

***Enchelys micrographica* nov. spec., a New Ciliate (Protista, Ciliophora) from Moss of Austria**

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SUMMARY

Enchelys micrographica nov. spec. was discovered in tree moss near a stream (Felberbach) in the surroundings of the town of Salzburg, Austria. It was investigated by live observation and protargol silver impregnation. The new ciliate has a size of about 120 x 45 µm and is obpyriform with the oral bulge about 11 µm wide and 3 µm high. It possesses more than 100 macronucleus nodules and several micronuclei. The extrusomes (toxicysts) are bluntly fusiform and about 4 x 0.7 µm in size. The cortical granulation is very dense. There is an average of 35 ciliary rows, each with three oralized somatic monokinetids. The dorsal brush is three-rowed, occupies an average of 22% of body length, and row 1 is usually slightly shortened anteriorly. *Enchelys micrographica* belongs to the multinucleate group of the genus and differs from closely related congeners mainly by the shape and size of the extrusomes and oral bulge.

Key words: *Enchelys farcimen*, resting cysts, Salzburg, soil ciliates.

***Enchelys micrographica* nov. spec., ein neues Moos-Ciliat (Protista, Ciliophora) von Österreich**

ZUSAMMENFASSUNG

Enchelys micrographica nov. spec. wurde im Moos eines Baumes vom Ufer des Felberbaches am Stadtrand von Salzburg entdeckt. Sie wurde in vivo und in Silberpräparaten untersucht. Das neue, obpyriforme Ciliat ist etwa 120 x 45 µm groß. Der Mundwulst hat einen Durchmesser von etwa 11 µm und eine Höhe von 3 µm. *Enchelys micrographica* hat über 100 Makronucleus-Teile und mehrere Mikronuclei. Die Extrusome (Toxicysten) sind stumpf spindelförmig und 4 x 0.7 µm groß. Die corticale Granulation ist sehr dicht. Jede der im Mittel 35 Wimpernreihen hat drei oralisierte somatische Monokinetiden am anterioren (oralen) Ende. Die Dorsalbürste ist dreireihig und erstreckt sich über 22% der Körperlänge; Reihe 1 ist am Vorderende meist leicht verkürzt. *Enchelys micrographica* gehört zur polynucleaten Gruppe der Gattung und unterscheidet sich von den nächst verwandten Arten hauptsächlich in der Form und Größe der Extrusome und des Mundwulstes.

Schlüsselwörter: *Enchelys farcimen*, Dauer-Cysten, Salzburg, Boden-Ciliaten.

INTRODUCTION

Since Müller (1786), the family Enchelyidae acts as a sink for many inconspicuous, holotrichously ciliated infusorians with ellipsoidal body and simple oral apparatus. Presently, the type genus, *Enchelys*, consists of about 60 nominal species and circa 40 combinations. Thus, it is not surprising that the diagnosis of the family varies highly, depending on the genera included and personal taste (Corliss 1979, Foissner and Foissner 1988, Jankowski 2007, Lynn 2008).

Foissner and Foissner (1988) confined the Enchelyidae to genera having so-called oralized somatic monokinetids composing the oral basket, because a dikinetidal circumoral kinety is lacking. This important feature, which is recognizable in silver preparations, still is the best character for a phenetic classification and family/genus recognition.

The nomenclatural problems surrounding the genus *Enchelys* have been solved by Aescht (2001). Unfortunately, the type species, *Enchelys farcimen*, has not yet investigated with modern methods. This makes all present classifications uncertain. However, *E. gasterosteus* Kahl, 1926 is possibly a junior synonym of *E. farcimen*. If this is accepted, the redescription of *E. gasterosteus* by Foissner (1984) can be used to diagnose the Enchelyidae as suggested by Foissner and Foissner (1988).

In the present paper, I describe a new "typical" *Enchelys* species, showing that their diversity is far from being exhausted. Indeed, we described seven new *Enchelys* species, mainly from soil, during the past 25 years. Most of the limnetic species are poorly known, needing detailed redescription.

