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# Morphology and ciliary pattern of some rare haptorid ciliates, with a description of the new family Kamburophryidae (Protists, Haptoria)

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## Abstract

We investigated three rare haptorid ciliates, viz., *Lagynophrya gibba* Kahl (1935), *Enchelys lajacola* nov. spec., and *Spathidium implicatum* Kahl (1930), using live observation, silver impregnation, morphometry, and scanning electron microscopy. *Lagynophrya gibba*, which was rediscovered in peatland soil from Iceland, is referred to a new genus, *Kamburophrys*, and a new family, Kamburophryidae, based on a unique organelle, the brush membranoid. This structure is near the dorsal brush and composed of very narrowly spaced cilia, about 5 µm long. The genus *Kamburophrys* has a unique combination of features, viz., an oral cone on the oral bulge, an oblique circumoral kinety, and a subapical hump carrying the three-rowed dorsal brush and the brush membranoid. The Kamburophryidae possibly belong to the order Spathidiida. *Enchelys lajacola* was discovered in mud from granitic rock-pools (Lajas) in Venezuela, South America. The new species is characterized by a bottle-like shape, a macronucleus with the shape of a curved strand, a heterostichad dorsal brush, and rod-shaped toxicysts. *Spathidium implicatum*, which was rediscovered in an ephemeral meadow puddle near Salzburg city centre, is neotypified and referred to the genus *Apertospathula* because it has an open circumoral kinety.


***Keywords:*** *Apertospathula implicata* nov. comb.; *Enchelys lajacola* nov. spec.; *Kamburophrys gibba* nov. gen., nov. comb.; Laja; *Spathidium implicatum*; Venezuela

## Introduction

Haptorids are predaceous ciliates with toxicysts used to paralyse and/or lyse the prey, usually other heterotrophic protists but sometimes also flagellated autotrophs and micrometazoans, such as rotifers and nematodes. Predators are often highly specialized, and thus show a great morphological diversity. This applies also to the haptorids, of which we have investigated well above 100 species during the past decade (Foissner 2003; Kreutz and Foissner 2006; Foissner and Xu 2007; Foissner et al. 1995, 1999, 2002). Many of these represented new species and genera, suggesting that a considerable proportion of the haptorid diversity is still undiscovered. A good example of unrecognized diversity is provided by the ciliates from a small mire in Germany, where most undescribed species belonged to the haptorids (Kreutz and Foissner 2006). Likewise, marine habitats are full of undescribed haptorids (Lin et al. 2008 and literature cited therein).

At first glance, many haptorids have a simple organization and look alike. However, more detailed

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investigations reveal a great diversity in shape of body and organelles. Diversity of body shape is demonstrated by genera like *Enchelys*, *Enchelyodon*, *Myriokaryon*, *Spathidium*, *Legendrea*, *Teuthophrys*, *Dileptus*, and *Loxophyllum* (Kahl 1930a, b; Kreutz and Foissner 2006), while organelle diversity is most impressively shown by the shape and size of the toxicysts, the main functional feature of the group (Foissner and Xu 2007; Foissner et al. 1999, 2002). Last but not least, one group of haptorids, the Trachelophyllida, has evolved epicortical scales of considerable diversity, so far embracing at least 10 genera (Foissner et al. 2002; Foissner 2005, and unpubl.).

In the present study, we describe three haptorids which show some of the diversity mentioned above. These are possibly rare species, because the senior author has found them only once or twice during 40 years of intense alpha-taxonomic research.

## Materials and methods

For the origin of the samples, see the individual species descriptions. Two of the three species were reactivated from resting cysts using the non-flooded Petri dish method (NFPM). Briefly, the NFPM involves placing 50–500 g litter and soil in a Petri dish (13–18 cm wide, 2–3 cm high) and saturating, but not flooding it, with distilled water. Such a culture is analysed for ciliates by inspecting about 2 ml of the run-off on days 2, 7, 14, 21, and 28; for a detailed description of the NFPM, see Foissner et al. (2002).

All species were observed in vivo and in protargol preparations; *Apertospathula implicata* was investigated also with the scanning electron microscope (SEM). See Foissner (1991) for description of methods. Counts and measurements on silvered specimens were conducted at a magnification of $\times 1000$. In vivo measurements were performed at magnifications of $\times 40-1000$. Drawings of live specimens were based on free-hand sketches; those of impregnated cells were made with a drawing device. Terminology is according to Corliss (1979) and, especially, Foissner and Xu (2007).

## Results and discussion

### Family Kamburophryidae nov. fam.

**Diagnosis**: Spathidiida (?) with brush membranoid.

**Type genus**: *Kamburophrys* nov. gen.

**Comparison with other families and genera**: *Kamburophrys gibba*, type of the family and genus (see below), has several peculiarities rarely found in other haptorids, suggesting distinctness at genus and family level: a subapical hump carrying the dorsal brush and the brush membranoid, a bulge cone, and an oblique circumoral kinety. While a subapical hump with dorsal brush bristles occurs in some spathidiid haptorids (Kahl 1930a; Foissner and Xu 2007), the brush membranoid is an almost unique organelle. Possibly, the brush membranoid is a strongly modified, fourth dorsal brush row because its structure resembles the monokinetidal tail of brush row 3 found in many haptorids. However, haptorids with four or more brush rows are rare; when present, the rows have an ordinary structure like rows 1–3 of *Kamburophrys*, except possibly for the genus *Papillorhabdos* (Fig. 1h, i), which has row 4 composed of very narrowly spaced mono- or dikinetids (Foissner 1984). *Papillorhabdos* belongs to the Enchelyina, a quite different group of haptorids lacking a circumoral kinety and thus making the oral basket from nematodesmata originating from oralized somatic monokinetids in the anterior region of the ciliary rows (Foissner and Foissner 1988). *Kamburophrys* has a circumoral kinety and thus an ordinary oral basket. Accordingly, we assume that the brush membranoid originated convergently. This is supported by the observation that the brush membranoid of *Kamburophrys* does not contact somatic kineties, while kinety 4 of *Papillorhabdos* continues as an ordinary somatic ciliary row (Fig. 1h, i).

The third unusual feature of *Kamburophrys* is the bulge cone resembling the *palpus oralis* of the spathidiid genus *Rhinothrix* (for a review, see Foissner and Xu 2007). A spathidiid relationship is also indicated by the oblique circumoral kinety, an unusual feature in cylindroidal haptorids, but quite typical for most spathidiids (Kahl 1930a; Foissner et al. 2002; Foissner and Xu 2007). Thus, *Kamburophrys* might belong to the spathidiids, representing a distinct family with the brush membranoid as a main character. This organelle, which does not contact the somatic ciliary rows, must be generated by a specific ontogenetic process, probably similar to that forming the preoral kineties of *Dileptus* (Vd'ačný and Foissner 2009).

Kahl (1935) classified *K. gibba* in *Lagynophrya*, which has a retractile oral dome and an ordinary dorsal brush (for a review with SEM micrographs, see Foissner et al. 1999). Interestingly, *Lagynophrya* has, like *K. gibba*, an oblique circumoral kinety. Unfortunately, the systematic position of *Lagynophrya* is not known. Except for the oblique circumoral kinety, there is no indication of a relationship with the order Spathidiida.

### Genus *Kamburophrys* nov. gen.

**Diagnosis**: Spathidiida (?) with oral cone, oral bulge, oblique circumoral kinety, and a subapical hump carrying the three-rowed dorsal brush and the brush membranoid.

