

This work is licensed under a Creative Commons Attribution 3.0 License.

#### Research article

urn:lsid:zoobank.org:pub:43278833-B695-4375-B1BD-E98C28A9E50E

# Seven scuticociliates (Protozoa, Ciliophora) from Alabama, USA, with descriptions of two parasitic species isolated from a freshwater mussel *Potamilus purpuratus*

Xuming PAN 1,2

<sup>1</sup>College of Life Science and Technology, Harbin Normal University, Harbin 150025, China. <sup>2</sup>School of Fisheries, Aquaculture & Aquatic Sciences, College of Agriculture, Auburn University, Auburn, AL 36849, USA. Email: pppppp206@126.com

urn:lsid:zoobank.org:author:B438F4F6-95CD-4E3F-BD95-527616FC27C3

**Abstract.** Isolates of *Mesanophrys* cf. *carcini* Small & Lynn *in* Aescht, 2001 and *Parauronema* cf. *longum* Song, 1995 infected a freshwater mussel (bleufer, *Potamilus purpuratus* (Lamarck, 1819)) collected from Chewacla Creek, Auburn, Alabama, USA. Free-living specimens of *Metanophrys similis* (Song, Shang, Chen & Ma, 2002) 2002, *Uronema marinum* Dujardin, 1841, *Uronemita filificum* Kahl, 1931, *Pleuronema setigerum* Calkins, 1902 and *Pseudocohnilembus hargisi* Evans & Thompson, 1964, were collected from estuarine waters near Orange beach, Alabama. Based on observations of living and silver-impregnated cells, we provide redescriptions as well as comparisons with original descriptions for the seven species. We also comment on the geographic distributions of known populations of these aquatic ciliate species and provide a table reporting some aquatic scuticociliates of the eastern US Gulf Coast.

Keywords. Ciliates, scuticociliates, morphology, freshwater mussel, Alabama, USA.

Pan X. 2016. Seven scuticociliates (Protozoa, Ciliophora) from Alabama, USA, with descriptions of two parasitic species isolated from a freshwater mussel *Potamilus purpuratus*. *European Journal of Taxonomy* 249: 1–19. <u>http://dx.doi.org/10.5852/ejt.2016.249</u>

# Introduction

Ciliates of the subclass Scuticociliatia are common inhabitants of freshwater, brackish, and marine environments (Foissner & Wilbert 1981; Parker 1981; Wiackowski *et al.* 1999; Wang *et al.* 2008a, 2008b, 2009; Foissner *et al.* 2009; Gao *et al.* 2010, 2012a, 2012b, 2013; Foissner *et al.* 2014; Mallo *et al.* 2014; Ofelio *et al.* 2014), wherein they may be free-living, commensals, benign symbionts, or likely pathogens of fishes and invertebrates (Puytorac *et al.* 1974; Kaneshiro & Holz 1976; Al-Marzouk & Azad 2007; Castro *et al.* 2014; Gao *et al.* 2016, 2016; Gao & Katz 2014; Zhan *et al.* 2014; Pan *et al.* 2016). Because of their small size and the high degree of similarity in the infraciliature, many scuticociliates are identified based upon a combination of characters observed *in vivo* and after silver staining (Foissner *et al.* 1994; Song & Wilbert 2002; Ma *et al.* 2004; Miao *et al.* 2008, 2009; Wilbert & Song 2008; Song *et al.* 2009; Fan *et al.* 2011a, 2011b, 2014; Lobban *et al.* 2011; Pan *et al.* 2011, 2013, 2015a, 2015b, 2015c, 2016).

Many new species and populations probably have yet to be discovered in the eastern US Gulf Coast, and many species reported from the region are insufficiently described (Calkins 1902; Noland 1925, 1937; Bovee 1960; Borror 1963a, 1963b, 1973; Jones 1974). More specifically, little information is available on the biodiversity and distribution of parasitic and free-living ciliates in Alabama's streams, rivers, and coastal environments, however, there are many reports about distribution and morphology of freshwater mussel *Potamilus purpuratus* Lamarck, 1819 (Hopkins 1934; Haag *et al.* 1993; Haggerty *et al.* 2005; Gangloff *et al.* 2006; Garner *et al.* 2009; McElwain & Bullard 2014). However, very little information exists on the specific identities of mussel ciliates (Grizzle & Brunner 2007). Similarly, collections of ciliates from rivers and streams of Alabama are infrequently reported, thus little is known about levels of ciliate biodiversity and endemnicity in these aquatic habitats.

Herein, we provide supplemental morphological information on seven nominal scuticociliate species based on microscopy of living and silver-impregnated specimens collected in Alabama. Records of aquatic scuticociliates of the eastern US Gulf Coast are given in Table 1.