MATERIAL AND METHODS

Enchelys micrographica was discovered in tree moss from the surroundings of the town of Salzburg, Austria. See section on occurrence and ecology for a more detailed site description. The moss was air-dried for a month and then used to set up a "non-flooded Petri dish culture". Briefly, this involves placing 50–500 g dry litter, moss, and/or 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 non-flooded Petri dish method, see Foissner et al. (2002). *Enchelys micrographica* was observed in vivo and in protargol silver preparations, as described by Foissner (1991). Counts and measurements on silvered specimens were conducted at a magnification of x 1000. In vivo measurements were performed at magnifications of x 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

Description of *Enchelys micrographica* nov. spec.

Diagnosis: Size about 120 x 45 μm in vivo. Usually slightly obpyriform with oral bulge about 11 μm wide and 3 μm high. On average more than 100 ellipsoidal macronucleus nodules and more than 10 micronulei each about 4 μm across. Extrusomes scattered in oral bulge, bluntly fusiform and slightly curved, about 4 x 0.7 μm in size. Cortical granulation very dense, plate-like. On average 35 ciliary rows, each with 3 oralized somatic monokinetids.

Dorsal brush three-rowed occupying 22% of body length on average, row 1 usually more or less shortened anteriorly.

Type locality: Tree moss from the surroundings of the town of Salzburg, 47° 47' N 13° 02' E.

Type material: 1 holotype slide and 3 paratype slides 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 "Mikroskopische (formerly Micrographische) Gesellschaft Wien" on occasion of its 100th birthday.

Description: Size 100–150 x 35–65 µm in vivo, usually near 120 x 45 µm, length: width ratio 2:1–3:1, on average about 2.6:1 both in vivo and in protargol preparations (Table 1). Usually slightly obpyriform and asymmetrical due to a more or less distinct ventral shoulder disappearing in most prepared specimens; about 1.5:1 flattened laterally; rarely elliptical or distinctly obpyriform and/or rather conspicuously curved (Figs 1, 2, 4, 13–16). On average 117 globular to elongate ellipsoidal macronucleus nodules scattered throughout cytoplasm, except of cell margin; individual nodules on average 10 x 6.5 µm in size and with several ordinarily-sized nucleoli in protargol preparations; in most specimens some dividing nodules, frequently also some rather long, strand-like pieces (excluded from morphometry). At least an average of 10 spherical to broadly ellipsoidal micronuclei scattered between macronucleus nodules; individual micronuclei about 4 µm across in vivo and thus rather large as compared with macronucleus nodules, deeply and smoothly impregnated with the protargol method used (Figs 1, 5, 24). Contractile vacuole in posterior body end with several excretory pores in pole area (Figs 1, 5, 15). Extrusomes scattered in oral bulge, do not impregnate with the protargol method used, bluntly fusiform and slightly curved, 3–4 x 0.5–0.8 µm in size; when exploded about 8 µm long and of typical toxicyst structure, some with a tube anteriorly and posteriorly (Figs 1, 3, 6, 8, 9). Cortex conspicuous because about 1.5 µm thick due to comparatively large, densely arranged granules, producing a bright, plate-like layer; individual granules colourless, about 1.2 x 0.6 µm in size, arranged in about eight rows between two kineties each; underneath granule layer densely spaced, ellipsoidal mitochondria (Figs 1, 18, 19). Cytoplasm colourless, appears dark at low magnification when packed with food vacuoles up to 30 µm across, containing mainly *Drepanomonas pauciciliata* and *Leptopharynx costatus*, rarely *Frontonia depressa*; prey taken whole and thus recognizable in young food vacuoles; in posterior region sometimes a defecation vacuole with loose contents and about 20 µm across (Figs 1, 13, 24). Many specimens studded with refractive, conical inclusions about 3 x 2 µm in size (Figs 7, 24), possibly spines used to cover the wall of the resting cyst, as described by Foissner (1984) and Foissner and Al-Rasheid (2007) in *E. polynucleata*. Movement without peculiarities.

