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A New Marine Ciliate, *Erniella wilberti* sp. n. (Ciliophora: Hypotrichida), from Shrimp Culturing Waters in North China

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Summary. The morphology and infraciliature of a new marine hypotrichous ciliate, *Erniella wilberti* sp. n., collected from a shrimp culturing pond near Qingdao (Tsingtao), China, are investigated using living observations and protargol silver impregnation method. The new species is characterized by: 80-180 × 25-70 µm *in vivo* with elongated body shape and thin hyaline margin encircling the whole cell; arc-shaped cortical structures arranged in 3 rows on dorsal side; bipartite adoral zone each with 8-12 and 16-20 membranelles respectively; 4-6 frontal, 1 buccal and 2-5 transverse cirri; 2 ventral cirral rows extending to posterior half of body; 3 dorsal kineties; 2 macronuclear nodules and 1-3 micronuclei.

Key words: *Erniella wilberti* sp. n., Hypotrichida, marine ciliate, morphology.

INTRODUCTION

The hypotrichous genus *Erniella* was established by Foissner (1987), which is characterized by: bipartite adoral zone of membranelles; with one left and one right marginal rows; two to several ventral rows, several frontal and transverse cirri present, no caudal cirri (Foissner 1987). It differs from other related genera in the following combined features of: (1) AZM in 2 clearly separated parts, (2) apart from several differentiated frontal cirri, most somatic ciliary organelles are in rows (ventral rows) and (3) transverse cirri present. Until now, only one species is known in this genus, *E. filiformis* Foissner, 1987, which was found in soil.

During a survey of the ciliate fauna in mariculture waters in northern China, an unknown ciliate was isolated from a shrimp-culturing pond near Qingdao in the summer of 2002. Subsequent observations and studies demonstrated that it represents a new member of the monotypic genus *Erniella*. Its morphology and infraciliature are documented as follows.

MATERIALS AND METHODS

Samples were collected on August 10, 2002 from a shrimp-culturing pond near Qingdao (Tsingtao, 36°08'N; 120°43'E), China.

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The ciliate was isolated and examined under a compound microscope with bright field and differential interference contrast equipments. The protargol silver staining method (Wilbert 1975) was used to reveal the infraciliature.

All drawings were performed at a magnification of 1250× with the aid of a camera lucida. Measurements were carried out with an ocular micrometer. Terminology mainly follows Corliss (1979) and Foissner (1982).

RESULTS

Erniella wilberti sp. n. (Figs 1-23; Table 1)

Diagnosis: Marine *Erniella*, with elongated body shape and thin hyaline margin encircling the whole cell; *in vivo* 80-180 × 25-70 μm; with arc-shaped cortical structures arranged in 3 rows on dorsal side; bipartite adoral zone each with 8-12 and 16-20 membranelles respectively; 4-6 frontal, 1 buccal and 2-5 transverse cirri; 2 ventral cirral rows extending to posterior half of body; 3 dorsal kineties; 2 macronuclear nodules and 1-3 micronuclei.

Dedication: We dedicate this species to Prof. Dr. Norbert Wilbert, Zoological Institute of the Bonn University, Germany, to express our special respects for his great contribution to the studies on the ciliatology and fatherly kindness to the junior author.

Type location and ecological features: Clear coastal water, salinity 18.5‰; water temperature 27°C; pH about 8.0.

Type slides: One holotype and one paratype slides of protargol impregnated specimens (No. Lin-02-08-10-A, B) are deposited in the Laboratory of Protozoology, Ocean University of China, P. R. China.

Morphology and infraciliature (Figs 1-9, 14-23): Size rather variable, 80-180 × 25-70 μm *in vivo*, but mostly about 120 μm long; body shape elongated with length: width about 3-4:1; both ends rounded, body widest in anterior 1/4-1/3; dorsoventrally flattened *ca* 2.5:1 (Figs 1-5). Body non-flexible and not contracted. Characteristically, cell clearly divided into 2 areas when observed dorsoventrally: central and marginal areas, of which the thin and hyaline margin is *ca* 5-8 μm in width, while the central portion is opaque and about twice to triple as thick as the margin area (Figs 1-3, 14). Buccal field narrow, about 2/5 to 1/4 of body length (Fig. 1). Pellicle conspicuously rigid, no typical cortical granules observed, but on dorsal side, some colorless arc-shaped structures loosely arranged in 3 rows, which are about 10-15 μm long and conspicuously recognizable even at low magnification (Figs 2, 18, 23, arrowheads). Cytoplasm colorless to grayish, with many large and differently-sized globules (3-10 μm across) making the central area opaque to completely dark (Figs 1, 4, 5, 14). Contractile vacuole not observed.

