Acta Protozool. (2002) 41: 263 - 278

ACTA
PROTOZOOLOGICA

Comparative Studies on Two Closely Related Species *Uronemella filificum* (Kahl, 1931) and *Uronema elegans* Maupas, 1883 with Redescription of *Paranophrys marina* Thompson et Berger, 1965 (Ciliophora: Scuticociliatida) from China Seas

Weibo SONG, Honggang MA, Mei WANG and Mingzhuang ZHU

Laboratory of Protozoology, MRL, Ocean University of Qingdao, Qingdao, P. R. China

Summary. The living morphology, infraciliature and silverline system of 3 known marine scuticociliates, *Uronemella filificum* (Kahl, 1931) Song et Wilbert, 2002 (formerly *Uronema filificum* Kahl, 1931), *Uronema elegans* Maupas, 1883 and *Paranophrys marina* Thompson et Berger, 1965 are reinvestigated and described. Based on the populations isolated from China seas, improved diagnoses for all three species are supplied. Diagnosis for *Paranophrys marina*: marine slender *Paranophrys in vivo ca* 35-45 x 10-15 μm with sharp apical and narrowed posterior end; membranelle 1 two-rowed and dominant long, almost conjoint to membranelle 2; scutica with several singled basal bodies arranged in line; 10 densely ciliated somatic kineties; contractile vacuole large and caudally positioned, opens at the posterior end of the somatic kinety 2; one oval macro- and one small micronucleus. The well-known *Uronemella filificum* can be recognized by the following characters: marine pear-shaped *Uronemella in vivo ca* 25-45 x 12-30 μm with conspicuously large apical plate and typically thigmotactic living behaviour; membranelle 1 one- or partly two-rowed, only slightly away from apical area; 16-23 somatic kineties with densely packed cilia; contractile vacuole pore at posterior end of somatic kinety 2; one macro- and one micronucleus; extrusomes present. *Uronema elegans* is characterized by cylindrical or kidney-like body shape *in vivo ca* 30-50 x 20-30 μm with small apical plate and conspicuously reticulate pellicle ridges; membranelle 1 one- or partly two-rowed, which is considerably away from the apical area; 23-26 somatic kineties; contractile vacuole positioned sub-caudally near ventral margin with its pore at posterior end of somatic kinety 2; one macro- and one micronucleus; extrusomes bar-like, densely distributed.

Key words: marine ciliates, morphology, *Paranophrys marina*, Scuticociliatida, taxonomy, *Uronema elegans*, *Uronemella filificum.*

INTRODUTION

Morphological researches on the order Scuticociliatida using modern methods have demonstrated that diversity in this specialized group exhibits a greater richness -not only in species number, but also in ecological or physiological phenotypes- than considered before. Accordingly, more and more work has revealed that taxonomic studies based only on the silver impregnations have lead to new problems in species identification even for the well-known scuticociliates (Berger and Thompson 1960; Borror 1963; Dragesco 1963; Small 1967; Thompson 1972; Grolière 1974, 1980; Agamaliev 1978; Small and Lynn 1985; Fernadez-Leborans and Novillo 1994). This is mainly because many criteria used for species sepa-

Address for correspondence: Weibo Song, College of Fisheries, Ocean University of Qingdao, 266003 Qingdao, P. R. China; Fax: [+86] 532 - 203 2283; E-mail: wsong@ouqd.edu.cn

ration, e.g. the number of somatic kineties, body shape and size *et al*., are confluent or overlap among many morphologically-similar organisms. Thus, it is difficult to recognize a species merely depending on data which derive from merely few specimens or a certain population (Wilbert 1986, Foissner *et al*. 1994, Petz *et al*. 1995, Foissner 1996).

In some of our recent work, many little-known marine forms have been reinvestigated (Song 1993, 2000; Xu and Song 1999; Song and Wilbert 2000), and as a part of that series, three scuticociliates collected from the Yellow Sea and the South China Sea are described in the present paper.

MATERIALS AND METHODS

Paranophrys marina was collected in summer of 1998 from an open off-shore maricultural pond for mollusc-farming (*Argopecten irradians*) near Qingdao (Tsingtao, 36° 08' N; 120° 43' E); *Uronema elegans* was isolated from the mantle cavity of a marine mollusc (*Sinonovacula constricta*) near Qingdao in June 2001, while *Uronemella filificum* was found in July 2001 from coastal water in the suburb area of Zhanjiang (21° 12' N; 110° 18' E), Guangdong Province (Fig. 1).

Observations on living cells were carried out with a microscope equipped with Normarski differential interference optics. Protargol (Wilbert 1975) and Chatton-Lwoff method (Corliss 1953) were used for revealing the infraciliature and silverline system.

Drawings of impregnated specimens were conducted with the help of camera lucida; measurement was performed under the 1250 x magnification. Terminology is mainly according to Corliss (1979).

RESULTS AND DISCUSSION

About the definition of the genus *Uronemella* Song et Wilbert, 2002 (in press)

The newly-established genus *Uronemella* Song et Wilbert, 2002 differs from the well-known *Uronema* Dujardin, 1841 basically in living features: (1) the former exhibits a dominant frontal plate, and hence has an inverted pear-shaped or stout-oval body shape (*vs*. oval, ellipsoid to cylindrical); (2) the cytostome is positioned post-equatorially (*vs*. located equatorially) and (3) thigmotactic locomotion (*vs*. non-thigmotactic in the latter). The movement of *Uronema* demonstrate a particular thigmotactic manner, the "rotation-movement": with help of a thread-like structure which derives from the caudal cilium and hence attaching temporarily to substrate and making continuously a rotation behaviour.

