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Weibo Song ^a

^a Laboratory of Protozoology, KLM , Ocean University of China , Qingdao 266003, P.R. China E-mail:

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Taxonomic description of two new marine oligotrichous ciliates (Protozoa, Ciliophora)

WEIBO SONG

Laboratory of Protozoology, KLM, Ocean University of China, Qingdao 266003, P.R. China; e-mail: wsong@ouc.edu.cn

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Abstract

The morphology and ciliary pattern of a new marine oligotrichous ciliate, *Strombidium wilberti* nov. spec., collected from the littoral area off Qingdao, North China, are described from live observations and after protargol impregnation. This new species is characterized by: about $45\text{--}60 \times 35\text{--}50\text{ }\mu\text{m}$ *in vivo* with truncated obconical body shape and conspicuous distended cell surface which covers the posterior half of the cell; buccal cavity extending posteriorly to about one-third of body length; about eight buccal and 15 collar membranelles; girdle kinety completely closed, consisting of *ca* 60 dikinetids; ventral kinety conspicuously long with about 30 densely packed dikinetids, which extends anteriorly to girdle area; extrusomes about $20\text{ }\mu\text{m}$ long, grouped in rows and arranged anterior to girdle kinety; single globular macronucleus. Related nominal congeners are compared and discussed. The Antarctic morphotype described by Petz et al. (1995) as *Strombidium emergens* (Leegaard, 1915) Kahl, 1932 is not conspecific with the original form and is hence recognized as a new member of this genus, *Strombidium petzi* nov. spec. The diagnosis for this new organism: large marine *Strombidium ca* $95\text{--}110 \times 60\text{--}65\text{ }\mu\text{m}$ *in vivo* with elongate elliptical body shape and conspicuously shallow oral cavity. Girdle kinety continuous on ventral side with *ca* 100 dikinetids; ventral kinety extending from posterior area almost to girdle kinety, consisting of more than 20 dikinetids; four to five buccal and 14–15 collar membranelles. Macronucleus oval in shape; one subequatorial girdle of extrusomes.

Keywords: *Oligotrichida*, *marine ciliate*, *Strombidium wilberti* nov. spec., *Strombidium petzi* nov. spec.

Introduction

Most naked or aloricate oligotrichs such as strombidiids are highly motile ciliates and often dominant in microzooplankton communities in both marine and freshwater habitats (Dragesco 1960; Borror 1972; Fenchel and Jonsson 1988; Lynn and Montagnes 1988; Krainer 1991). Since many of these species are minute and fragile, they have often been investigated rather superficially, based largely or entirely on preserved or live material (Fauré-Fremiet 1924; Kahl 1932; Maeda and Carey 1985). For this reason, numerous ambiguities concerning the identification of taxa have accumulated over the last century and hence, most nominal species need to be redefined or re-investigated using not only live observations but also impregnation methods. This is especially true for the marine forms, which were relatively poorly considered as revealed in some recent studies (Lynn et al.

1988; Montagnes et al. 1990; Martin and Montagnes 1993; Montagnes and Taylor 1994; Petz et al. 1995; Agatha and Riedel-Lorjé 1997).

Faunistic research on plankton ciliates from coastal waters in north China seas has been carried out in recent years and various little-known or new oligotrichs have been isolated and studied (Song and Packroff 1997; Song and Bradbury 1998; Lei et al. 1999; Song et al. 2000). As a new contribution to our knowledge of these organisms, a further unknown species in the genus *Strombidium* is identified and described in the present paper.

Materials and methods

Sampling sites and cell culture. Samples were collected in December 1993 from several sites used for mollusc-farming in coastal regions of the Yellow Sea near Qingdao, China. Cells were cultured at room temperature in Petri dishes with the water in which they were isolated. These cultures were usually maintained for several days and then used for morphological studies.

Observations and staining. Specimens were observed *in vivo* with phase contrast and differential interference contrast microscopy. The infraciliature was revealed by protargol impregnation (Wilbert 1975). Counts, drawings (with help of a camera lucida) and measurements were performed at a magnification of $\times 1250$. Terminology is mainly according to Corliss (1979) and Lynn et al. (1988).

