

Pelagostrobilidium wilberti n. sp. (Oligotrichea, Choreotrichida): Morphology and Morphogenesis

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ABSTRACT. Morphology, infraciliature, morphogenetic features, and some ecological data for *Pelagostrobilidium wilberti* n. sp. are described. This new species was collected from a temporary pond in Magdalena, Buenos Aires province, Argentina, which was sampled monthly from August 2003 to July 2005. The species was found in autumn and winter. Observations were made in vivo and after staining with protargol. *Pelagostrobilidium wilberti* n. sp. measures 63–84 × 42–49 μm in vivo and is conical in shape, with a posterior spine-like cytoplasmic process. It possesses 6 somatic kineties, with kinety 2 sinistrally curved and shorter than the others. The oral apparatus is composed of 25–32 external and two internal membranelles. The macronucleus is horseshoe-shaped and located beneath the oral apparatus; two or three spherical micronuclei lie dorsally. There is a posterior contractile vacuole. Morphogenesis is hypo-apokinetal and begins dorsally between the curved kinety 2 and kinety 3. After the discovery of this new species, the diagnosis of the genus *Pelagostrobilidium* was amended.

Key Words. Argentina, Ciliophora, freshwater ciliate, new species, Protozoa, temporary pond.

TEMPORARY ponds represent heterogeneous environments with diverse fauna and flora. The organisms inhabiting such habitats are physiologically adapted to the changing conditions of their environment (Williams 1987). The ciliate biota from temporary shallow ponds has scarcely been investigated at all, despite their importance in the food webs arising from these transient habitats (Andrushchynshyn, Magnusson, and Williams 2003). Although oligotrichid and aloricate choreotrichid ciliates are very well known in marine environments or in brackish waters, only a few investigations have been conducted in freshwaters in the last decades (Foissner, Skogstad, and Pratt 1988; Krainer 1991, 1995; Petz and Foissner 1992, 1993).

The aim of the present contribution is to describe the morphology, infraciliature, and morphogenetic events of *Pelagostrobilidium wilberti* n. sp. from a temporary pond from Argentina, complemented with some ecological data.

MATERIALS AND METHODS

Samples were taken monthly from August 2003 to July 2005 from a temporary pond located in Magdalena, Buenos Aires province, Argentina (35°05'S, 57°48'W) (Fig. 1). The water supply to this pond comes mainly from rainfall, and the pond goes through drought periods several times during an annual cycle, depending on the climatic conditions. Temperature, pH, and electrical conductivity were recorded using a multiparameter sensor and dissolved oxygen was estimated by the Winkler method (Eaton, Clesceri, and Greenberg 1995).

Live sub-samples, taken from the pond with a 5-L bottle, were cultured at room temperature (~ 15 °C) in Petri dishes with wheat infusion, in order to have large numbers of ciliates for morphological and morphogenetic studies. Specimens were observed in vivo and after revealing argentophilic structures by the protargol method (Wilbert 1975), under the light microscope at magnifications of 100X, 400X, and 1,000X. Descriptions of morphogenesis, as well as drawings and photographs, were made from permanent and brighter, non-permanent slides (i.e. avoiding the alcohol series), which allowed the structures to be distinguished more

clearly. Measurements of dividing specimens are from protargol-impregnated cells and percentage of body length is on average. The drawings were made using a tracing device at 1,000X. Photographs were taken with a Leica Wild MPS52. Terminology is according to Corliss (1979) and Montagnes and Lynn (1991).

RESULTS

Pelagostrobilidium wilberti n. sp.

Description (Table 1 and Fig. 2–8, 18–22). The size in vivo is 63–84 μm long by 42–49 μm wide. The conical-shaped body is round in cross-section with a short posterior cytoplasmic spine-like process. The cytoplasm is colorless and it lacks symbiotic algae. Somatic extrusomes are absent. There are six somatic kineties. Kineties 1 and 3–6 are almost longitudinal, with kineties 3 and 4 being slightly directed toward kinety 2. These five kineties extend from just underneath the external zone of membranelles to near the posterior pole, with kinety 1 being the longest. Kinety 2 is curved sinistrally and anteriorly, and is shorter than the others. It begins one-third of the way from the oral end and near to kinety 1, and extends to the posterior third of the cell (Fig. 3–7, 18–21). The length of the somatic kineties increases from kinety 2 to kinety 1, but several individuals possessed shorter kineties 2–4 (Fig. 8). The ciliary rows are composed of monokinetids that bear cilia directed to the left and parallel to the body surface (Fig. 22). A pair of ciliated kinetosomes is located in the vicinity of the anterior end of the somatic kineties in some non-dividing organisms. The cell surface has cytoplasmic flaps that partially cover the somatic kineties.