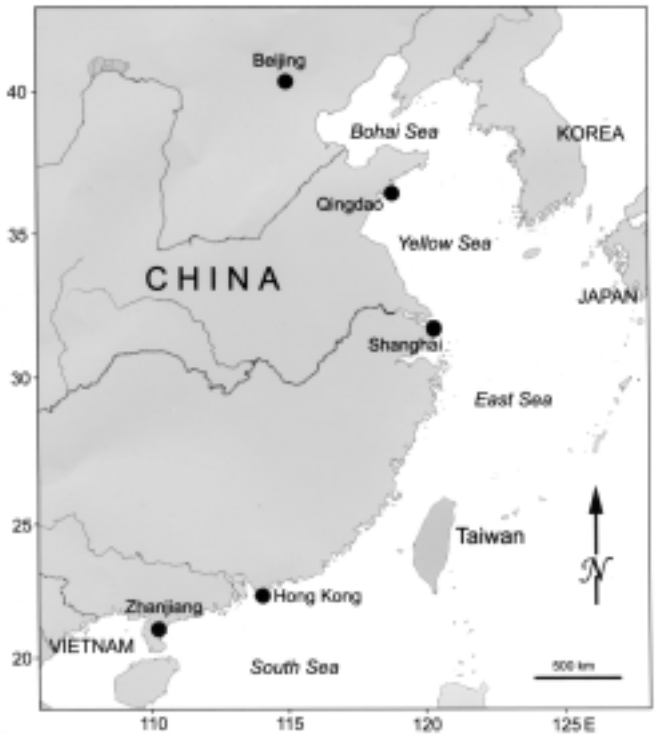


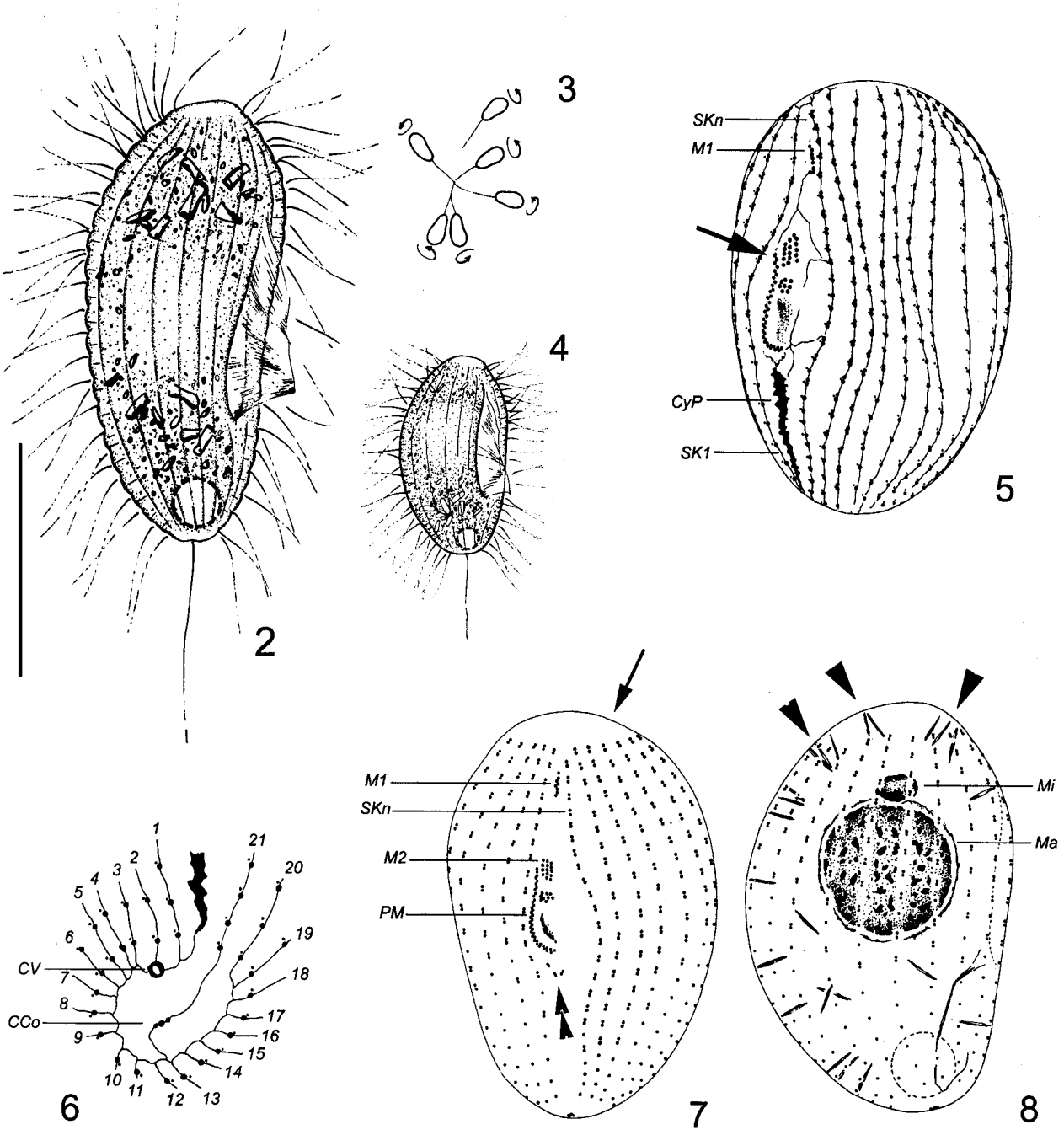
Fig. 1. Map of sampling sites (Qingdao and Zhanjiang)

Kahl (1931, p. 356) described the movement for *Uronema filificum* as follows: "…nach einiger Zeit der Ruhe sieht man auf dem Objektträger mehr und mehr Individuen, die sich an Detritus angeheftet haben und einen Faden langsam bis auf 1/2 mm ausziehen, an dem sie rotierend pendeln; sie lösen sich aber oft ab…" According to this definition, *Uronema filificum* Kahl, 1931 was transferred into the genus *Uronemella* by Song and Wilbert (2002).

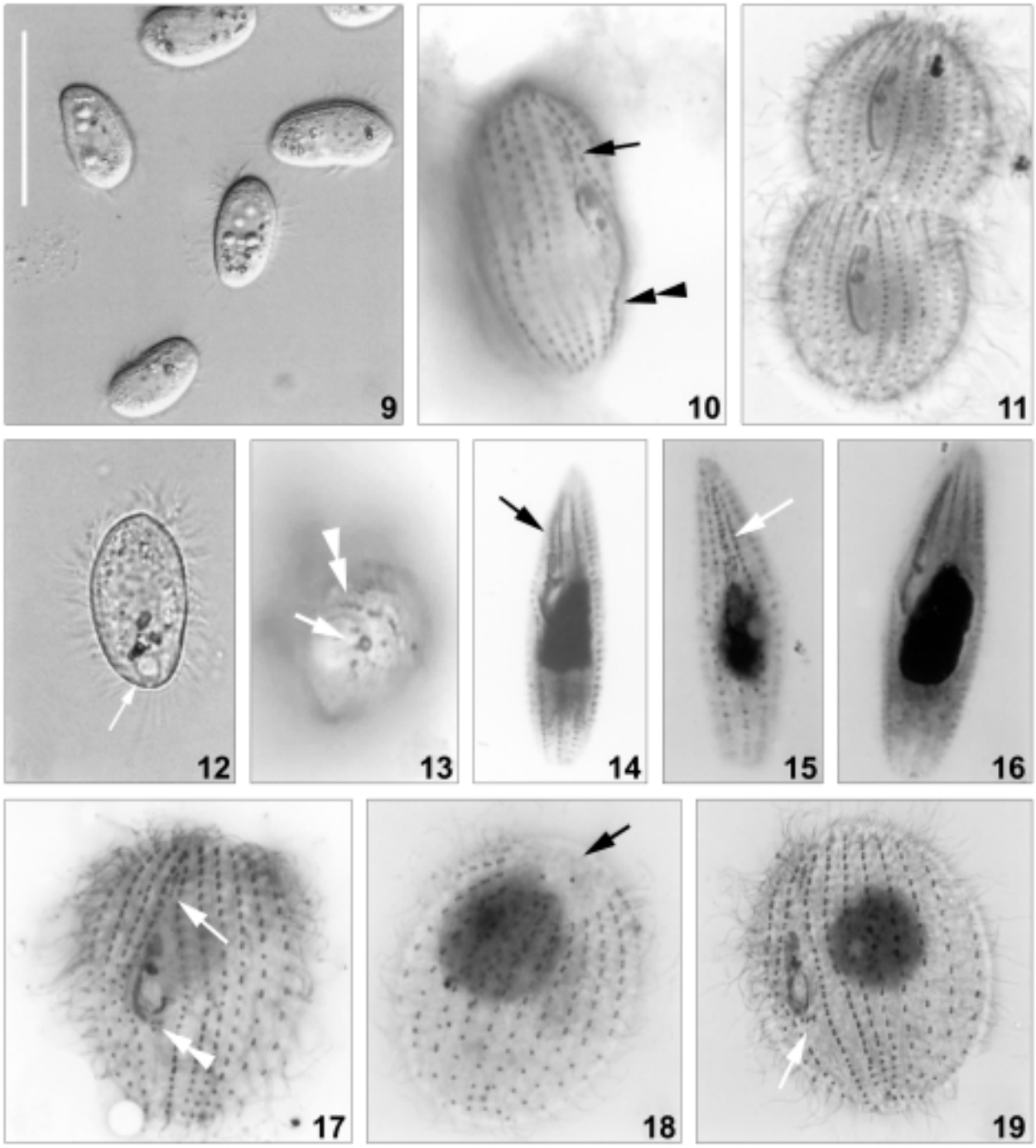
In 1980, Jankowski proposed a new genus *Uronemita* with *U. filificum* as its type species (Jankowski 1980). However, according to art 13 of the Code of Zoological Nomenclature (ICZN 1999), *Uronemita* is an invalid name because no definition or description has been supplied in the original report for this new taxon.

Uronemella filificum (Kahl, 1931) Song et Wilbert, 2002 (Figs 2-8, 9-13, 17-19, 28, 33; Table 1, 2)

Syn. 1931 *Uronema filificum* - Kahl, *Tierwelt Dtl.,* **21:** 356 (original)
1963 *Uronema filificum* - Borror, *Trans. Am. Microsc.* Soc. **82:** 125
1968 *Uronema elegans* - Czapik, *Acta Protozool*. **5:** 225
1968 *Uronema filificum* - Thompson et Kaneshiro, *J. Protozool*. **15:** 141
1981 *Uronema filificum* - Wilbert et Kahan, *Arch. Protistenkd.* **124:** 78
1985 *Urocyclon filificum* - Small et Lynn, In: An Illustrated Guide to the Protozoa
1986 *Uronema elegans* - Dragesco et Dragesco-Kernéis, *Faune Trop.* **26:** 347
1996 *Uronema filificum* - Pérez-Uz, Song et Warren, *Europ. J. Protistol*. **32:** 263



Figs 2-8. *Uronemella filificum in vivo* (2-4), after silver nitrate (5, 6) and protargol impregnations (7, 8). **2** - right lateral view of a representative individual; **3** - motion scheme, to show 4 cells conjoint together with the caudal thread attached on the bottom; **4** - a well-fed specimens after culture; **5** - ventro-lateral view of silverline system; arrow marks the anterior end of the paroral membrane; **6** - caudal view, to show the silverline pattern; **7** - ventral view of infraciliature, note the conspicuous cilia-free apical plate (arrow) and the small scutica with only 3 pairs of basal bodies (arrowheads); **8** - right lateral view of infraciliature, to show the extrusomes (arrowheads) and nuclear apparatus. Abbreviations: CCo - caudal cilium complex, CV - pore of contractile vacuole, CyP - cytopyge, M1-2 - membranelle 1-2, Ma - macronucleus, Mi - micronucleus, PM - paroral membrane, SK1, n - the first and the last somatic kinety. Scale bar - 20 μm