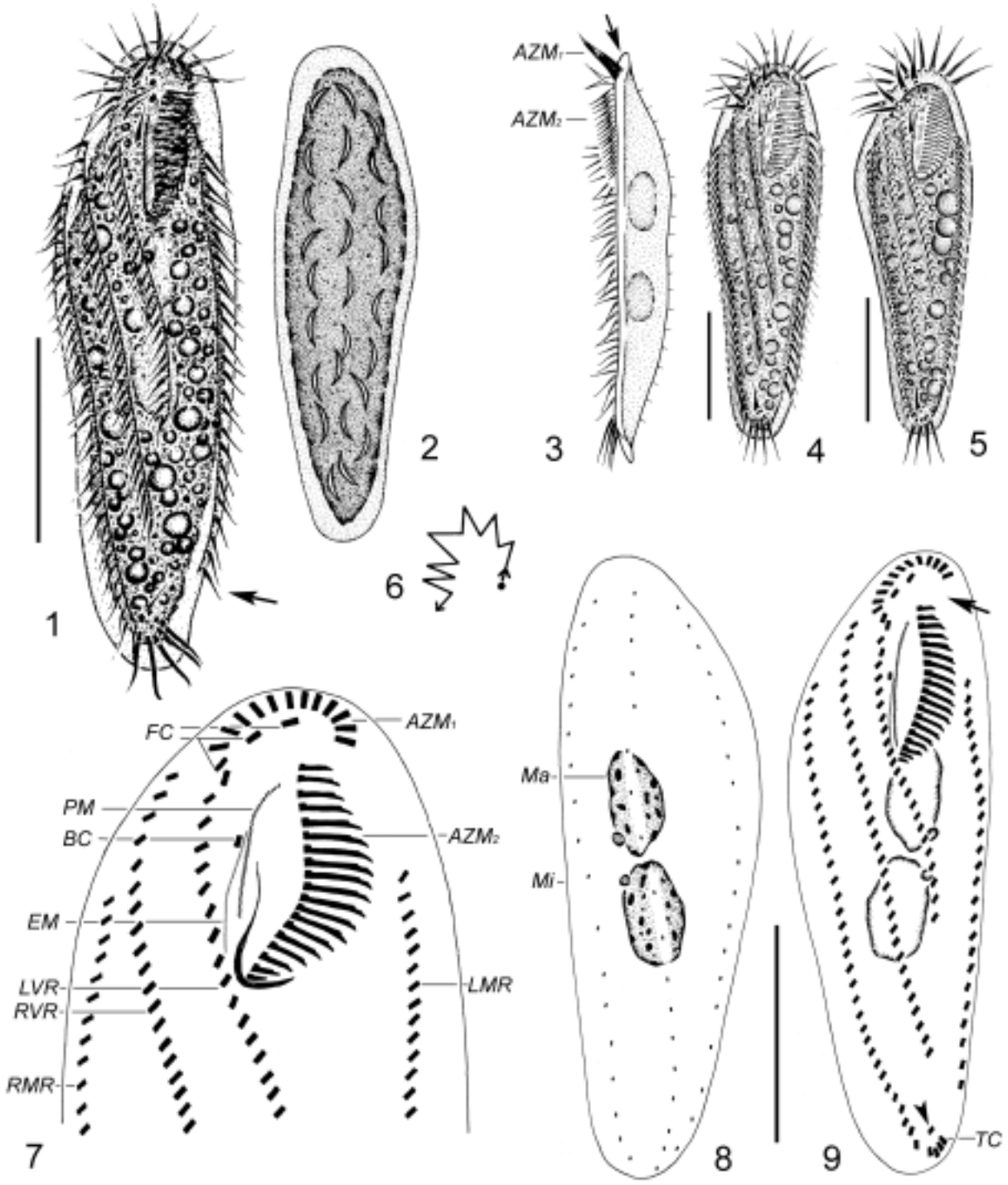
Two large macronuclear nodules (Ma), ovoid to ellipsoid, about 20 × 10 μm in size after protargol impregnation, lying in mid-body, containing many large spherical nucleoli (Fig. 8); 1-3 micronuclei (Mi) globular, about 4 μm in length, adjacent to macronuclei (Figs 8, 22, arrowheads).