Results

Strombidium wilberti nov. spec. (Figures 1, 2; Table I)

Diagnosis. Marine *Strombidium*, about $45\text{--}60 \times 35\text{--}50\ \mu\text{m}$ *in vivo* with truncated obconical body shape and conspicuous distended cell surface which covers the posterior half of the cell; buccal cavity extending posteriorly to about one-third of body length; about eight buccal and 15 collar membranelles; girdle kinety completely closed, consisting of *ca* 60 dikinetids; ventral kinety prominently long with about 30 densely packed dikinetids, extends anteriorly to girdle area; extrusomes about $20\ \mu\text{m}$ long, grouped in rows and arranged anterior to girdle kinety; single globular macronucleus.

Ecological characteristics for sampling sites. Sandy coastal area with clean water, salinity was 33, pH about 8.1, water temperature ranged from 5 to 9°C .

Type deposition. One permanent slide as holotype with protargol-impregnated specimens is deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao.

Dedication. I dedicate this species to Prof. Dr Norbert Wilbert (Institute of Zoology, Bonn University, Germany), a well-respected and great German protozoologist, who has made significant contributions to ciliate taxonomy and ecology during the last 30 years.

Description. Size mostly about $50 \times 40\ \mu\text{m}$ *in vivo*. Body shape generally invariable, broadly obconical and circular in cross-section, widest at anterior third of cell length (Figure 1A),

Figure 1. *Strobilidium wilberti* nov. spec. from life (A–C, E) and after protargol impregnation (D, F–H). (A) A typical individual, ventral view; (B) detailed structure of perilemma and extrusomes, arrow marks the outer ends of the grouped extrusomes; (C) swimming trace; (D) buccal apparatus; (E) specimen with evident ventral groove (arrow) and slightly asymmetric body; (F) ventral view of infraciliature, arrow indicates the anterior end of the ventral kinety; (G) dorsal view of the same specimen as (F), arrow marks the posterior end of the ventral kinety; (H) ventral view of an early divider, arrow marks the newly formed (subcortical) oral primordium. BM, buccal adoral membranelles; CM, collar adoral membranelles; CyP, cytopharynx; Ex, extrusomes; ExG, extrusome girdle; GK, girdle kinety; Ma, macronucleus; Pe, perilemma; PM, paroral membrane; VK, ventral kinety. Scale bars: 30 μ m.

while in some individuals body might be slightly asymmetric when viewed dorso-ventrally (Figure 1E). Apical protrusion or collar inconspicuous *in vivo*, usually undetectable after fixation (Figure 1F, H). Buccal cavity relatively shallow and inconspicuous, extending posteriorly to about 25–30% of cell length (Figure 1A, E). Ventral groove conspicuous and extending from girdle region to posterior cell end, containing long ventral kinety (Figure 1F). Cilia in collar membranelles about 25–30 μ m long, stretching anteriorly when swimming, cilia in buccal membranelles *ca* 10 μ m in length (Figure 1A).

Perilemma remarkable, always as conspicuously distended ‘lorica-like’ structure covering the posterior half of live cell (Figure 1A, E), but only about 40% after protargol impregnation where the anterior cell portion is swollen (Figure 1F). No cortical platelets recognizable either *in vivo* or in silvered specimens. Extrusome girdle conspicuous, extrusomes about 20 μ m long, bar-like and regularly grouped (each group with about six extrusomes), which are closely spaced anterior to the girdle kinety (Figure 2C). Outer ends

Figure 2. Micrographs of *Strombidium wilberti* nov. spec. after protargol impregnation. (A) Ventral view showing general appearance of buccal and somatic ciliature, arrow and arrowhead mark the buccal and collar membranelles, respectively. Note that the proximal end of the buccal zone is far away from the girdle kinety; (B) macronucleus in early stage of cell division, note that it is oval rather than globular in shape; (C) dorsal view, to show the girdle kinety (lower) and the extrusome girdle (upper); (D) ventral view, double-arrowheads mark the paroral membrane, arrowhead indicates the membranelles, while the arrow refers the anterior end of the ventral kinety; (E) ventral view, to demonstrate the ventral kinety; (F) posterior portion of cell, to show the anterior end of the ventral kinety (arrowhead); (G) ventral view, arrow marks the newly formed oral primordia, which are beneath the girdle kinety. Scale bars: 30 μm (A); 40 μm (G).

of extrusomes arranged in oblique rows (anchored obliquely beneath pellicle), which make cell surface granulated and slightly punctated (Figure 1B).