The prominent oral apparatus is in the anterior end of the body, and is composed of a closed ring of 25–32 external and two internal oral polykinetids, the shortest of them being to the right. Three to five external membranelles extend into the eccentric buccal cavity. All oral polykinetids have three rows of kinetosomes except for the shortest internal one, which is composed of only two rows. The right-most row of kinetosomes of the largest internal membranelle, is shorter than the other two (Fig. 8). The adoral cilia are ~ 35 μm long in living cells (estimated from light micrographs). The endoral kinety is composed of a single row of kinetosomes, extending from the bottom of the buccal cavity to the right external margin of the oral field (Fig. 3, 6, 8). Cytopharyngeal fibers reach the aboral third of the body.

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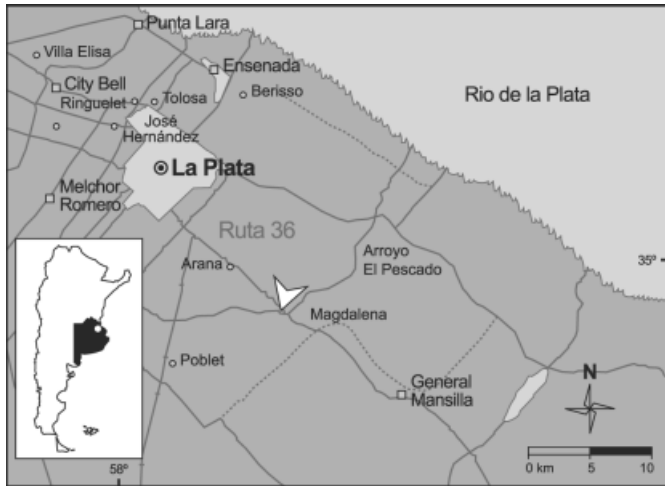


Fig. 1. Location of the sampling site in Buenos Aires Province, Argentina.

The macronucleus is horseshoe-shaped, and located beneath the adoral zone of membranelles, with its opened end to the ventral side of the body. Two or three spherical micronuclei are located dorsally, sometimes faintly or not impregnated at all, and indented in the macronucleus below dorsal membranelles (Fig. 8, 20, 28). The contractile vacuole is located at the posterior end of the body, above the caudal process (Fig. 2). Whether it empties ventrally or dorsally could not be determined.

Morphogenesis (Fig. 9–17, 23–29). Stomatogenesis is hypo-apokinetal and begins dorsally, in a supra-equatorial position. An anarchic field of kinetosomes proliferates on the cell surface, between the anterior ends of kineties 2 and 3 (Fig. 9, 23). These kinetosomes sink into a sub-surface pouch where the future external oral polykinetids of the opisthe differentiate. The endoral kinety also forms de novo, between the open ends of the anlage for the adoral zone, and extends towards the anterior end of the cell (Fig. 10, 24). At this stage, the oral primordium resembles a ‘‘D.’’ The external oral polykinetids continue their development until the adoral zone of membranelles closes, but several anarchic kinetosomes still remain. Some polykinetids (3–5; $n = 13$) of the posterior end of the oral primordium extend into the future oral cavity of the opisthe, and the two separate and short internal oral polykinetids form beneath these (Fig. 11, 25). The adoral membranelles lie perpendicular to the cell surface and their curvature becomes evident (Fig. 12, 13). The diameter of the fully developed oral apparatus ranges between 17 and 41 μm ($n = 14$), and its polykinetids appear concave along the inner surface of the sub-surface pouch, when the cell is viewed dorsally. The body length increases by $\sim 16\%$ (63–91 μm ; $n = 23$). Then, the new oral apparatus faces the opening of the sub-surface pouch in the cortex, with adoral cilia below the cell surface (Fig. 12). During the differentiation of the oral primordium, the somatic kineties lengthen by 12%–14% through intrakinetal proliferation of kinetosomes, but whether this proliferation occurred at a specific end or among the parental kinetosomes could not be determined. Subsequently, the oral apparatus slightly increases in diameter as the membranelles spread (28–45 μm ; $n = 19$), and as soon as the ciliature is totally formed, they become convex and the cilia emerge through a wider cortical opening (Fig. 14, 15, 28). The whole evaginating structure is bordered by a cytoplasmic rim. Somatic kineties lengthen by a further 10%–13%.

