Linzer biol. Beitr.	29/1	349-368	31.7.1997

Morphology and infraciliature of *Microthorax pusillus* ENGELMANN 1862 and *Spathidium deforme* KAHL 1928, two ciliates (Protozoa, Ciliophora) from activated sludge

A. R. LEITNER & W. FOISSNER

Abstract: Two populations each of *Microthorax pusillus* ENGELMANN 1862 and *Spathidium deforme* KAHL 1928 were found in well-performing activated sludge plants. Both species are first records for the activated sludge biotope and possibly prefer alpha-mesosaprobic conditions. They are re-defined with neotypes deposited in (LI), using live observation, silver impregnation, and morphometry. The body shape of *M. pusillus* is highly variable and depends on environmental conditions, whereas the cortical sculpturing and the infraciliature remain rather constant. Separation of *M. pusillus* from *M. transversus* FOISSNER 1985 and *M. australis* FOISSNER & O'DONOGHUE 1990 requires silver impregnation and live observation. *Spathidium deforme* has blunt, thorn-shaped extrusomes, an oblong macronucleus, and 25 somatic kineties on average. It differs from typical congeners in that the ciliary rows are completely separated from the circumoral kinety, as in *Arcuospathidium*.

1 Introduction

Ciliates from activated sludge have been studied extensively during the past three decades, but mainly from an ecological point of view (AESCHT & FOISSNER 1992; CURDS 1975; LEE & WELANDER 1996; MADONI 1988; RATSAK et al. 1996; SALVADÓ et al. 1995). Taxonomy was largely neglected, in spite of many obvious misidentifications and its importance for community analysis and, in turn, evaluation of sludge quality (CINGOLANI et al. 1991; CURDS 1975; MADONI 1994; SALVADÓ et al. 1995); furthermore, activated sludge is an excellent source for new, rare, and insufficiently known species (e. g. AESCHT & FOISSNER 1992; AUGUSTIN & FOISSNER 1989, 1992; BECARES & FOISSNER 1994; LEITNER & FOISSNER 1997; OBERSCHMIDLEITNER & AESCHT 1996). The species redescribed in this paper reaffirm our previous claims regarding the combination of thorough live observations with various silver techniques. Such descriptions are not only valuable for taxonomists but also for ecologists because they provide characters recognizable in live specimens, i.e. reliable identifications can usually be obtained without applying the often rather complicated silver techniques.

2 Material and Methods

Microthorax pusillus and *Spathidium deforme* were found in June 1995 in activated sludge of a two stage sewage-treatment plant at Siggerwiesen, Salzburg, Austria. Small quantities of sludge were placed in one-way Petri-dishes and enriched with a sterilised, crushed wheat grain. Both species developed abundantly in these raw cultures after about 14 days.

A second population each of *Microthorax pusillus* and *Spathidium deforme* developed in February 1996 under the same laboratory conditions from activated sludge of a plant at Bad Waltersdorf, Styria, Austria. These populations were not studied in detail but served for checking some key characters.

Cells from populations I and II were studied in vivo using a high-power oil immersion objective and differential interference contrast. The infraciliature and other cytological details of the Siggerwiesen populations were revealed with protargol. Specimens from both populations of *Microthorax pusillus* were also impregnated with the "dry" silver nitrate method. See FOISSNER (1991) for detailed description of the methods used.

Counts and measurements on silvered specimens were performed at a magnification of X 1,000. In vivo measurements were conducted at a magnification of X 250 - 1,000. Although these provide only rough estimates, it is convenient to give such data as specimens usually shrink in preparations and/or contract during fixation. Statistics were calculated according to textbooks. Illustrations of live specimens were based on free-hand sketches and micrographs, those of impregnated cells were made with a camera lucida. All figures are orientated with the anterior end of the organism directed to the top of the page. Terminology is according to CORLISS (1979) and FOISSNER (1984, 1985), who provided detailed schemata of the spathidiid and microthoracid infraciliature.

3 Results

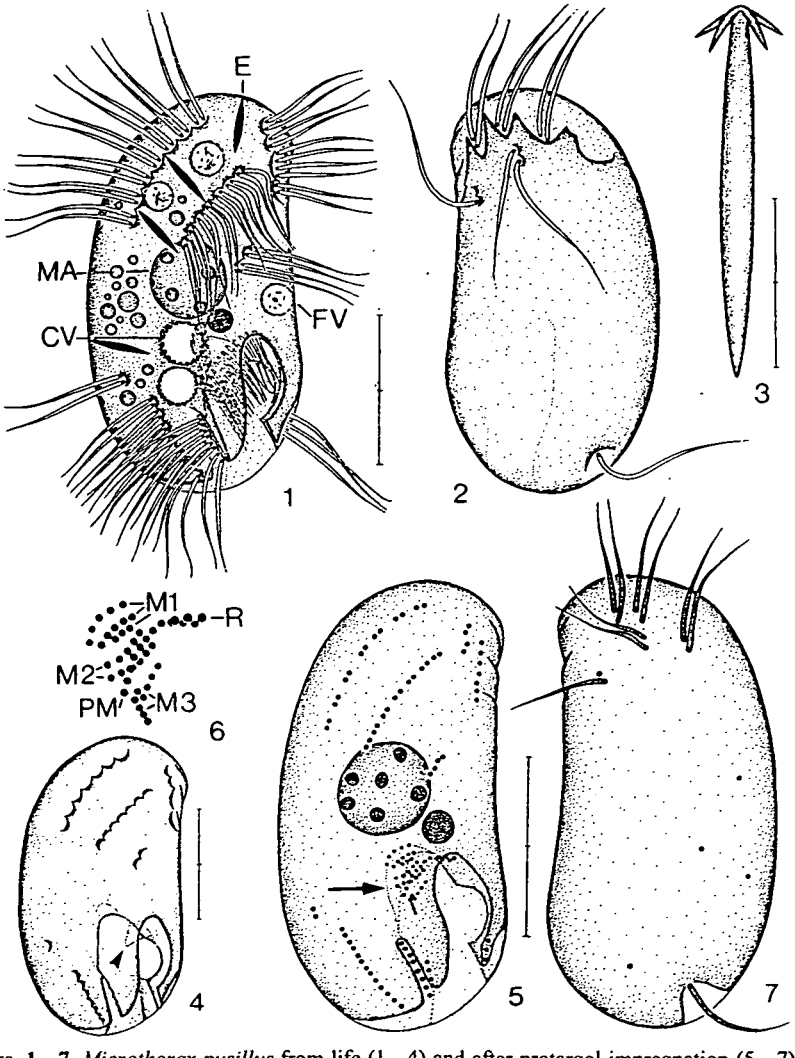
3.1 Redescription of *Microthorax pusillus* ENGELMANN 1862

Neotype material

Two slides each with protargol (WILBERT technique) and silver nitrate-impregnated (KLEIN-FOISSNER method) cells have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens are marked by a black ink circle on the cover glass. Accession numbers: 1997/17-20.

