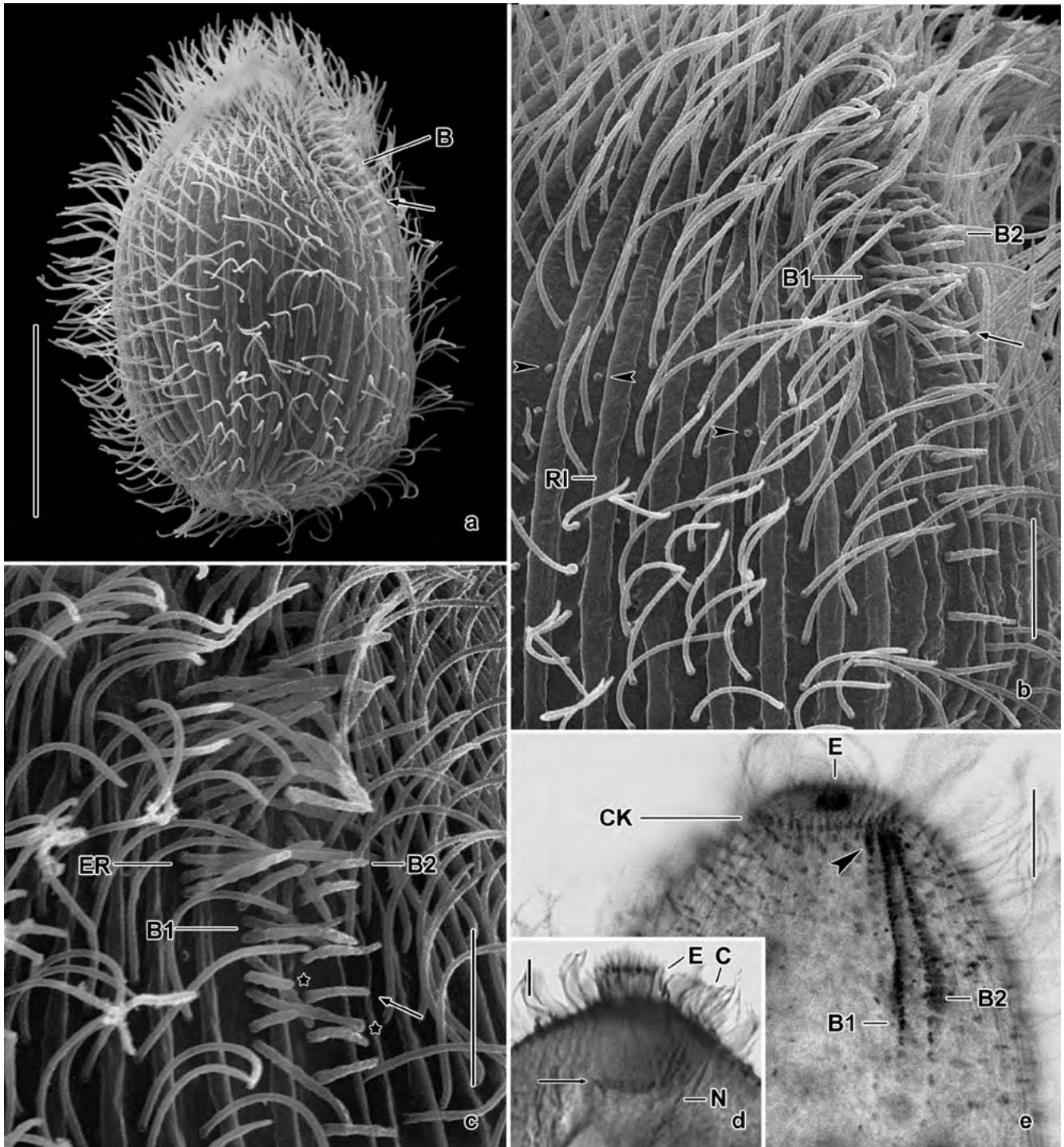
Figs 7m–q. *Fuscheria nodosa salisburgensis* after protargol impregnation. **m** – a 95 μm long specimen with nematodesmata originating from the circumoral dikinetids, 5 to 14 ciliated basal bodies in the anterior region of all somatic kineties, and the posterior basal body of the dorsal brush dikinetids; **n**, **o**, **q** – specimens with food vacuoles containing the telotroch of a peritrich, *Dileptus* sp., and *Tetrahymena* sp., respectively; **p** – when feeding on the hypotrich *Psilotricha succisa*, the mouth opens widely, length without prey 80 μm. B(1–2) – dorsal brush (rows), CK – circumoral kinety, FV – food vacuoles, MA – macronucleus, N – nematodesmata, SK – somatic kinety. Scale bars: 30 μm.