In late dividers, the diameter of the oral apparatus of the opisthe ranges between 35–52 μm ($n = 9$), and the body length increases

by 24% (70–112 μm ; $n = 9$). The somatic kineties separate into two segments (Fig. 16, 29). They continue to lengthen during separation of the opisthe and are positioned at their final location just before cytokinesis. Once the kineties are split, proter kinety 2 lies almost longitudinally while opisthe kinety 2 is somewhat curved (Fig. 16). This stage is followed by cytokinesis.

Nuclear changes. The horseshoe-shaped macronucleus shows two replication bands that migrate from the ends toward a dorsal position (Fig. 11). Micronuclei increase in diameter and become elliptical. Later on, the macronucleus becomes a rounded mass, and the micronuclei divide into four or six (Fig. 14, 28). As the macronucleus becomes elliptical and lies longitudinally in the parental cell, the divided micronuclei migrate near to the newly formed oral apparatus. The macronucleus elongates even more and protrudes into the developing opisthe (Fig. 15). Subsequently, it divides into two rounded masses and each of them reorganizes below the proter’s and opisthe’s oral apparatus (Fig. 16, 29).

Post-dividers (size 56 \times 58 μm , $n = 1$) still have the macronucleus as a rounded mass, and the posterior cytoplasmic process is absent. The curvature of kinety 2 is not very conspicuous, although it can be recognized (Fig. 17). In advanced post-dividers (size 62 \times 49 μm , $n = 3$), the macronucleus elongates below the external membranelles. At this stage, non-ciliated kinetosomes are visible at the anterior ends of the somatic kineties, possibly due to their further elongation. Kinety 2 lies almost transversely, and its anterior end, which also shows non-ciliated kinetosomes, is still distant from kinety 1.

The oral ciliature of the parental cell does not dedifferentiate during ontogenesis.

Occurrence and some ecological data. *Pelagostrobilidium wilberti* n. sp. was found in Magdalena for the first time in the winter of 2003 and again after two periods of drought in autumn 2004 and 2005. The species was also collected in winter 2005 in a temporary pool from Punta Lara, Buenos Aires Province (34°52’S, 57°54’W). During the period it was studied in Magdalena, the pH ranged from 5.09 to 8.76, electrical conductivity from 120 to 321 $\mu\text{S}/\text{cm}$, temperature from 5 °C to 9 °C, and dissolved oxygen from 4.05 to 11.24 mg/L. Food vacuoles were observed to contain ingested diatoms and other algae. It could also be maintained in laboratory cultures at room temperature (± 15 °C), feeding mainly on bacteria. While swimming, this species orients with adoral membranelles raised upwards, gyrating in the same place. When disturbed, it swims in a straight line, and then, it abruptly stops.

DISCUSSION

The genus *Pelagostrobilidium* was created by Petz, Song, and Wilbert (1995) for those species that have longitudinal and transversely arched somatic kineties that do not form a caudal spiral. Agatha et al. (2005) improved the genus diagnosis as follows ‘‘Strobilidiidae with kinety 3 and 4 posteriorly shortened and abutting on curved kinety 2. Oral primordium develops left laterally anterior to kinety 2 and right of kinety 3.’’ With this criterion, these authors transferred several species belonging to the genus *Strobilidium* Schewiakoff 1892 to *Pelagostrobilidium*.

Although Lynn and Montagnes (1988) and Montagnes and Taylor (1994) stated that differences in kinetal structure, such as the presence of cytoplasmic flaps and mono- and dikinetids, are used to diagnose genera within the family Strobilidiidae, while the arrangement of somatic kineties is more likely to show convergences, several other authors have used this latter character to establish new genera within this family (Agatha et al. 2005; Petz and Foissner 1992; Petz et al. 1995). This suggests that the features being used for this purpose may need reconsideration, but while this is unresolved, the species in the present study belongs to the genus *Pelagostrobilidium*.

Table 1. Morphometric Data on *Pelagostrobilidium wilberti* n. sp.