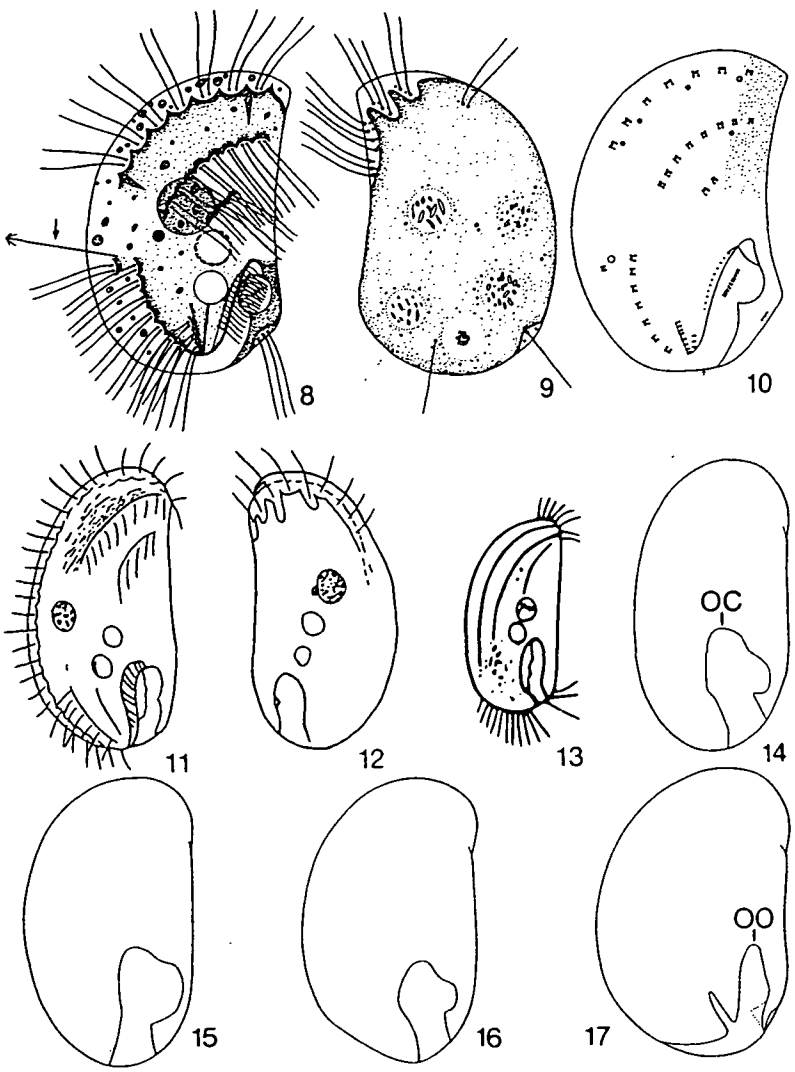
Redescription (Figs. 1 - 32, Table 1)

Size in vivo 25 - 33 x 13 - 17 µm, hardly changed by preparation procedures (Tab. 1), laterally flattened 2:1 to 3:1. Shape highly dependent on culture conditions, specimens from young, flourishing dishes oblong to slightly reniform (Figs. 1, 2, 4,

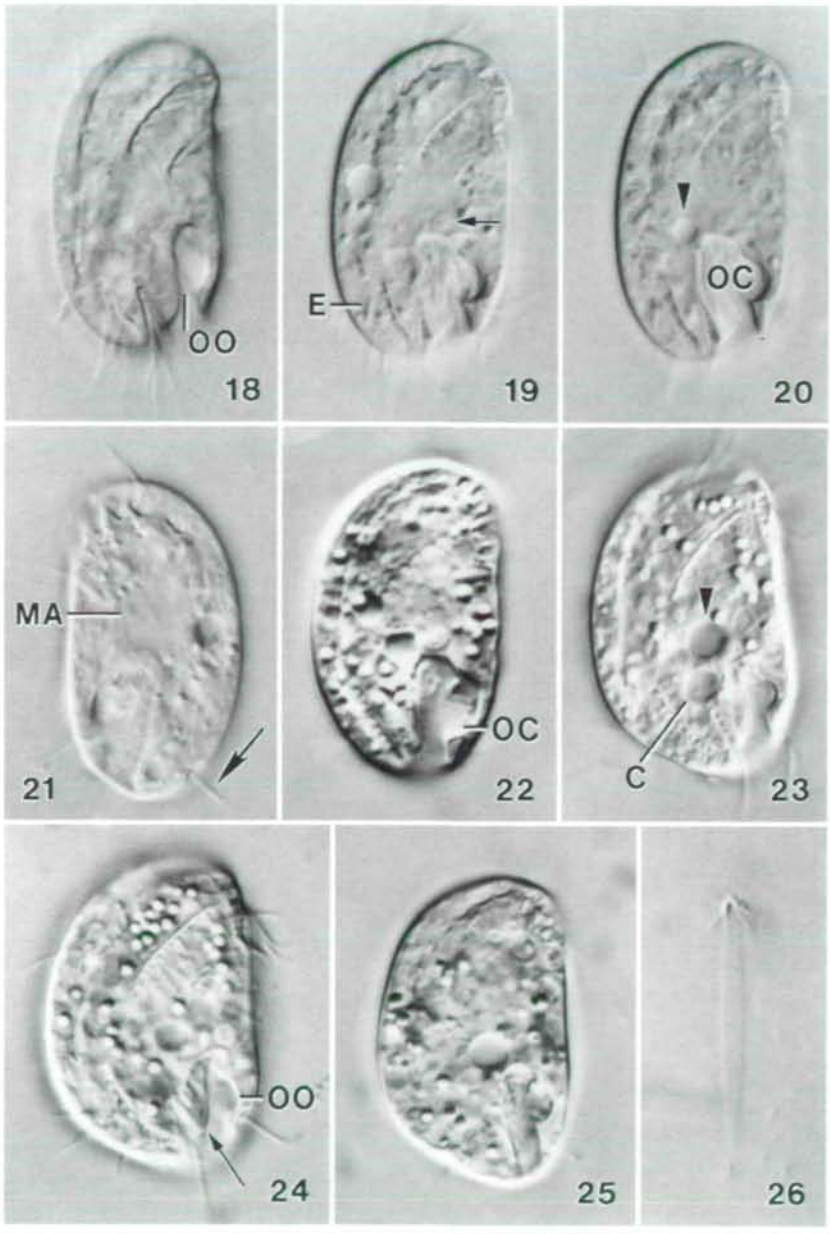


Figs. 1 - 7. *Microthorax pusillus* from life (1 - 4) and after protargol impregnation (5 - 7). **1, 2:** Right and left lateral view of typical specimen from a young culture. **3:** Discharged extrusome. **4:** Right lateral view showing somatic and oral cortical sculpturing. Arrowhead marks triangular process within oral cavity. **5 - 7:** Infraciliature of right and left side. Arrow denotes oral infraciliature shown in detail in Fig. 6. CV – contractile vacuole, E – extrusome, FV – food vacuole, MA – macronucleus, M1 - 3 – adoral membranelles, PM – paroral membrane, R – row of granules above left end of paroral membrane. Scale bar division: 5 µm.

14, 18 - 21), those from old, extincting cultures almost semicircular because broader and with more distinctly curved left margin; furthermore, such specimens often have a rather distinctly protruding oral area (Figs. 15 - 17, 22 - 25). Macronucleus in mid-body, globular, with some irregularly distributed, roundish nucleoli. Micronucleus adjacent to macronucleus, usually globular, in 8 % of specimens (n = 83) elongate-ellipsoidal.



Figs. 8 - 17. *Microthorax pusillus* from life (8, 9, 11 - 17) and after dry silver nitrate impregnation (10); Figs. 8 - 10 from FOISSNER (1979), Figs. 11, 12 from KAHL (1931), Fig. 13 from ENGELMANN (1862), Figs. 14 - 17 originals. **8, 9, 11 - 13:** Right and left lateral views, length 27 - 32 µm. Arrow marks extrusome. **10:** Infraciliature of right side and silverline system (stippled). **14 - 17:** Shape variability in specimens from a young culture (14) and an extincting culture (15 - 17) of population I. OC – oral cavity, OO – oral opening.



Figs. 18 - 26. *Microthorax pusillus* from life. **18 - 21:** Specimen from a young, flourishing culture of population I shown in successive focus planes from right to left side. Small arrow marks micronucleus. Large arrow denotes cilium at posterior end of kinety 6. Arrowhead indicates contractile vacuole. **22 - 24:** Shape variants of specimens from an aged culture of population I. Arrow marks roof of oral cavity. Arrowhead denotes contractile vacuole. **25:** Typical cell from population II. **26:** Discharged extrusome, C – cytopyge, E – extrusome, MA – macronucleus, OC – oral cavity, OO – oral opening.