# **Material and methods**

Invertebrates and environmental samples were collected during October through December 2013 in rivers or earthen aquaculture ponds in central Alabama as well as along the Gulf Coast of Alabama and Florida (Fig. 1). Specimens of *Mesanophrys* cf. *carcini* Small & Lynn in Aescht, 2001 and *Parauronema* cf. *longum* Song, 1995 were isolated from a bleufer, *Potamilus purpuratus* (Lamarck, 1819) collected from Chewacla Creek, Auburn, Alabama, USA (32°36′56″ N, 85°28′58″ E) on 8 Oct. 2013. Specimens of *Metanophrys similis* (Song, Shang, Chen & Ma, 2002), *Uronema marinum* Dujardin, 1841, *Uronemita filificum* Kahl, 1931, *Pleuronema setigerum* Calkins, 1902 and *Pseudocohnilembus hargisi* Evans & Thompson, 1964 were collected from Orange beach, Alabama (30°16′44″ N, 87°33′35″ E) on 24 Oct. 2013. Only one of the seven ciliates, *Mesanophrys* cf. *carcini*, was previously reported as parasitic.



**Fig. 1.** Sampling map. **A**. Chewacla Creek, Auburn, Alabama (32°36′56″ N, 85°28′58″ E). **B**. Orange Beach, Alabama (30°16′44″ N, 87°33′35″ E).

Samples were examined after being maintained in a plastic container filled with site water from the original sample locality and exchanged weekly. Ciliates were detached with a pipette and maintained as pure or raw cultures in Petri dishes in the laboratory for days to weeks, with ciliate-free animal tissues as a food source to enrich bacteria for ciliate maintenance. The freshwater mussel *Potamilus purpuratus* (Lamarck, 1819), commonly known as bluefer or purpleshell, was identified according to Williams *et al.* (2008) (having 150 mm shell length; outline subtriangular; anterior margin rounded; dorsal margin with low wing; umbo broad; pseudocardinal teeth triangular, two divergent teeth in left valve, one tooth in right valve; lateral teeth moderately long and slightly curved, two in left valve, one in right valve; nacre purple). The freshwater mussel was released into Chewacla Creek after observation. Observations of living specimens were conducted with the aid of light microscopy. Silver carbonate impregnation was performed according to Ma *et al.* (2003). Specimens were photographed and measured using a compound microscope equipped with a digital camera. Systematics of ciliates follows Lynn (2008).

Abbreviations in the text are list in the following :

- M1 = Membranelle 1
- M2 = Membranelle 2
- M3 = Membranelle 3
- Ma = macronucleus
- PM = paroral membrane
- Sc = scutica

# Results

Two parasitic species isolated from freshwater mussel Potamilus purpuratus

Subclass Scuticociliatia Small, 1967 Order Philasterida Small, 1967 Family Orchitophryidae Cépède, 1910 Genus *Mesanophrys* Small & Lynn, 1985

*Mesanophrys* cf. *carcini* Small & Lynn *in* Aescht, 2001 Fig. 2A–C; Table 1

#### Description

Description based on Alabama population: body (*in vivo*)  $35-55 \times 15-25 \mu m$ , spindle-shaped (Fig. 2A) or pyriform (Fig. 2B), having sharply pointed anterior end and narrowly rounded caudal end (Fig. 2A); variability in shape likely attributable to nutritional conditions (Fig. 2A–B) or division (Fig. 2C). Buccal field 10–15  $\mu$ m in length or 25–30% of body length. Somatic cilia distributed in a dense lateral field, 5–8  $\mu$ m long (Fig. 2A). Extrusomes undetectable (Fig. 2A–C). Cytoplasm transparent or grey, granulated; cytoplasmic granules 3–5  $\mu$ m long, 1–1.5  $\mu$ m wide, refractive. Macronucleus single, large, 8–13  $\mu$ m long or 20–30% of body length, 8–12  $\mu$ m wide or 40–50% of body width, spheroid, centrally located. One micronucleus attached to macronucleus. Locomotion by moving on substrate or swimming in water. Somatic cilia 7 to 10  $\mu$ m long, ten somatic kineties composed of dikinetids in anterior  $\frac{2}{3}$  of cell. Membranelle 1 (M1) slightly separated from apex of anterior end, comprising two rows of kinetids each with seven to nine basal bodies (Fig. 2D). M2 bearing three to five longitudinal rows of cilia, with each longitudinal row having six to eight basal bodies (Fig. 2D). M3 posterior to M2, comprising three rows of kinetids (Fig. 2D).

Family Parauronematidae Small & Lynn, 1985 Genus *Parauronema* Thompson, 1967

Parauronema cf. longum Song, 1995 Fig. 3E–J; Table 1

#### Description

Description based on Alabama population: body  $60-85 \times 25-35 \ \mu m$  *in vivo*, elongate oval with a large, truncated apical plate (Fig. 3E–G). Body shape generally constant. Posterior end rounded, ventral side straight, dorsal side convex (Fig. 2E–F). Buccal field 35–40% of body length. Pellicle slightly indented at bases of cilia. Extrusomes rod-shaped, ca 2–3  $\mu m$  long. Cytoplasm colourless to grayish, containing several to many large (ca 5  $\mu m$  across) food vacuoles and dumbbell-shaped crystals (ca 1–2  $\mu m$  long) concentrated in anterior and posterior ends of body (Fig. 3H). Single ellipsoid to spherical macronucleus, 10  $\mu m$  in diameter, micronucleus not identified. Single contractile vacuole, 8  $\mu m$  across during diastole, at posterior end of cell. Somatic cilia 6  $\mu m$ -long, densely arranged (Fig. 3F, arrows); single caudal cilium