In vivo	X	M	SD	CV	n
Body					
Length	75 (63–84)	73.5	7	9.3	14
Width	44 (42–49)	42	3.3	7.5	14
After protargol					
Body					
Length	67 (42–77)	70	7.9	11.8	30
Width	46.5 (38–53)	46	4.1	8.8	30
External membranelles					
Number	28 (25–32)	28	1.4	5	30
Enlarged into the oral cavity					
Number	4 (3–5)	4	0.6	15	13
Polykinetid length	14 (13–15)	14	0.7	5	10
Polykinetid width	1.4	1.4	0	0	10
Internal membranelles					
Number	2	2	0	0	16
Adoral cilia					
Length	32 (22–41)	32	6.2	19.3	15
Distance from apex to cytostome	15.5 (14–20)	15	1.8	11.6	15
Cytopharyngeal fibers					
Length	22.2 (20–24)	22	1.4	6.3	9
Somatic kineties					
Number	6	6	0	0	30
Length K1	40 (25–52)	41	6.3	15.7	23
Length K2	28 (21–38)	27.5	4.1	14.6	24
Length K3	29 (20–41)	28	5.6	19.3	25
Length K4	31.5 (23–43)	31	5.7	18.1	25
Length K5	35 (22–43)	36	5.1	14.5	23
Length K6	34 (22–46)	35	6.5	19.1	23
Somatic cilia					
Length	2.7 (2–3.5)	2.8	0.5	18.5	10
Macronuclear figure					
Length	43.3 (34–53)	43	4	9.2	30
Width	9.6 (7–13)	9.5	1.8	18.7	30
Micronucleus					
Number	2 (2–3)	2	0.5	25	30
Diameter	2.5 (2.1–2.8)	2.8	0.3	12	18

Measurements are given in μm .

X, arithmetic mean (minimum and maximum values are between brackets); M, median; SD, standard deviation; CV, coefficient of variation; n, number of observations.

All *Pelagostrobilidium* spp. described to date were found in marine or brackish waters (Agatha et al. 2005; Lynn and Montagnes 1988; Montagnes and Taylor 1994; Ota and Taniguchi 2003; Pettigrosso 2003; Petz et al. 1995). *P. wilberti* is the first record of the genus in freshwaters. Unlike the other members of the genus, the kineties 3 and 4 of this new strobilidiid do not abut onto curved kinety 2, since this is shorter than in other described species, and kineties 3 and 4 are not conspicuously shortened. Thus, this species departs from the kinety arrangement previously considered a generic trait by Agatha et al. (2005).

Morphogenetic events began in a dorsal position, as in several other strobilidiids (Agatha et al. 2005). As in *Pelagostrobilidium neptuni* (Montagnes and Taylor, 1994) Petz, Song, and Wilbert, 1995, the morphogenesis of which was studied by Agatha et al. (2005), the formation of the oral primordium begins between kineties 2 and 3, but in *P. neptuni* the oral primordium originates in mid-body, laterally on the left. This could be a species-specific character, as suggested by Petz and Foissner (1992). Unlike the

observations of Agatha et al. (2005), the elongation and shaping of the macronucleus occurred in post-dividers, although only a few specimens were observed in this stage. The addition of kinetosomes to the opisthe's somatic kineties seems to occur at their anterior ends, as was also observed by Petz and Foissner (1993) for tintinnids. During the whole ontogenetic process, the kinety arrangement previously considered a generic trait by Agatha et al. (2005) did not occur, suggesting that the elongation of kinety 2 and the shortening of kineties 3 and 4, eventually abutting on kinety 2, could be derived characters in other species of *Pelagostrobilidium*. It is noteworthy that in late post-dividers of *P. wilberti* n. sp., the elongation of kineties 3 and 4 is delayed in comparison to the other kineties. A further elongation of kinety 2 is also seen in *P. neptuni* and *Pelagostrobilidium spirale* (Lynn and Montagnes, 1988) Petz, Song, and Wilbert, 1995, in which it forms a semicircular area with kinety 1, thus, supporting the preceding hypothesis. To accommodate these variations in pattern, an amendment of the diagnosis of the genus is necessary.