Cytoplasm basically colourless to slightly greyish, containing numerous light-reflecting, lipid-like granules (ca 2–5 μm in diameter) and food vacuoles containing diatoms, flagellates etc., which often render cells rather opaque or even completely dark when observed at lower magnifications (Figure 1E). Macronucleus about 15–20 μm across, spherical in shape and centrally located, usually contains many large roundish nucleoli as demonstrated after impregnation (Figure 1F); no micronucleus recognized.

Locomotion very fast and changing direction frequently, never stops swimming (Figure 1C). This organism seems fragile for it readily bursts especially when a cover glass is used for observing under the microscope.

Buccal apparatus typical of genus, consisting of 14–16 collar membranelles (CM), eight buccal membranelles (BM) and one single-rowed paroral membrane (Figure 1D). Bases of CM about 10 μm long, each composed of three equal-length basal body rows (Figure 2A, D: arrowheads); BM being continuous with CM, similar to CM in structure but shorter in length of bases (about 5–7 μm) (Figure 2A: arrow), which invaginate ventrally into centre of cell.

Table I. Comparison of some marine, morphologically similar *Strombidium* species.

Characters	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13
Cell length <i>in vivo</i>	45–60	30–50	–	–	50–70	–	<i>ca</i> 40	–	–	–	–	32–46	–
Cell length after impregnation	51–64	30–47	28–52	13–18	43–90	26–44	17–28	21–29	21–37	25–52	21–33	14–32	28–42
Length of buccal field: body length	<i>ca</i> 1/3	<i>ca</i> 1/3	<i>ca</i> 1/4?	<i>ca</i> 1/4?	<i>ca</i> 1/2	<1/5?	<i>ca</i> 1/4?	<i>ca</i> 1/4?	>1/3?	<i>ca</i> 1/3?	<i>ca</i> 2/5?	<i>ca</i> 1/3?	<i>ca</i> 2/5
No. of buccal membranelles	8	7–9	10–22 †	6–9	13–19	A few, inconspicuous	6–8	A few, inconspicuous	12–15	7–9	5–7	7–9	8–11
No. of collar membranelles	14–16	13–16	12–15	10–15	16–19	13–17	12–15	12–15	13–16	15–17	14–16	12–15	12–15
No. of dikinets in girdle kinety	<i>ca</i> 60	<i>ca</i> 50	–	–	<i>ca</i> 115	–	–	–	–	–	–	–	–
No. of dikinets in ventral kinety	<i>ca</i> 30	5–12	Not observed	Not observed	9–15	Not observed	Inconspicuous	–	–	27–29	<10?	7–9	–
Shape of macronucleus	Globular to oval	Oval to ellipsoid	Oval	Oval	Spherical	Oval	Oval	Oval	Sausage-like	Oval	Oval	Oval	Oval
Position of girdle kinety	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 4/5 of cell length	<i>ca</i> 1/2 of cell length	>2/3 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 2/3 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length	<i>ca</i> 1/2 of cell length
Length of extrusomes	<i>ca</i> 20	12–15	–	–	<i>ca</i> 17	–	–	–	–	–	–	<i>ca</i> 15	–
Grouping feature of extrusomes (Ex)	One- rowed, <i>ca</i> 6 Ex per group	One- rowed, several Ex per group	–	–	–	–	–	–	–	–	–	–	–
Appearance of ventral kinety	Long, extending anteriorly to GK	Short, caudally positioned	Inconspicuous	Inconspicuous	Conspicuous	Inconspicuous	Short, in posterior third of body	Long, extending almost to GK	Very short, caudally positioned	Long, extending to GK	Short, sub- caudally positioned	Short, caudally positioned	Long

Table I. (Continued).