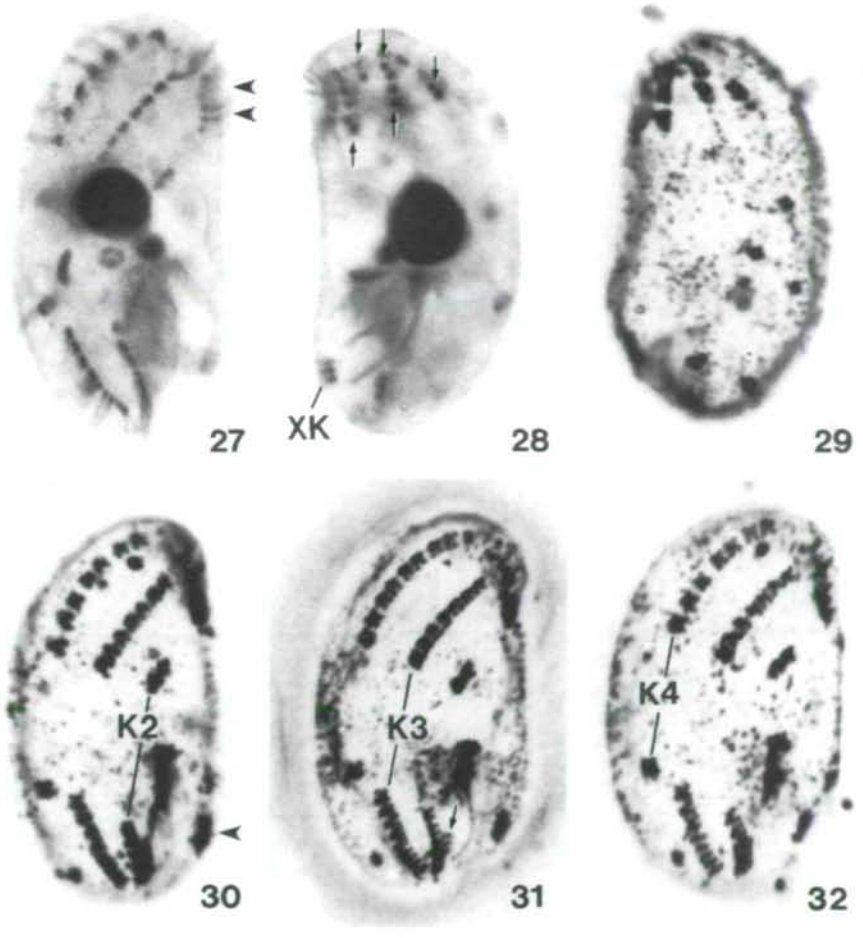
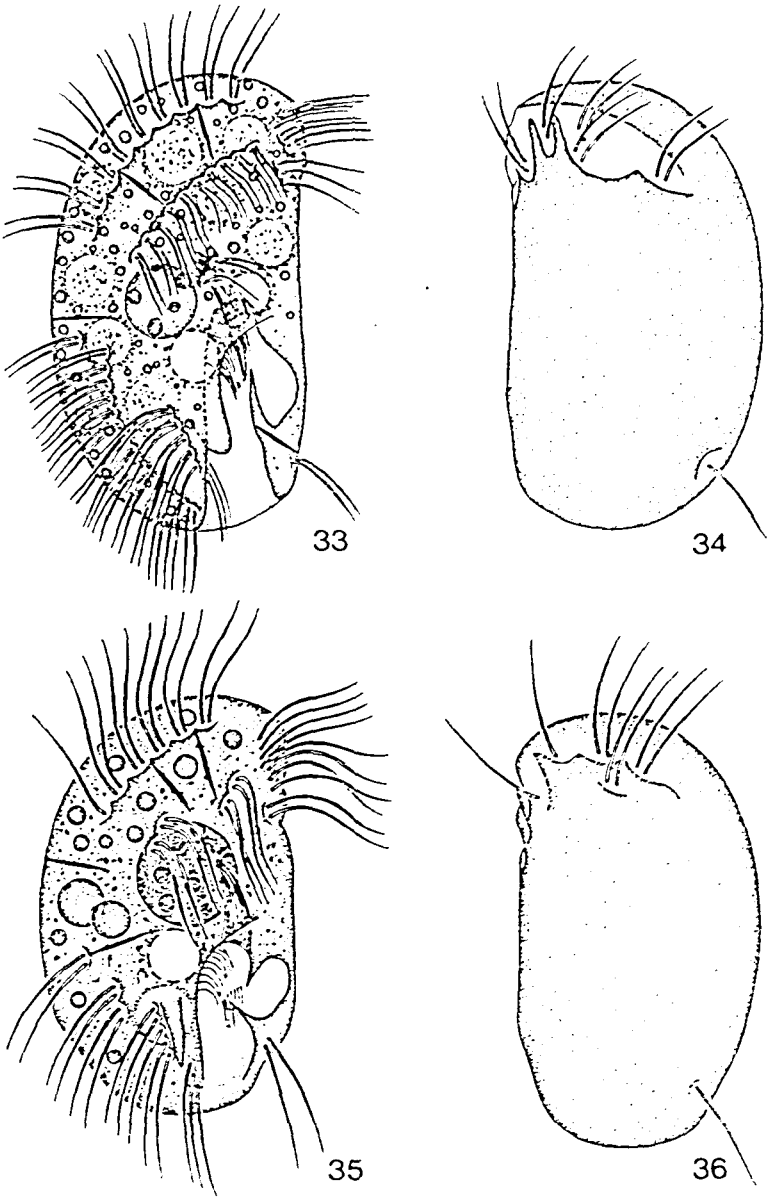


Fig. 27 - 32. *Microthorax pusillus* after protargol (27, 28) and dry silver nitrate (29 - 32) impregnation. **27, 28:** Infraciliature of right and left side. Downward arrows denote dikinetids from kinety 5, upward arrows mark dikinetids of kineties 6 and 7. Arrowheads mark preoral kineties 1 and 2. **29 - 32:** Infraciliature of left and right side. Arrow denotes kinety 1 (K1). Arrowhead marks x-kinety. K2 - 4 – somatic kineties 2 - 4, XK – x-kinety.

Contractile vacuole at upper right of oral cavity, about 2.5 µm across. Cytopyge close underneath contractile vacuole and of similar size, pulsates at long intervals. Trichocysts fusiform, about 4 - 5 x 0.5 - 0.7 µm, often attached to small protrusions separating individual kinetids (cilia), but also scattered in cytoplasm (Fig. 1); extruded organelles about 21 x 2 µm, with four anchor-like processes at distal end, each about 3 µm long and difficult to recognize because very hyaline (Figs. 3, 26). Cortex rigid and colourless. Cytoplasm transparent, contains yellowish and colourless globules, 0.5 - 1.5 µm across. Food vacuoles about 2 µm in diameter, often difficult to recognize because almost as transparent as cytoplasm and

containing only few, colourless bacteria. Rotates about main body axis when swimming, usually, however, crawls rapidly on sludge flocks.



Figs. 33 - 36. *Microthorax* species similar to *M. pusillus*. **33, 34:** *Microthorax transversus*, right and left lateral view from life and after protargol impregnation, length 35 µm. **35, 36:** *Microthorax australis*, right and left lateral view from life and after protargol impregnation, length 20 µm.

Most somatic cilia 7 - 10 µm long, those of kineties 6 and 7 about 12 µm long, invariably arranged in 7 rows interrupted at mid-body. Ciliary rows at margin of crenelated furrows, except for anterior portion of kinety 5, where dikinetids are in lobe-like cavities; crenelation of anterior portions of kineties 2, 3, 6, and 7 directed to left, that of posterior portions and of other kineties directed to right (Figs. 1, 2, 4, 18, 19, 21). Somatic kinety 1 (K1) with 3 - 5 basal bodies (BB); K2 anterior with 4, posterior with 8 - 11 BB; K3 anterior with 12 - 20, posterior with 9 - 16 BB; K4 anterior with 14 - 18, posterior with 2 BB; K5 anterior with 6, posterior with 1 - 2 BB; K6 anterior with 2, in middle with 1 (sometimes lacking) and posterior usually with 1, rarely 2 BB (one parasomal sac?); K7 anterior with 2, posterior with 1 BB (Figs. 5, 7, 27 - 32; Tab. 1). Kinety 1 and single basal bodies in K5, K6 and K7 unciliated, except for posterior K6. Anterior dikinetid of K6 and K7 with only one cilium each, in protargol preparations cilium often lacking (lost during fixation?); however, K6 shows some natural variability, i.e. in 2 out of 66 specimens both basal bodies were ciliated, in 7 cases only the posterior and in 2 cases only the anterior basal body was ciliated. Preoral kinety 1 (P1) with three basal bodies, of which only the anterior two are ciliated, P2 and P3 with four ciliated basal bodies each. X-kinety usually with 3, in 1 out of 30 specimens with 4 ciliated basal bodies (Figs. 5, 7, 27 - 32; Tab. 1).