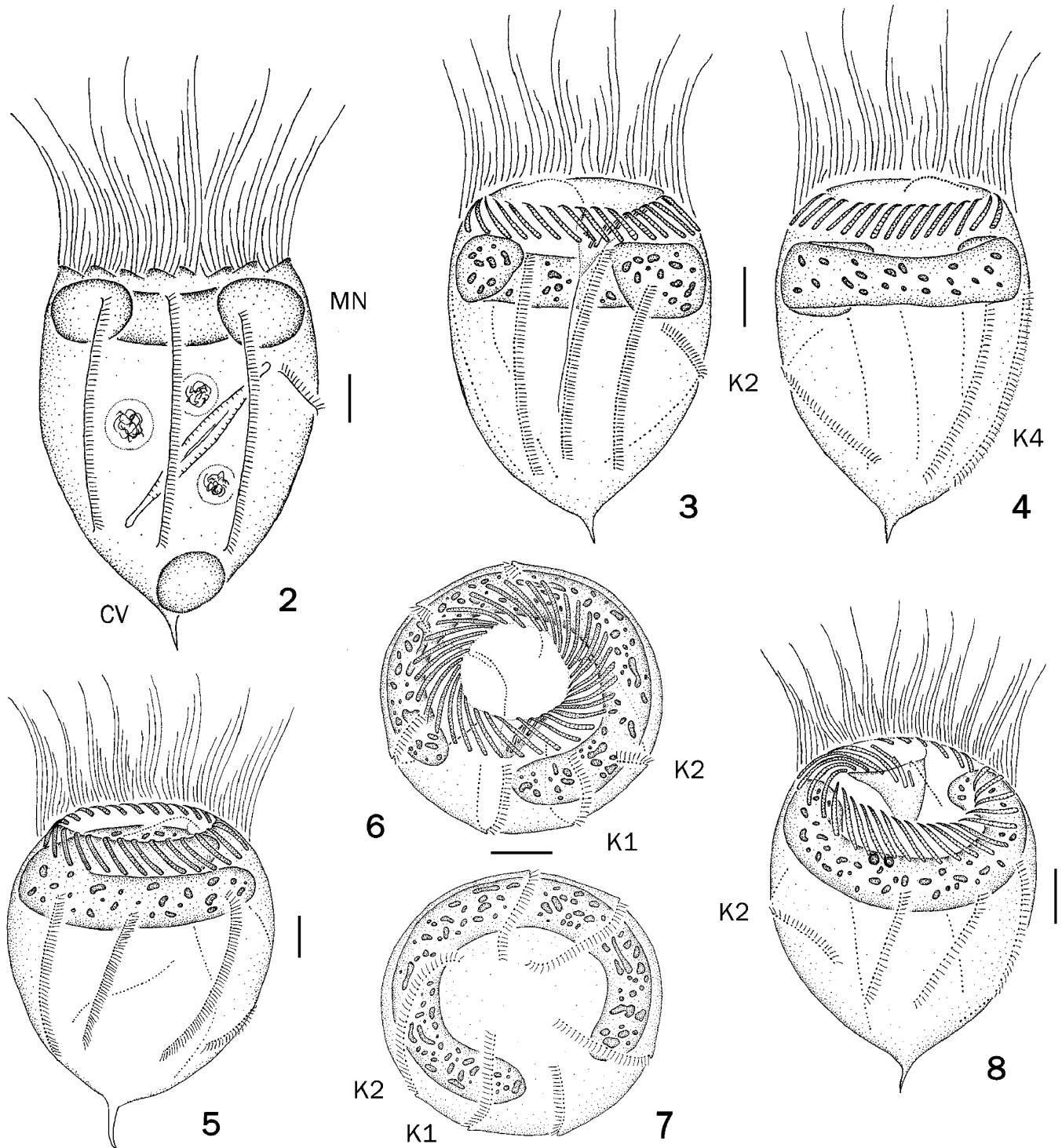


Fig. 2–8. *Pelagostrobilidium wilberti* n. sp., from life (2) and after protargol impregnation (3–8). 2. Ventral view. Food vacuoles inside the cell. 3. Ventral view. 4. Dorsal view. Kinity 2 sinistrally curved. 5. Right lateral view. 6. Oral view. 7. Aboral view. 8. Dorsal view. Specimen with shorter kineties 2–4. Note oral cavity and micronuclei. CV, contractile vacuole; K1, kinity 1; K2, kinity 2; K4, kinity 4; K6, kinity 6; MN, macronucleus. Scale bars = 10 μ m.

Concerning the species status of *P. wilberti* n. sp., *Pelagostrobilidium epacrum* (Lynn and Montagnes, 1988) Agatha, Strüder-Kypke, Beran, and Lynn, 2005 differs from *P. wilberti* in having five somatic kineties, with the typical arrangement of kineties 2–4. The number of external membranelles is higher

(33–40 vs. 25–32), although Pettigrosso (2003) mentions a slightly different number (30–31). Lynn and Montagnes (1988) observed four independent internal membranelles, while two were observed by Pettigrosso (2003). The number of micronuclei is not mentioned by these authors.