Locomotion relatively fast, crawling on the bottom of Petri dish or on debris, with short and frequent pauses and then changing the moving direction (Fig. 6).

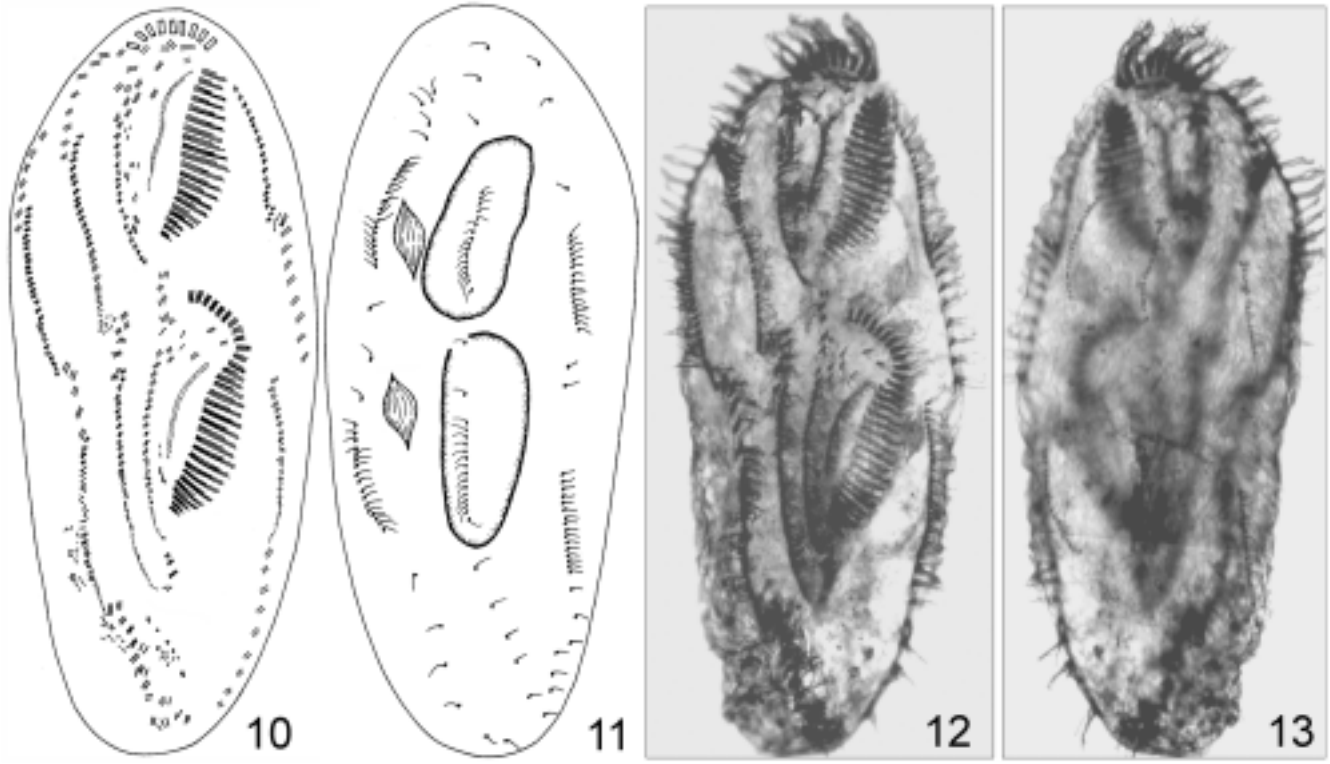
Adoral zone of membranelles (AZM) genus typic: one huge gap between bipartite AZMs (Fig. 9, arrow). Bases of membranelles in AZM_1 about 3 μm long, which are conspicuously shorter than that in AZM_2 (*vs.* 4-8 μm) and locating on ventral side (Fig. 3, arrow). Cilia of AZM_1 about 15 μm long. Paroral membrane (PM) long and slightly curved; endoral membrane (EM) relatively short and terminated anteriorly at about mid-PM (Figs 7, 17). 4-6 frontal cirri (FC); less than half of specimens (6 out of 16) having 4 slightly enlarged FC (Fig. 7), in which the posterior two (Fig. 17, arrowheads) are continuous with the distal end of AZM_1. In most cases, two more frontal cirri positioned posterior to the 4 anterior ones, which are located near the anterior end of the left ventral row and hence often difficult to detect (Fig. 19, arrows). Single buccal cirrus (BC) situated near mid-way of paroral membrane (Figs 7, 17, arrow). 2-5 transverse cirri (TC) (Fig. 20, arrow) with cilia *ca* 12-15 μm long, only slightly enlarged and close to the posterior end of right marginal row (Fig. 9). Two ventral rows, of which the left row (LVR) consists of 20-36 cirri, terminating posteriorly at 2/5 of cell length, while the right one (RVR) is composed of 31-47 cirri and terminates subcaudally (Figs 9, 15, 20). Usually 1-2 ventral cirri located near transverse ones (Figs 9, 20, arrowhead). Two marginal rows separated posteriorly, the left row (LMR) composed of 21-36 cirri and terminating subcaudally (Fig. 1, arrow), while the right one (RMR) with 18-39 cirri, extending to almost the cell end (Figs 9, 15).