**Fig. 2. A–D**. *Mesanophrys* cf. *carcini* Small & Lynn *in* Aescht, 2001. **E–G**. *Metanophrys similis* Song *et al.*, 2002. A–C, E–F. *In vivo*. D, G. After silver impregnation. **A**, **E**. Ventral views of typical individuals, arrow in (E) shows caudal cilia. **B**, **F**. Different individuals, showing different body shapes. **C**. Individual in morphogenesis. **D**, **G**. Detailed structure of the buccal area. Abbreviations: M1, 2, 3 = membranelle 1, 2 and 3; Ma = macronucleus; PM = paroral membrane. Scale bars: 30 μm.

**Table 1.** Morphometric characterization of seven species from Alabama populations. Data based on silver carbonate-impregnated specimens. Measurements in  $\mu$ m. Abbreviations: CV = coefficient of variation in %; n = number of specimens investigated; Max = maximum; Mean = arithmetic mean; Min = minimum; SD = standard deviation.

Character	Species	Min	Max	Mean	SD	CV	n
Body length	Mesanophrys cf. carcini	43	74	61.2	11.9	16.3	15
	Uronema marinum	21	32	27.8	9.6	5.2	15
	Metanophrys similis	48	56	51.2	5.8	6.7	15
	Uronemita filificum	40	46	43.8	3.7	5.6	15
	Pleuronema setigerum	72	80	76.0	7.3	8.0	15
	Pseudocohnilembus hargisi	43	57	49.1	6.7	8.9	15
	Parauronema cf. longum	86	92	89.1	9.3	7.5	15
Body width	Mesanophrys cf. carcini	20	31	23.2	16.4	10.2	15
	Uronema marinum	12	18	15.4	7.2	3.7	15
	Metanophrys similis	31	38	33.5	10.9	7.1	15
	Uronemita filificum	27	30	28.1	7.9	2.1	15
	Pleuronema setigerum	35	45	41.3	6.5	7.2	15
	Pseudocohnilembus hargisi	11	16	14.4	3.3	5.6	15
	Parauronema cf. longum	38	47	44.7	7.1	4.3	15
Number of somatic kineties	Mesanophrys cf. carcini	10	10	10.0	0	0	13
	Uronema marinum	12	13	12.3	5.2	4.7	14
	Metanophrys similis	12	12	12.0	0	0	15
	Uronemita filificum	23	24	23.2	2.9	2.8	13
	Pleuronema setigerum	13	14	13.7	8.5	3.4	12
	Pseudocohnilembus hargisi	6	7	6.4	6.7	7.1	14
	Parauronema cf. longum	18	21	20.1	2.4	5.6	13
Macronucleus length	Mesanophrys cf. carcini	8	13	10.7	19.3	16.5	15
	Uronema marinum	8	10	9.2	12.5	14.7	15
	Metanophrys similis	12	15	13.2	3.2	1.3	15
	Uronemita filificum	15	17	15.9	7.2	3.9	15
	Pleuronema setigerum	15	18	16.6	4.5	7.6	15
	Pseudocohnilembus hargisi	6	8	7.3	6.1	2.6	15
	Parauronema cf. longum	15	18	16.2	4.3	4.6	15
Macronucleus width	Mesanophrys cf. carcini	8	12	11.1	14.7	11.7	15
	Uronema marinum	10	11	10.7	5.1	2.5	15
	Metanophrys similis	14	15.3	4.3	5.7	15	16
	Uronemita filificum	16	18	17.8	4.1	3.6	15
	Pleuronema setigerum	15	17	15.7	7.8	6.7	15
	Pseudocohnilembus hargisi	6	7	6.4	11.3	13.4	15
	Parauronema cf. longum	14	17	15.9	5.0	4.1	15
Kinetosomes in kinety 1, number*	Mesanophrys cf. carcini	27	31	28.8	5.7	2.0	15
	Uronema marinum	17	22	20.2	2.4	1.2	15
	Metanophrys similis	24	26	25.2	9.8	3.9	15
	Uronemita filificum	18	24	22.4	6.4	2.8	15
	Pleuronema setigerum	-	-	-	-	-	15
	Pseudocohnilembus hargisi	16	19	18.4	10.1	5.6	15
	Parauronema cf. longum	22	25	23.4	1.2	0.5	15

\* Basal body pairs counted as single ones

approximately 15  $\mu$ m long (Fig. 31). Swims straight ahead in slightly helical path. Crawls slowly with frequent changes of direction when feeding on surface of debris or stays quiet for long period. Eighteen to 24 somatic kineties, somatic kinety 1 with about 32 basal bodies. Somatic cilia about 5 to 7  $\mu$ m long. M1 long, consisting of two longitudinal rows of kinetids, each with seven to ten kinetosomes (Fig. 3J). M2 well-separated from M1, composed of three rows of kinetosomes, each row containing six kinetosomes (Fig. 3J). M3 much shorter than M1 and M2, comprising two or three short, irregularly arranged rows of kinetosomes (Fig. 3J). PM on right of buccal cavity, terminating anteriorly at level of mid-region of M2. Scutica Y-shaped comprising four pairs of kinetosomes (Fig. 3J).