On average 35 ordinarily spaced and ciliated somatic kineties, each with 2–4, usually 3 oralized somatic monokinetids difficult to recognize at curved anterior end of rows, as typical for *Enchelys* (Foissner et al. 2002); basal bodies usually rather irregularly spaced within kineties due to pair-like (reserve?) kinetids having an about 10 µm long cilium associated with only the posterior basal body (Figs 1, 4, 10–12, 21; Table 1). Dorsal brush heterostichad and, basically, isomorphic, rather short occupying 22% of body length on average; dikinetids ordinarily spaced bearing 2–3 µm long, cylindroidal bristles; frequently with irregularities, such as minute breaks and/or shifts, missing or supernumerary dikinetids, or some interspersed monokinetids. Brush row 1 slightly to distinctly shortened anteriorly in about 60% of specimens, composed of an average of 24 dikinetids, shortened area occasionally occupied by some monokinetids or scattered dikinetids; row 2 slightly longer than row 1, composed of an average of 29 dikinetids, posterior region more or less distinctly heteromorphic, anterior end with some monokinetids in a few specimens; brush row 3 on average considerably shorter than rows 1 and 2, composed of an average of 11 dikinetids

Table 1. Morphometric data on *Enchelys micrographica*.

| Characteristics ^a | Mean | M | SD | SE | CV | Min | Max | n |
|--|-------|-------|------|-----|------|------|-------|----|
| Body, length | 107.2 | 103.0 | 14.0 | 3.1 | 13.1 | 90.0 | 135.0 | 21 |
| Body, width | 42.4 | 41.0 | 7.2 | 1.6 | 17.0 | 35.0 | 65.0 | 21 |
| Body length:width, ratio | 2.6 | 2.5 | 0.3 | 0.1 | 10.4 | 2.0 | 3.1 | 21 |
| Oral bulge, width | 9.7 | 9.0 | 1.4 | 0.3 | 14.6 | 7.0 | 13.0 | 21 |
| Oral bulge, height | 2.4 | 2.0 | 0.5 | 0.1 | 22.4 | 2.0 | 3.5 | 21 |
| Oral basket, length ^b | 29.3 | 30.0 | – | – | – | 15.0 | 40.0 | 21 |
| Anterior body end to first macronucleus nodule, distance | 13.4 | 13.0 | 4.4 | 1.0 | 32.8 | 7.0 | 27.0 | 21 |
| Macronucleus nodules, number ^c | 116.7 | 100.0 | – | – | – | 50.0 | 210.0 | 21 |
| Macronucleus nodules, length | 6.5 | 6.0 | 2.3 | 0.5 | 36.1 | 3.0 | 12.0 | 21 |
| Macronucleus nodules, width | 4.1 | 4.0 | 1.2 | 0.3 | 27.8 | 3.0 | 7.0 | 21 |
| Micronuclei, number ^c | 9.9 | 10.0 | – | – | – | 5.0 | 15.0 | 21 |
| Micronuclei, length | 3.4 | 3.0 | 0.5 | 0.1 | 16.1 | 3.0 | 5.0 | 21 |
| Micronuclei, width | 3.3 | 3.0 | – | – | – | 3.0 | 4.0 | 21 |
| Somatic ciliary rows, number | 34.9 | 35.0 | 2.0 | 0.4 | 5.7 | 32.0 | 39.0 | 21 |
| Basal bodies in a lateral kinety, number ^d | 84.2 | 78.0 | 17.0 | 3.7 | 20.2 | 50.0 | 128.0 | 21 |
| Dorsal brush rows, number | 3.0 | 3.0 | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 21 |
| Dorsal brush row 1, length | 22.5 | 23.0 | 4.1 | 0.9 | 18.3 | 17.0 | 35.0 | 21 |
| Dorsal brush row 2, length ^e | 23.4 | 22.0 | 4.5 | 1.0 | 19.1 | 17.0 | 34.0 | 21 |
| Dorsal brush row 3, length | 9.0 | 9.5 | 1.7 | 0.4 | 19.3 | 6.0 | 12.0 | 20 |
| Dorsal brush row 1, number of dikinetids | 23.7 | 22.0 | 4.9 | 1.1 | 20.8 | 18.0 | 38.0 | 21 |
| Dorsal brush row 2, number of dikinetids ^e | 28.9 | 30.0 | 6.2 | 1.3 | 21.3 | 19.0 | 40.0 | 21 |
| Dorsal brush row 3, number of dikinetids | 11.4 | 12.0 | 3.1 | 0.7 | 27.4 | 6.0 | 17.0 | 19 |