caused by fibres extending to bulge centre and forming the internal basket; bulge margin occasionally nicely crenelated (Figs. 7a, 8e, l, m, u–w). Circumoral kinety at base of oral bulge, composed of obliquely arranged dikinetids (Figs 7b, f, m, p, 8e, l, m). Nematodesmata originate from circumoral dikinetids, 5 to 14 ciliated basal bodies in anterior region of all somatic kineties ("oralized somatic monokinetids"), and from posterior basal body of dorsal brush dikinetids (Fig. 7m); inconspicuous *in vivo*, while long and thus prominent in protargol preparations, where the rods form a conspicuous basket extending about half of body length, possibly even further because the end of the fine fibres might have impregnated too lightly to be recognizable (Figs 7b, m–q, 8d, u–w; Table 4). Internal basket conical or obconical, less than 10 μm and thus inconspicuous (Figs 7l, m, 8u–w).

Ontogenesis: Some dividers show that somatic ontogenesis and cell fission proceed as described by Berger *et al.* (1983) in *F. terricola*. The newly formed oral dikinetids are obliquely disposed (Figs 7i, l, 8n).

Resting cyst: Three days resting cysts are possibly mature and have an average size of 49.9 × 51.6 μm without the mucous envelope (n = 15, length M = 50 μm, SD = 3.4 μm, SE = 0.9 μm, CV = 6.9%, Min = 45 μm, Max = 56 μm; width M = 50 μm, SD = 7.3 μm, SE = 1.9 μm, CV = 14.2%, Min = 45 μm, Max = 75 μm). However, when they are slightly pressed by the coverslip, the contents starts to rotate showing that the cilia have not yet resorbed (Figs 7j, 8f–h). Likewise, the macronucleus appears unchanged and the oral extrusome bundle is opposed to the contractile vacuole, showing the structural integrity of the encysted cell (Fig. 7j). When rotating, a space containing cell debris appears between the cyst wall and the cortex (Fig. 8h, k).