Figs 9-19. Photomicrographs of *Uronemella filificum* (9-13, 17-19) and *Paranophrys marina* (14-16) from life (9, 12), after silver nitrate (10, 13) and protargol impregnations (11, 14- 19). **9** - slightly pressed cells; **10** - lateral view, to show the buccal apparatus (arrow marks the membranelle 1) and the cytopyge (arrowheads); **11** - ventral view of a late divider; **12** - lateral view, arrow indicates the contractile vacuole; **13** - caudal view, arrow marks the contractile vacuole pore, while arrowheads indicate the last somatic kinety which extends through the caudal cilia complex; **14, 15** - lateral and ventral view, to show the prolonged membranelle 1 (arrows in 14, 15); **16** - lateral view, to show macronucleus; **17** - ventral view, to show the buccal apparatus; arrow indicates the membranelle 1, while the arrowheads mark the scutica; **18** - dorsal view, to show the dominant apical plate (arrow); **19** - ventral view, arrow demonstrates the scutica. Scale bar - 40 μm

Table 1. Morphometrical characterization of *Uronemella filificum* population I (first line, original), *U. filificum* population II (second line, original), *Uronema elegans* (third line, original) and *Paranophrys marina* (fourth line, data after Xu and Song 1999). Data based on protargol impregnated specimens (according to the present authors). All measurements in µm. CV- coefficient of variation, Max - maximum, Mean - arithmetic mean, Min - minimum, n - sample size, SD - standard deviation, SE - standard error of the mean.

Character	Min	Max	Mean	SD	SE	CV	n
Body length	20	36	31.9	8.19	2.05	25.7	16
	28	43	34.4	3.31	0.74	9.6	20
	33	45	36.8	3.45	0.92	9.4	14
	35	53	46.6	4.05	1.08	8.7	14
Body width	10	25	21.1	8.18	2.04	38.8	16
	16	28	23.2	3.64	0.81	15.7	20
	19	25	20.9	1.77	0.47	8.5	14
	11	16	12.9	1.44	0.39	11.2	14
Length of buccal field	11	23	17.1	3.87	1.12	22.7	12
	18	24	21.0	1.75	0.44	8.3	16
	18	24	20.3	2.01	0.58	9.9	12
	23	29	26.6	2.30	0.73	8.7	10
Number of somatic kineties	21	22	21.3	0.48	0.12	2.2	16
	22	23	22.8	0.41	0.09	1.8	20
	23	24	23.1	0.36	0.10	1.6	14
	10	10	10	0	0	0	14
Number of basal bodies in somatic kinety 1*	17	23	19.6	2.15	0.14	11.0	7
	18	23	20.3	1.56	0.45	7.6	12
	ca 23	-	-	-	-	-	-
	-	-	-	-	-	-	-
Number of basal bodies in somatic Kinety n	21	28	23.3	1.82	0.49	7.8	14
	23	32	27.6	3.28	0.88	7.6	14
	ca 28	-	-	-	-	-	-
	-	-	-	-	-	-	-
Number of basal bodies in membranelle 1	7	9	-	-	-	-	3
	ca 6	-	-	-	-	-	-
	ca 7	-	-	-	-	-	-
	20	27	-	-	-	-	-
Number of macronucleus	1	1	1	0	0	0	>100
	1	1	1	0	0	0	>100
	1	1	1	0	0	0	>100
	1	1	1	0	0	0	>100
Length of macronucleus	7	20	13.0	4.80	0.30	36.9	15
	10	14	11.5	1.37	0.34	11.9	16
	11	18	13.8	2.68	0.74	19.5	13
	12	18	15.0	1.95	0.59	13.0	11

* Dikinetids counted as single ones

Though this "well-known" species has been mentioned or redescribed for several times after Kahl (Borror 1963, Thompson and Kaneshiro 1968, Wilbert and Kahan 1981, Small and Lynn 1985), all these studies supplied little living information which is, in authors' opinion, insufficient for species identification. On the basis of the previous descriptions as well as the data obtained from the Chinese population, an improved diagnosis is added hence.

Improved diagnosis: marine *Uronemella in vivo ca* 25-45 x 12-30 µm with conspicuously large apical plate and typically thigmotactic living behaviour; membranelle 1 one- or partly two-rowed, slightly away from apical area; 16-23 somatic kineties with densely packed cilia; contractile vacuole pore at posterior end of somatic kinety 2; one macro- and one micronucleus; extrusomes present.

Description of Chinese populations: cells *in vivo* about 25-35 x 15-25 µm in the Zhanjiang population, while 30-45 x 20-30 µm in the Qingdao population. Body shape rather constant, inverted pear-shaped or oval when well-fed with ventral side slightly concave, while

Table 2. Morphological and morphometrical characterization of *Uronemella filificum* and *Uronema elegans*. All measurements in µm. [CVP - contractile vacuole pore; EP - about equatorially positioned; Ex - extrusomes; BM - behind mid-body; SK - somatic kinety(ies); SS - silverline system; ? - data not available]

Character	*Uronemella filificum*	*U. filificum*	*U. filificum*	*U. filificum*	*U. filificum*	*Uronema elegans*	*U. elegans*	*U. elegans*	*U. filificum**	*U. elegans*
Cell size *in vivo*	*ca* 30 (length)	23-43 x 12-27	27-32 x 12-20	25-35 x 15-25	30-45 x 20-30	35-50 x 20-25	?	?	?	40-90
Cell size after impregnation	?	23-31 x 14-21	?	20-36 x 10-25	28-43 x 16-28	33-45 x 19-25	40-50 (length)	35-58 x 20-34	33-37 x 24-31	?
Number of SK	*ca* 18	16-19	16-17	21-22	22-23	23-24	25-26	23-24	*ca* 21	?
Position of CVP	end of SK2	end of SK2	end of SK2	end of SK2	end of SK2	end of SK2	?	?	?	?
Reticulate -stripes on cell surface (*in vivo*)	?	?	?	absent	absent	present	?	?	?	present
Body shape	oval, inverted pear-shaped	inverted pear-shaped	inverted pear-shaped	slender, inverted pear-shaped	plump D-shaped	cylindrical	?	elongate cylinder-shaped	oval	elongate, cylinder-shaped
Apical plate	very large	very large	very large	very large	very large	relatively small	?	relatively small	?	relatively small
Glutinous thread extended from caudal cilium	present	present	present	present	present	absent	?	absent	?	absent
Buccal field	?	?	?	shallow and long	shallow and long	very deep and short	?	?	?	?
Position of cytostome	BM	BM	BM	BM	BM	EP	EP	EP	BM	EP
Extrusomes	?	?	?	present	present	present	?	?	?	?
Structure / position of membranelle 1	one-rowed slightly sub-apical	one-rowed slightly sub-apical	one-rowed slightly sub-apical	partly two-rowed slightly sub-apical	one-rowed slightly sub-apical	partly two-rowed considerably sub-apical	one-rowed considerably sub-apical	one-rowed considerably sub-apical	one-rowed slightly sub-apical sub-apical	?
Sample location	Red Sea, Israel	Virginia coast, USA	Florida coast, USA	South China Sea Zhanjiang, China	Yellow Sea, Qingdao, China	Yellow Sea, Qingdao, China	Caspian Sea, Georgia	Virginia coast, USA	Cotonou, Africa	North Sea, Germany
Data resource	Wilbert, Kahan 1981	Thompson, Kaneshiro1968	Borror1963	original	original	Song, Wilbert 2002	Agamaliev1974	Thompson, Kaneshiro 1968	Dragesco, Dragesco-Kernéis 1986	Kahl 1931

* Described as *Uronema filificum*

Table 3. Morphological comparison of *Uronemella filificum* and *Uronema elegans*.