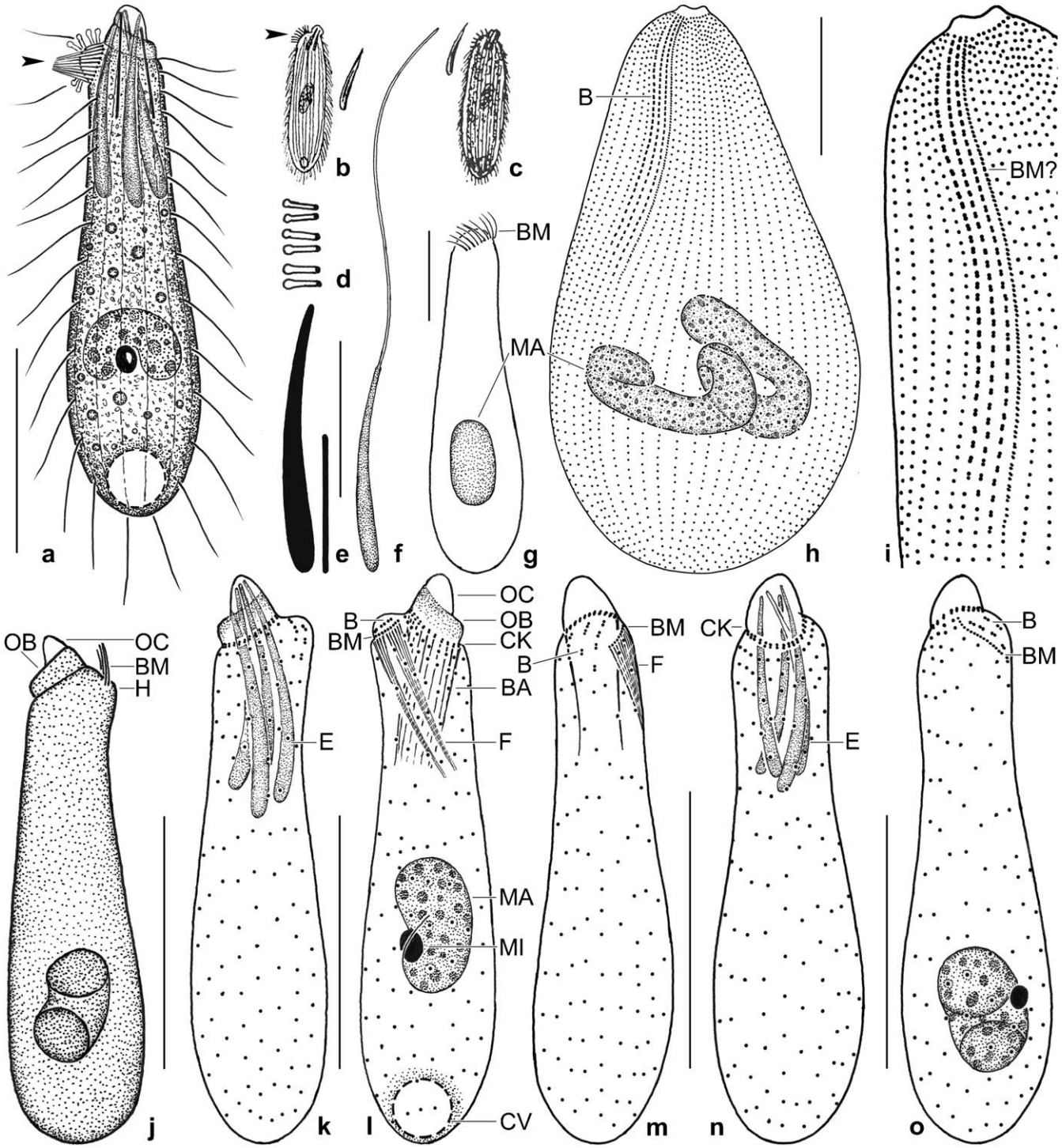


**Fig. 1. a–o**. *Kamburophrys gibba* (a–g, j–o) and *Papillorhabdos carchesii* (h, i) from life (a–f) and after protargol impregnation (g–o). **a**. A representative specimen from Iceland population. Arrowhead marks dorsal hump with brush and brush membranoid. **b**, **c**. *Lagynophrya gibba*, overview and extrusome of a German specimen, length 80 μm (from Kahl 1935, 1943). Arrowhead marks dorsal hump with dorsal brush. **d**. Dorsal brush bristles are slightly inflated distally and about 2 μm long. **e**. Types I and II extrusome, drawn to scale. **f**. Exploded type I extrusome, length 30 μm. **g**. Dorsal view showing brush membranoid. **h**, **i**. *Papillorhabdos*, dorsal view showing ciliary pattern and the supposed brush membranoid (from Foissner 1984). **j**. The cell impregnates rather deeply with protargol, except of the oral cone. **k**, **l**. Left and right side view of ciliary pattern and nuclear apparatus of main voucher specimen. **m**, **n**. Dorsal and ventral view of ciliary pattern. **o**. Dorsolateral view showing dorsal brush and brush membranoid. B – dorsal brush, BA – oral basket, BM – brush membranoid, BM? – supposed brush membranoid, CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, F – fibres, H – dorsal hump, MA – macronucleus, MI – micronucleus, OB – oral bulge, OC – oral cone. Scale bars 10 μm (e, g) and 20 μm (a, h, j–o).

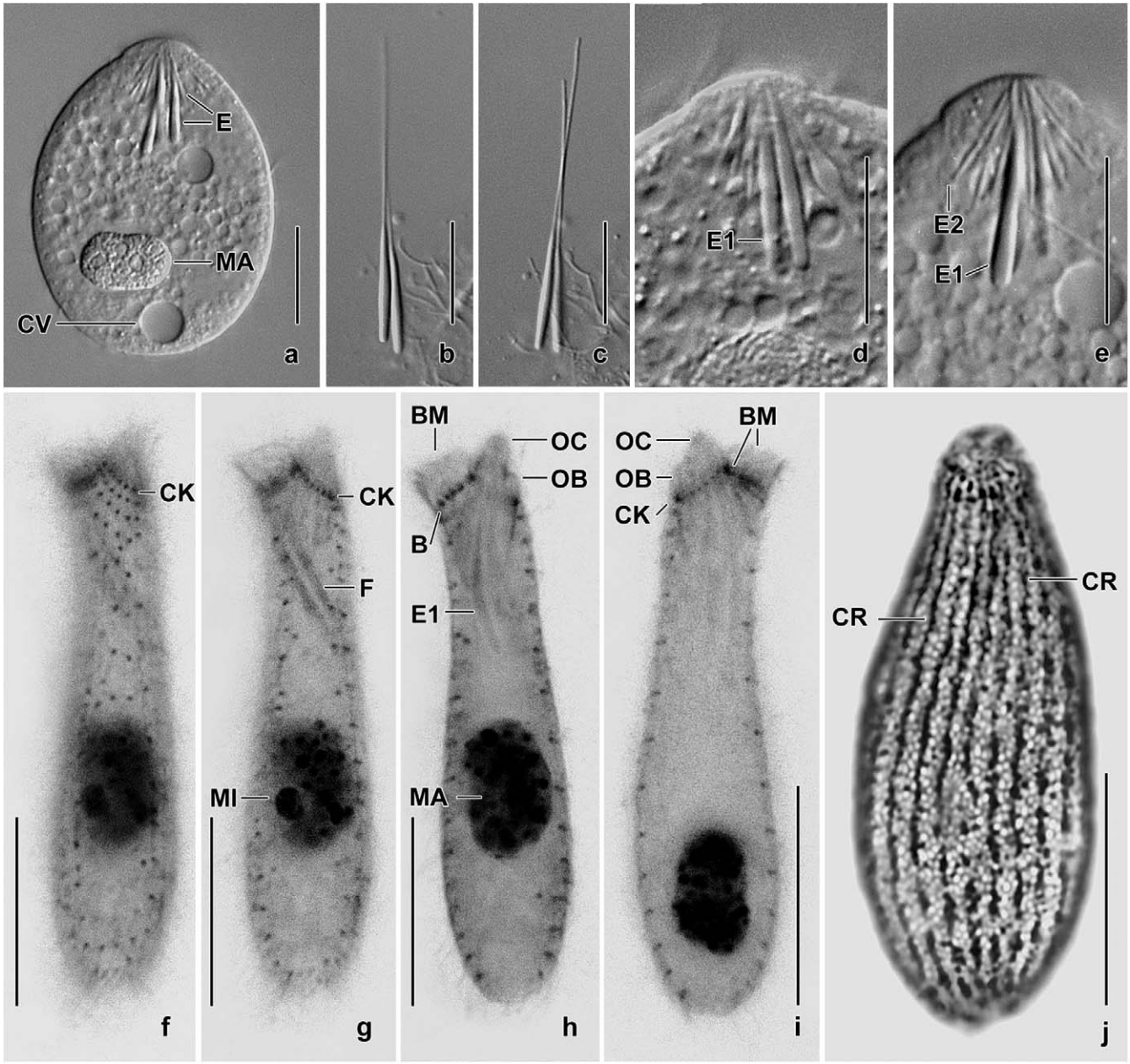


**Fig. 2. a–j**. *Kamburophrys gibba*, Iceland specimens from life (a–e), after protargol impregnation (f–i), and after Klein–Foissner silver nitrate impregnation (j). **a**. A strongly squashed specimen showing main cell organelles. **b**, **c**. Exploded type I extrusomes in two focal planes. **d**, **e**. Type 1 extrusomes are massive, i.e., about 17 × 1.4 µm, while type 2 extrusomes are filiform and about 9 µm long. **f–h**. Three focal planes of the specimen shown in Fig. 1k, l, demonstrating main organelles. **i**. Longitudinal optical section showing body shape and details of oral apparatus and dorsal brush. **j**. Overview showing the very narrowly-meshed silverline pattern. B – dorsal brush, BM – brush membranoid, CK – circumoral kinety, CR – ciliary rows, CV – contractile vacuole, E – extrusomes, E1, 2 – types I and II extrusomes, F – fibres originating from brush membranoid, MA – macronucleus, MI –micronucleus, OB – oral bulge, OC – oral cone. Scale bars 15 µm (b–j) and 20 µm (a).