The cyst is covered by an about 5 μm thick, mucous envelope, which is so hyaline that it usually becomes recognizable only due to adhering bacteria and debris (Figs 7j, 8f, i). The distinct part of the cyst wall is about 5 μm thick and consists of two layers separated by a sheet of lower refractivity. The outer layer is 2–4 μm thick and less dense (refractive) than the 1–1.5 μm thick inner layer. This structure of the cyst wall becomes prominent under bright field illumination, where the cell's cortex and the inner cyst layer are well separated from the outer layer by a bright middle zone (Fig. 8g). Interestingly, the cyst has some sort of escape apparatus, i.e., a small area of the outer wall layer separates to form a distinct convexity; the resulting space contains some debris (Figs 7j, 8f, g, i). The cytoplasm is finely
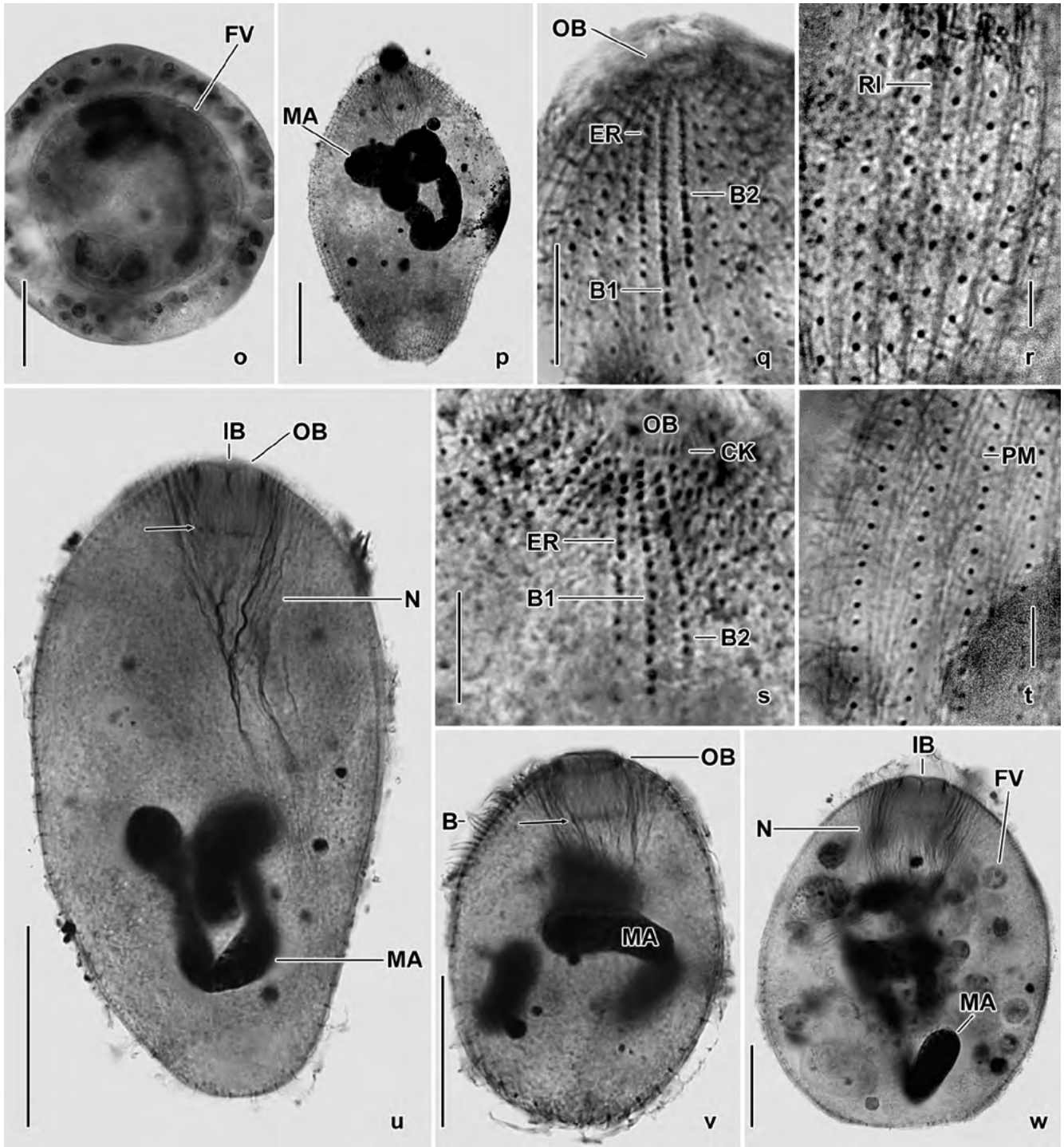
Figs 8a–e. *Fuscheria nodosa salisburgensis* in the SEM (a–c) and after protargol impregnation (d, e). **a, b** – dorsolateral overview and detail of dorsal brush area. Note the broad cortex ridges and the begin (arrow) of the tail of brush row 2. Arrowheads mark unciliated kinetids scattered throughout the rows, a typical feature of haptorid ciliates; **c** – dorsal brush of a specimen with an extra row on the left. Arrow marks monokinetidal tail of brush row 2. Asterisks mark a dikinetid of row 1, where the anterior bristle is much shorter than the posterior one; **d** – the central extrusome bundle is occasionally extruded as a whole. The arrow marks the dark line formed by the proximal globule of the extrusomes; **e** – *Fuscheria nodosa salisburgensis* has two brush rows, but a more or less long third row (arrowhead) may be present, especially in cultivated specimens. B(1–2) – dorsal brush (rows), C – cilia, CK – circumoral kinety, E – extrusomes, ER – extra dorsal brush row, N – nematodesmata, RI – cortical ridge. Scale bars: 5 μm (b, c, d), 10 μm (e), and 20 μm (a).