Oral opening almost parallel to main body axis and roughly dumb-bell shaped due to curved right margin and thorn-like cortical thickening projecting inwards from left margin (Figs. 1, 4, 5, 17 - 19, 24). Oral cavity roughly morel-shaped, extends, gradually deepening and widening, from posterior left end to near cell centre, its right half thus covered roof-like by right side somatic cortex (Figs. 4, 14 - 16, 19, 20, 25); roof appears as comparatively large, triangular lobe because bordered at right by deep groove containing posterior portion of somatic kinety 2 (Figs. 1, 4, 5, 18, 24). In mid-anterior of oral cavity, a small, triangular process distinctly vibrating when adoral cilia beat (Fig. 4). Right margin of buccal cavity thickened. Adoral membranelles at bottom of right anterior portion of oral cavity, entirely covered by right side somatic cortex (roof), tiny, details thus difficult to recognize (Figs. 5, 6). Membranelle 1 very likely made up of three closely spaced kineties; membranelle 2 parallel to membranelle 1 and composed of only two kineties; membranelle 3 also made up of two kineties, which, however, form an angle of about 130° to each other, producing peculiar, hook-like pattern. Paroral membrane composed of 9 - 12 single basal bodies forming slightly curved row, in surface view above adoral membranelle 2; at its left end 3 - 4 heavily impregnated granules forming a minute row, which very likely does not belong to the paroral because it completely or partially covers the paroral's left end, i.e. is in a slightly different (nearer to the cell surface) focus level (Figs. 5, 6). Oral basket neither recognizable in vivo nor after protargol impregnation. Silverline system granular (Figs. 29 - 32).

Table 1. Morphometric characteristics from *Microthorax pusillus*[1)]

Character	P	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length (in vivo)	I	26.9	26.0	2.3	0.5	8.7	25.0	33.0	20
Body, length (in vivo)	II	28.2	28.0	2.4	0.5	8.4	25.0	32.0	20
Body, length	I	23.6	24.0	2.5	0.5	10.7	19.0	30.0	30
Body, width (in vivo)	I	14.8	15.0	1.3	0.3	8.8	13.0	17.0	20
Body, width (in vivo)	II	18.4	19.0	1.9	0.4	10.5	15.0	21.0	20
Body, width	I	12.6	13.0	1.6	0.3	12.9	10.0	17.0	30
Macronucleus, diameter (in vivo)	I	5.3	5.0	1.0	0.3	19.4	4.0	7.0	11
Macronucleus, diameter (in vivo)	II	6.7	6.5	0.9	0.3	13.5	5.0	8.0	13
Macronucleus, length	I	4.9	5.0	0.6	0.1	11.6	4.0	6.3	30
Macronucleus, width	I	4.5	4.0	0.7	0.1	15.9	3.8	6.0	30
Micronucleus, diameter (in vivo)	I	1.6	1.5	-	-	-	1.5	2.0	11
Micronucleus, length	I	1.6	1.3	0.6	0.1	35.2	1.3	4.0	30
Micronucleus, width	I	1.4	1.3	0.2	0.3	11.3	1.2	1.8	30
Distance between anterior end of cell and upper end of macronucleus	I	8.5	8.5	1.2	0.2	14.3	6.5	11.5	30
Somatic kineties, no. on right side	I	4.0	4.0	0.0	0.0	0.0	4.0	4.0	30
Somatic kineties, no. on left side	I	3.0	3.0	0.0	0.0	0.0	3.0	3.0	30
Somatic kinety 1, no. of basal bodies	I	3.4	3.0	0.6	0.1	18.3	3.0	5.0	30
Somatic kinety 2, no. of basal bodies	I	13.2	13.0	0.9	0.2	6.9	12.0	15.0	30
Somatic kinety 3, no. of basal bodies	I	27.4	27.0	2.9	0.5	10.5	23.0	36.0	30
Somatic kinety 4, no. of basal bodies	I	16.3	16.0	1.1	0.2	6.5	16.0	20.0	30
Somatic kinety 5, no. of basal bodies	I	7.9	8.0	-	-	-	7.0	8.0	30
Somatic kinety 6, no. of basal bodies	I	4.0	4.0	0.5	0.1	11.4	3.0	5.0	30
Somatic kinety 7, no. of basal bodies	I	2.9	3.0	-	-	-	2.0	3.0	30
Preoral kineties, number	I	3.0	3.0	0.0	0.0	0.0	3.0	3.0	30
Preoral kinety 1, no. of basal bodies	I	3.0	3.0	0.0	0.0	0.0	3.0	3.0	30
Preoral kinety 2, no. of basal bodies	I	4.0	4.0	0.0	0.0	0.0	4.0	4.0	30
Preoral kinety 3, no. of basal bodies	I	4.0	4.0	0.0	0.0	0.0	4.0	4.0	30
x-kinety, no. of basal bodies	I	3.0	3.0	-	-	-	3.0	4.0	30
Paroral membrane, no. of basal bodies	I	9.8	9.0	1.0	0.2	9.9	9.0	12.0	17

[1)] All data based, if not stated otherwise, on randomly selected, protargol impregnated specimens. Measurements in µm. CV — coefficient of variation in %, M — median, Max — maximum, Min — minimum, n — number of specimens investigated, P — population (I — from Siggerwiesen, II — from Bad Waltersdorf), SD — standard deviation, SE — standard error of mean, $\bar{x}$ — arithmetic mean.

Occurrence and ecology

Microthorax pusillus reproduced well in putrescent activated sludge cultures and was associated with several other ciliates, e. g. *Acineria uncinata*, *Cinetochilum margaritaceum*, *Dexiotricha* sp., *Litonotus lamella*, *Spathidium deforme* (described below), *Spirostomum teres*, *Stentor coeruleus*, *Stentor roeselii*, *Thigmogaster oppositevacuolatus*, and *Trithigmostoma cucullulus*. *Microthorax pusillus* has been classified as a sharp indicator of alpha-mesosaprobity by FOISSNER et al. (1994). This is supported by our records from well-performing activated sludge plants, where *M.*

pusillus has never yet been found, possibly because it is too small to be recognized in routine investigations. The populations survived for weeks in the sludge cultures, where saprobity slowly decreased, supporting the beta-mesosaprobic portion (2 points) in its valency spectrum.

3.2 Redescription of *Spathidium deforme* KAHL 1928

Improved diagnosis

Size in vivo about 95 x 40 µm, barrel-shaped to inversely pyriform. Oral bulge cuneate to elongate-ellipsoidal, inconspicuous because short, flat and narrow. Macronucleus usually slenderly reniform, 26 x 8 µm on average. Extrusomes 4 x 0.6 µm, thorn-shaped, form single row in oral bulge. 25 somatic kineties on average, distinctly separate from circumoral kinety.