**Type species**: *Kamburophrys gibba* (Kahl, 1935) nov. comb. (basionym: *Lagynophrya gibba* Kahl, 1935).

**Etymology** : Composite of the Greek words *kambura* (hump) and *ophrys* (eyelash–cilium–ciliate), meaning a ciliate with a hump. Feminine gender.

## Redescription *of Kamburophrys gibba* (Kahl, 1935) nov. comb. (Figs 1a–g, j–o, 2a–j)

**Improved diagnosis**: Size about 50 × 13 µm in vivo. Dorsal hump hemispherical, body thus slightly dumbbell-shaped or

**Table 1.** Morphometric data on *Kamburophrys gibba*.

| Characteristics[a] | $\bar{x}$ | *M* | SD | SE | CV | Min | Max | *n* |
|---|---|---|---|---|---|---|---|---|
| Body, length | 42.8 | 43.0 | 3.4 | 1.0 | 7.9 | 36.0 | 48.0 | 11 |
| Body, width at brush hump | 7.2 | 7.0 | 0.6 | 0.2 | 8.4 | 6.0 | 8.0 | 11 |
| Body, maximum post-brush width | 11.2 | 11.0 | 0.9 | 0.3 | 7.8 | 10.0 | 13.0 | 11 |
| Anterior end to macronucleus, distance | 23.7 | 24.0 | 4.4 | 1.3 | 18.4 | 16.0 | 29.0 | 11 |
| Anterior end to end of dorsal brush, distance | 6.2 | 6.0 | 0.8 | 0.2 | 12.1 | 5.7 | 7.0 | 11 |
| Oral bulge, width | 4.8 | 5.0 | 0.6 | 0.2 | 12.5 | 4.0 | 6.0 | 11 |
| Oral bulge, height | 4.4 | 4.0 | – | – | – | 4.0 | 5.0 | 10 |
| Macronucleus, length[b] | 10.7 | 11.0 | – | – | – | 10.0 | 11.0 | 11 |
| Macronucleus, width | 6.0 | 6.0 | 0.8 | 0.2 | 12.9 | 5.0 | 7.0 | 11 |
| Micronucleus, maximum length | 2.6 | 2.5 | – | – | – | 2.0 | 3.0 | 11 |
| Long extrusomes, length | 14.9 | 15.0 | 1.1 | 0.3 | 7.6 | 13.0 | 17.0 | 11 |
| Ciliary rows, number | 17.3 | 17.0 | 0.8 | 0.2 | 4.6 | 16.0 | 18.0 | 11 |
| Cilia in a right side row, number | 14.4 | 14.0 | 2.6 | 0.8 | 18.0 | 10.0 | 18.0 | 11 |

[a]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, $\bar{x}$ – arithmetic mean.
[b]Not corrected for curved ends.

slenderly obpyriform, depending on side viewed. Macronucleus usually reniform or ellipsoidal. Two types of oral extrusomes: type I very narrowly cuneate, conspicuous because about 17 × 1.4 µm in size; type II filiform, about 9 µm long. On average 17 ciliary rows. Dorsal brush minute, each row consisting of about 4 dikinetids with bristles 2 µm long; brush membranoid composed of about 13 basal bodies with cilia 4–5 µm long. Oral bulge discoidal, ventrally slightly lower than dorsally; oral dome conical occupying dorsal half of oral bulge.

**Type locality**: Not given in the original description, but likely in the Hamburg area (Germany), where Kahl lived and worked. Our material is from peatland in SW-Iceland, i.e., from the surroundings of the town of Thingvellir.

**Type material**: Not available. We have deposited 6 slides (five with protargol-impregnated specimens; one impregnated with the Klein–Foissner silver nitrate method to show the silverline pattern) in the Biology Centre of the Museum of Upper Austria, Linz (LI). Relevant specimens are marked by black ink circles on the coverslip.

**Etymology**: Not given in the original description. The Latin adjective *gibba* (gibbous) obviously refers to the dorsal hump.

**Description of German population** (translated from Kahl 1935, p. 809; Fig. 1b): Length 80 µm; easy to recognize by the dorsal hump carrying a dorsal brush with long cilia, and the long trichocysts thickened to 2.5 µm posteriorly. Found only once in low numbers between *Utricularia*.

**Description of Iceland population**: Size and shape fairly constant. Size 40–55 × 10–15 µm in vivo, usually near 50 × 13 µm (Table 1). Outline slenderly obpyriform in ventral and dorsal view (Fig. 1m–o), while slightly dumbbell-shaped in lateral view due to the dorsal hump and a slight widening in third quarter containing the nuclear apparatus (Figs 1a, j, k, 2f–i). Macronucleus reniform to ellipsoidal, rarely horseshoe-shaped, dumbbell-shaped, or in two globular nodules (post-conjugants?); nucleoli numerous, of ordinary size. Micronucleus usually in curved centre of macronucleus, globular to broadly ellipsoidal, in vivo about 3 µm across (Figs 1a, g, j, l, o, 2a, f–i; Table 1). Contractile vacuole in posterior body end. Two types of oral extrusomes, probably toxicysts because type I shows a filament about 30 µm long when exploded (Figs 1a, e, f, k, n, 2a–e, h; Table 1): about 3–6 type I extrusomes attached to oral cone, very narrowly cuneate and slightly curved (straight in Venezuelan specimens), highly conspicuous because strongly refractile and 15–18 × 1.2–1.5 µm in size; about 10 type II extrusomes, filiform, 9–10 µm long. Cortex rather rigid, specimens thus not very flexible. Cortical granules hardly recognizable in vivo because colourless, loosely arranged, and $<0.5$ µm in size; frequently impregnated with protargol disturbing analysis of ciliary pattern. Cytoplasm bright, finely granular, contains some lipid droplets 1–2 µm across. Nutrition not known, but likely to be predaceous (massive toxicysts!); food vacuoles not present in over 100 specimens checked, indicating fast digestion or lysis of prey outside of cell. Swims moderately fast rotating about main body axis.

Cilia about 8 µm long in vivo, ordinarily spaced, arranged in an average of 17 narrowly-spaced, meridional rows most abutting to circumoral kinety, some to brush membranoid, and three are modified anteriorly to a minute dorsal brush occupying about 10% of body