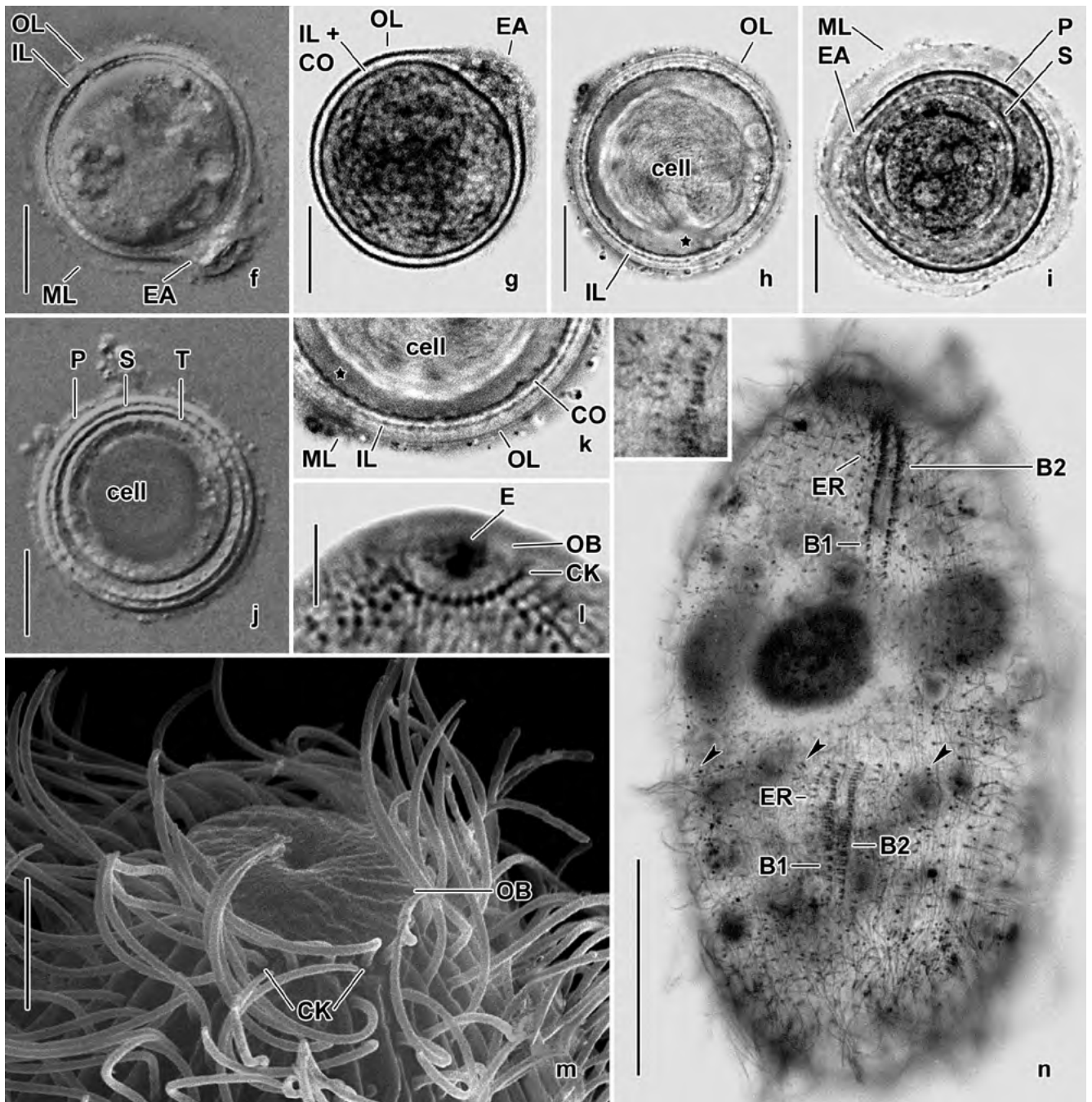
Figs 8f–n. *Fuscheria nodosa salisburgensis* from life (f–k), after protargol impregnation (l, n), and in the SEM (m). **f–h**, **k** – three days resting cysts with escape apparatus, diameter about 50 μm. Under bright field, the inner layer of the cyst wall and the ciliate cortex become indistinguishable producing, together with the outer layer, a conspicuous bipartition of the wall (g). Under slight coverslip pressure, cells start to rotate within the cyst (h) and the wall structure becomes distinct (k). Asterisks mark space between cyst wall and rotating cell; **i**, **j** –a secondary and a tertiary cyst wall are produced in the cyst within ten days; however, the encysted cell starts to rotate when the cyst is slightly pressed by the coverslip (j); **l**, **m** – oblique frontal views showing, *inter alia*, the thin oral bulge, which has a diaphragm-like sur-face pattern and a central concavity. The circumoral kinety, which consists of dikinetids, is very distinct in protargol preparations (l), while indistinct in the SEM (m) because only one basal body of the dikinetids is ciliated; **n** – overview of an early divider with three brush rows. The inset shows the opisthe extra row. A dikinetid each is generated at the anterior end of the opisthe´s ciliary rows (arrowheads). B 1, 2 – dorsal brush rows, CK – circumoral kinety, CO – cortex, E – central extrusome bundle, EA – escape apparatus, ER – extra dorsal brush row, IL – inner layer, ML – mucus layer, OB – oral bulge, OL – outer layer, P, S, T– primary, secondary, and tertiary cyst wall. Scale bars: 5 μm (l, m), 20 μm (f–j), and 30 μm (n).