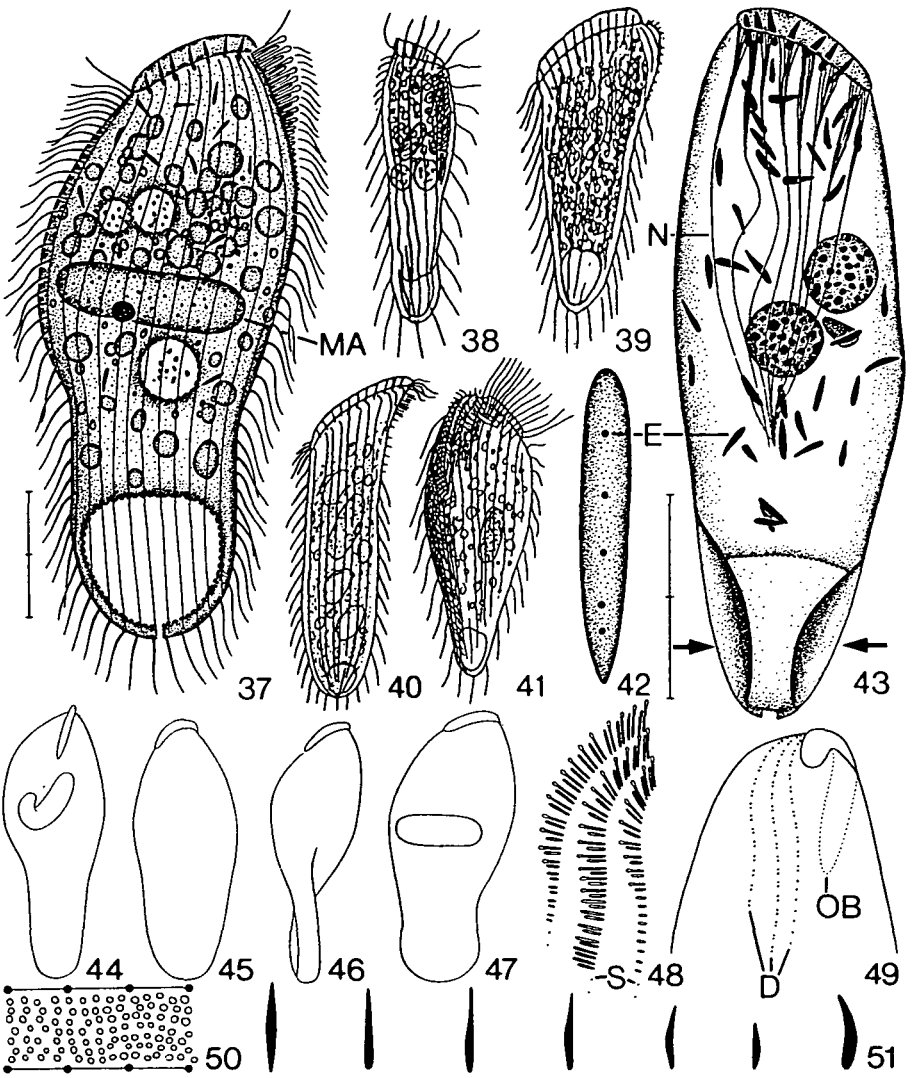
Neotype material

Two slides with protargol (WILBERT technique) impregnated cells have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens are marked by a black ink circle on the cover glass. Accession numbers: 1997/21, 22.

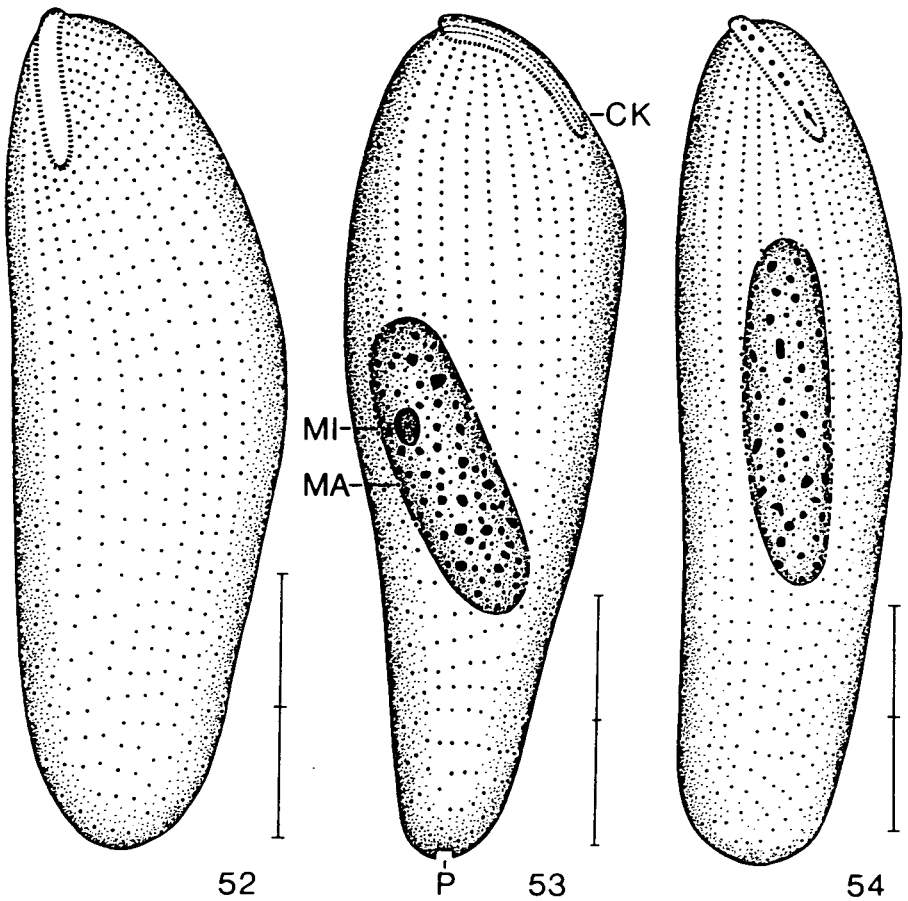
Redescription (Fig. 37, 38, 42 - 62, Table 2)

The two populations studied highly resembled each other, especially in having a very similar body and macronucleus shape as well as identical shape, size and arrangement of the extrusomes. The following description is based, unless otherwise stated, on individuals from population I.

Size in vivo 80 - 115 x 30 - 55 µm, population II more variable, viz. 60 - 140 µm long. Well-fed specimens barrel-shaped to inversely pyriform and circular in cross section; starving cells of similar shape but distinctly flattened and occasionally wrinkled and/or twisted in posterior body half, giving cells a somewhat deformed appearance; distressed individuals and some of the small specimens roughly ellipsoidal (Figs. 37, 43 - 47, 57, 58). Nuclear apparatus rather variable, in or near centre of cell, of 81 specimens investigated 87 % had one oblong macronucleus, 10 % had two globular pieces, and 3 % had four; very likely, these were reorganizers. Macronucleus highly variable in shape and length : width ratio (2.5 - 5.5:1, average 3:1; Tab. 2), in 25 % (n = 69) of specimens elongate-ellipsoidal (Figs. 37, 47, 53, 54, 58), in 75 % reniform or roughly C-shaped (Figs. 44, 55); in population II usually slightly less slender than in population I (Figs. 47, 58); contains many minute granules (nucleoli?) and, in protargol preparations, many 0.3 - 2.0 µm sized (chromatin?) patches. Micronucleus ellipsoidal, in vivo about 4 - 5 µm across, recognizable only when attached to macronucleus, usually indistinguishable from cytoplasmic inclusions, in 40 % (n = 81) of specimens not