#### Five free-living scuticociliates

Family Orchitophryidae Cépède, 1910 Genus *Metanophrys* de Puytorac, Grolière, Roque & Detcheva, 1974

*Metanophrys similis* (Song, Shang, Chen & Ma, 2002) Fig. 2E–G; Table 1

#### Description

Description based on Alabama population: body *in vivo* 35–40 × 20–25  $\mu$ m, plump pyriform, anteriorly tapering, posteriorly rounded; no apical plate formed (Fig. 2E). Ventral side slightly straight, dorsal side slightly convex (Fig. 2E–F). Somatic cilia 7–8  $\mu$ m long, densely arranged (Fig. 2F). Single caudal cilium approximately 10  $\mu$ m in length (Fig. 2E). Extrusoms bar-shaped, approximately 2  $\mu$ m in length, arranged in rows between the somatic kineties. Endoplasm colourless to grayish, and contained abundant food vacuoles (2–5  $\mu$ m in across) and many bar- or dumbbell-shaped crystals 1–2  $\mu$ m in length (Fig. 2E). One large, spherical to ovoid macronucleus, approximately 10  $\mu$ m in length and 12  $\mu$ m in width, centrally located. Swimming aimlessly, without pause, or sometimes crawling on substrates. Twelve somatic kineties. M1 near apex, comprising three longitudinal rows of kinetids with six basal bodies each (Fig. 2G). M2 three-rowed, as long as M1, composed of six basal bodies in each longitudinal row (Fig. 2G). M3 located close to M2, usually comprises three short arranged rows of basal bodies.

Family Pseudocohnilembidae Evans & Thompson, 1964 Genus *Pseudocohnilembus* Evans & Thompson, 1964

**Pseudocohnilembus hargisi** Evans & Thompson, 1964 Fig. 3A–D; Table 1

## Description

Description based on Alabama population: Size *in vivo*  $20-30 \times 8-12 \mu m$ , elongate-elliptical in outline, with cell width becoming wider toward posterior end (Fig. 3A–C). Cytoplasm colourless to grayish, containing several to many shining granules (Fig. 3A–B). Extrusomes not observed. One caudal cilium 10  $\mu$ m long or approximately 30% of body length. Single macronucleus. Somatic cilia approximately 5  $\mu$ m long. Twelve to 13 somatic kineties. M1 and 2 parallel to the cell's longitudinal axis, each formed by a single row of kinetosomes (Fig. 3D). M2 started anteriorly near the second paired kinetids of somatic kinety 1, terminated posteriorly near the mid-body region (Fig. 3D). Length of M2 80% of buccal field; M3 consisting of three rows of kinetids.

Family Uronematidae Thompson, 1964 Genus *Uronemita* Song & Wilbert, 2002

> Uronemita filificum Kahl, 1931 Fig. 4A–E; Table 1

#### Description

Description based on Alabama population: body  $30-45 \times 15-20 \ \mu m$  *in vivo*, inverted pear-shaped with large, conspicuous apical plate (Fig 4A–B). Dorsal side conspicuously convex. Length of buccal field 60% of body. Extrusomes appromately 2  $\mu m$  long, rod-shaped, closely beneath pellicle. Cytoplasm colourless to grayish, containing several (ca 3  $\mu m$  across) food vacuoles and dumbbell-shaped crystals (ca 1–2  $\mu m$  long) often concentrated in anterior end of body (Fig. 4C). Single macronucleus larg (Fig. 4E). Locomotion by swimming aimlessly, sometimes rotates while attached to substratum by caudal cilium. Eighteen or 19 somatic kineties, anterior third of each composed of dikinetids (Fig. 4E). M1 single-rowed with five or six kinetosomes (Fig. 4D); M2 three-rowed; M3 smaller and close to M2 (Fig. 4D). PM on right of shallow buccal cavity, with zigzag row of basal bodies, extending anteriorly to the middle of M2. Scutica consisting of three or four basal body pairs (Fig. 4D).



**Fig. 3.** A–D. *Pseudocohnilembus hargisi* Evans & Thompson, 1964. E–J. *Parauronema* cf. *longum* Song, 1995. A–C, E–I. *In vivo*. D, J. After silver impregnation. A, E. Ventral views of typical individuals, arrow in (A) shows caudal cilia. B–C, F–G. Different individuals, showing varying body shapes, arrowheads in (F) mark somatic kineties. D, J. Detailed structure of the buccal area. H. Ventral view, arrow refers to dumbbell-shaped crystals. I. Posterior end, arrow marks caudal cilium. Abbreviations: M1, 2, 3 = membranelle 1, 2 and 3; PM = paroral membrane; Sc = scutica. Scale bars: A–B = 15 µm; C, E = 40 µm; G = 60 µm.