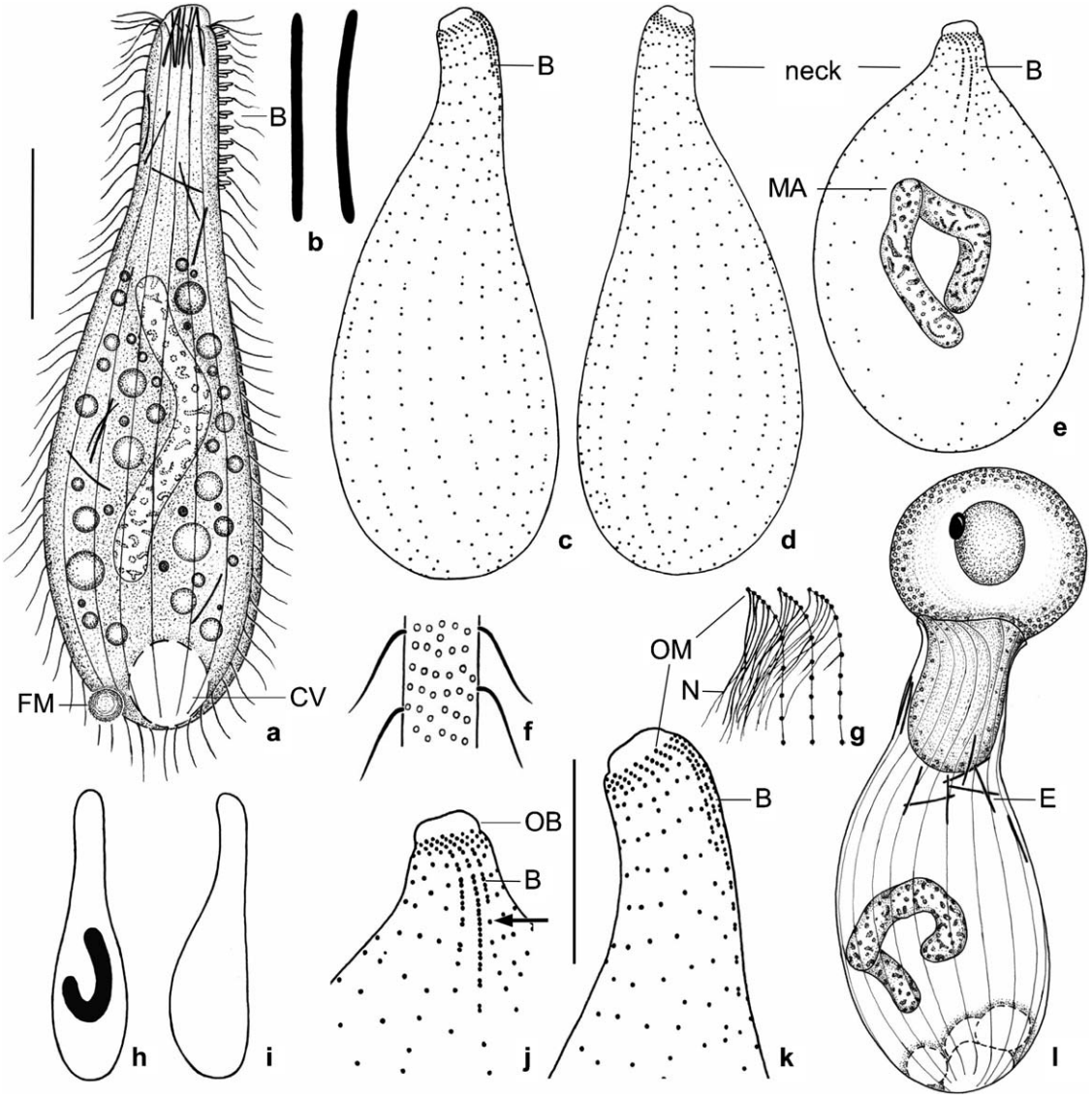


**Fig. 3. a–l**. *Enchelys lajacola* from life (a, b, f, h, i) and after protargol impregnation (c–e, g, j–l). **a**. Left side view of a representative specimen with many lipid droplets. **b**. Extrusomes are rod-shaped, slightly curved, and about 10 μm long. **c**, **d**, **k**. Ciliary pattern of right and left side of an exconjugant. **e**, **j**. Dorsal view of holotype specimen, i.e., of a trophont with curved macronucleus strand and a distinctly heterostichad dorsal brush. Note the distinct condensation of the oralized somatic monokinetids in the curved anterior region. The ciliary pattern is not impregnated in the central area. The arrow marks the start of the monokinetidal bristle tail of brush row 3. **f**. Cortical granulation. **g**. Semischematic illustration of the oral infraciliature, showing the oralized somatic monokinetids and their nematodesmata. **h**. Specimen with semicircular macronucleus. **i**. An asymmetrically lageniform specimen. **l**. A specimen engulfing a *Colpoda inflata*. B – dorsal brush, E – extrusomes, FM – faecal mass, MA – macronucleus, N – nematodesmata, OB – oral bulge, OM – oralized somatic monokinetids. Scale bars 30 μm (a, c–e, l) and 20 μm (j, k).

length (Figs 1a, k–o, 2f, g, j; Table 1). Brush rows each composed of 3–5, usually 4 dikinetids with bristles about 2 μm long and inflated distally. Brush membranoid extends obliquely along left base of dorsal hump, composed of about 10–15 very narrowly-spaced, rod-shaped cilia, 4–5 μm long, forming a membrane-like structure in vivo; basal bodies associated with long fibres extending obliquely to mid-body close underneath cell cortex (Figs 1a, d, g, j–m, o, 2h, i; Table 1). Silverline pattern narrowly reticular, as in all haptorids (Fig. 2j).

Oral structures fragile and minute, i.e., distance between peak of oral cone and end of dorsal brush on average only 6.2 μm in protargol preparations (Figs 1a, j–o, 2f–i; Table 1). Circumoral kinety distinctly oblique (~35°), composed of dikinetids associated with fine nematodesmata, forming a cylindroidal oral basket extending to second quarter of body. Oral bulge discoidal and slightly higher dorsally than ventrally, emphasizing oblique circumoral kinety. Oral cone difficult to recognize in vivo and in protargol

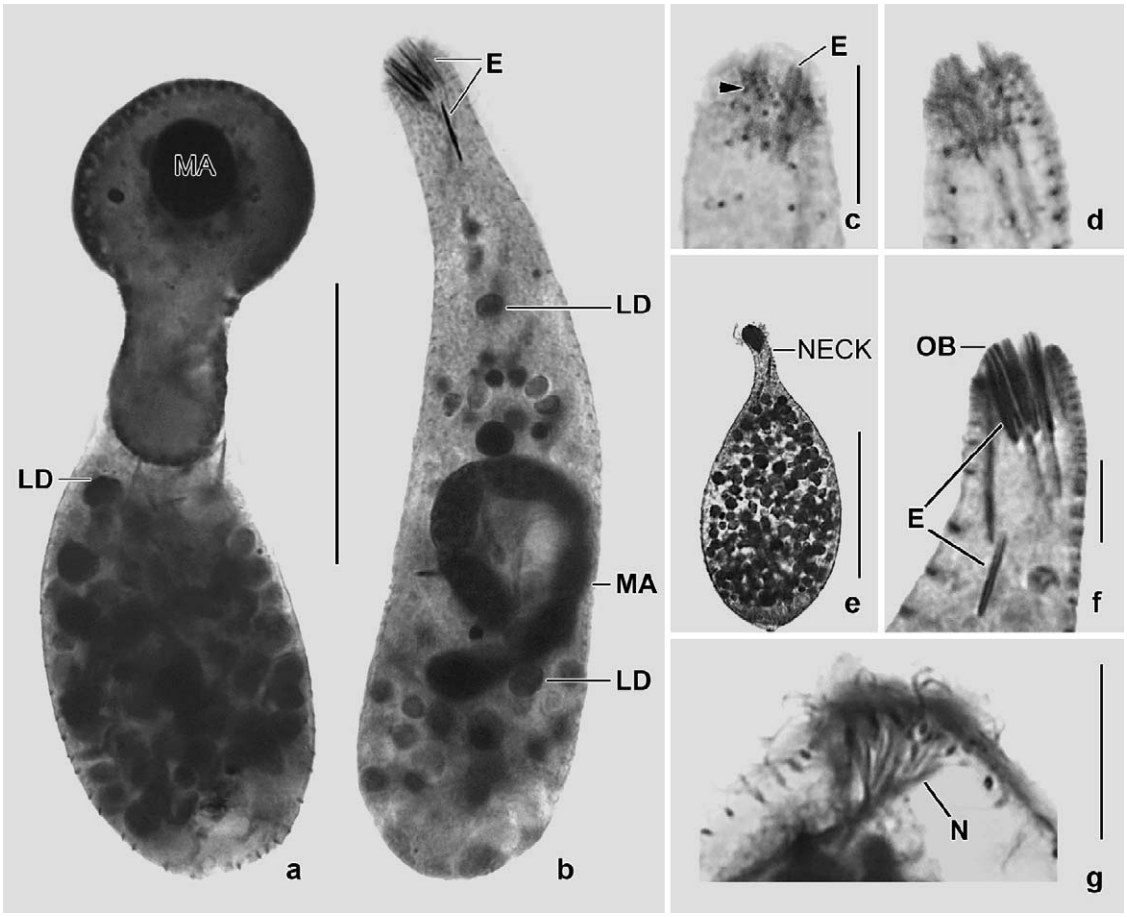


**Fig. 4. a–g**. *Enchelys lajacola* after protargol impregnation. **a**. A specimen engulfing a *Colpoda inflata*. **b**. An ordinary specimen showing shape of body and macronucleus. **c**, **d**. Ciliary pattern of right and left side in oral region, where the cilia of the somatic kineties are condensed forming a circumoral girdle (arrowhead). **e**. The narrow neck of *E. lajacola* is recognizable even in trophonts packed with lipid droplets. **f**. Anterior body region showing extrusomes. **g**. Anterior body region showing nematodesmata originating from the anterior basal bodies of the ciliary rows (oralized somatic monokinetids). E – extrusomes, LD – lipid droplets, MA – macronucleus, N – nematodesmata, OB – oral bulge. Scale bars 10 μm (c, d, f, g) and 40 μm (a, b, e).

preparations because hyaline and not impregnated, respectively; occupies dorsal half of oral bulge, contains tip of extrusomes, not retractable (Figs 1a, j–o, 2f–i).

**Occurrence and ecology**: Kahl (1935) found low numbers of *K. gibba* in a colony of bladderwort (*Utricularia*). In Iceland, large numbers of *K. gibba* developed in a non-flooded Petri dish culture set up with peatland soil composed of rotting moss and grass roots (pH 4.5 in water). In Venezuela, low numbers of *K. gibba* developed in a non-flooded Petri dish culture set up with benthic litter and mud from dry granitic rock-pools (lajas) near to the airport of Puerto Ayacucho. The specimens of this population were in vivo highly similar to those from Iceland, both in size and cytology.

**Comparison with original description**: *Lagynophrya gibba* has never been redescribed. Our population matches the brief description of Kahl (1935) only partially, suggesting that it could be a distinct species. Specifically, Kahl's specimens were 80 μm long (vs. up to 55 μm) and elongate ellipsoidal (vs. slenderly obpyriform to slightly dumbbell-shaped). Thus, we do not neotypify *K. gibba* with the Iceland population. On the other hand, the Venezuelan specimens match well those from Iceland, suggesting wide distribution and superficial observation by Kahl. This is supported by the figure he provided in 1943 (reprinted in Acta Protozoologica 43: 3–69, 2004): both the hump and the oblique, distinct oral bulge are hardly recognizable (Fig. 1c). If we have had only this figure, we would not have identified our species as *Lagynophrya gibba* Kahl! We assume that the figure from 1943 is a failed redrawing of that provided in 1935 (Fig. 1b).