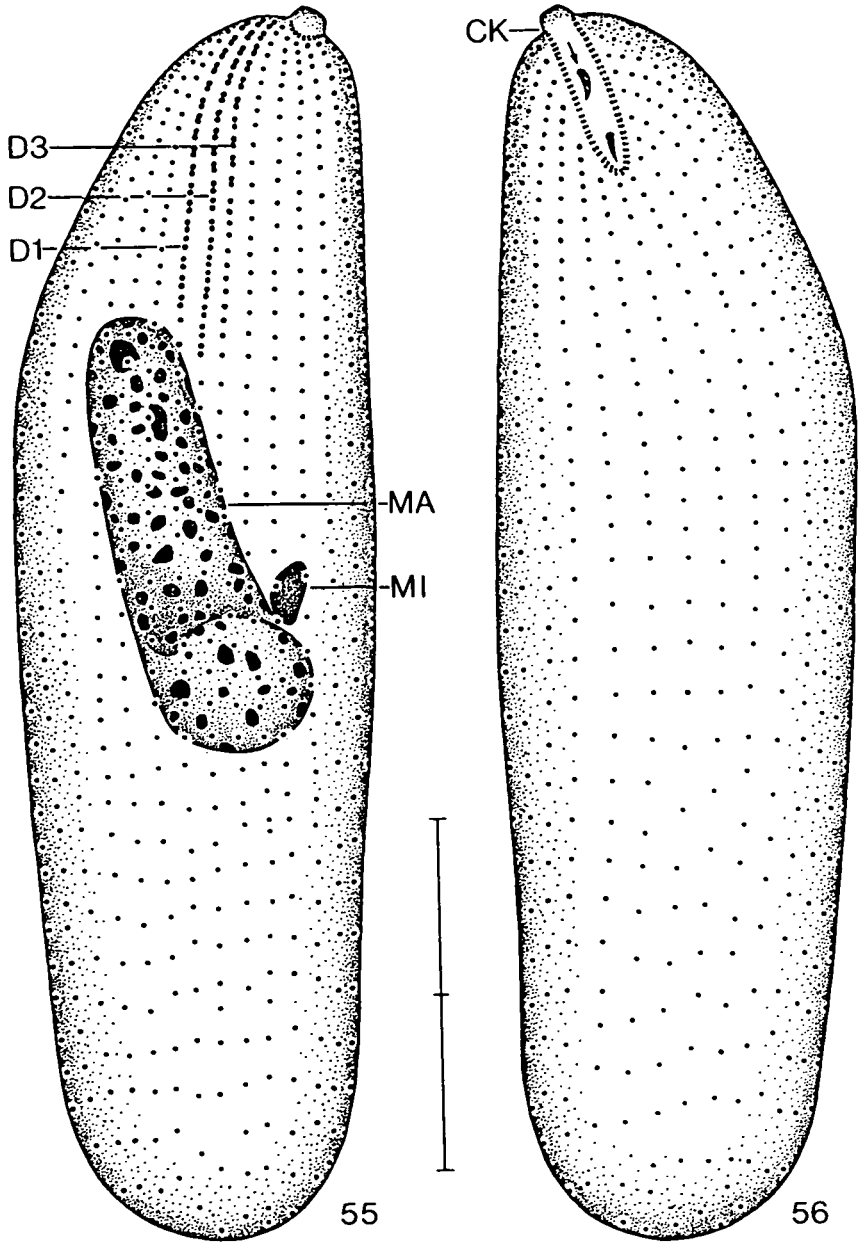


Figs. 37 - 51. *Spathidium deforme* (37, 38, 42 - 51) and related species (39 - 41) from life (37 - 42, 44 - 51) and after protargol impregnation (43, 49). **37, 38:** Left (original) and right lateral view from KAHL (1928), length 70 - 80 µm. **39 - 41:** *Spathidium gibbum*, *S. paucistriatum*, and *S. pectinatum* from KAHL (1926, 1930a,b), length 70 - 100 µm. **42:** Frontal view of oral bulge. **43:** Right lateral view of specimen with two macronuclear nodules. Arrows denote wrinkled posterior body portion. **44:** Ventral view. **45, 46:** Shape of a distressed and a starving individual. **47:** Specimen from population II. **48:** Dorsal brush. **49:** Spatial relationship between dorsal brush and oral bulge. **50:** Cortical granulation. **51:** Variability of extrusomes, drawn to scale. D – dorsal brush, E – extrusomes, MA – macronucleus, N – nematodesmata, OB – oral bulge, S – somatic monokinetids with normal cilia. Scale bar division: 10 µm.

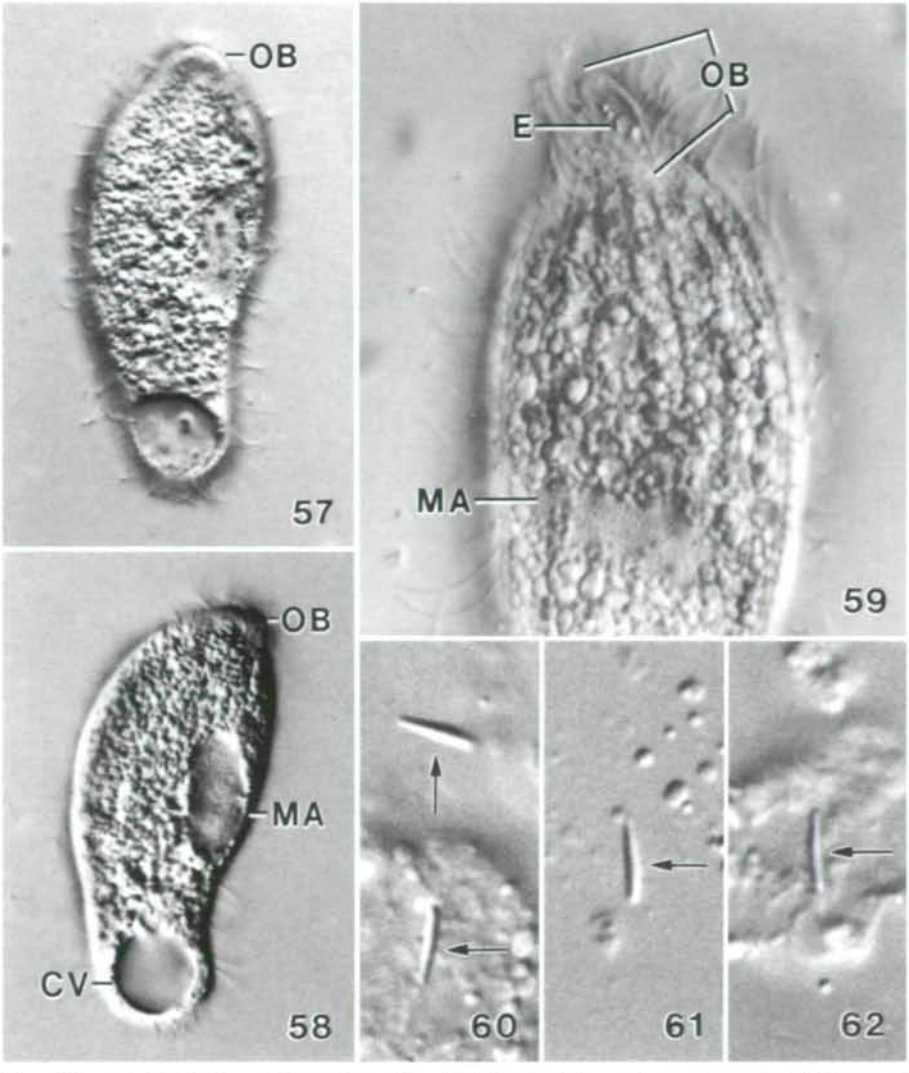


Figs. 52 - 54. *Spathidium deforme*, infraciliature after protargol impregnation. **52:** Ventral view. **53:** Right lateral view. **54:** Ventro-lateral view. Arrow marks single row of extrusomes in oral bulge. CK – circumoral kinety, MA – macronucleus, MI – micronucleus, P – excretory pore of contractile vacuole. Scale bar division: 10 µm.

impregnated with protargol; in 23 out of 32 individuals investigated located in slight indentation of macronucleus, in others slightly distant. Contractile vacuole in rear end, sometimes rather huge (up to 25 µm), with single excretory pore in centre of posterior pole. Extrusomes 2.5 - 5 x 0.5 - 0.7 µm, i.e. rather blunt, usually straight and thorn-shaped, rarely colon-shaped, fusiform, or slightly clavate; arranged in single, loose row in oral bulge and irregularly distributed in cytoplasm, often difficult to recognize in well-fed specimens (Figs. 37, 42, 43, 51, 54, 56, 59 - 62). Cortex flexible, contains many minute (≤ 0.5 µm), colourless granules arranged in 6 - 8 indistinct rows between two kineties each (Fig. 50). Cytoplasm of well-fed specimens conspicuously dark at X 100 due to innumerable, highly refractile, 1 - 6 µm sized fat globules and some 5 - 10 µm sized food vacuoles with indefinable contents. Swims moderately fast, rotating about main body axis.



Figs. 55, 56. *Spathidium deforme*, infraciliature of dorsal and ventral side after protargol impregnation. Arrow marks row of extrusomes in oral bulge. CK – circumoral kinety, D1 - 3 – kineties of dorsal brush, MA – macronucleus, MI – micronucleus. Scale bar division: 10 µm.



Figs. 57 - 62. *Spathidium deforme* from life. **57, 58:** Typical specimens from Bad Waltersdorf population. The cytoplasm is packed with small fat globules. **59:** Ventral view of anterior body half showing single row of extrusomes (E) in oral bulge (OB). **60 - 62:** Shape variability of extrusomes (arrows). CV – contractile vacuole, E – extrusomes, MA – macronucleus, OB – oral bulge.

Cilia 8 - 10 µm long. Somatic kineties anteriorly slightly more densely ciliated than posteriorly, distinctly separate from circumoral kinety with which they form almost right angles dorsally on both sides of the oral bulge, and acute angles ventrally, more pronounced on right than on left side (Figs. 37, 52 - 56). Dorsal brush composed of three rows of dikinetids, kineties extend in flat grooves and continue posteriorly as normal somatic ciliary rows (Figs. 49, 55; Tab. 2); anterior cilium of dikinetids up to

6 µm long and markedly inflated distally, posterior cilium up to 4 µm long and rod-shaped, length of cilia decreasing in rear portion of kineties 1 and 3; row 3 continues with single, 1 - 2 µm long bristles to level of posterior end of row 2 (Fig. 48).