Character	*Uronemella filificum*	*Uronema elegans*
Body shape	inverted pear-shaped	cylindrical to kidney-shaped
Apical plate	conspicuously large, about 1/3 of body width	small, definitely <1/3 of body width
Anterior end of membranelle 1	slightly away from apical plate	conspicuously away from apical plate
Pellicle feature	slightly notched	strongly notched with reticulate ridges on cell surface
Extrusomes	positioned within kineties, not highly developed, generally not recognizable after silver nitrate impregnation	highly developed and densely arranged, positioned often slightly away from kineties; always clearly to discern after silver nitrate impregnation
Number of somatic kineties	16-23 (mostly *ca* 18)	23-27
Arrangement of basal bodies within somatic kinety n and n-1	closely packed till posterior end	loosely distributed (especially in the posterior portion)
Buccal field (buccal apparatus) after impregnation	area between somatic kinety 1 and n proportionally wide and dominant	(as an area) small and narrow, relatively inconspicuous
Buccal cavity *in vivo* (1) when viewed from side	inconspicuous (shallow and often difficult to recognize)	as a clear area, small but very deep; clearly to discern
(2) when viewed ventrally	slender water-drop-shaped, long and always as a hyaline area	almost not recognizable
Position of cytostome	posterior to equatorial level	on the equatorial level
Cytopyge	relatively long	proportionally short
Position of contractile vacuole	caudally positioned	sub-caudally near ventral side
Behaviour	typical thigmotactic, always making "*filificum*"- movement	non-thigmotactic, completely quiet on substrate
Sticky thread deriving from caudal cilium	present	absent

dorsally conspicuously convex; cross section more or less bilaterally flattened. Frontal end flat, truncated with conspicuous apical plate, which is about 1/3 of body width, while posteriorly narrowly rounded (Fig. 2). In culture, cells usually plump and more oval than pear-shaped (Fig. 4). Buccal field about 3/5 of body length; cytostome conspicuously posterior to mid-body level (Figs 2, 4), which is as a narrow field (or slender-water-drop-shaped) when viewed from ventral side. Pellicle thin and inconspicuously notched. Extrusomes about 1.5 μm long, bar-shaped, closely beneath pellicle, *ca* 3 μm long after protargol impregnation (Figs 2, 8).

Cytoplasm colourless to slightly greyish, contains many to numerous granules *ca* 0.5-1 μm across and crystals of different size and shape (Fig. 2). Macronucleus large, round to oval, located centrally. Contractile vacuole caudally positioned (Figs 2, 4, 12).

Cilia about 8 μm long, densely arranged; caudal cilium *ca* 15 μm long. Two manners of locomotion: (1) as attaching to substrate, making typical rotation movement. In this case, cells often several together conjoined with their threads (about 10 μm long) deriving from caudal cilium, or sticking to debris while slowly rotating around longitudinal axis of body in both cases (Fig. 3); (2) when disturbed, cells moving very quickly, swimming in zig-zag pattern.

Somatic ciliature of the Zhanjiang population as shown in Figs 7 and 8: mostly 21 ciliary rows extending over entire length of body, composed of close-set dikinetids in anterior half of body, while monokinetids loosely-arranged in posterior half. As exception, left-most kineties (or SKn, SKn-1; to left of buccal field) conspicuously more densely ciliated than other nearby ones with almost entirely dikinetids (Figs 7, 17, 19).

Buccal apparatus similar to its congeners. Membranelle 1 positioned near apical plate and clearly separated from other membranelles, consisting of *ca* 7-9 basal bodies, which form often 2-rowed structure in the middle portion (Figs 7, 19). Membranelle 2 and 3 short, near to each other, each consisting of 3 rows of basal bodies. Paroral membrane on right of shallow buccal cavity, composed zigzaging row of basal bodies, extending anteriorly to about middle of membranelle 2. Scutica consisting of 3 to 4 pairs of basal bodies (Figs 7, 17; arrowheads).

Silverline system as shown in Figs 5, 6, 10 and 13: pore of contractile vacuole at posterior end of somatic kinety 2 (Fig. 13, arrow); line from somatic kinety n (left-most one to buccal field) extending posteriorly through caudal complex (CCo) and connecting dorsally with about kinety 12-13 (Fig. 6). Cytopyge (CyP) long, irregularly shaped (Fig. 5).

Ecological features: This species was isolated from a shrimp-incubating pond, salinity was about 20 ‰, pH and temperature were 8.2 and *ca* 30°C respectively.

Comparison: With establishment of *Uronemella*, Song and Wilbert (2002) included three morphotypes in the genus: *U. binucleata* (Song, 1993) Song et Wilbert, 2002 (= *Homalogastra binucleata* Song, 1993), *U. filificum* (Kahl, 1931) Song et Wilbert, 2002 and *U. cymruensis* (Pérez-Uz et Hope, 1997) Song et Wilbert, 2002 (=*Urocyclon cymruensis* Pérez-Uz et Hope, 1997).

As a widely distributed species, *Uronemella filificum* has been redescribed for many times during last decades from various geographical regions (Borror 1963, Thompson and Kaneshiro 1968, Wilbert and Kahan 1981, Pérez-Uz *et al*. 1996). But considering its identification, there is always some disagreement remained: e.g. the number of somatic kineties seems highly variable and population-dependent (Table 2), which is usually relatively constant in most other scuticociliates (Foissner and Wilbert 1981, Wilbert 1986, Foissner *et al*. 1994, Petz *et al*. 1995). This character is hence somehow confluent in some cases between *U. filificum* and *Uronema elegans* (Table 2), and this is also the main reason why these two forms were repeatedly confused in previous reports (Borror 1963, Czapik 1968, Agamaliev 1974, Dragesco and Dragesco-Kernéis 1986).

Based on the information obtained in the present work as well as in previous papers, *Uronemella filificum* can be separated from *Uronema elegans* in many "minor" dissimilarities: (1) the body size in the former is usually smaller (25-40 *vs.* 35-50 μm in length); (2) the body shape *in vivo* is basically inverted pear-like with broadest region in anterior portion (vs. elongate or cylindrical with small apical plate in *U. elegans*); (3) cell surface is only inconspicuously notched (vs. conspicuously reticulate stripes and ridges on cell surface - even at low magnification); (4) the former possesses thigmotactic manner with sticky thread deriving from the caudal cilium (vs. lacking such thread and non-thigmotactic as observed by the authors recently); (5) buccal ciliary organelles are clearly to recognize from life (vs. within the large buccal cavity and somewhat difficult to discern from outside); (6) contractile vacuole is located at the posterior-most end (vs. near ventral side sub-caudally); (7) membranelle 1 only slightly away from the apical plate (vs. far away in *U. elegans*); (8) usually lower number of somatic kineties (16-23 *vs.* 23-27).

In addition, both forms demonstrate different appearances of the buccal apparatus (buccal region conspicuously wider in *U. filificum* than in *U. elegans*) and silverline system (e.g. extrusomes usually not recognizable after silver nitrate impregnation *vs.* highly developed and usually slightly away from kineties in the latter) (Table 3) (Borror 1963, Czapik 1968, Thompson and Kaneshiro 1968, Agamaliev 1974, Wilbert and Kahan 1981, Small and Lynn 1985, Dragesco and Dragesco-Kernéis 1986).

Song and Wilbert (2002) described a morphotype found from Qingdao, which differs from the Zhanjiang population only in possessing slightly higher number of somatic kineties (22-23 *vs.* 21-22) and a plump body shape. Considering the variation of these features among different populations, it should be conspecific, as an extreme form, with *U. filificum* according to the new understanding.

With reference to the position of cytostome, the body shape, the general appearance of living morphology, the living behaviour, the number of somatic kineties and other infraciliatural features, the following two morphotypes were very possibly misidentified and hence should be also conspecific with *Uronemella filificum*: *Uronema elegans* sensu Dragesco et Dragesco-Kernéis, 1986 and *Uronema elegans* sensu Czapik, 1968.

Uronema elegans Maupas, 1883 (Figs 20-26, 27, 29-32, 37-46; Table 1, 2)

Syn. 1883 *Cryptochilum elegans* - Maupas, *Arch. Zool. Exp. Gén. 1:* 663 (original)
1931 *Uronema elegans* - Kahl, *Tierwelt Dtl.,* **21:** 357
1968 *Uronema elegans* - Thompson et Kaneshiro, *J. Protozool.* **15:** 142
1974 *Uronema elegans* - Agamaliev, *Acta Protozool.* **13:** 69

This species was often confused with its morphologically similar form, *Uronemella filificum* considering the taxonomic definition and species separation. Based on the data obtained, we supply here a new definition for this "well-known" organism.

Improved diagnosis: Marine cylindrical or kidney-shaped *Uronema in vivo* mostly *ca* 30-50 x 20-30 μm with small apical plate and conspicuously reticulate pellicle ridges; membranelle 1 short and one- or partly two-rowed, which is considerably away from the apical area; 23-26 somatic kineties; contractile vacuole subcaudally positioned near ventral margin with its opening pore at posterior end of somatic kinety 2; one macro- and one micronucleus; extrusomes bar-like, densely distributed.