Family Uronematidae Thompson, 1964 Genus *Uronema* Dujardin, 1841

Uronema marinum Dujardin, 1841 Fig. 4F–I; Table 1

#### Description

Description based on Alabama population: Size in vivo  $10-25 \times 6-10 \mu m$ , elongate-elliptical in outline



**Fig. 4. A**–**E**. Uronemita filificum Kahl, 1931. **F**–**I**. Uronema marinum Dujardin, 1841. **J**–**N**. Pleuronema setigerum Calkins, 1902. A–C, F–H, J–L. In vivo. D–E, M–N. After silver impregnation. **A**, **F**, **J**. Ventral views of typical individuals. **B–C, G–H, K–L**. Different individuals, showing variation in body shape, arrow in (B) shows the conspicuous apical plate, arrows in (C, H) mark contractile vacuoles. **D, I, M–N**. Detailed structures of buccal area, arrow in (M) indicates the ring-like posterior end of M2a, arrowheads in (M) mark preoral kineties. **E**. Ventral view, arrowheads show somatic kineties. Abbreviations: M1, 2, 3 = membranelle 1, 2 and 3; M2a = the anterior part of membranelle 2; M2b = the posterior part of membranelle 2; Ma = macronucleus; PM = paroral membrane. Scale bars: A–B = 20 µm; F, H, N = 10 µm; G = 5 µm, J–L = 30 µm.

(Fig. 4F). Anterior end flat, with an apical plate, dorsal posterior area slightly rounded (Fig. 4F–H). Buccal field 50% of body length. Pellicle smooth (Fig. 4F–I). Extrusomes approximately 1  $\mu$ m long, rod-shaped, closely beneath pellicle. One large globular to ellipsoidal macronucleus centrally located with numerous tiny (approximately 1×1  $\mu$ m) irregularly-shaped peripheral nucleoli (Fig. 4F–I). Contractile vacuole about 3  $\mu$ m in diameter at posterior end of cell (Fig. 4H). Movement unremarkable, either swimming continuously or resting on the bottom. Twelve to 13 ciliary rows. Buccal apparatus typical of genus: M1 positioned near apical plate and composed of 5–7 kinetosomes; M2 slightly longer than M1; M3 very short (Fig. 4I). PM on right of shallow buccal cavity, composed zigzaging row of basal bodies, extending anteriorly to middle of M2 (Fig. 3I).

Family Pleuronematidae Kent, 1881 Genus *Pleuronema* Dujardin, 1836

Pleuronema setigerum Calkins, 1902 Fig. 4J–N, Table 1

#### Description

Description of present specimens: Size *in vivo* 50–70 × 25–35 µm, slender oval in outline, widest at  $\frac{1}{3}$  posterior end of body (Fig. 4J–L). Ventral side almost flat, dorsal side conspicuously convex. Buccal field 80% of body length (Fig. 4J–L). Extrusomes 4–5 µm long, closely beneath pellicle. Cytoplasm colourless to grayish containing numerous granules (3–6 µm across) and cytoplasmic crystals of varying size and shape (Fig. 4J–L). One spherical macronucleus. Thirteen to 15 elongated caudal cilia about 25 µm in length (Fig. 4J–L). Locomotion by swimming while rotating on main body axis, or briefly lying motionless on debris. Twelve to fifteen somatic kineties. Five preoral kineties to left of buccal field (Fig. 4M–N). Oral apparatus similar to congeners: M1 with one short and two longer rows; M2a mostly two-rowed but with a middle section that is single-rowed in a 'zigzag' pattern, with its posterior end characteristically ring-like. M2b V-shaped, distinctly separated from M2a; M3 three-rowed (Fig. 4M–N). PM 80% of body length.

# Discussion

# Remarks on Mesanophrys cf. carcini and Parauronema cf. longum

#### Infection sites and pathogenic effects

In the case of *Mesanophrys* cf. *carcini* and *Parauronema* cf. *longum*, they were isolated from the surface of an old live mussel's gut. To be specific, epithelia of the gut were scraped with a glass-slide or a glass-pipette, and then the scrape content was left in a petri dish, in which adding a small amount of distilled water. Two species were observed attached to the epithelia of the gut or surrounded by mucus or debris. Given no ciliates were found in other parts of the mussel, we concluded that the mussel might have been lightly infected with the ciliates only occurring in some areas of the gut.

# Differences in morphology of free-living vs parasitic forms of *Mesanophrys carcini* and *Parauronema* longum

#### Mesanophrys cf. carcini Small & Lynn in Aescht, 2001

The newly observed specimens resemble those of free-living forms of *Mesanophrys carcini* Small & Lynn in Aescht, 2001 in all respects except: numbers of somatic kineties (10 in parasitic forms vs 11 in free-living forms) and body shapes (variable in parasitic forms vs constant slim and spindle-shaped to long fusiform in free-living forms) (Grolière & Leglise 1977; Song & Wilbert 2000). These differences may be due to different life-styles and abundance of food sources.