Dorsal cilia bristle-like, about 3-5 μm long, consistently arranged in 3 rows, which extend over entire length of body (Figs 3, 8, 16, 21, arrowheads).

Some morphogenetic features during binary division (Figs 10-13): As a less-commonly occurring genus, the morphogenetic process of *Erniella* remains unknown. In the present work, one specimen in the



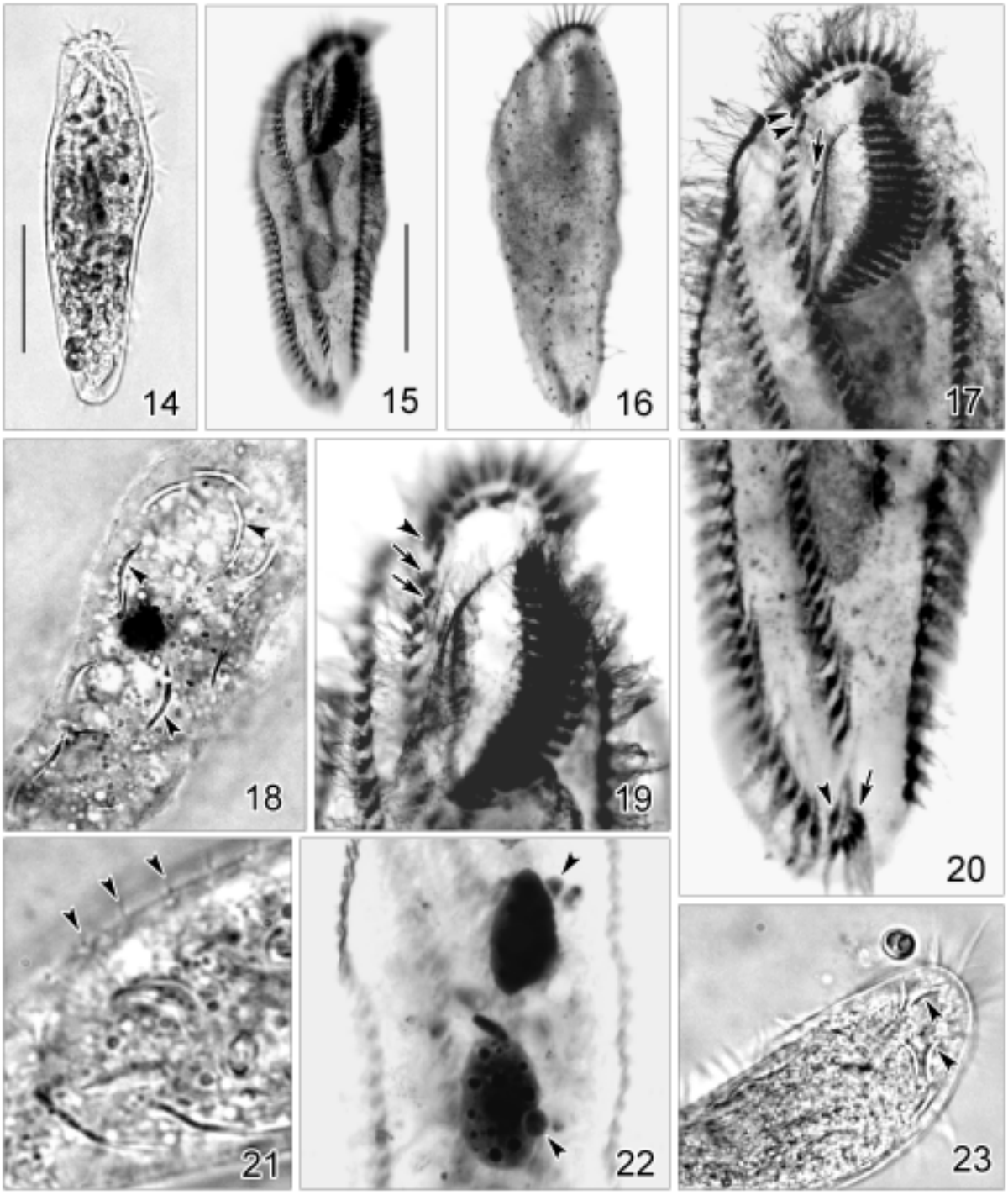
Figs 1-9. Morphology (1-6) and infraciliature (7-9) of *Erniella wilberti* sp. n., from life (1-5) and after protargol impregnation (7-9). **1 -** ventral view of a typical individual, arrow marks the end of left marginal row; **2 -** dorsal view, showing the arrangement of the arc-shaped cortical structures and hyaline cell margin; **3 -** lateral view, arrow indicates the cell margin, note that the membranelles in AZM_1 are located on the ventral side; **4, 5 -** ventral views, to show different body shapes; **6 -** diagram, to illustrate the movement; **7 -** infraciliature of the anterior part, to show the buccal apparatus; **8, 9 -** infraciliature of dorsal (8) and ventral (9) sides of the same specimen, arrow and arrowhead in Fig. 9 mark the gap between AZMs and the ventral cirrus anterior to the transverse cirri, respectively. AZM_1 - frontal part of adoral zone of membranelles, AZM_2 - posterior part of adoral zone of membranelles, BC - buccal cirrus, EM - endoral membrane, FC - frontal cirri, LMR - left marginal row, LVR - left ventral row, Ma - macronuclear nodules, Mi - micronuclei, PM - paroral membrane, RMR - right marginal row, RVR - right ventral row, TC - transverse cirri. Scale bars 40 µm.



Figs 10-13. Ventral (10, 12) and dorsal (11, 13) views of a specimen in the middle morphogenetic stage.

Table 1. Morphometrical data of *Erniella wilberti* sp. n. All data are based on protargol impregnated specimens. Measurements in µm. Abbreviation: AZM_1 - frontal part of the adoral zone of membranelles; AZM_2 - posterior part of the adoral zone of membranelles; CV - coefficient of variation; M - median, Max - maximum, Mean - arithmetic mean, Min - minimum, n - sample size, SD - standard deviation.