Oral bulge slightly convex and inclined ventrally, broadly cuneate to elongate-ellipsoidal, inconspicuous because flat and narrow and on broadened anterior end of body, appears as knob-like protrusion in dorsal view (Figs. 37, 42, 44, 49, 55, 59). Circumoral kinety continuous, i.e. not fragmented, composed of closely spaced dikinetids associated with long, fine nematodesmata forming wedge-shaped bundles extending beyond mid-body (Figs. 43, 52 - 54, 56).

Table 2. Morphometric characteristics from *Spathidium deforme*[1)]

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length (in vivo)	96.7	95.0	9.4	2.5	9.7	80.0	115.0	14
Body, length	73.9	73.5	9.1	1.7	12.3	50.0	90.0	30
Body, width (in vivo)	40.4	38.5	6.5	1.7	16.0	30.0	55.0	14
Body, width	26.5	27.0	3.6	0.7	13.7	20.0	35.0	30
Oral bulge, length	13.3	13.0	1.7	0.4	12.5	11.0	17.0	19
Oral bulge, width	2.4	2.5	-	-	-	2.0	2.5	15
Oral bulge, height	2.3	2.4	-	-	-	2.0	2.5	12
Macronucleus, length (in vivo)	31.6	31.0	1.7	0.7	5.4	30.0	34.0	7
Macronucleus, length	26.6	26.0	4.8	0.9	18.0	19.0	42.0	30
Macronucleus, width (in vivo)	9.7	9.0	1.7	0.6	17.5	8.0	12.0	7
Macronucleus, width	7.8	7.5	1.0	0.2	12.7	6.0	10.0	30
Micronucleus, length	2.9	3.0	0.4	0.07	13.0	2.5	3.8	30
Micronucleus, width	2.1	2.2	0.5	0.09	23.2	1.3	3.0	30
Extrusomes, length (in vivo)	3.9	3.9	0.6	0.2	15.5	3.0	5.0	10
Extrusomes, length	3.5	3.8	0.6	0.1	16.2	2.5	4.5	30
Macronuclei, number	1.2	1.0	-	-	-	1.0	4.0	81
Somatic kineties, number in mid-body	24.9	25.0	1.5	0.3	6.1	22.0	27.0	30
Basal bodies in a ventral somatic kinety, number	38.8	38.0	10.4	2.5	26.7	26.0	64.0	17
Brush kinety 1, length	12.8	13.3	1.4	0.4	11.0	10.0	14.0	10
Brush kinety 2, length	15.4	15.8	1.4	0.6	9.4	12.5	17.0	10
Brush kinety 3, length of dikinetidal portion	7.6	7.3	0.9	0.3	12.8	6.0	9.0	10
Dikinetids in brush kinety 1, number	16	17	-	-	-	14.0	17.0	3
Dikinetids in brush kinety 2, number	19	20	-	-	-	17.0	20.0	3
Dikinetids in brush kinety 3, number	9.7	10	-	-	-	8.0	11.0	3

[1)] All data based, if not stated otherwise, on randomly selected, protargol impregnated specimens from Siggerwiesen. Measurements in µm. CV — coefficient of variation in %, M — median, Max — maximum, Min — minimum, n — number of specimens investigated, SD — standard deviation, SE — standard error of mean, $\bar{x}$ — arithmetic mean.

Occurrence and ecology

KAHL (1928) discovered *S. deforme* in small, brackish (15 - 20 ‰ salinity) puddles on the northern coast of Germany. It occurred in rather large numbers in late autumn. No other records are known, but *Spathidium* spp. are rather frequently mentioned in ecological papers on activated sludge (BARKER 1943; CINGOLANI et al. 1991; SALVADÓ 1994), and some of these records might belong to *S. deforme*. Our populations persisted for about three weeks in the sludge cultures and disappeared when saprobity decreased. *Spathidium deforme* might even have some tolerance to H_2S because it has been observed in very putrid cultures. See *Microthorax pusillus* for species associated with *S. deforme*.

4 Discussion

4.1 Identification and comparison of *Microthorax pusillus* with related species

Microthorax pusillus was redefined by FOISSNER (1979) because of the incompleteness of the original description and several redescriptions (KAHL 1931; KLEIN 1928; ROUX 1901; WRZEŚNIOWSKI 1870). Later, FOISSNER et al. (1994) provided a detailed photographic documentation of another population. However, protargol impregnated cells and morphometrics were never investigated in detail, suggesting complete redescription.

Microthorax pusillus came to our attention because the specimens of the first population were slightly larger (25 - 33 vs. 20 - 30 µm) and more slender (13 - 17 vs. 15 - 25 µm) than those figured by FOISSNER (1979; Figs. 8, 9) and FOISSNER et al. (1994), and thus their contoures perfectly matched those shown by ENGELMANN (1862; Fig. 13) and KAHL (1931; Figs. 11, 12). Accordingly, we supposed that these could even be different species. However, when the cultures became older, the shape of the specimens changed markedly (Figs. 14 - 18, 23 - 25), becoming more and more similar to those figured by FOISSNER (1979) and FOISSNER et al. (1994). Finally, silver impregnation revealed only very small differences between the present and FOISSNER's (1979) population, suggesting conspecificity (Figs. 5, 7 - 10, 27 - 32). In fact, the only definite difference, verified by a reinvestigation of FOISSNER's original notes, concerns the posterior kinetid in kinety 7, which is ciliated in FOISSNER's population and unciliated in the one described here.

Both shape variants of *M. pusillus* occur in nature, and not only in cultures, as indicated by the findings of ENGELMANN (1862), KAHL (1931), FOISSNER (1979), and FOISSNER et al. (1994). The shapes are very likely rather constant and/or dependent on certain environmental conditions because we found, like FOISSNER (1979) and FOISSNER et al. (1994), only broad specimens in population II (Fig. 25). The

infraciliature, as revealed by the dry silver impregnation method, of population II perfectly matched that of population I and the specimens investigated by FOISSNER (1979).

It is a rather difficult task to separate *M. pusillus* from some recently described congeners. *Microthorax transversus* FOISSNER 1985 (Figs. 33, 34) is very similar in shape and infraciliature, although the number of basal bodies is rather different in kineties 3 (39 - 54 vs. 23 - 36) and 4 (20 - 24 vs. 16 - 20; Figs. 1, 33). However, the main distinctive character is the deep transverse furrow near the anterior left end (Fig. 34), which is markedly different from the small lobes found in *M. pusillus* (Figs. 2, 21). Furthermore, the thorn on the left wall of the oral cavity is more distinct and more anteriorly directed in *M. transversus* than in *M. pusillus* (Figs. 1, 4, 14 - 20, 22 - 25, 33). *Microthorax pusillus* also highly resembles *M. australis* FOISSNER & O'DONOGHUE 1990 (Figs. 35, 36), which, however, has distinctly fewer basal bodies in kineties 2 and 3, i.e. on average 9 vs. 13 in K2 and 8 vs. 27 in K3 (Figs. 1, 5, 35; Tab. 1). Certainly, the differences between *M. pusillus*, *M. transversus*, and *M. australis* are rather inconspicuous, suggesting that subspecies rank would probably more accurately reflect reality, i.e. their very close relationship.

Of the more incompletely defined *Microthorax* species, *M. hungaricus* HORVÁTH 1935 has an almost identical overall appearance to *M. pusillus*. However, HORVÁTH (1935) definitely states that the left side of *M. hungaricus* is unciliated. Another similar species is *M. ovinucleatus* ŠRÁMEK-HUŠEK 1957, which, however, has symbiotic algae (zoochlorellae).

4.2 Identification and comparison of *Spathidium deforme* with related species

This species was difficult to identify because of its variable shape and the rather incomplete original description (KAHL 1928): "Length 70 - 80 µm, cross section circular; oral bulge short and broad, contains few short, blunt trichocysts. Anterior portion of cell with conspicuous accumulation of blackishgreen granules, posterior portion colourless, empty and wrinkled. Loosely ciliated. Moves very slowly". Our identification is mainly based on the extrusomes and the wrinkled posterior portion, characters exactly matching KAHL's description (Figs. 37, 38, 43, 46); furthermore size and shape agree rather well. The only problem concerns the macronucleus which is, according to KAHL's figure (Fig. 38), less distinctly elongate and shorter than in our specimens. However, KAHL (1928) obviously did not observe the macronucleus in detail (because he did not mention it in the description), and it showed considerable variation in our populations (Tab. 2). A further difference is the habitat, brackish waters vs. activated sludge. But, because activated sludge is sometimes also a rather concentrated (saline) environment, we again do not rate this as a decisive difference.