#### Parauronema cf. longum Song, 1995

The morphological characters of parasitic *Parauronema* cf. *longum* correspond well with free-living *P. longum* except the body size (60–85 × 25–35  $\mu$ m *in vivo* in parasitic forms vs 30–55 × 12–25  $\mu$ m in in free-living forms), proportion of buccal field length to body length (35–40% in parasitic forms vs almost 50% in free-living forms) and extrusomes (present in parasitic forms vs absent in free-living forms). Characters of larger body size and smaller cytostome may be consistent with its parasitic life-style.

#### **Remarks on five free-living species**

#### Metanophrys similis Song et al., 2002

The specimens described herein generally resemble those of the original species description (Song *et al.* 2002b) except: a slight, possibly insignificant, difference in shape (plump pyriform vs more slender).

#### Pseudocohnilembus hargisi Evans & Thompson, 1964

Our specimens are similar to those of Song & Wilbert (2002) except: the specimens in Song & Wilbert (2002) have a smaller body ( $20-30 \times 8-12 \mu m$  *in vivo* vs  $35-55 \times 10-15 \mu m$ ) and fewer somatic kineties (12-13 somatic kineties vs 13-14 somatic kineties).

# Uronemita filificum Kahl, 1931

Fig. 4A–E; Table 1

Subsequent to Kahl's (1931) original description, Pérez-Uz *et al.* (1996), Song & Wilbert (2002), and Song *et al.* (2002a) have provided supplemental information on the species. The Alabama population is identical to that of Kahl (1931) and Pérez-Uz *et al.* (1996) but differs from that of Song & Wilbert (2002) and Song *et al.* (2002a) in the number of somatic kineties (18 or 19 vs 20–23).

#### Uronema marinum Dujardin, 1841

#### **Remarks and comparison**

Kahl's (1931) original illustrations of *Uronema marinum*, based on live cells, showed that the cell shape is pyriform and elongate-elliptical in outline. Specimens detailed by Kahl (1931) apparently have distinctive, long extrusomes rather than short and inconspicuous extrusomes as in our specimens. Borror (1963a) first gave a simple illustration of infraciliature of *U. marinum*, showing more variability (12–15) in the number of somatic kineties than observed in the present study (12–13). Thompson (1964) reported a small marine ciliate, identified as *U. marinum*, with an even wider range (13–16). Czapik (1964) reported a form named *U. marinum*, with body dimensions 30–40 µm long vs 10–25 µm long in our population. Our specimens are nearly identical with those of the type population described by Pan *et al.* (2010) regarding body size (approximately 20 ×10 µm in length), and shape (elongate-elliptical in outline; anterior end flat, with an apical plate, dorsal posterior area slightly rounded).

#### Pleuronema setigerum Calkins, 1902

#### **Remarks and comparison**

Kahl (1931) redescribed this species, and Borror (1963a) described its infraciliature but gave only a diagram of the buccal morphology. Pan *et al.* (2010) offered improved diagnoses of this species based on a Chinese population. Cells of the North American population matched previous descriptions of

the species in all morphological characters, except body size *in vivo* (50–70 × 25–35  $\mu$ m vs 30–50 × 15–30  $\mu$ m).

### Conclusion

All populations having been reported for each species and records of scuticociliates of the eastern US Gulf Coast (Fig. 5; Table 2)

There is much evidence to reveal that ciliates thrive wherever they find a specific combination of environmental conditions, that the same species will be discovered wherever this combination occurs, and that ciliates appear therefore to be cosmopolitan (Finlay 1997; Finlay & Esteban 1998; Foissner *et al.* 2009; Lobban *et al.* 2011). More specifically, an individual species is perhaps represented by only a few individuals, or even as cysts in similar combinations of environmental conditions, but when appropriate conditions are supplied, that species flourishes and becomes abundant. Similarly, species may be present in very thin populations in particular circumstances of space and time or even only be represented by dormant stages (Fenchel *et al.* 1997; Finlay 1997; Finlay & Esteban 1998). Scuticociliates are common members of ecosystems in habitats worldwide and they often act as symbionts or even pathogens of aquatic animals (Wang *et al.* 2009a, 2009b not in ref maybe 2009; Fan *et al.* 2011a, 2011b;



Fig. 5. All reported populations for the following species. A. Uronema marinum Dujardin, 1841.
B. Pseudocohnilembus hargisi Evans & Thompson, 1964. C. Metanophrys similis Song et al., 2002.
D. Pleuronema setigerum Calkins, 1902. E. Uronemita filificum Kahl, 1931.