^a Data based on mounted, protargol-impregnated (Foissner 1991, protocol A), 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.

^b Rough values because faintly and very likely incompletely impregnated.

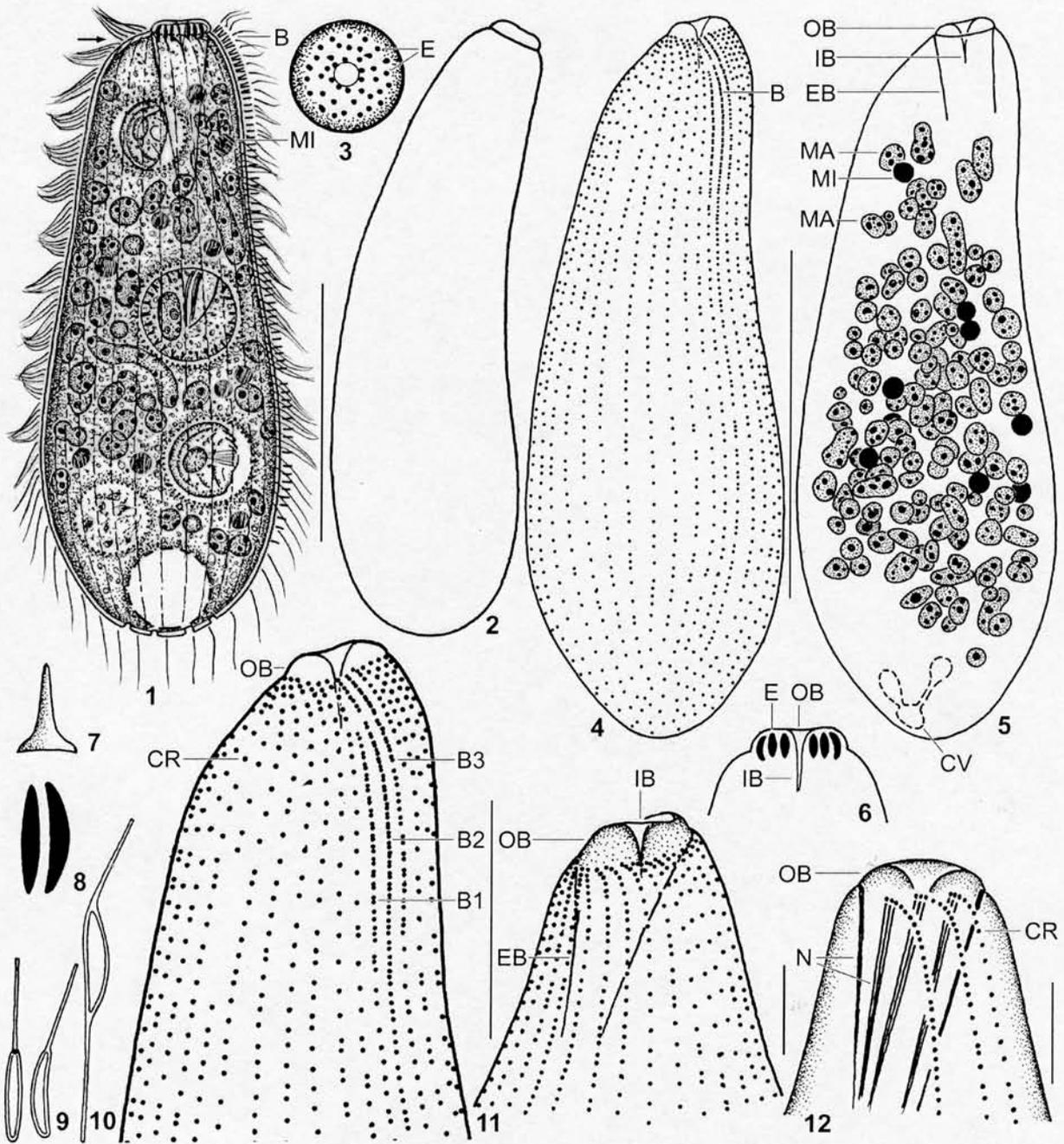
^c Rough values because difficult to count.