Figs 8o–w. *Fuscheria nodosa salisburgensis* after protargol impregnation. **o** – transverse view of a specimen with a large food vacuole containing a ciliate; **p** – a specimen with long, tortuous macronucleus; **q**, **s** – three-rowed dorsal brushes. Possibly, the extra brush row is usually left of row 1; **r**, **t** – broad cortical ridges and long postciliary microtubule ribbons extend between the ciliary rows (cp. Fig. 8b); **u–w** – these specimens show: the dorsal brush bristles, the internal oral basket, the long nematodesmata forming the external basket (u), the tortuous macronucleus, and the oral bulge extrusomes whose globular proximal end forms a fairly distinct, dark line (arrows). B(1–2) – dorsal brush (rows), CK – circumoral kinety, E – extrusomes, ER – extra dorsal brush row, FV – food vacuoles, IB – internal oral basket, MA – macronucleus, N – nematodesmata, OB – oral bulge, PM – postciliary microtubule ribbons, RI – cortical ridge. Scale bars: 5 µm (r, t), 10 µm (q, s), 20 µm (v, w), and 30 µm (o, p, u).

Table 5. Number of brush dikinetids in *Fuscheria nodosa salisburgensis* specimens with 3 dorsal brush rows. Data based on mounted, protargol-impregnated (Foissner's method) specimens from a non-flooded Petri dish culture (NFP) and a semi-pure limnetic culture (LC). ER – extra brush row supposed to be left of rows 1 and 2, Max – maximum, Min – minimum, n – number of individuals investigated, x̄ – arithmetic mean.