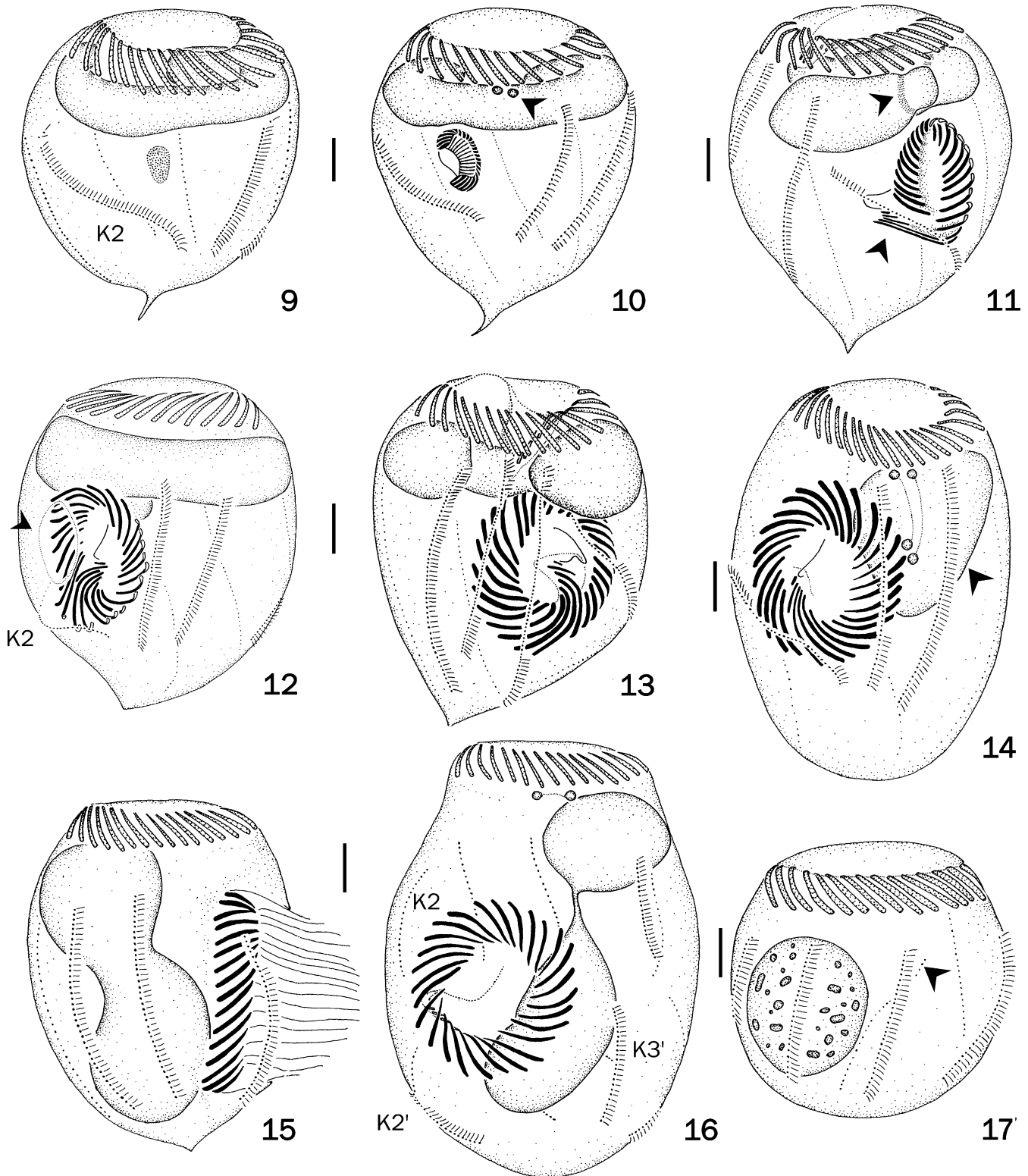


Fig. 9–17. *Pelagostrobilidium wilberti* n. sp., morphogenesis after protargol impregnation. 9. Dorsal view. Anarchic field of kinetosomes between kineties 2 and 3. 10. Dorsal view. Development of membranelles and the endoral kinety. Micronuclei (arrowhead). 11. Left lateral view. Replication bands (anterior arrowhead). Differentiation of internal membranelles (posterior arrowhead). 12. Dorsal view. Opening of the sub-surface pouch (arrowhead). Note concave disposition of membranelles. 13. Ventral view of the new oral apparatus. 14. Dorsal side. Macronucleus as a rounded mass (arrowhead). Note dividing micronuclei. 15. Left lateral view. 16. Dorsal view of a late divider. 17. Right lateral view of a post-divider. Kinety 2 (arrowhead). Some somatic cilia in Fig. 11–15 were not illustrated, in order to observe the oral primordium more clearly. K2, kinety 2; K2', K3', opisthe's kineties 2 and 3. Scale bars = 10 μ m.