^d Ciliated and non-ciliated basal bodies.

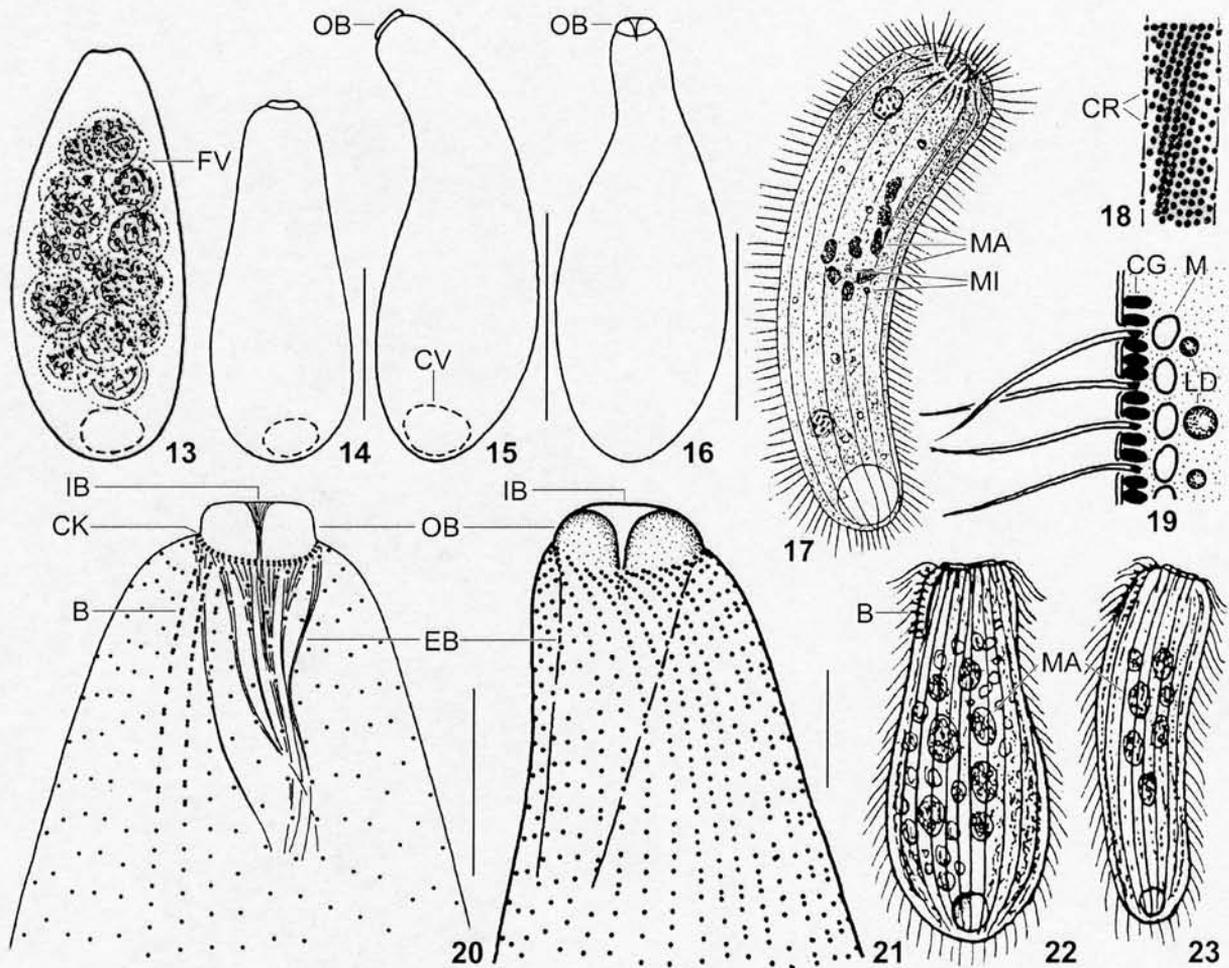
^e Without heteromorphic posterior end.