Characters	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13
Data source	Present work	Montagnes <i>et al.</i> , 1990; Lei <i>et al.</i> , 1996; Song <i>et al.</i> , 2000	Lynn <i>et al.</i> , 1988	Lynn <i>et al.</i> , 1988	Petz <i>et al.</i> , 1995	Lynn <i>et al.</i> , 1988	Leegaard, 1915; Lynn <i>et al.</i> , 1988	Lynn <i>et al.</i> , 1988	Martin and Montagnes, 1993	Martin and Montagnes, 1993	Martin and Montagnes, 1993	Montagnes <i>et al.</i> , 1990; Modeo <i>et al.</i> , 2003	Lynn <i>et al.</i> , 1988

Measurements in μm . GK, girdle kinety. No. 1: *S. wilberti* n. sp.; No. 2: *S. sulcatum* Claparède and Lachmann, 1858; No. 3: *S. acutum* Leegaard, 1915; No. 4: *S. dahum* Lynn *et al.*, 1988; No. 5: *S. crassulum* (Leegaard, 1915); No. 6: *S. rhynchum* Lynn *et al.*, 1988; No. 7: *S. compressum* (Leegaard, 1915) Kahl, 1932; No. 8: *S. tressum* Lynn *et al.*, 1988; No. 9: *S. taylori* Martin and Montagnes, 1993; No. 10: *S. basimorphum* Martin and Montagnes, 1993; No. 11: *S. ventropinnum* Martin and Montagnes, 1993; No. 12: *S. inclinatum* Montagnes *et al.*, 1990; No. 13: *S. emergens* (Leegaard, 1915). [†]Incorrect data (?), unlikely a normal intra-species variation; ?, assumed from the illustrations or photograph(s) of impregnated specimens; –, data unavailable.

Paroral membrane (PM) along right border of buccal cavity with densely packed kinetosomes. Pharyngeal fibres up to 20 μm long after protargol impregnation (Figure 1D).

Somatic ciliature as shown in Figure 1F, G. Girdle kinety (GK) completely closed and posterior to extrusome girdle (Figure 2E, F), composed of approximately 60 dikinetids (Figure 1F). Ventral kinety (VK) conspicuously long, with about 30 densely spaced dikinetids in shallow groove, commencing underneath girdle kinety on ventral side and extending across posterior pole to dorsal side (Figure 1F, G).

Comparison and discussion

Comparison with related species

As in many other oligotrichs, species identification within the genus *Strombidium* is problematic. This is largely because these small organisms share many characters among congeners with respect to their infraciliature and living features. This problem is usually exacerbated as many descriptions are based only on live observations or preserved material, in which many characters are not recognizable.

To date, approximately 15 *Strombidium* species with similar cell shape and size inhabiting marine biotopes have been reported, of which about 12 have been described using silver-impregnation methods (Lynn et al. 1988; Montagnes et al. 1988, 1990; Montagnes and Lynn 1991; Lynn and Gilron 1993; Martin and Montagnes 1993; Petz et al. 1995; Lei et al. 1996; Song and Packroff 1997; Song et al. 2000) (Table I).

As accepted by most taxonomists today (Krainer 1991; Petz et al. 1995; Agatha and Riedel-Lorjé 1997; Lei et al. 1999; Song et al. 2000), the following criteria, which are observed either *in vivo* or from silvered specimens, are important in species separation in the genus *Strombidium*:

- (1) the buccal and somatic ciliature, especially the length and position of the ventral kinety (e.g. fragment-like near the caudal pole or as a long row positioned ventrally/laterally) and the appearance of membranelles in buccal and collar zones (clearly separated or in a continuous structure, differences in length of bases etc.);
- (2) biometric data of ciliature (the number of kinetosomes in girdle and ventral kineties, membranelles in collar and buccal zones etc.);
- (3) length, distribution and organization (i.e. grouped or sparsely distributed) of extrusomes;
- (4) appearance of the cell surface (presence/absence of polygonal structure etc.);
- (5) length of cilia and bases of membranelles, the orientation or beating characteristics of membranelles *in vivo*;
- (6) habitat (marine or freshwater) and swimming behaviour (highly species-specific!);
- (7) body shape, size, length of the buccal cavity, prominence of the anterior protrusion or collar etc. (though the significance of these characters was/is often over-evaluated, in my opinion);
- (8) characteristics of nuclear apparatus (shape and size of macronucleus, number of micronuclei etc.) and cytoplasmic inclusions (symbionts etc.).