Description of Chinese population: Cells *in vivo* usually about 35-45 x 20-25 μm in newly-sampled specimens, body shape rather constant, generally cylindrical or kidney-shaped when viewed from lateral with ventral side slightly concave in mid-body, while dorsally convex (Figs 20, 26, 38); cross section rounded. Frontal end truncated, that is, with small apical plate (Figs 20, 26, 40). In culture, cells might be slightly plumper and more oval (Figs 26, 38). Buccal field about 1/2 of body length, with cytostome at bottom of deep buccal cavity (Fig. 25). Pellicle thick and strongly notched on outline with conspicuous reticulate ridges (Figs 24, 37). Extrusomes bar-shaped, about 2 μm long and closely arranged beneath pellicle (Figs 21, 25, 44).

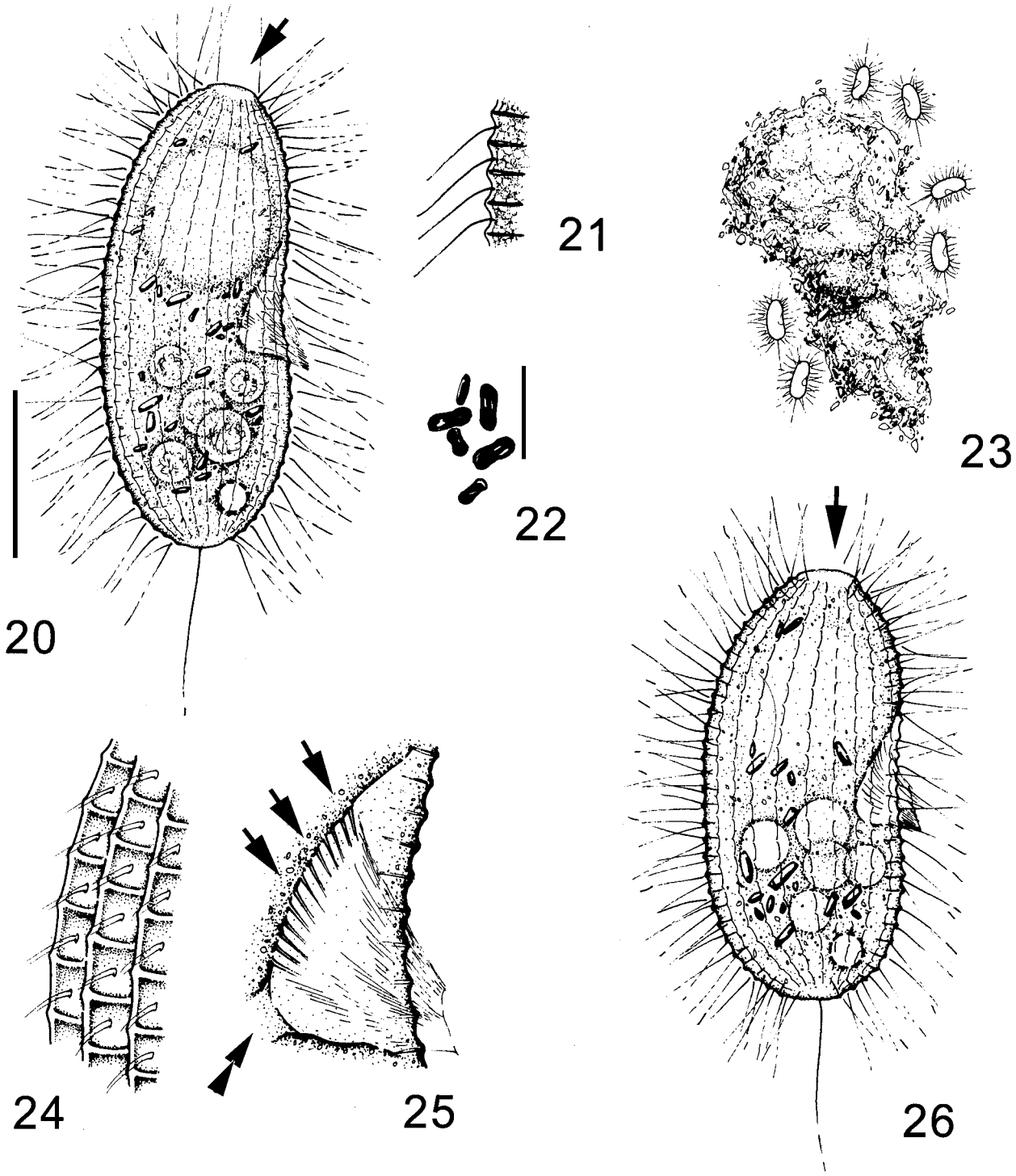
Cytoplasm colourless to greyish, containing several to many large (*ca* 5 μm across) food vacuoles and bar- or dumbbell-like crystals, which are usually 2-3 μm in size (Figs 25, 44). Macronucleus large and rounded, located mostly in anterior region (Figs 20, 26). Contractile vacuole small, sub-caudally near ventral side positioned (Figs 20, 26, 39).

Cilia about 8-10 μm long, densely arranged; caudal cilium *ca* 15-18 μm long. Cilia within buccal cavity about 6-8 μm long, usually difficult to recognize (Figs 20, 25). Swimming behaviour generally slow with no peculiarities. But mostly, when no disturbing, always completely quiet on the bottom. In this case, cells seem to attach to substrate using any part of cell region (i.e. no special thigmotactic area) (Figs 23).

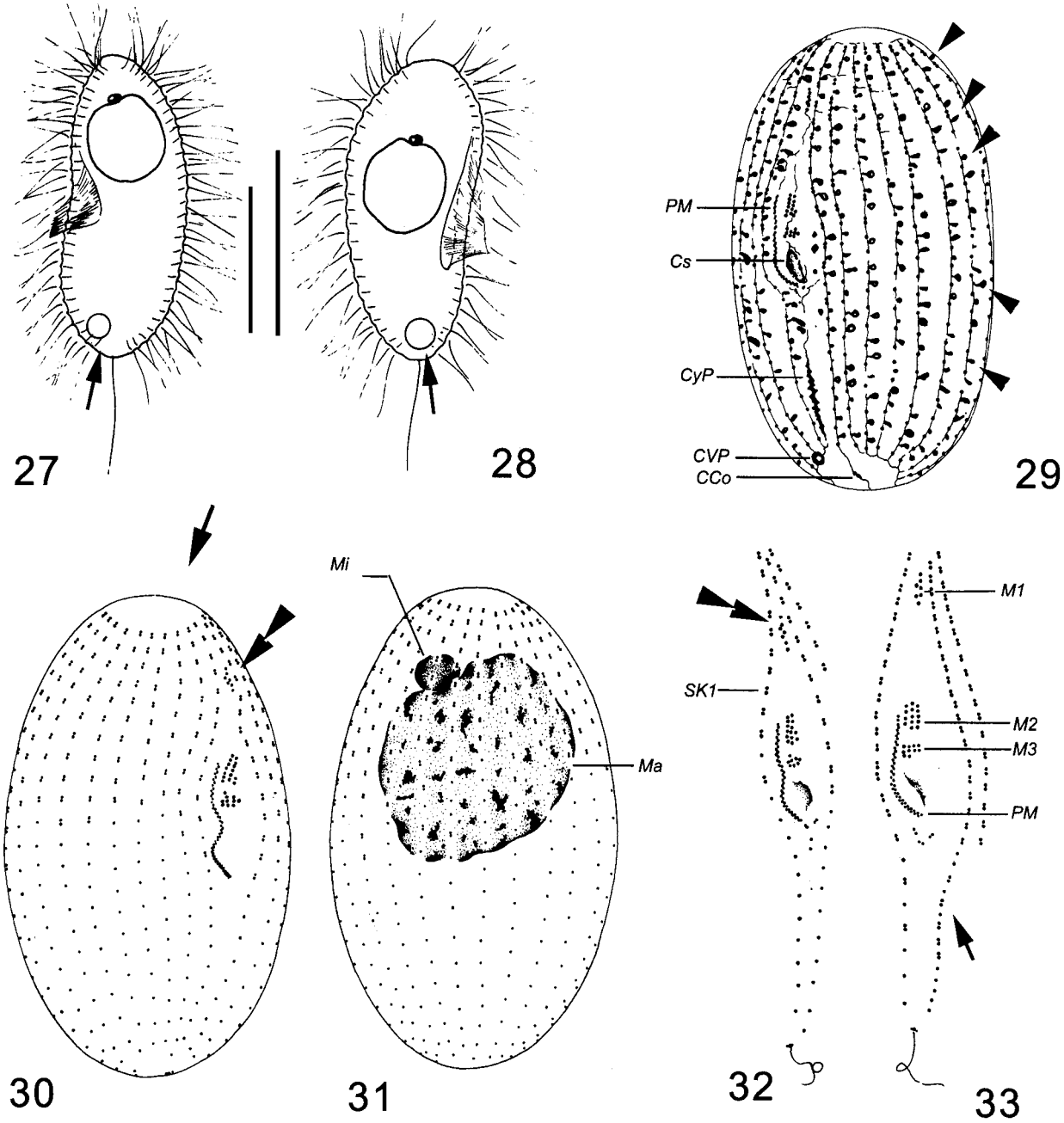
Somatic ciliature as shown in Figs 30 and 31: mostly 23 somatic kineties extending over entire length of body, composed of dikinetids in anterior 40 % of body length, while loosely-arranged monokinetids in posterior portion (Fig. 45).