# Table 2 (continued on next page). Records of aquatic scuticociliates of the eastern US Gulf Coast.

Species	Family	Order	Locality	References	
Cohnilembus verminus (Müller, 1786)	Cohnilembidae	Philasterida	A tidal marsh in New Hampshire Mobile Bay, Alabama	Borror 1972 Jones 1974	
			Virginia	Borror 1973	
Conchophthirus anodontae Cattaneo, 1889	Conchophthiridae	Thigmotrichida	Mountain lake region, Giles	Bovee 1960	
Cristigera sp.	Pleuronematidae	Pleuronematida	County, Virginia Mountain lake region, Giles	Bovee 1960	
Ctedoctema acanthocrypta Stokes, 1884	Ctedoctematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
				Stokes 1988	
Protocyclidium citrullus Cohn, 1886	Pleuronematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
			Wisconsin	Noland 1925	
Cyclidium glaucoma Müller, 1786	Pleuronematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
			Wisconsin	Noland 1925	
Cyclidium litomasum Stokes, 1884	Pleuronematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
			County, Virginia	Stokes 1988	
Cyclidium marinum Borror, 1963	Pleuronematidae	Pleuronematida	A tidal marsh in New Hampshire	Borror 1963a	
Cyclidium oblongum Kahl, 1926	Pleuronematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
Cyclidium plouneouri Dragesco, 1963	Pleuronematidae	Pleuronematida	A tidal marsh in New Hampshire	Borror 1972	
Glauconema trihymene Thompson, 1966	Parauronematidae	Philasterida	Virginia coast	Thompson 1966	
Histiobalantium semisetatum Noland, 1937	Histiobalantiidae	Pleuronematida	Lemon Bay, Florida	Noland 1937	
Histiobalantium agile Stokes, 1886	Histiobalantiidae	Pleuronematida		Stokes 1988	
Miamiensis avidus Thompson, 1964	Parauronematidae	Philasterida	Virginia	Thompson 1964	
Paralembus hargisi Evans & Thompson, 1964	Paralembidae	Philasterida	A tidal marsh in New Hampshire	Borror 1972	
Paralembus marinus (Thompson, 1966)	Paralembidae	Philasterida	A tidal marsh in New Hampshire	Borror 1972	
Paranophrys magna Borror, 1972	Orchitophryidae	Philasterida	A tidal marsh in New Hampshire	Borror 1972	
Paranophrys marina Thompson, 1965	Orchitophryidae	Philasterida	San Juan Archipelago, Washington	Thompson 1965	
Pleuronema chrysalis Perty, 1852	Pleuronematidae	Pleuronematida	Mountain lake region, Giles	Bovee 1960	
Pleuronema coronatum Kent, 1881	Pleuronematidae	Pleuronematida	County, Virginia Alligator Harbor, Florida	Borror 1963a	
			Lemon Bay, Florida	Noland 1937	
Pleuronema marinum Borror,1963	Pleuronematidae	Pleuronematida	Alligator Harbor, Florida	Borror 1963a	
Pleuronema marinum Noland, 1937	Pleuronematidae	Pleuronematida	Lemon Bay, Florida	Noland 1937	
Pleuronema setigerum Calkins, 1902	Pleuronematidae	Pleuronematida	Alligator Harbor, Florida	Borror 1963a	
			Lemon Bay, Florida	Noland 1937	
			Woods Hole	Calkins 1902	
Pleuronema smalli Dragesco, 1968	Pleuronematidae	Pleuronematida	A tidal marsh in New Hampshire	Borror 1972	
Paranophrys magna Borror, 1972	Uronematidae	Philasterida	A tidal marsh in New Hampshire	Borror 1972	
Pseudoconilembus longisetus Tompson, 1965	Uronematidae	Philasterida	A tidal marsh in New Hampshire	Borror 1972	
Uronema acutum Buddenbrock, 1920	Uronematidae	Philasterida	A tidal marsh in New Hampshire	Borror 1963b	
Uronema filificum Kahl, 1931	Uronematidae	Philasterida	A tidal marsh in New Hampshire	Borror 1965	

Species	Family	Order	Locality	References
Uronema marinum Dujardin, 1841	Uronematidae	Philasterida	A tidal marsh in New Hampshire	Borror 1963a
			Woods Hole	Calkins 1902
			Cedar river, Virginia	Thompson 1964
			A tidal marsh in New Hampshire	Borror 1972
Uronema pluricaudatum Noland, 1937	Uronematidae	Philasterida	Lemon Bay, Florida	Noland 1937
Uronema filificum Kahl, 1931	Uronematidae	Philasterida	Sipperwisett Marsh, Massachusetts	Kaneshiro & Holz 1976
Uronema sp.	Uronematidae	Philasterida	Barnstable Town beach,	Kaneshiro & Holz
Uropedalium pyriforme Kahl, 1928	Uronematidae	Philasterida	Massachusetts A tidal marsh in New Hampshire	1976 Borror 1972

PAN X., Seven scuticociliates from Alabama, USA

Pan *et al.* 2011, 2014 not in ref, 2015 a,b or c). In our present work, records from investigations of all populations of seven scuticociliates (*Mesanophrys* cf. *carcini, Metanophrys similis, Parauronema* cf. *longum, Pleuronema setigerum, Pseudocohnilembus hargisi, Uronema marinum, Uronemita filificum*) are highlighted in Fig. 5, with emphasis on the generally cosmopolitan distribution of scuticociliates. As shown in Fig. 4, *Uronema marinum* ranges in coastal areas of the southern and eastern parts of the North America, Europe, southern and eastern China, Korea and Antarctica, mainly between 20° N and 60° N. *Pseudocohnilembus hargisi* occurs in coastal areas of southern China, the Gulf of Mexico and the Great Salt Lake. *Metanophrys similis* occurs in coastal areas of northern China and the Gulf of Mexico. *Pleuronema setigerum* occurs in the coastal areas of China, off European and eastern North America and the Gulf of Mexico. *Uronemita filificum* ranges in the coastal areas of China, off northern Europe and the Americas. The distributions of the last four species include the northeastern Pacific Ocean and northeast Atlantic Ocean, which also indicates a similar combination of environmental conditions in certain regions of northern hemisphere.