Compared with the well-known *Strombidium sulcatum*, the new species, *S. wilberti*, has a different pattern of ventral kinety: conspicuously long and extending across the whole post-equatorial ventral portion (versus fragment-like and very short in *S. sulcatum*) with higher number of basal body pairs (30 versus <10). In addition, *S. wilberti* is relatively large (45–60 versus about 40 μm in *S. sulcatum*) and has a more broadly and truncate conical body

shape in life (Figure 3H) (Fenchel and Jonsson 1988; Montagnes et al. 1990; Lei et al. 1996; Song et al. 2000).

Another similar morphotype, *Strombidium inclinatum* Montagnes et al., 1990, can also be easily separated from the new species by the characters of somatic ciliature mentioned above and its shorter body length (32–46 μm *in vivo*) (Figure 3F; Table I) (Montagnes et al. 1990; Modeo et al. 2003).

Considering the ciliary pattern, *Strombidium basimorphum* Martin and Montagnes, 1993 is also similar to *S. wilberti*. The former, however, can be identified by: (1) the ‘compressed’ conical body shape (usually width > length) and (2) the longer bases of collar membranelles, i.e. about twice as long as those of the buccal membranelles, as shown in Figure 7G of the original report (Martin and Montagnes 1993) (Figure 3B; Table I).

Figure 3. Some related *Strombidium* species, arrows mark the anterior end of the ventral kinety. (A) *Strombidium acutum* (from Lynn et al. 1988); (B) *S. basimorphum* (from Martin and Montagnes 1993); (C) *S. dalum* (from Lynn et al. 1988); (D) *S. crassulum* (from Leegaard 1915); (E) *S. epidemum* (from Lynn et al. 1988); (F) *S. inclinatum* (from Montagnes et al. 1990); (G) *S. compressum* (from Lynn et al. 1988); (H) *S. sulcatum* (from Song et al. 2000); (I) *S. taylora* (from Martin and Montagnes 1993); (J) *S. emergens* (from Lynn et al. 1988); (K) *S. rhynchum* (from Lynn et al. 1988); (L) *S. tressum* (from Lynn et al. 1988); (M) *S. ventropinnum* (from Martin and Montagnes 1993); (N) *S. petzi* (from Petz et al. 1995); (O, P) *S. crassulum* (from Petz et al. 1995). Scale bars: 20 μm (A, D, G, H, J, K, M, N); 30 μm (B); 15 μm (C, E, F, I, L); 40 μm (O, P).

With reference to the body size, shape and general appearance from life, *Strombidium crassulum* (Leegaard, 1915) Kahl, 1932 should be most similar to the new organism. The infraciliature of *S. crassulum* was described by Petz et al. (1995) based on an Antarctic strain (Figure 3O, P). According to this redescription, *S. crassulum* can be separated from the new species by its highly developed buccal membranelles (13–19 versus eight in *S. wilberti*) which extends to the equatorial area, and the structure of the somatic ciliature (9–15 versus about 30 dikinetids in ventral kinety and *ca* 115 versus *ca* 60 dikinetids in girdle kinety, respectively) (Table I).

Strombidium compressum (Leegaard, 1915) Kahl, 1932 also has a similar body shape (Figure 3G) to *S. wilberti*, but according to the redescription by Lynn et al. (1988), *S. compressum* differs in its short ventral kinety which is positioned in the posterior third of the cell with only several dikinetids and is considerably shorter (17–28 μm after protargol impregnation) (Table I).

Compared to *Strombidium dalum* and *S. epidemum*, both found/described from the Isles of Shoals by Lynn et al. (1988), the new species described here is much larger (over 50 versus <20 μm after impregnation) and has a conspicuously longer ventral kinety which extends from girdle kinety to posterior end of the cell (versus not observed, possibly reduced) (Lynn et al. 1988; Figure 3C, E; Table I).