Buccal apparatus slightly different from its congeners: membranelle 1 conspicuously sub-apically positioned and "far away" from other membranelles, consisting of *ca* 7 basal bodies, which are often arranged somehow in 2 rows in the middle portion (Figs 32, 43). Membranelle 2 and 3 relatively small, each consisting of 3 rows of basal bodies. Paroral membrane on right of narrow buccal field, with zig-zag rows of basal bodies, extending anteriorly to about middle of membranelle 2. Scutica with *ca* 3 pairs of basal bodies, closely behind posterior end of paroral membrane (Fig. 32).

Silverline system rather unique: extrusomes always densely distributed after silver nitrate impregnation (arrowheads in Fig. 29), which are often more or less away from direct silverline (thus between kineties)



Figs 20-26. *Uronema elegans* from life. **20** - a slender specimen, note that the contractile vacuole is located near ventral side caudally; arrow marks the small apical plate; **21** - portion of pellicle, to show extrusomes and the ridges; **22** - crystals; **23** - six specimens on the detritus , note that cells may attach on the debris using any part of the body; **24** - top view of a portion of the pellicle, to show the reticulate ridges; **25** - portion of the buccal field, arrows mark the cilia of the membranelles on the bottom of the buccal cavity; note that the cytostome is located at the deepest area of the buccal cavity (arrowheads); **26** - a plump specimen, arrow indicates the apical plate. Scale bars -15 μm (in 20), 5 μm (in 22)



Figs 27-33. *Uronema elegans* (27, 29-32) and *Uronemella filificum* (28, 33) from life (27, 28), after silver nitrate (29) and protargol impregnations (30-33). **27, 28** - lateral views, to show the typical body shapes of two species, note that the widest part of the body are in different regions; arrow marks the contractile vacuole; **29** - silverline system of ventral side, note that the extrusomes are arranged slightly away from the somatic kineties (arrowheads); **30, 31** - ventral and dorsal view of infraciliature; arrow marks the apical plate, arrowheads indicate the sub-apically positioned membranelle 1; **32, 33** - comparison of buccal apparatus. Note that the position of the membranelle 1 is considerably anteriad positioned in filificum than in elegans (arrowheads); arrow in 33 refers to the close-set basal bodies in the last somatic kinety. Abbreviations: CCo - caudal cilium complex, Cs - cytostome, CVP - pore of contractile vacuole, CyP - cytopyge, M1-3 - membranelle 1-3, Ma - macronucleus, Mi - micronucleus, PM - paroral membrane, SK1, - the first somatic kinety. Scale bars - 20 μm

(Figs 29, 42, 46). Contractile vacuole pore positioned at posterior end of somatic kinety 2. Cytopyge (CyP) proportionally short, irregularly shaped (Fig. 29).

Ecological features: This species was isolated from the mantle cavity of marine mollusc (*Sinonovacula constricta*), salinity was about 32 ‰, pH was 8.0 and temperature was *ca* 24°C.

Comparison and discussion: We recognized the Qingdao population basically due to the following features: body shape including the small apical plate, appearance of the pellicle, high number of ciliary rows, ventrally positioned contractile vacuole, small but deep buccal cavity, and habitat. Thus, we believe that the identification is correct. As to the only exception, i.e. the body size, which was claimed to be up to 90 μm long in some cases (Maupas 1883, Kahl 1931), a reasonable explanation is that Maupas might mix this species with other forms or due to an optical misinterpretation as many other researchers did in that time.

As mentioned above, because of the variable number of somatic kineties (23-26 in *U. elegans vs.* 16-23 in *U. filificum*), this species has been repeatedly confused with *Uronemella filificum* (see comparison part for *Uronemella filificum*). A summarized comparison between these two organisms is hence supplied in Table 3.

The African population described by Dragesco and Dragesco-Kernéis (1986) under the name of *Uronema elegans* is likely a misidentification. According to the position of membranelle 1, it should be a population of *Uronema filificum*.

Paranophrys marina Thompson et Berger, 1965 (Figs 14-16, 34-36; Table 1)

This species was described first by Thompson and Berger (1965) and has never been reinvestigated ever since. As no adequate living data were supplied in original work, a comprehensive redescription based on the Chinese population is hence given here.

Improved diagnosis: Marine slender *Paranophrys in vivo ca* 30-45 x 10-15 μm with pointed apical and narrowly rounded posterior end; membranelle 1 very long and two-rowed, almost conjoint to membranelle 2; 10 somatic kineties with densely spaced cilia; scutica composed of several singled basal bodies arranged in line; contractile vacuole large and caudally positioned, opens at posterior end of somatic kinety 2; one oval macro- and one small micronucleus.

Redescription: Body shape generally constant, slim and spindle-shaped with sharply pointed anterior end and narrowly rounded caudal end (Fig. 34). Cell size *in vivo* about 30-45 x 10-15 μm, in some giant forms (not uncommon in fresh samples) length up to 55 μm. Buccal cavity inconspicuous and about 2/5 of body length. Pellicle smooth, no extrusomes observed. Cytoplasm colourless to greyish, often filled with many small (*ca* 2-3 μm across) light-reflecting granules (inactive food vacuoles) and several bar-shaped crystals (Fig. 34). One large oval macronucleus centrally located with many nucleoli on surface; one small micronucleus anteriorly attached to macronucleus (Fig. 35). Contractile vacuole large, terminally located at posterior end of cell (Fig. 34).

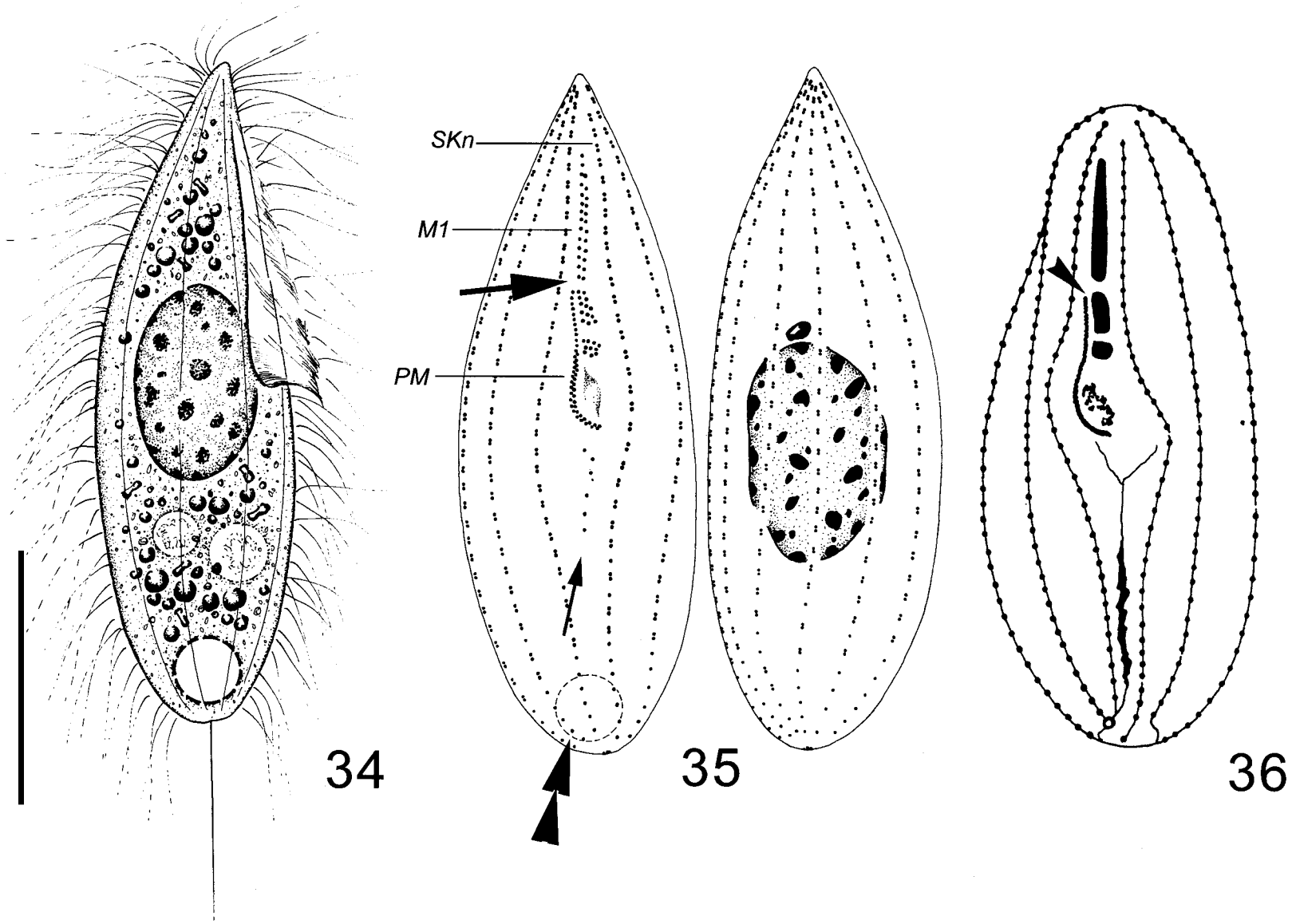
Cilia densely arranged, *ca* 8 μm long, one single caudal cilium about 15 μm in length. Movement active, or pretty quiet while crawling on bottom of petri dish.