followed by 2 μm long, monokinetidal bristles extending to near posterior body end, in some specimens with a short anterior tail of ordinary cilia (Figs 1, 4, 10; Table 1).

Oral bulge usually inconspicuous at low magnification – although about 11 μm wide – because only 3–4 μm high in vivo, appears as a plate-like, slightly concave projection inconspicuously higher dorsally than ventrally; in some specimens almost as distinct as in *Echelyodon* or simply transverse truncate; outline circular to very broadly elliptical, slightly



Figs 1–12. *Enchelys micrographica* from life (1–3, 6–9) and after protargol impregnation (4, 5, 10–12). 1, 2 – Left side and ventral view of a representative specimen, which has a size of 120 x 45 μm and has ingested a *Frontonia depressa* (mid) and a *Leptopharynx costatus* (rear). The arrow marks the ventral shoulder. 3 – Frontal view of oral bulge. 4, 5, 10 – Dorsolateral view of holotype specimen, showing the ciliary pattern and nuclear apparatus; length 103 μm . The enlarged anterior portion (Fig. 10) shows the dorsal brush. Brush row 1 is shortened anteriorly in about 60% of specimens. Only the distal half of the oral basket (Fig. 5, EB) is impregnated. 6 – Location and shape of extrusomes in oral bulge. 7 – Many specimens have countless conical inclusions about 3 μm in size (cp. Fig. 24). 8 – Extrusomes are slightly curved and about 4 x 0.7 μm in size. 9 – Exploded extrusomes (length about 8 μm), the right one has a tube at both ends. 11 – The oral bulge surface is slightly curved like a propeller blade. 12 – Oral body portion, showing the nematodesmata originating from three oralized somatic monokinetids at anterior end of ciliary rows; for clearness, each second row has been deleted. B – dorsal brush, B1–3 – dorsal brush rows, CR – ciliary row, CV – contractile vacuole, E – extrusomes, EB – external oral basket, IB – internal oral basket, MA – macronucleus nodules, MI – micronuclei, N – nematodesmata, OB – oral bulge. Scale bars 10 μm (Figs 11, 12), 20 μm (Fig. 10), and 50 μm (Figs 1, 2, 4, 5).



Figs 13–23. *Enchelys micrographica* (13–16, 18, 19, 21) and related species (17, 20, 22, 23) from life (13, 14, 17–19, 22, 23) and after protargol impregnation (15, 16, 20, 21). **13** – When containing many food vacuoles, specimens appear dark at low magnification ($\leq \times 100$). **14–16** – A frequent (14, cp. with Fig. 22) and two rare shape variants. **17** – *Enchelys mutans*, length 113 μm (from Mermod 1914), differs from *E. micrographica* by the lower number of macronucleus nodules (6–12 vs. an average of 117) and ciliary rows (about 16 vs. 35). **18, 19** – Surface view and optical section showing the dense cortical granulation. The individual granules are about $1.2 \times 0.6 \mu\text{m}$ in size. **20, 21** – Oral portion of *Enchelyodon* (from Foissner 1984) and *Enchelys micrographica*, which greatly differ in the oral ciliary pattern: the oral basket rods of the former originate from a dikinetidal circumoral kinety (CK), while those of the latter originate from some oralized somatic monokinetids at the anterior end of the ciliary rows (see also Fig. 12!). The oral bulge may be similar in both genera, but is usually less conspicuous in *Enchelys*. **22, 23** – *Enchelys mutans* according to Kahl (1930). The left specimen is moderately nourished and 100 μm long, while the right one is hungry and only 60 μm long. Note the few macronucleus nodules (6–12), a main difference to *E. micrographica* (on average 117 nodules). B – dorsal brush, CG – cortical granules, CK – circumoral kinety, CR – ciliary row, CV – contractile vacuole, EB – external oral basket, FV – food vacuoles, IB – internal oral basket, LD – lipid droplets, M – mitochondrion, MA – macronucleus nodules, MI – micronuclei, OB – oral bulge. Scale bars 10 μm (Figs 20, 21) and 50 μm (Figs 14–16).