## *Enchelys lajacola* nov. spec. (Figs 3a–l, 4a–g; Table 2)

**Diagnosis** : Size about 120 × 45 μm in vivo; lageniform (theront) to ovate (trophont). Macronucleus curved cylindroidal. Extrusomes rod-shaped, about 10 × 0.5 μm

**Table 2.** Morphometric data on *Enchelys lajacola*.

| Characteristics[a] | $\bar{x}$ | *M* | SD | SE | CV | Min | Max | *n* |
|---|---|---|---|---|---|---|---|---|
| Body, length | 95.7 | 96.0 | 14.8 | 3.1 | 15.4 | 71.0 | 125.0 | 22 |
| Body, width | 39.2 | 39.0 | 6.3 | 1.3 | 16.1 | 27.0 | 50.0 | 22 |
| Body length: width, ratio | 2.5 | 2.5 | 0.6 | 0.1 | 22.9 | 1.4 | 3.7 | 22 |
| Oral bulge, width | 5.8 | 6.0 | – | – | – | 5.0 | 6.0 | 19 |
| Oral bulge, height | 2.2 | 2.5 | 0.4 | 0.1 | 15.7 | 1.2 | 2.5 | 19 |
| Macronuclear figure, length | 28.8 | 27.5 | 7.8 | 1.8 | 27.2 | 16.0 | 42.0 | 19 |
| Macronucleus, length[b] | 51.9 | 50.0 | 10.2 | 2.3 | 19.7 | 37.0 | 75.0 | 19 |
| Macronucleus, width | 6.1 | 6.0 | 0.8 | 0.2 | 13.8 | 5.0 | 7.0 | 19 |
| Somatic ciliary rows, number | 17.0 | 16.0 | 1.2 | 0.2 | 6.8 | 15.0 | 19.0 | 24 |
| Kinetids in a ventral kinety, number[c] | 40.9 | 43.0 | 6.9 | 1.6 | 16.9 | 31.0 | 57.0 | 19 |
| Oralized monokinetids, number/row | 4.9 | 5.0 | 0.8 | 0.2 | 16.5 | 4.0 | 6.0 | 16 |
| Dorsal brush rows, number | 3.1 | 3.0 | – | – | – | 3.0 | 4.0 | 20 |
| Dikinetids in brush row 1, number | 7.5 | 8.0 | 1.4 | 0.4 | 18.7 | 5.0 | 10.0 | 14 |
| Dikinetids in brush row 2, number | 12.4 | 12.0 | 1.7 | 0.4 | 13.5 | 9.0 | 16.0 | 19 |
| Dikinetids in brush row 3, number | 3.7 | 3.0 | 0.9 | 0.3 | 24.3 | 3.0 | 5.0 | 11 |
| Brush row 1, length[d] | 14.2 | 13.3 | 3.9 | 1.0 | 27.2 | 9.0 | 22.0 | 16 |
| Brush row 2, length[d] | 20.0 | 20.0 | 3.8 | 0.7 | 19.1 | 14.0 | 25.0 | 27 |
| Brush row 3, length[d] | 5.2 | 5.0 | 1.1 | 0.4 | 21.8 | 4.0 | 8.0 | 8 |
| Extrusomes, length | 9.8 | 10.0 | 1.2 | 0.3 | 12.6 | 7.0 | 13.0 | 19 |

[a]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, $\bar{x}$ – arithmetic mean.
[b]If curved, length of spread macronucleus was estimated; values thus approximations.
[c]Including oralized monokinetids as well as ciliated and unciliated basal bodies.
[d]Anterior kinety end to end of dikinetidal portion of brush row.

in size. On average 17 ciliary rows, 3 anteriorly differentiated to a distinctly heterostichad dorsal brush.

**Type locality** : Mud and algal crusts from a roadside laja about 150 km NE of Puerto Ayacucho, Venezuela, i.e., in the savanna between the towns of La Urbana and Las Ventanas, 7°N, 66°56′W.

**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** : Named after the site where found, i.e., a temporary pool (lithotelma) on a granitic outgrowth, called "Laja" in Venezuela.

**Description** : Size and shape rather variable because slender theronts and broad trophonts occur. Size about 80–150 × 30–60 µm in vivo, usually about 120 × 45 µm, length:width ratio 1.3–3.7:1, on average 2.4:1 in protargol preparations (Table 2). Asymmetrically lageniform, obclavate or ovate with distinct neck (Figs 3a, d, e, h, i, 4a, b, e). Very flexible and slightly contractile under coverslip. Macronucleus usually in posterior two thirds of cell, curved cylindroidal, rarely semicircular or rod-shaped, very rarely moniliform, about 52 × 6 µm in size in protargol preparations, in vivo stands out brightly from granular cytoplasm; nucleoli scattered (Figs 3a, e, h, l, 4b). Micronuclei not recognizable among lipid droplets. Contractile vacuole in rear end, several excretory pores in pole area; cytopyge near excretory pores; faecal mass dense, about 7 µm across (Fig. 3a). Extrusomes rod-shaped and slightly curved, about 7–13 × 0.5 µm in vivo; 6–20 extrusomes attached to oral bulge and few to 30 scattered in cytoplasm (Figs 3a, b, l, 4b, c, f); impregnate with the protargol method used, a rare feature, at least for mature toxicysts anchored to the oral bulge. Cortical granules ordinarily arranged, inconspicuous because about 0.5 µm across and of usual refractivity (Fig. 3f). Cytoplasm colourless, usually opaque due to many lipid droplets 1–8 µm across, neck hyaline (Figs 3a, 4a, b, e). Engulfs middle-sized ciliates, e.g., *Colpoda inflata* digested in food vacuoles up to 40 µm in size (Figs 3l, 4a).

Cilia about 10 µm long in vivo, ordinarily spaced, except for curved anterior end bearing about five narrowly-spaced, oralized somatic monokinetids, forming a conspicuous ciliary girdle (Figs 3a, c–e, j, k, 4c, d). On average 17 equidistantly spaced, longitudinally to slightly spirally extending ciliary rows, three, rarely four anteriorly modified to a distinctly heterostichad dorsal brush occupying about 20% of body length (Fig. 3a, c, e, j, k; Table 2). Brush row 1 shorter than row 2, composed of 5–10 dikinetids; longest row 2 composed of 9–16 dikinetids; row 3 only half as long as row 1, composed of 3–5 dikinetids followed by a short

monokinetidal bristle tail. Posterior bristle of dikinetids shorter (1 μm) than anterior one (2–2.5 μm).

Oral bulge small as compared to size of cell but may open widely when ingesting large prey (Figs 3l, 4a), in vivo slightly oblique, obovate, and about 8 × 3 μm in size. Nematodesmata about 13 μm long, originate from about five densely spaced oralized somatic monokinetids at anterior end of ciliary rows (Figs 1a, c–e, g, j, k, 4b–d, f, g; Table 2). No circumoral kinety. Details of oral structures hardly recognizable in most specimens due to 6–20 strongly impregnated extrusomes anchored in oral bulge (Fig. 4a).

**Occurrence and ecology** : As yet found only at type locality. *Enchelys lajacola* became moderately abundant in the non-flooded Petri dish culture one week after rewetting the sample. It is probably a limnetic species.

**Comparison with related species** : *Enchelys lajacola* differs from the congeners by the bottle-like shape, i.e., the distinct neck, and the curved, strand-like macronucleus (37–75 × 5–7 μm), which is the most important feature. Few *Enchelys* species have been described from soil (Foissner 1998; Foissner et al. 2002), and none is similar to *Enchelys lajacola*. However, it is fairly close to several limnetic species, especially *Enchelys gasterosteus*, as redescribed by Foissner (1984) and Foissner et al. (1995). Both differ in body shape (with vs. without neck) and the macronucleus (a tortuous strand vs. reniform). Further, although *Enchelys lajacola* is larger than *Enchelys gasterosteus* (120 × 45 vs. 40–70 × 20–40), it has fewer ciliary rows (17 vs. 17–30): compared to body width, *Enchelys gasterosteus* should have only about 11 ciliary rows. We carefully checked the absence of oral dikinetids to exclude a relationship with the Acropisthiina, as defined by Foissner and Foissner (1988).

## *Apertospathula implicata* (Kahl, 1930a, b) nov. comb. (Figs 5a–t, 6a–d, 7a–i; Table 3)

1930 *Spathidium implicatum* Kahl, Arch. Protistenk., 70: 369.

1930 *Spathidium implicatum* Kahl, 1930a, b – Kahl, Tierwelt Dtl., 18: 159.