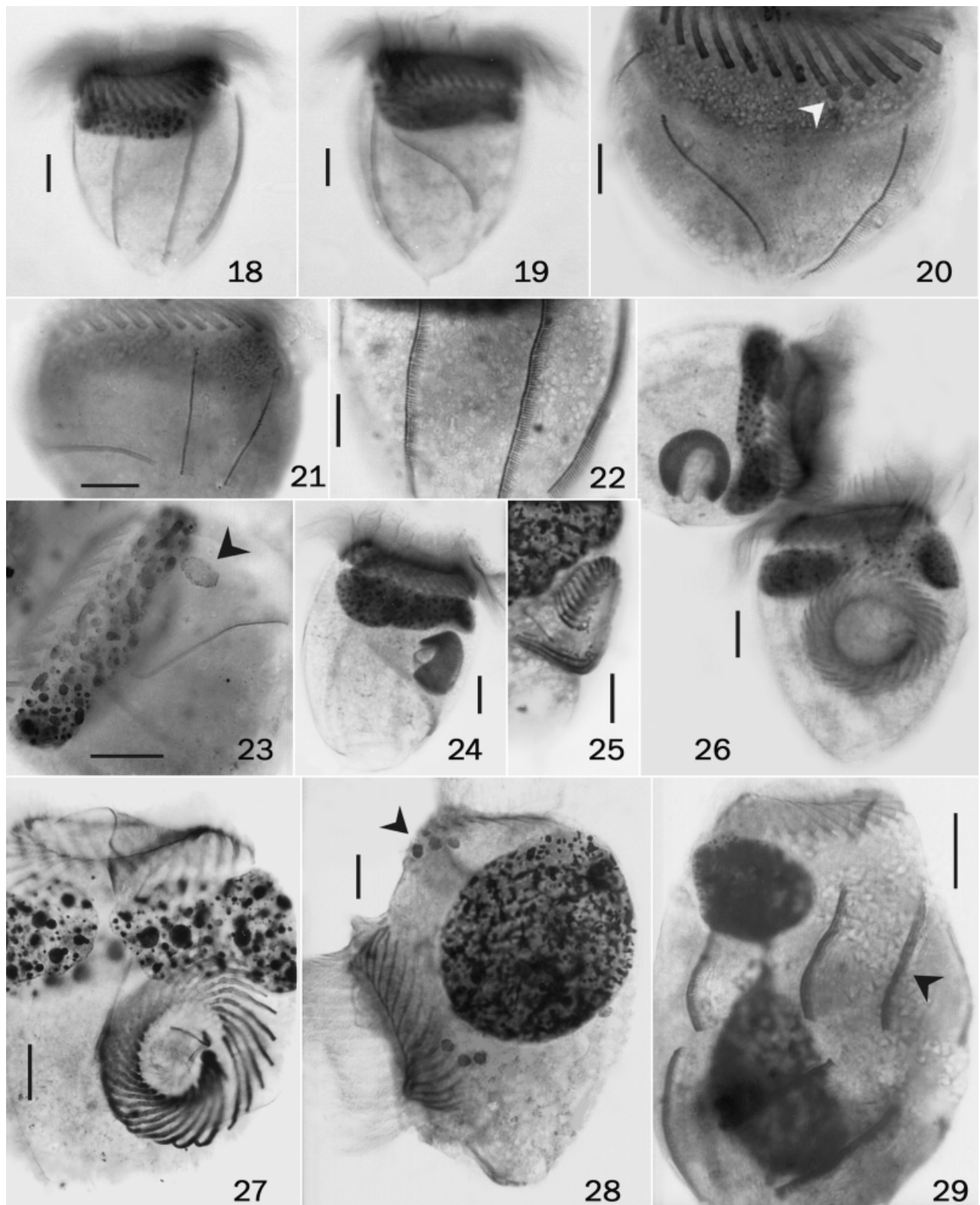


Fig. 18–29. *Pelagostrobilidium wilberti* n. sp., morphology (18–22) and morphogenesis (23–29) after protargol impregnation. 18. Right lateral view. 19. Dorsal view. 20, 21. Higher magnification of dorsal view showing micronuclei (20, arrowhead) and disposition of kineties 2–4 (21). 22. Higher magnification of somatic kineties 4–6 (from left to right). 23. Higher magnification of anarchic field of kinetosomes (arrowhead). 24. Dorsal view showing developing polykinetids and endoral kinety. 25. Detail of oral apparatus, perpendicular to cell surface, showing enlarged external and internal polykinetids. 26. Ventral view showing an earlier stage (cell on the left) and a fully developed oral apparatus (cell on the right). 27. Higher magnification of the fully developed oral apparatus, ventral view. 28. Dorso-lateral view. Proter's micronuclei (arrowhead). 29. Ventral view. Kinity 1 (arrowhead). Scale bars = 10 μ m.