Spathidium paucistriatum KAHL 1930a (Fig. 40) is possibly a junior synonym of *S. deforme*, as indicated by the similar shape and size of the body and the extrusomes. However, it is strongly flattened and the oral bulge projects more distinctly above the dorsal side. Other species which could be confused with *S. deforme* are *S. pectinatum* KAHL 1926 (Fig. 41) and *S. gibbum* KAHL 1930b (Fig. 39). However, these species have the dorsal brush on a rather distinct tuberosity and the extrusomes are fine and 8 - 15 µm long, which is definitely different from the short, blunt extrusomes found in our material (Figs. 37, 43, 51, 60 - 62). *Spathidium striatum* VUXANOVICI 1962 is smaller (60 µm) and has distinctly fewer (16) somatic kineties.

The generic classification of *S. deforme* is also difficult because its circumoral kinety is completely separate from the somatic kineties, as is characteristic for *Arcuospathidium* FOISSNER 1984. However, the kineties of *Arcuospathidium* form very steep angles on *both* sides of the oral bulge, which differs from *S. deforme*, where the kineties of the left side are slightly inclined ventrally, as in *Spathidium* (FOISSNER 1984). Thus, we keep it with this genus at the present state of knowledge.

Acknowledgements

We thank Uwe SCHIFTNER for providing the culture from Bad Waltersdorf and appreciate the technical assistance of Mag. Eric STROBL and Mr. Andreas ZANKL.

References

AESCHT E. & FOISSNER W. (1992): Biology of a high-rate activated sludge plant of a pharmaceutical company. — Arch. Hydrobiol. Suppl. **90**: 207-251.

AUGUSTIN H. & FOISSNER W. (1989): Morphologie einiger Ciliaten (Protozoa: Ciliophora) aus dem Belebtschlamm. — Lauterbornia **1**: 38-59.

AUGUSTIN H. & FOISSNER W. (1992): Morphologie und Ökologie einiger Ciliaten (Protozoa: Ciliophora) aus dem Belebtschlamm. — Arch. Protistenk. **141**: 243-283.

BARKER A. N. (1943): The protozoan fauna of sewage disposal plants. — Naturalist, Hull, year **1943** (no. 806): 65-69.

BECARES E. & FOISSNER W. (1994): Redescription of *Chilodonatella minuta* DRAGESCO 1966 (Protozoa, Ciliophora). — Linzer biol. Beitr. **26**: 512-530.

CINGOLANI L., COSSIGNANI M. & MILIANI R. (1991): The role of microfauna in the prediction and control of the activated sludge disfunctions of a municipal plant. — In MADONI P. (ed.): Biological approach to sewage treatment process: current status and perspectives, pp. 93-96. Centro „Luigi Bazzucchi", Perugia.

CORLISS J. O. (1979): The ciliated protozoa. Characterization, classification and guide to the literature. 2nd ed. — Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt. 455 pp.

CURDS C. R. (1975): Protozoa. — In CURDS C. R. & HAWKES H. A. (eds.): Ecological aspects of used-water treatment. Volume 1 – The organisms and their ecology, pp. 203-268. Academic Press, London, New York, San Fransisco.

ENGELMANN T. W. (1862): Zur Naturgeschichte der Infusionsthiere. — Z. wiss. Zool. **11**: 347-393.

FOISSNER W. (1979): Taxonomische Studien über die Ciliaten des Großglocknergebietes (Hohe Tauern, Österreich). Familien Microthoracidae, Chilodonellidae und Furgasoniidae. — Sber. Akad. Wiss. Wien **188**: 27-43.

FOISSNER W. (1984): Infraciliatur, Silberliniensystem und Biometrie einiger neuer und wenig bekannter terrestrischer, limnischer und mariner Ciliaten (Protozoa: Ciliophora) aus den Klassen Kinetofragminophora, Colpodea und Polyhymenophora. — Stapfia **12**: 1-165.

FOISSNER W. (1985): Morphologie und Infraciliatur der Genera *Microthorax* und *Stammeridium* und Klassifikation der Microthoracina JANKOWSKI 1967 (Protozoa: Ciliophora). — Zool. Anz. **214**: 33-53.

FOISSNER W. (1991): Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. — Europ. J. Protistol. **27**: 313-330.

FOISSNER W. & O'DONOGHUE P. J. (1990): Morphology and infraciliature of some freshwater ciliates (Protozoa: Ciliophora) from Western and South Australia. — Invert. Taxon. **3**: 661-696.

FOISSNER W., BERGER H. & KOHMANN F. (1994): Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band III: Hymenostomata, Prostomatida, Nassulida. — Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft **1/94**: 1-548.

HORVÁTH P. (1935): Egy új Hymenostomata véglény. (*Microthorax hungaricus* nov. spec.) Szeged környyékéröl. — Acta biol., Szeged. **3**: 167-189 (in Hungarian with German summary).

KAHL A. (1928): Die Infusorien (Ciliata) der Oldesloer Salzwasserstellen. — Arch. Hydrobiol. **19**: 50-123.

KAHL A. (1930a): Neue und ergänzende Beobachtungen holotricher Infusorien. II. — Arch. Protistenk. **70**: 313-416.

KAHL A. (1930b): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. — Tierwelt Dtl. **18**: 1-180.

KAHL A. (1931): Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 2. Holotricha außer den im 1. Teil behandelten Prostomata. — Tierwelt Dtl. **21**: 181-398.

KLEIN B. M. (1928): Die Silberliniensysteme der Ciliaten. Weitere Resultate. — Arch. Protistenk. **62**: 177-260.

LEE N. M. & WELANDER T. (1996): Use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment. — Biotechnol. Lett. **18**: 429-434.

LEITNER A. R. & FOISSNER W. (1997): Taxonomic characterization of *Epicarchesium granulatum* (KELLICOTT 1887) JANKOWSKI 1985 and *Pseudovorticella elongata* (FROMENTEL 1876) nov. comb., two peritrichs (Protozoa, Ciliophora) from activated sludge. — Europ. J. Protistol. **33**: 13-29.

MADONI P. (1988): I protozoi ciliati nel controllo di efficienzia dei fanghi attivi. — Centro Italiano Studi di Biologia Ambientale, Reggio Emilia. 82 pp.

MADONI P. (1994): A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. — Wat. Res. **28**: 67-75.

OBERSCHMIDLEITNER R. & AESCHT E. (1996): Taxonomische Untersuchungen über einige Ciliaten (Ciliophora, Protozoa) aus Belebtschlämmen oberösterreichischer Kläranlagen. — Beitr. Naturk. Oberösterreichs **4**, 3-30.

RATSAK C. H., MAARSEN K. A. & KOOIJMAN S. A. L. M. (1996): Effects of protozoa on carbon mineralization in activated sludge. — Wat. Res. **30**: 1-12.

ROUX J. (1901): Faune infusorienne des eaux stagnantes des environs de Genève. — Mém. Inst. natn. génev. **19**: 1-148.

SALVADÓ H. (1994): Effect of mean cellular retention time on ciliated protozoan populations in urban wastewater treatment plants based on a proposed model. — Wat. Res. **28**: 1315-1321.

SALVADÓ H., GRACIA M. P. & AMIGÓ J. M. (1995): Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants. — Wat. Res. **29**: 1041-1050.

ŠRÁMEK-HUŠEK R. (1957): K poznání nálevniku ostravského kraje (Zur Kenntnis der Ciliaten des Ostrauer-Gebietes [Tschechoslovakei]). — Věst. čsl. zool. Spol. **21**: 1-24 (in Czech with German summary).

VUXANOVICI A. (1962): Contributii la sistematica ciliatelor (Nota I). — Studii Cerc. Biol. (Biol. Anim.) **14**: 197-215 (in Rumanian with Russian and French summaries).

WRZEŚNIOWSKI A. (1870): Beobachtungen über Infusorien aus der Umgebung von Warschau. — Z. wiss. Zool. **20**: 467-511.

Address of the authors: Andreas Rodolfo LEITNER and Wilhelm FOISSNER,
Universität Salzburg, Institut für Zoologie,
Hellbrunnerstrasse 34, A-5020 Salzburg, Austria.