**Improved diagnosis** (includes the original data and those from the Austrian neotype): Size about 130 × 30 μm in vivo. Narrowly spatulate slightly widening anteriorly; oral bulge cuneate and about as long as widest body portion. Macronucleus highly tortuous, forming a knot-like structure in mid-body. Extrusomes narrowly cuneate, 4–8 μm long. On average 17 ciliary rows, 3 anteriorly differentiated to a distinctly heterostichad dorsal brush; rows 1 and 2 of same length, each composed of an average of 19 dikinetids, row 3 composed of 10 dikinetids and followed by a monokinetidal tail comprising about 8 bristles.

**Type locality** : The neotype is from the upper mud and soil layer of a shallow meadow puddle near the centre of the town of Salzburg, Austria, 47°47′N, 13°02′E. Kahl (1930a, b) discovered *Apertospathula implicata* in a pond of the botanical garden in Hamburg city, Germany, 53°33′N, 9°51′E.

**Type material** : No type material is available from Kahl's specimens. Thus, we fix the Austrian population as a neotype (see below). Five slides with eight well impregnated and marked specimens have been deposited in the Biology Centre of the Museum of Upper Austria, Linz (LI).

**Etymology** : The Latin adjective *implicatus* (tortuous) refers to the main feature of the species, that is, the highly tortuous, strand-like macronucleus.

**Description of Austrian neotype population** : Size 90–150 × 25–45 μm in vivo, usually about 130 × 30 μm; length:width ratio near 4:1 in vivo and 3:1 in protargol preparations, where specimens are rather distinctly inflated in mid-body losing the typical shape (Fig. 5i; Table 3). Two main shape variants, both slightly and gradually widening anteriorly, occur in vivo: about 65% of specimens narrowly spatulate and only very slightly widening anteriorly (Fig. 5a, n), while 30% widen rather distinctly (Fig. 5o); rarely specimens have an inconspicuous neck or a cylindrical body (Figs 5p, 7a); oral region laterally flattened, anterior end slanted by about 45°, posterior end rounded (Figs 5a, g–j, n–q, 6a–d, 7a, b). Macronucleus slightly above, rarely close underneath mid-body, highly tortuous usually forming a globular, knot-like mass 20–35 μm across, about 90 μm long in "uncoiled" condition; nucleoli globular, numerous (Figs 5a, g, i, 6a; Table 3). Micronuclei indistinguishable from cytoplasmic inclusions in protargol preparations. Contractile vacuole in rear end, excretory pore(s) not impregnated. Mature extrusomes rather distinctly impregnated in some specimens; about 20 extrusomes scattered each in cytoplasm and dorsal half of oral bulge, while about 10 form an indistinct row each in right and left half of ventral bulge portion (Figs 5f, i, m, 6c, d); individual extrusomes very narrowly cuneate and slightly curved, 4–5 × 0.8 μm in size (Figs 5r, 7c), when exploded of typical toxicyst structure and about 10 μm long (Figs 5t, 7d), when partially exploded obclavate and about 6 μm long (Fig. 5s). Cortex slightly furrowed by ciliary rows (Figs 5a, 7a, i), about 1 μm thick and distinctly separate from cytoplasm, contains about three widely spaced rows of minute (≤0.5 μm) but very densely spaced granules between each two kineties (Fig. 5b). Cytoplasm colourless, packed with highly refractive granules 1–3 μm in size and of various shapes (fusiform, ovate, hemispherical, ellipsoidal; Figs 5u, 7e); few to many lipid droplets (Figs 5a, 7e) and corroded starch grains from the wheat kernels added to the culture.

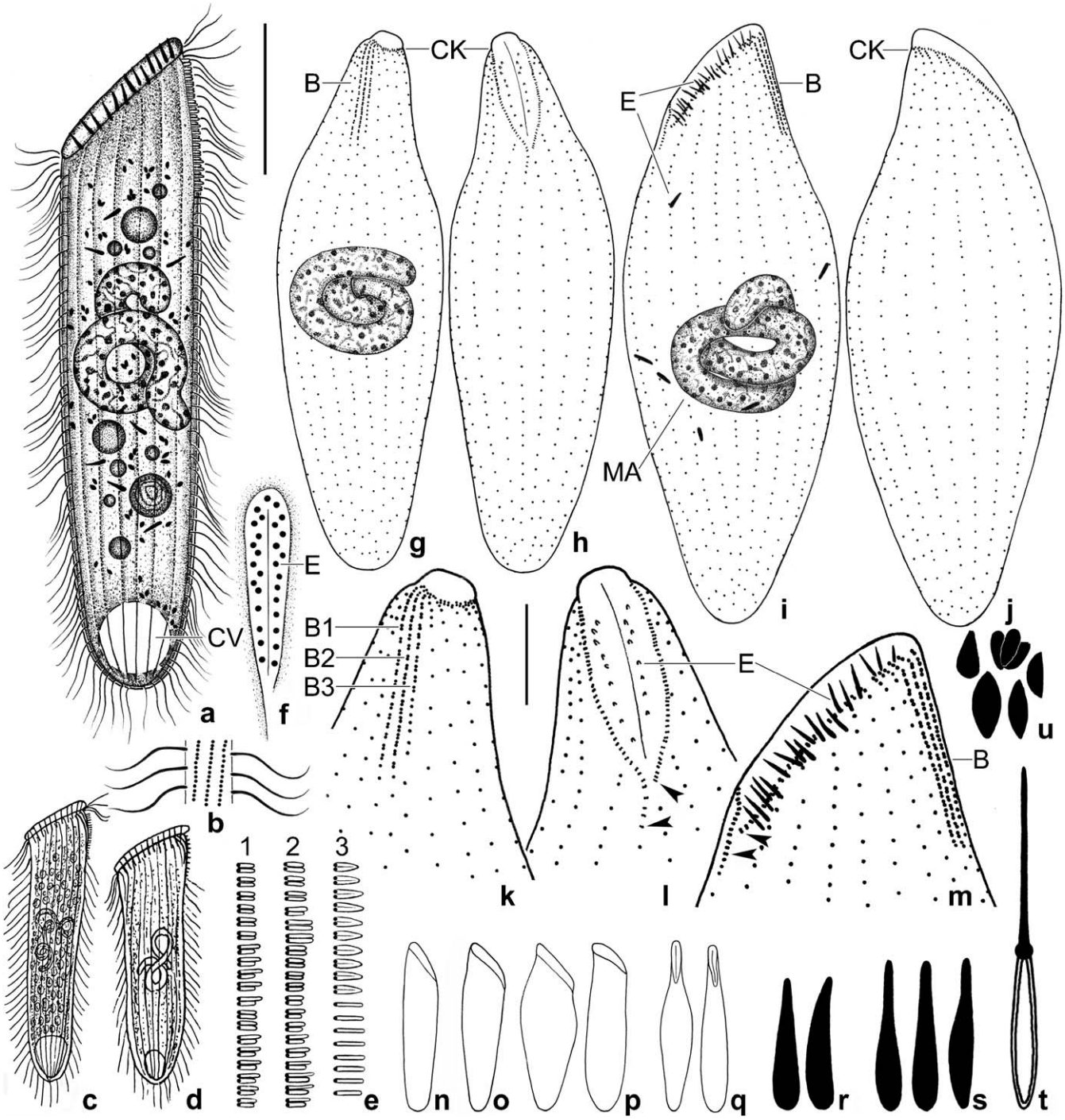


**Fig. 5. a–u**. *Apertospathula implicata*, Austrian neotype (a, b, e–u) and German (c, d) specimens from life (a–f, n–u) and after protargol impregnation (g–m). **a**. Left side view of a representative specimen, length 130 µm. **b**. Surface view showing cortical granulation. **c**, **d**. Left side views of specimens from Hamburg population, 130–160 µm long (from Kahl 1930a, b). **e**. Dorsal brush, composite from live and SEM observations. The dikinetidal bristles of row 3 stick together. **f**. Frontal view of oral bulge showing the *Apertospathula* pattern and the arrangement of the extrusomes (cp. Fig. 7f). **g**, **h**. Ciliary pattern of dorsal and ventral side and nuclear apparatus of main neotype specimen. **i**, **j**. Ciliary pattern of left and right side of a neotype specimen. Note the highly tortuous macronucleus strand in mid-body, an important feature of this species. **k–m**. Dorsal, ventral, and right side views of anterior part of the neotype specimens shown in Fig. 5g, h, i. The right half of the circumoral kinety is five dikinetids longer than the left one (arrowheads). **n**, **o**. Dominating shape variants. **p**. Rare shape variants. **q**. Ventral view of shape variants. **r**. Oral bulge extrusomes, length 5 µm. **s**. Partially exploded extrusomes, length 6 µm. **t**. Fully exploded extrusome, length 30 µm. **u**. Granules occurring in cytoplasm. B (1–3) – dorsal brush (rows), CK – circumoral kinety, CV – contractile vacuole, E – extrusomes, MA – macronucleus. Scale bars 30 µm (a, g–j) and 10 µm (k–m).