Infraciliature as shown in Fig. 35. Buccal apparatus consisting of 3 closely distributed membranelles on left side of buccal cavity and one paroral membrane on right side (Figs 35, 15). Membranelle 1 slightly away from apex, consisting of 2 long rows of kinetids with about 10-12 basal bodies each, hence about 2.5 - 3 times as long as membranelle 2; membranelle 2 with 2 long and 1 short longitudinal rows (sometimes difficult to distinguish), each containing about 2-6 basal bodies; membranelle 3 located close to membranelle 2, with normally 3 short rows of basal bodies (Figs 35, 15). Paroral membrane characteristic of genus extending anteriorly to about anterior level of membranelle 2, composed of zig-zag rows of basal bodies (Figs 10, 15). Scutica generally in linear form, consisting of about 6 basal bodies (Fig. 35).

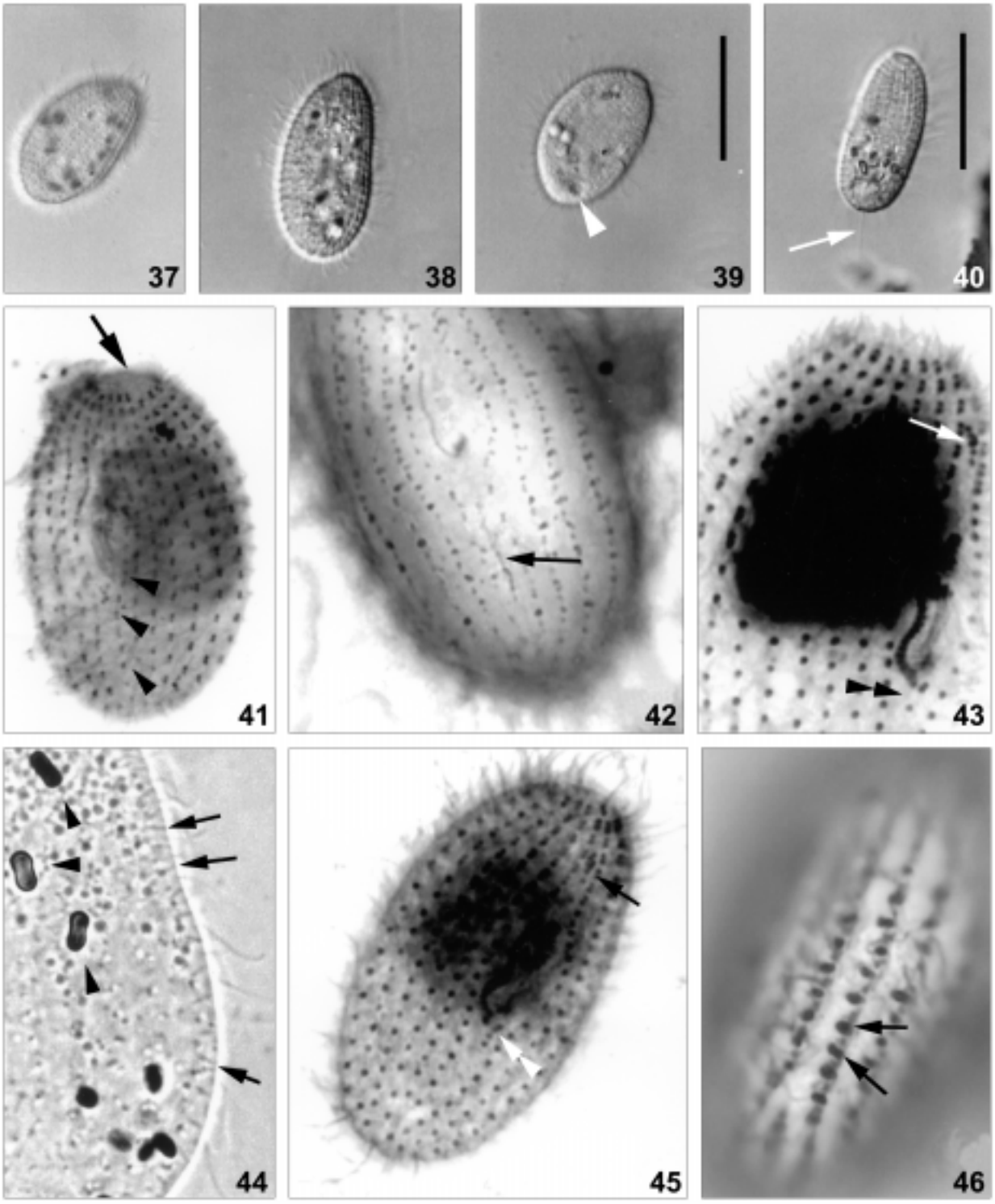
Invariably 10 bipolar somatic kineties composed of densely-arranged dikinetids over usually more than 4/5 of the length, only in caudal portion with monokinetids (Fig. 35).

Ecological features: This species was found as an ectocommensal within the mantle cavity of farmed scallop (*Argopecten irradians*) near Qingdao, where the water was clean (salinity about 31‰, pH 8.2, water temperature *ca* 25°C). This ciliate can be easily maintained as free-living one in seawater with rice grains.

Discussion: Thompson and Berger (1965) described *Paranophrys marina*, which was isolated from hydroids (*Plumularia* sp.) in the waters near Friday Harbor (Washington, USA), as follows: "... the body is round in cross-section and bluntly tapered in the anterior third of the body. Both the anterior and posterior ends are rounded. The anterior ventral surface, containing the buccal apparatus, is somewhat flattened...". Since no detailed living information was supplied, we identified our organism more or less inferentially. In the original



Figs 34-36. *Paranophrys marina in vivo* (34), after protargol (35) and silver nitrate impregnations (36, redrawn from Thompson et Berger, 1965). **34** - right lateral view of a representative specimen; **35** - ventral and dorsal view of the same specimen; small arrow marks the scutica where basal body pairs are aligned in a long row; arrowheads indicate the large contractile vacuole. Note that the membranelle 1 is almost conjoined with membranelle 2 (large arrow); **36** - ventral view of silverline system, arrowhead marks the anterior end of paroral membrane which extends to the anterior level of membranelle 2. Abbreviations: CV - contractile vacuole, M1 - membranelle 1, PM - paroral membrane, SKn - somatic kinety n. Scale bar - 20 μm



Figs 37-46. Photomicrographs of *Uronemella elegans* from life (37-40, 44), after protargol (41, 43, 45) and silver nitrate impregnations (42, 46). **37** - a slightly pressed specimen, focusing on the cell surface to show the reticulate appearance of the pellicle; **38** - a plump form, note the strongly notched surface; **39** - to show the contractile vacuole (arrowhead); **40** - slender form, note the small apical plate; arrow marks the caudal cilium; **41** - ventral view of infraciliature, arrow marks the small apical plate, while the arrowheads indicate the somatic kinety n; **42** - ventral view, to show the narrow buccal field and the short cytopyge (arrow); **43** - anterior portion of right-lateral side, arrow indicates the membranelle 1, while the arrowheads mark the scutica; **44** - detail of cortex to show the extrusomes (arrows) and crystals (arrowheads); **45** - general view of infraciliature, note that the basal bodies within kineties are relatively loosely arranged; arrow marks the membranelle 1, arrowheads refer to the scutica; 46 - to demonstrate extrusomes (arrows), which are away from the kineties. Scale bars - 20 μm

description, *Paranophrys marina*, as an ectocommensal form, possesses (1) constant 10 somatic kineties; (2) long and dominant membranelle 1, which is close to the other membranelles; (3) conspicuously small cilia-free apical area and (4) paroral membrane begins at anterior end of membranelle 2. All these features resemble our form perfectly (Figs 35, 36).