twisted making bulge shaped like a propeller blade, respectively, like a recumbent number 8 (Figs 1, 2, 4, 10–16, 21; Table 1). Oral basket obconical, weakly impregnated and thus possibly longer than measured, composed of about 30 μm long nematodesmata originating from anteriormost kinetids of somatic ciliary rows, i. e., from the oralized somatic

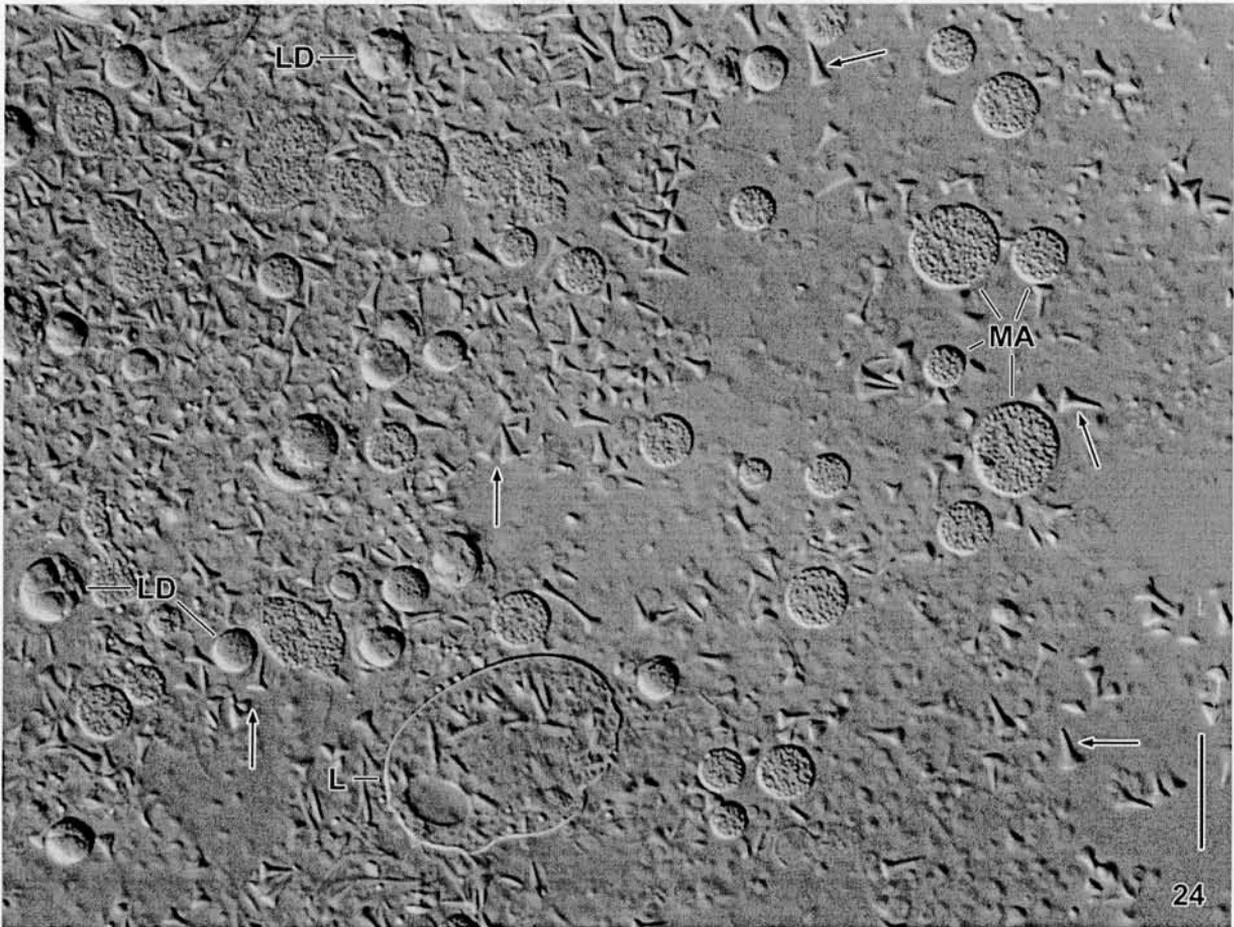


Fig. 24. *Enchelys micrographica*, a squashed specimen showing the cell contents, i. e., macronucleus nodules (MA), lipid droplets (LD), a hardly digested *Leptopharynx costatus* (L), and countless conical structures (arrows), which are possibly deposited on the surface of the resting cyst. Scale bar 10 μ m.

monokinetids described above. Internal oral basket inconspicuous, about 6 μ m long (Figs 1, 5, 11, 12, 21; Table 1).

Occurrence and ecology: As yet found only at type locality, where it was rather abundant in the non-flooded Petri dish culture. The type locality is the bank of the Felberbach, where tree mosses were collected, air-dried, stored in a plastic bag for a month, and then used to set up a non-flooded Petri dish culture. The Felberbach is a stream in the SE region of the town of Salzburg, i. e., in the surroundings of the church of the village of Aigen.

DISCUSSION

Three congeners are similar to *Enchelys micrographica* nov. spec., namely, *E. mutans* (Mermod, 1914) Kahl, 1930 (Figs 17, 22, 23); *E. multinucleata* Dragesco and Dragesco-Kernéis, 1979; and *E. polynucleata* (Foissner, 1984) Foissner et al., 2002.

Enchelys mutans differs from *E. micrographica* mainly by the number of macronucleus nodules: 6–12 vs. over 100. Mermod (1914) investigated the nuclear apparatus with acetic