With reference to the general infraciliature, *Strombidium tressum* Lynn et al., 1988 also seems to be related to *S. wilberti*. However, the former can be distinguished by the extremely long cilia in its collar membranelles (over 40 μm , about twice as long as body size after protargol impregnation), reduced buccal membranelles (only 'a few' in number in small buccal groove) and the conspicuous perilemma, which covers almost three-quarters of body length (Figure 3L). In addition, *S. tressum* is significantly smaller (21–29 μm long after fixation) (Table I).

Strombidium rhynchum Lynn et al., 1988 differs from *S. wilberti* in its slender body shape, highly developed perilemma which covers almost four-fifths of posterior body and reduced buccal zone of membranelles (very short and located in the inconspicuous buccal cavity) (Figure 3K; Table I).

Two other marine species, *S. taylori* Martin and Montagnes, 1993 and *S. ventropinnum* Martin and Montagnes, 1993, should also be compared with *S. wilberti*. The former is characterized by: (1) the unique sausage-like macronucleus (versus oval in *S. wilberti*), (2) the higher number of buccal membranelles (12–15 versus eight) and (3) the short, fragment-like ventral kinety (Figure 3I); *S. ventropinnum* is diagnosed with slender body shape, five to seven small buccal membranelles (versus eight in *S. wilberti*), prominent buccal cavity which extends to about two-fifths of cell length and conspicuously shorter ventral kinety (Figure 3M; Table I).

According to the original report and the redescription by Lynn et al. (1988), *Strombidium acutum* Leegaard, 1915 could be similar to the new species as well, but can, however, be distinguished by: (1) the highly reduced ventral kinety, which was undetectable (possibly extremely inconspicuous) as mentioned in the redescriptions, and (2) having very shallow buccal cavity (Lynn et al. 1988). In addition, Lynn et al. (1988) defined this organism possessing 10–22 buccal membranelles, which is an unusually wide range of variation (Figure 3A; Table I).

Strombidium emergens (Leegaard, 1915) Kahl, 1932 was redescribed by Lynn et al. (1988) under the name *Strombidium sulcatum*, which was later determined as *S. emergens* by Montagnes et al. (1990). It differs from *Strombidium wilberti* in its slender body shape *in vivo*, smaller size (28–42 versus 44–53 μm in length after protargol impregnation) and the

presence of the conspicuously shorter ventral kinety (extending anteriorly to about a quarter of cell length) (Figure 3J; Table I).

Lynn and Gilron (1993) described a Jamaican population under the name of *Strombidium inclinatum* Montagnes et al., 1990, which is possibly a misidentification because it has a long ventral kinety extending anteriorly almost to the equatorial region. Since no statistical data are available about the number of kinetosomes in either girdle or ventral kineties, it is difficult to compare it with the species described here. There remains the possibility that this organism is conspecific with *S. wilberti*.

Establishment of a new species

***Strombidium petzi* nov. spec.**

Synonym. *Strombidium emergens* sensu Petz, Song and Wilbert, 1995.

Petz et al. (1995) described a large form from sea ice of the Weddell Sea, Antarctica as *Strombidium emergens* (Leegaard 1915), which was characterized by its extremely large size (*in vivo* 95–110 × 60–65 µm, after protargol impregnation 90 × 49–52 µm versus 30–40 µm in preserved material in original report) and ‘reduced’ buccal membranelles (only a few very short membranelles in shallow oral cavity) (Figure 3N). Considering the combination of body size, shape and pattern of infraciliature, the Antarctic organism is clearly different from all known congeners described hitherto. Hence, it is reasonable to recognize it as a new species, *Strombidium petzi* nov. spec.

Diagnosis. Large marine *Strombidium* about 95–110 × 60–65 µm *in vivo* with elongate elliptical body shape and conspicuously shallow oral cavity. Girdle kinety continuous on ventral side with *ca* 100 dikinetids; ventral kinety extending from posterior area almost to girdle kinety, consisting of more than 20 dikinetids; four to five buccal and 14–15 collar membranelles. Macronucleus oval in shape; one subequatorial girdle of extrusomes.

Type deposition. One slide as holotype with protargol-impregnated specimens is deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum, A-4040 Linz, Austria.

Dedication. This species is dedicated to Dr Wolfgang Petz (Institute of Zoology, University of Salzburg, Austria), to express my respects to his great contributions in various fields of protozoological research.

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