Unlike *P. wilberti* n. sp., *Pelagostrobilidium simile* Song and Bradbury, 1998 has five somatic kineties and, in addition to the arrangement of kineties 2–4, kinety 5 also abuts on to kinety 2, which is transversely arched around the caudal area of the cell. As in *P. wilberti*, kinety 1 seems to be the longest. The number of external membranelles is similar (29–31), but only one internal membranelle was observed by these authors. The presence of a single conspicuous micronucleus is also a difference from *P. wilberti*.

Pelagostrobilidium sp. sensu Ota and Taniguchi (2003) also presents the arrangement of kineties observed in *P. simile*, as could be inferred from their illustrations and taking into consideration that somatic kineties remain unchanged during conjugation, but its kinety 6 also abuts on to the curved kinety 2.

Pelagostrobilidium spirale and *P. neptuni* also show the same arrangement of somatic kineties 2–4, but unlike *P. wilberti*, kinety 5 presents a particular arrangement, which in *P. neptuni* seems to be slightly variable among the populations studied by different authors. As described by Montagnes and Taylor (1994), kinety 5 originates one-third of the way from the aboral end of the cell and runs anteriorly, parallel to kinety 1, while it resembles an inverted L and is parallel to kinety 1 along its anterior half, according to Petz et al. (1995) and Agatha et al. (2005). In *P. spirale*, kinety 5 takes the form of a partial circle.

Based on the criteria proposed by Agatha (2004a, b) and Agatha et al. (2005) to infer the evolution of ciliary patterns in oligotrichid ciliates, the ancestral form of choreotrichids is presumed to have more or less meridional kineties. Thus, *Rimostrobilidium* Jankowski 1978 would be ancestral to the forms with spiral kineties, with *P. wilberti* being transitional to *P. epacrum*, which was also suggested by Agatha et al. (2005) before the discovery of this new strobilidiid. One could speculate on an evolution of the ciliary patterns among the extant species of the genus *Pelagostrobilidium*, where an elongation of the curved kinety 2 occurred. In this way, *P. wilberti*, with its short kinety 2, would be at a basal stage, followed by the arrangement observed in *P. epacrum*, with an elongated kinety 2 and kineties 3 and 4 posteriorly shortened and abutting on to kinety 2. In *P. neptuni* and *P. spirale*, kinety 2 elongates even more towards the posterior end of kinety 1, thus, forming a semicircular area with this kinety, and in *P. simile* kinety 2 extends around the caudal area, causing kinety 5 to also abut on to it. This proposed evolution of the ciliary pattern could be tested through phylogenetic analysis using gene sequences from these different species.

Thus, *P. wilberti* n. sp. is sufficiently distinct in a number of features to be justified as a new species as proposed below, along with the amended diagnosis of the genus *Pelagostrobilidium*.

Amended genus diagnosis. Strobilidiidae with kineties 3 and 4 posteriorly shortened, these abut on curved kinety 2 or, when the latter is posteriorly shortened, extend close to kinety 2. Oral primordium develops left laterally or dorsally anterior to kinety 2 and left of kinety 3.

Pelagostrobilidium wilberti n. sp.

Diagnosis. Size in vivo 63–84 × 42–49 μm. Body conical-shaped, with a posterior spine-like cytoplasmic process. With six somatic kineties that are almost longitudinal, except for kinety 2 that is sinistrally curved and is shorter than the others. Twenty-five to 32 external and two internal membranelles; 3 to 5 external membranelles extend into the oral cavity. Macronucleus horse-shoe-shaped, beneath the oral apparatus. Two or three dorsal micronuclei. Posterior contractile vacuole.

Type location. Temporary pond in Magdalena, Buenos Aires province, Argentina (35°05'S, 57°48'W).

Type specimens. Three permanent protargol-stained slides, representing hapantotypes, are deposited in the Smithsonian

Institution, Washington, USA (registration numbers USNM 1088408 and USNM 1088409), and one paratype slide in the Invertebrates Collection of the Museo de La Plata, Argentina (registration number MLP 022). Relevant organisms are marked.

Dedication. The species is dedicated to Professor Dr. Norbert Wilbert, Institut für Zoologie der Universität Bonn, Germany, to show him our appreciation and gratitude for sharing his knowledge and kind friendship with the senior author.

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