methyl-green in over 100 specimens and reached the following conclusion: "Ils sont en nombre variable". Unfortunately, Mermod (1914) did not provide any number, but illustrated eight nodules and about 16 ciliary rows in the type specimen (Fig. 17). Kahl (1930), who reinvestigated *E. mutans*, found 6-12 rather large macronucleus nodules and about 25 ciliary rows (Figs 22, 23). These data show that the number of macronucleus nodules is indeed very different in *E. mutans* and *E. micrographica*; further, the number of ciliary rows (16–25 vs. 32–39) is considerably higher in the latter. Possibly, the shape and size of the extrusomes is also different.

Enchelys multinucleata, which was redescribed by Berger et al. (1984), is considerably larger (\bar{x} 186 vs. 107 μm in protargol preparations) and twice as slender (~5:1 vs. 2.6:1) as *E. micrographica*. Further the extrusomes are rod-shaped (vs. bluntly fusiform) and longer (8 μm vs. 4 μm).

Enchelys polynucleata differs from *E. micrographica* mainly by the extrusomes (14 μm long rods vs. 4 μm long and bluntly fusiform) and the shape and size of the oral bulge (distinctly elliptical and about 20 x 5.7 μm vs. circular and about 11 μm). See Foissner (1984) and Foissner and Al-Rasheid (2007) for detailed figures of the oral bulge of *E. polynucleata*.

ACKNOWLEDGEMENTS

I thank Prof. Erich Steiner, president of the "Mikroskopische Gesellschaft Wien", for inviting this contribution. Financial support was provided by the Austrian Science Foundation (FWF – project P-19699-B17) and a Salzburg Research Fellowship, project P-143001-04. The technical assistance of Mag. Barbara Harl, Manuela Pölsler, Robert Schörghofer, and Andreas Zankl is greatly acknowledged.

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