The only dissimilarity is possibly the body shape: the Qingdao population has a sharply narrowed anterior cell end, while Thompson and Berger depicted their form with a "rounded" one. We assume that their description was possibly deduced from the silver-impregnated specimens instead of deriving *in vivo* observations (as Thompson mentioned that this species was collected by the junior author several years before it was described). Thus the actual body form of this species was likely misinterpreted: its apical end is pointed but not rounded (this supposition might be confirmed by the photos in the original report, p.528, Figs 2, 3) (Thompson and Berger 1965).

Acknowledgements. This work belongs to the research projects supported by "the Natural Science Foundation of China" (project number: 39970098) and partly by the "Chueng Kong Scholars Programme". We also wish to extend our appreciation to Mr. Chen Guoliang, manager of the shrimp-incubating company in Zhanjiang, for his kind help in sampling. Thanks also due to Mr. Hu Xiaozhong and Gong Jun, graduate students in the Laboratory of Protozoology, OUQ for photographical assistance.

REFERENCES

Agamaliev F. G. (1974) Ciliates of the solid surface overgrowth of the Caspian Sea. *Acta Protozool.* **13:** 53-84 (in Russian, English summary)

Agamaliev F. G. (1978) Morphology of some free-living ciliates of the Caspian Sea. *Acta Protozool*. **17:** 419-444

Berger J., Thompson J. C. (1960) A redescription of *Cyclidium glaucoma* O. F. M., 1786 (Ciliata: Hymenostomatida), with particular attention to the buccal apparatus. *J. Protozool.* **7:** 256-262

Borror A. C. (1963) Morphology and ecology of some uncommon ciliates from Alligator Harbour, Florida. *Trans. Am. Microsc. Soc*. **82:** 125-131

Corliss J. O. (1953) Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. *Stain Technol*. **28:** 97-100

Corliss J. O. (1979) The Ciliated Protozoa: Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, Oxford

Czapik A. (1968) La morpholige de *Uronema elegans* Maupas et de *Uronema parva* sp. n. *Acta Protozool*. **5:** 225-228

Dragesco J. (1963) Compléments à la connaissance des ciliés mésopsammiques de Roscoff. I. Holotriches. *Cah. Biol. Mar*. **4:** 91-199

Dragesco J., Dragesco-Kernéis A. (1986) Ciliés libres de l'Afrique intertropicale. *Faune Tropicale* **26:** 1-559

Fernadez-Leborans G., Novillo A. (1994) Three new species of ciliate in the genera *Pseudocohnilembus, Pleuronema,* and *Urotricha* (Ciliophora). *Proc. Biol Soc. Wash*. **107:** 221-238

Foissner W. (1996) Faunistics, taxonomy and ecology of moss and soil ciliates (Protozoa, Ciliophora) from Antarctica, with description of new species, including *Pleuroplitoides smithi* gen. n., sp. n. *Acta Protozool.* **35:** 95-123

Foissner W., Wilbert N. (1981) A comparative study of the infraciliature and silverline system of the fresh-water scuticociliates *Pseudocohnilembus putrinus* (Kahl, 1928) nov. comb., *P. pusillus* (Quennerstedt, 1869) nov. comb., and the marine forms *P. marinus* Thompson, 1966. *J. Protozool*. **28:** 291-297

Foissner W., Berger H., Kohmann F. (1994) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems -Band III: Hymenostomata, Prostomatida, Nassulida. *Inform.-Ber. Bayer. Landesamt. Wass.-Wirtsch*. **1/94:** 1-548

Grolière C. A. (1974) Étude comparée de la stomatogenèse chez quelques ciliés hymenostomes des genres *Paralembus* Kahl, 1933, *Philaster* Fabre-Domergue, 1885, *Parauronema* Thompson, 1967, *Tetrahymena* Furgasson, 1940. *Protistologica* **10:** 319-331

Grolière C. A. (1980) Morphologie et stomatogenèse chez deux ciliés Scuticociliatida des genres *Philasterides* Kahl, 1926 et *Cyclidium* O. F. Müller, 1786. *Acta Protozool*. **19:** 195-206

ICZN (International Commission on Zoological Nomenclature) (1999) International Code of Zoological Nomenclature. 4 ed. International Trust for Zoological Nomenclature, London

Jankowski A.W. (1980) Conspectus of a new system of the phylum Ciliophora. *Trudy Zool. Inst. Leningrad* **94:** 103-121 (in Russian)

Kahl A. (1931) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 2. Holotricha. *Tierwelt Dtl*. **21:** 181-398

Maupas E. (1883) Contribution a l'étude morphologique et anatomique des infusoires ciliés. *Archs Zool. exp. gén.* **1:** 427-664

Pérez-Uz B., Hope S. (1997) *Urocyclon cymruensis* n. sp. (Ciliophora: Uronematidae). A new scuticociliate associated with decaying seaweed from the Welsh Coast of UK. *Europ. J. Protistol*. **33:** 316-322

Pérez-Uz B., Song W., Warren A. (1996) Morphogenetic processes in division of *Uronema filificum* Kahl, 1931 (Ciliophora: Scuticociliatida) and implications for its systematic position in the family Uronematidae. *Europ. J. Protistol*. **32:** 262-270

Petz W., Song W., Wilbert N. (1995) Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia* **40:** 1-223

Small E. B. (1967) The Scuticociliatida, a new order of the class Ciliatea (Phylum Protozoa, Subphylum Ciliophora). *Trans. Am. Microsc. Soc*. **86:** 345-370

Small E. B., Lynn D. H. (1985) Phylum Ciliophora. In: An Illustrated Guide to the Protozoa, (Eds. J. J. Lee, S. H. Hutner and E. C. Bovee) Lawrence, Kansas

Song W. (1993) Studies on the morphology of *Homalogastra binucleata* nov. spec. and a brief revision of its congeners (Ciliophora, Scuticociliatida). *J. Oceanol. Limnol. Sin*. **24:** 143-150 (in Chinese, English summary)

Song W. (2000) Morphological and taxonomical studies on some marine scuticociliates from China sea, with description of two new species, *Philasterides armatalis* sp. n. and *Cyclidium varibonneti* sp. n. (Protozoa: Ciliophora: Scuticociliatida). *Acta Protozool*. **39:** 295-322

Song W., Wilbert N. (2000) Redefinition and redescription of some marine scuticociliates from China. I, with report of a new species, *Metanophrys sinensis* nov. spec. (Ciliophora, Scuticociliatida). *Zool. Anz*. **239:** 45-74

Song W., Wilbert N. (2002) Reinvestigations of three "well-known" marine scuticociliates: *Uronemella filificum* (Kahl, 1931) nov. gen., nov. comb., *Pseudocohnilembus hargisi* Evans et Thompson, 1964 and *Cyclidium citrullus* Cohn 1865, with description of the new genus *Uronemella* (Protozoa, Ciliophora, Scuticociliatida). *Zool. Anz*. (in press)

Thompson J. C. (1972) Ciliated protozoa of the Antarctic Peninsula. In: Antarctic Terrestrial Biology, (Ed. G. A. Liano). *Antarctic Res. Ser*. **27:** 41-67

Thompson J. C., Berger J. (1965) *Paranophrys marina* n. g., n. sp., a new ciliate associated with a hydroid from the Northeast Pacific (Ciliata: Hymenostomatida). *J. Protozool.* **12:** 27-531

Thompson J. C., Kaneshiro E. S. (1968) Redescriptions of *Uronema filificum* and *Uronema elegans*. *J. Protozool*. **15:** 141-144
Wilbert N. (1975) Eine verbesserte Technik der Protargolimprägnation für Ciliaten. *Mikrokosmos* **64:** 171-179
Wilbert N. (1986) Ciliaten aus dem Interstitial des Ontario Sees. *Acta Protozool.* **25:** 379-396
Wilbert N., Kahan D. (1981) Ciliates of Solar Lake on the Red Sea shore. *Arch. Protistenkd.* **124:** 70-95
Xu K., Song W. (1999) Study on some pathogenetic ciliates from the mantle cavity of marine molluscs. *J. Fish. Sci. China* **6:** 41-45 (in Chinese, English summary)

Received on 20th September, 2001; accepted on 14th March, 2002