NFP-specimens					LC specimens					NFP and LC specimens				
Brush rows	x̄	Min	Max	n	Brush rows	x̄	Min	Max	n	Brush rows	x̄	Min	Max	n
ER	10.5	7	14	2	ER	9.9	6	13	12	ER	10.2	6	14	14
1	23.5	22	25	2	1	17.9	14	23	12	1	20.7	14	25	14
2	14.5	13	16	2	2	12.5	9	15	12	2	13.5	9	16	14

granulated and contains some 3 to 5 µm-sized globules, likely autophagosomes (Figs 7j, 8f).

A secondary and tertiary cyst wall develop within the cyst during two weeks. They have the same structure as the primary wall but are only 2 to 2.5 µm thick and separated from each other by distinct spaces containing cell debris (Figs 7k, 8i, j); all walls are flexible and honey-coloured. During this process, the diameter of the cell decreases from about 45 µm to 35 µm and less. However, still the cell starts rotating when the cyst is slightly pressed by the coverslip, showing that the cilia have been not resorbed (Fig. 8j).

Occurrence and ecology: As yet found only at type locality, where it was abundant in the non-flooded Petri dish culture. Possibly, this is mainly a limnetic species.

Comparison with related taxa: Morphologically, the population from Salzburg is quite similar to the populations of *F. nodosa* described by Foissner (1983) from the Austrian Central Alps and by Foissner and O'Donoghue (1990) from Australia. In contrast, several important morphometrics are moderately (length of macronucleus and oral basket, number of cilia in a kinety) to distinctly (40–50 ciliary rows in the Salzburg specimens vs. only 24–30 in the *F. nodosa nodosa* populations) different. Further, the Salzburg specimens are quite similar to *F. uluruensis* described above in all morphological and morphometric features, except of the macronucleus pattern (a long, tortuous strand vs. scattered nodules) and the resting cyst (with vs. without escape apparatus). Of the features mentioned in the diagnosis, the number of ciliary rows is the most important one.

Although hardly to fix unambiguously in this type of ciliates, the oral basket of *F. nodosa salisburgensis*

and *F. uluruensis* is much longer than that of *F. nodosa nodosa* and *F. terricola*. Whether or not this is related to body size and/or the number of ciliary rows needs further investigations.

Was the ancestral dorsal brush of the Acropisthiina Foissner and Foissner, 1988 two-rowed or three-rowed? Xu and Foissner (2005) suggested a two-rowed dorsal brush in ancestral spathidiids but emphasized that this may be not applicable to other haptorids. Indeed, the data from *F. nodosa salisburgensis* indicate a three-rowed brush in ancestral acropisthiids.

The Salzburg population of *F. nodosa salisburgensis* contains a comparatively high number of specimens with three dorsal brush rows: 3.1% in those from the non-flooded Petri dish culture and 31.1% in the cells from the limnetic culture. The latter value is very high considering the usual stability of this feature. We checked the data for the possibility to find out where the third row has been added: right or left of the two ordinary rows. A clear result was not obtained because of the similarity in the number of dikinetids composing the marginal brush rows (Table 5). In species with three rows, the middle row usually has slightly more dikinetids than row 1, which has about twice the number of dikinetids of row 3 (for examples, see Foissner *et al.* 2002). This method indicates that row 2 has been preserved but provides no information whether the extra row has been added right or left. Fortunately, there is another good marker: only the rightmost row has a monokinetidal bristle tail. In *F. nodosa salisburgensis*, a tail is present only in the rightmost row, showing that the extra row has been added to the left margin of the brush. Thus, row 1 has been lost in *Fuscheria*, just as in several spathidiids (see *Arcuospathidium namibiense* in Foissner and Xu 2007).

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