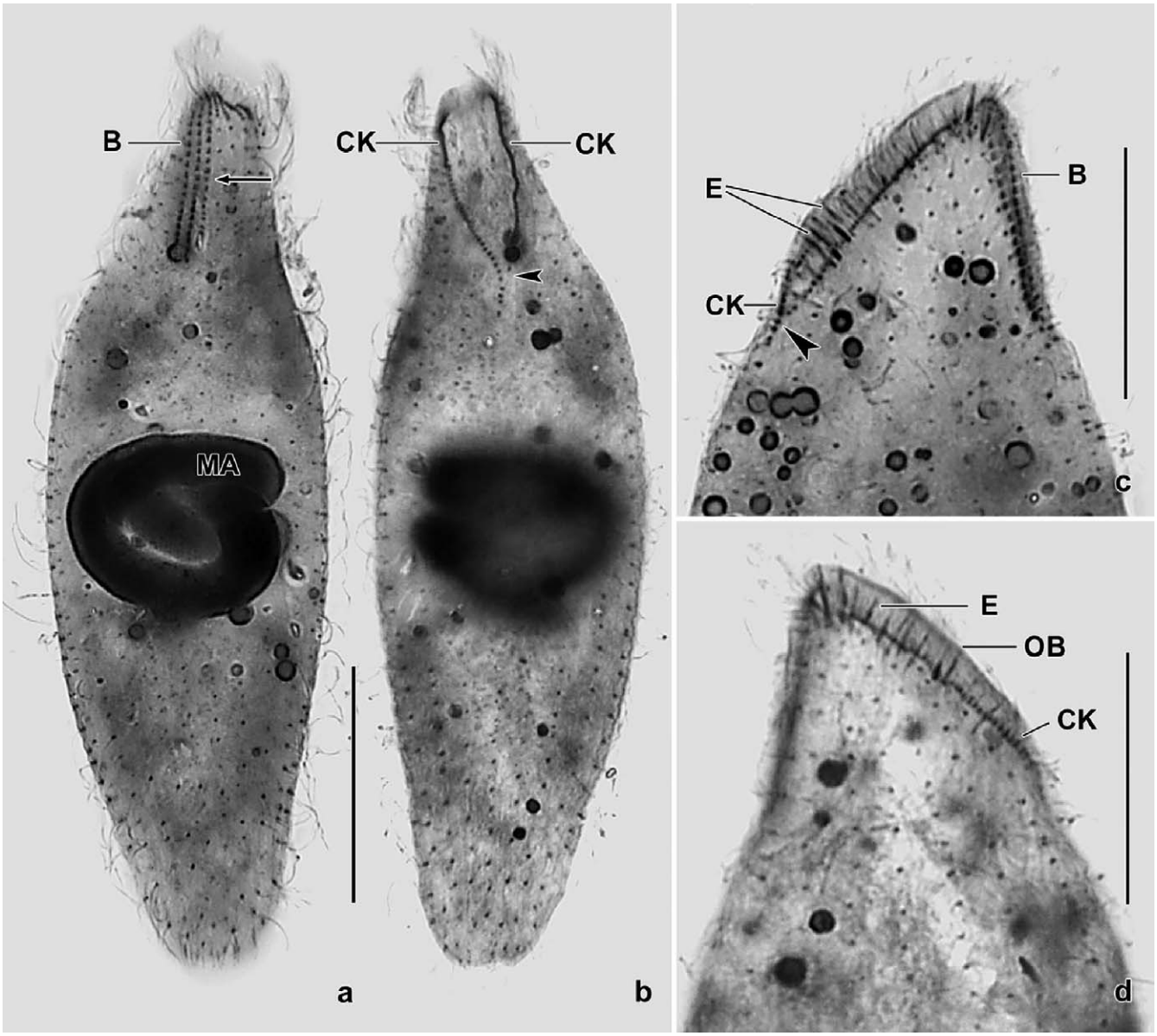


**Fig. 6. a–d**. *Apertospathula implicata*, Austrian neotype specimens after protargol impregnation. **a**, **b**. Ciliary pattern of dorsal and ventral side and nuclear apparatus of main neotype specimen (cp. Fig. 5g, h, k, l). Dorsal brush row 3 is only half as long as rows 1 and 2 (arrow denotes end of dikinetidal part of row 3). The right half of the circumoral kinety is five dikinetids longer than the left one (arrowhead). Note the strongly tortuous macronucleus. **c, d**. Ciliary pattern of left and right side of a neotype specimen (cp. Fig. 5i, j, m). Note the rather deeply impregnated extrusomes. The left branch of the circumoral kinety (arrowhead) is slightly longer than the right one. B – dorsal brush, CK – circumoral kinety, E – extrusomes, MA – macronucleus, OB – oral bulge. Scale bars 20 μm (c, d) and 30 μm (a, b).

Cilia 12 μm long in vivo, while shrunken to about 8 μm in SEM preparations and to 10 μm in protargol slides, densely to ordinarily spaced, arranged in an average of 17 ordinarily and equidistantly spaced rows; anterior end of up to four kineties right and left of dorsal brush more densely ciliated. Right side kineties slightly curved anteriorly and abutting to circumoral kinety, left side kineties anteriorly very slightly curved or straight abutting to circumoral kinety at a more acute angle than the right side ones (Figs 5g–m, 6a–d, 7a, b; Table 3). Three ciliary rows anteriorly differentiated to a heterostichad dorsal brush occupying an average of 20% of body length; rows parallel, equidistant, ordinarily spaced, and extending in furrows 2 μm deep producing distinct ridges in SEM preparations (Fig. 7g, h). Rows 1 and 2 of same length each composed of an average of 19 ordinarily spaced dikinetids with bristles 2–3 μm long; posterior bristle of dikinetids slightly