Character	Min	Max	Mean	M	SD	CV	n
Body length	92	160	121.1	124	21.37	17.6	16
Body width	28	60	42.9	44	10.01	23.4	16
Length of buccal field	20	32	26.8	27	3.29	12.3	16
Number of AZM_1	8	12	9.8	10	0.98	10.0	16
Number of AZM2	16	20	18.9	20	1.45	7.7	16
Number of frontal cirri	4	6	5.3	4	1.00	19.1	16
Number of buccal cirri	1	1	1	1	0	0	50
Number of transverse cirri	2	5	4.3	4	0.86	20.2	16
Number of cirri in left ventral row	20	36	25.2	25	3.92	15.6	16
Number of cirri in right ventral row	31	47	35.1	37	4.01	11.4	16
Number of cirri in left marginal row	21	36	29.4	31	3.93	13.4	16
Number of cirri in right marginal row	18	39	31.9	31	5.23	16.4	16
Number of ventral cirri anterior to transverse cirri	0	2	1.3	1	0.58	46.2	16
Number of macronuclei	2	2	2	2	0	0	50
Length of macronuclei	10	38	21.9	22	8.29	37.8	16
Width of macronuclei	7	20	11	11	3.98	36.2	16
Number of micronuclei	1	3	2	2	0.41	20.4	13
Length of micronuclei	3	6	3.8	3	1.09	28.6	16
Width of micronuclei	2	5	2.8	2	0.84	30.3	16
Number of dorsal kineties	3	3	3	3	0	0	50



Figs 14-23. Photomicrographs of the morphology and infraciliature of *Erniella wilberti* sp. n., from life (14, 18, 21, 23) and after protargol impregnation (15-17, 19, 20, 22). **14 -** general appearance of a living cell; **15, 16 -** infraciliature of the ventral (15) and dorsal (16) sides of the same specimen; **17 -** ventral view, to show the oral apparatus; arrowheads indicate two frontal cirri, which are continuous with the distal end of AZM_1, arrow marks the buccal cirrus; note the intersecting of paroral and endoral membrane; **18 -** dorsal side, to demonstrate the arc-shaped cortical structures (arrowheads); **19 -** infraciliature of the anterior portion; arrowhead indicates a pair of frontal cirri; arrows mark last pair of frontal cirri, which near the anterior end of the left ventral row; **20 -** ventral view of the posterior portion; arrowhead and arrow indicate the ventral and transverse cirri, respectively; **21 -** to show the bristle-like dorsal cilia (arrowheads); **22 -** nuclear apparatus, arrowheads mark the micronuclei; **23 -** dorsal view of the anterior portion; arrowheads indicate the cortical structures. Scale bars 40 µm.

middle morphogenetic stage was observed (Figs 10-13). Based on this stage, the following conclusions can be made: (1) the developing pattern of the fronto-ventral transverse cirri is very likely a 5-anlagen-mode; (2) the right ventral row seems developing independently within the parental structure and separated from other cirri; (3) the parental oral structure is possibly partly renewed (?, only the AZM_2 will be renewed) or completely retained for the proter and (4) one anlage develops within each of the 3 parental dorsal kineties in both proter and opisthe (Figs 11, 13).

DISCUSSION

Since the genus *Erniella* was established, only one terrestrial species, *E. filiformis* Foissner, 1987, has been reported (Foissner 1987). The new form, *E. wilberti* differs from it in the following features: (1) different habitat (marine *vs.* soil); (2) smaller size (80-180 × 25-70 µm *vs.* 200-300 × 25-35 µm); (3) lower number of macronuclei (2 *vs.* 31-61); (4) more dorsal kineties (3 *vs.* 1); (5) lower number of ventral rows (2 *vs.* 3) and (6) single buccal cirrus (1 *vs.* 3-6). In addition, the presence of the arc-shaped cortical structures in the new species is, as to authors' knowledge, unique. Hence, the two organisms can be clearly separated.

As mentioned in the description, most specimens possess 6 frontal cirri. In this case, the last 4 are arranged like two pairs of cirri, i.e. a minimum zig-zag pattern (Fig. 19). This arrangement might indicate that this organism (this genus?) could be an intermediate form between urostylids and stichotrichs: it possibly represents a primary pattern of mid-ventral rows, which are seen in most typical urostylids (Hemberger 1982, Song 1990, Eigner and Foissner 1994, Shi *et al*. 1999).

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