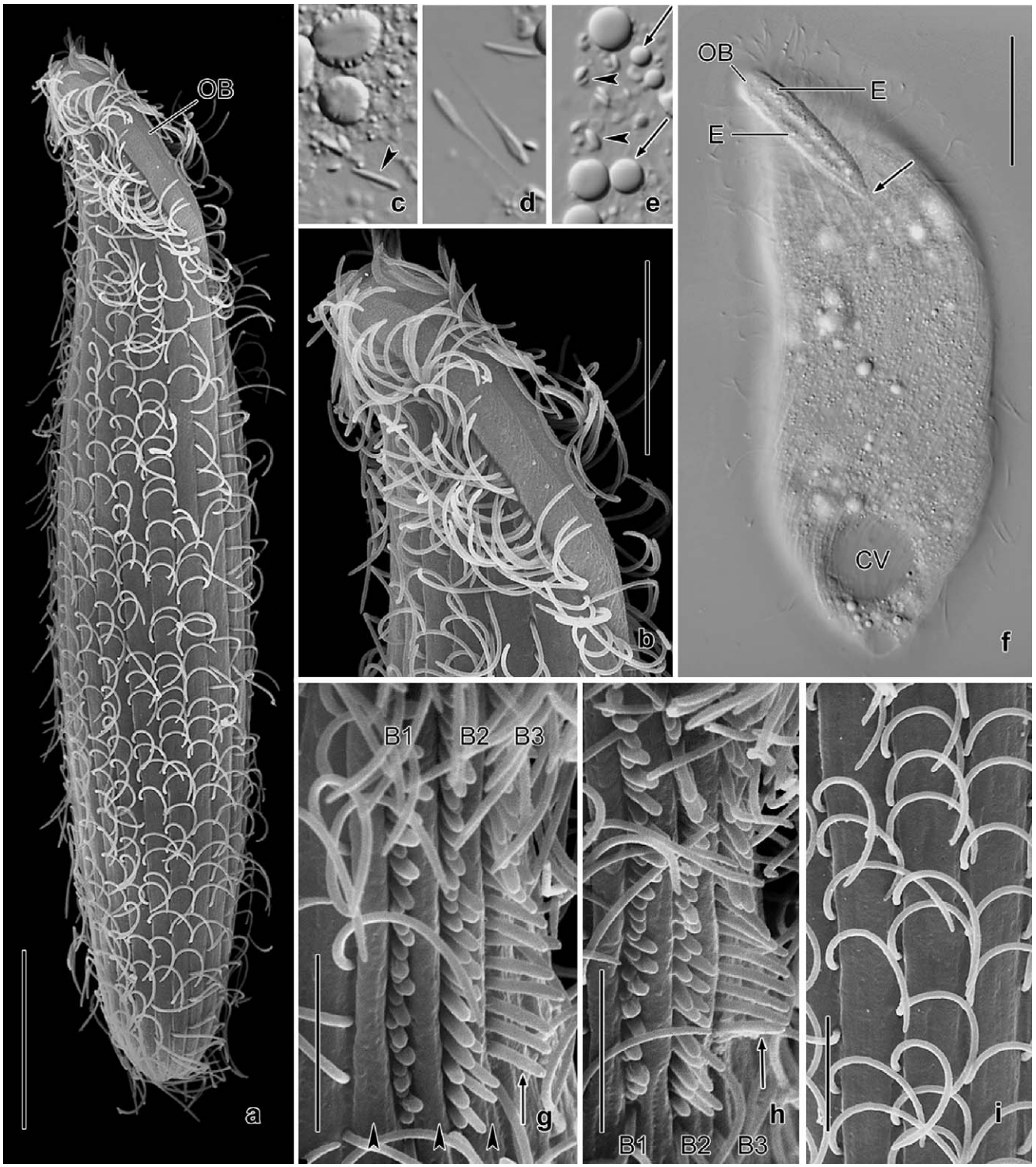


**Fig. 7. a–i**. *Apertospathula implicata*, Austrian neotype specimens in the scanning electron microscope (a, b, g–i) and from life (c–f). **a**, **b**. Ventrolateral view of a slender specimen (a) and detail of its anterior body portion (b). **c**, **d**. Resting (c, arrowhead) and exploded (d) toxicysts, length 4 µm and 10 µm. **e**. Cytoplasmic granules (arrowheads, about 3 µm) and lipid droplets (arrows). **f**. Ventral view of a squeezed specimen, showing the cuneate shape of the oral bulge whose posterior end is open due to the slightly shortened left bulge half (arrow). **g**, **h**. Dorsal brush of two specimens. The brush rows are separated by fairly distinct ridges (arrowheads), and the bristles of the monokinetidal tail (arrows) are slightly longer than those of the dikinetids. **i**. Surface view showing cortex and cilia of the specimen depicted in Fig. 7a. B (1–3) – dorsal brush rows, CV – contractile vacuole, E – extrusomes, OB – oral bulge. Scale bars 20 µm (a, f), 10 µm (b), and 5 µm (g–i).

**Table 3.** Morphometric data on *Apertospathula implicata*.

| Characteristics[a] | $\bar{x}$ | *M* | SD | SE | CV | Min | Max | *n* |
|---|---|---|---|---|---|---|---|---|
| Body, length | 113.8 | 114.0 | 9.1 | 3.2 | 8.0 | 103.0 | 130.0 | 8 |
| Body, width | 37.3 | 38.0 | 4.0 | 1.5 | 10.7 | 31.0 | 43.0 | 7 |
| Body length: width, ratio | 3.0 | 3.1 | 0.3 | 0.1 | 9.3 | 2.6 | 3.3 | 7 |
| Oral bulge, width | 6.0 | 6.0 | 0.0 | 0.0 | 0.0 | 6.0 | 6.0 | 7 |
| Oral bulge, height | 2.6 | 2.5 | – | – | – | 2.5 | 3.0 | 8 |
| Oral bulge, length | 24.8 | 25.0 | – | – | – | 24.0 | 25.0 | 6 |
| Body length:oral bulge length, ratio | 4.6 | 4.7 | 0.4 | 0.2 | 8.3 | 4.2 | 5.2 | 6 |
| Anterior body end to macronucleus, distance | 43.5 | 44.0 | 7.5 | 2.6 | 17.2 | 29.0 | 53.0 | 8 |
| Macronucleus figure, length | 26.1 | 25.5 | 5.7 | 2.0 | 22.0 | 19.0 | 35.0 | 8 |
| Macronucleus, length (spread; approximate) | 88.8 | 90.0 | – | – | – | 75.0 | 100.0 | 8 |
| Macronucleus, width | 6.6 | 6.0 | 0.8 | 0.3 | 11.8 | 6.0 | 8.0 | 8 |
| Somatic ciliary rows, number | 17.4 | 17.0 | 0.7 | 0.3 | 4.3 | 17.0 | 19.0 | 8 |
| Ciliated kinetids in a lateral kinety, number | 49.3 | 50.0 | 6.7 | 2.5 | 13.7 | 40.0 | 60.0 | 7 |
| Dorsal brush rows, number | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 8 |
| Dikinetids in brush row 1, number | 18.9 | 17.0 | 2.9 | 1.1 | 15.4 | 17.0 | 24.0 | 7 |
| Dikinetids in brush row 2, number | 19.2 | 17.5 | 3.1 | 1.3 | 16.0 | 17.0 | 24.0 | 6 |
| Dikinetids in brush row 3, number | 9.5 | 10.0 | 1.2 | 0.5 | 12.9 | 8.0 | 11.0 | 6 |
| Brush row 1, length[b] | 19.3 | 19.0 | – | – | – | 19.0 | 20.0 | 8 |
| Brush row 2, length[b] | 19.3 | 19.0 | – | – | – | 19.0 | 20.0 | 8 |
| Brush row 3, length[b] | 11.1 | 11.0 | 1.4 | 0.5 | 12.1 | 10.0 | 13.0 | 7 |
| Bristles in monokinetidal tail of row 3, number | 8.2 | 8.0 | 0.8 | 0.3 | 9.2 | 7.0 | 9.0 | 6 |

[a]Data based on mounted, protargol-impregnated (Foissner's method), and randomly selected specimens from a mud and soil infusion. 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, $\bar{x}$ – arithmetic mean.

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

shorter than anterior one. Row 3 half as long as rows 1 and 2, composed of an average of 10 ordinarily spaced dikinetids with bristles up to 3 µm long sticking together in vivo and forming a conical structure not maintained in SEM preparations; dikinetids followed by 7–9 monokinetids with bristles 3–4 µm long (Figs 5a, e, g, i, k, m, 6a, c, 7g, h; Table 3).

Oral bulge rather inconspicuous because only slightly set off from body proper and 3–4 µm high; 40–50° oblique with flat to slightly convex surface, on average about as long as body width, in ventral view cuneate with dorsal end 6–8 µm wide in vivo (Figs 5a, f, g–m, n–q, 6a–d, 7a, b, f; Table 3). Circumoral kinety composed of approximately 80–100 dikinetids, ventral opening about 2 µm wide, right branch longer than left one by about five dikinetids (Figs 5l, m, 6b); *Apertospathula* pattern recognizable also in vivo in optimally orientated specimens (Fig. 7f).

**Occurrence and ecology** : *Apertospathula implicata* was discovered by Kahl (1930a) in a pond of the botanical garden in Hamburg city, Germany, where it was temporarily abundant among fouling macrophytes, such as *Glyceria*, *Phragmites* and *Nymphaea*. The neotype population is from an infusion of the upper mud and soil layer of a shallow meadow puddle near to the so-called Henkerhaus (house of the hangman) in the Donnerbergpark (a municipal park near Salzburg city centre). The infusion was enriched with some wheat grains. Thus, it soon became micro- and anaerobic. A rich population of *Perispira* sp. developed in the microaerobic zone above the mud and soil mass. *Apertospathula implicata* occurred in very low abundance among the numerous *Perispira*. No other records are known. Both, Kahl's and our sites suggest that *A. implicata* is a rare, microaerobic species.

**Generic classification and neotypification** : Our reinvestigation shows that *Spathidium implicatum* belongs to the genus *Apertospathula* because it has an open circumoral kinety (Foissner and Xu 2007). We neotypify *A. implicata* with the Austrian population because (i) no type material is available, (ii) we do not have doubts on the identification, (iii) the identity of the species is endangered by several similar species, and (iv) it is from the same biogeographic region and a similar habitat.

**Comparison with original description and related species** : Our observations match the original description very well (Kahl 1930a, b). As usual, there are some small differences, most likely caused by insufficient observations. For instance, Kahl (1930a, b) did not note the fairly distinct ridges between the dorsal brush rows. Further, his specimens were slightly longer (130–160 µm vs. 90–150 µm) and had larger extrusomes (about 8 µm vs. 4–5 µm). *Apertospathula implicata* can hardly be

confused with other spathidiids because none of the congeners, and only few species from other spathidiid genera, show such conspicuous nuclear pattern, that is, a highly tortuous strand forming a knot-like structure in mid-body (Foissner and Xu 2007). In vivo, *Spathidium piliforme* and *Arcuospathidium muscorum* may be mistaken for *A. implicata* because they have a similar body size and macronucleus pattern. The former is obclavate to slenderly bursiform, while *A. implicata* is narrowed posteriorly (Kahl 1930a, b; Fig. 5a, n). The populations of *S. piliforme* investigated by Foissner and Didier (1981) and Foissner (1984) are likely misidentifications because they have a much higher number of ciliary rows (~30 vs. ~15). *Arcuospathidium muscorum* is usually stouter (3:1 vs. 4:1) and has a much longer oral bulge (ratio of body length to bulge length about 2–3:1 vs. 4–5:1). As concerns body shape and ciliary pattern, *A. implicata* is highly similar to *A. lajacola* Foissner and Xu, 2007, while the extrusomes are conspicuously different (cuneate and 4 µm long vs. filiform and 12 µm long).

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