In our work, seven scuticociliates were isolated from aquaculture ponds in central Alabama as well as along the Gulf Coast of Alabama and Florida, the results, as an additional contribution to the knowledge of protozoan fauna in the eastern US Gulf Coast. All records of scuticociliates of the eastern US Gulf Coast are listed in Table 1. Based on the information shown, *Cyclidium* sp., *Pleuronema* sp. and *Uronema* sp. can be considered as common/dominant groups among scuticociliates having been reported. Four species (*Mesanophrys* cf. *carcini*, *Metanophrys similis*, *Parauronema* cf. *longum* and *Pseudocohnilembus hargisi*), described in the current work, are reported in the eastern US Gulf Coast for the first time. Additionally, many species in Table 1 (including the four species mentioned above) were also isolated from coastal areas of eastern China, which indicates a most similar biological or geographical environment existing in two regions (Wang *et al.* 2009; Fan *et al.* 2011a, 2011b; Pan *et al.* 2011, 2014 not in ref, 2015).

#### Acknowledgments

This work was supported by the Natural Science Foundation of China (project number: 31501844). We thank Prof. Weibo Song (Ocean University, China) and Dr. Stephen A. Bullard (Auburn University, USA) for their help on this work.

# References

Al-Marzouk A. & Azad I.S. 2007. Growth kinetics, protease activity and histophagous capability of *Uronema* sp. infesting cultured silver pomfret *Pampus argenteus* in Kuwait. *Diseases of aquatic organisms* 76: 49–56. http://dx.doi.org/10.3354/dao076049

Borror A.C. 1963a. Morphology and ecology of some uncommon ciliates from Alligator Harbour, Florida. *Transactions of the American Microscopical Society* 82: 125–131.

Borror A.C. 1963b. Morphology and ecology of the benthic ciliated protozoa of Alligator Harbor, Florida. *Archiv für Protistenkunde* 106: 465–534.

Borror A.C. 1973. Marine flora and fauna of the northeastern United States. Protozoa: Ciliophora. NOAA Technical Report. NMFS, i–iii, 1–62. <u>http://dx.doi.org/10.5962/bhl.title.63126</u>

Bovee E.C. 1960. Protozoa of the mountain lake region, Giles County, Virginia. *Journal of Protozoology* 7: 352–361. <u>http://dx.doi.org/10.1111/j.1550-7408.1960.tb05982.x</u>

Calkins G.N. 1902. Marine Protozoa from Woods Hole. *Bulletin of the United States Fish Commission* 21: 413–468.

Castro L.A., Küppers G.C., Schweikert M., Harada M.L. & Paiva Tda S. 2014. Ciliates from eutrophized water in the northern Brazil and morphology of *Cristigera hammeri* Wilbert, 1986 (Ciliophora, Scuticociliatia). *European Journal of Protistology* 50: 122–133. <u>http://dx.doi.org/10.1016/j.ejop.2014.01.005</u>

Czapik A. 1964. La stomatogénèse du Cilié *Uronema marinum* Dujardin (Hymenostomatida, Tetrahymenina). *Acta Protozoologica* 2: 207–211.

Fan X., Hu X., Al-Farraj S.A., Clamp J.C. & Song W. 2011a. Morphological description of three marine ciliates (Ciliophora, Scuticociliatia), with establishment of a new genus and two new species. *European Journal of Protistology* 47: 186–196. <u>http://dx.doi.org/10.1016/j.ejop.2011.04.001</u>

Fan X., Lin X., Al-Rasheid K.A.S., Warren A. & Song W. 2011b. The diversity of scuticociliates (Protozoa, Ciliophora): a report on eight marine forms found in coastal waters of China, with a description of one new species. *Acta Protozoologica* 50: 219–234. http://dx.doi.org/10.4467/16890027AP.11.021.0021

Fan X., Al-Farraj S.A., Gao F. & Gu F. 2014. Morphological reports on two species of *Dexiotricha* (Ciliophora, Scuticociliatia), with a note on the phylogenetic position of the genus. *International Journal of Systematic and Evolutionary Microbiology* 64: 680–688. http://dx.doi.org/10.1099/ijs.0.059899-0

Fenchel T., Esteban G.F. & Finlay B.J. 1997. Local versus global diversity of microorganisms: crypticdiversity of ciliated protozoa. *Oikos* 80: 220–225. <u>http://dx.doi.org/10.2307/3546589</u>

Finlay B.J. 1997. The Diversity and Ecological Role of Protozoa in Fresh Waters. *In*: Sutcliffe D.W. (ed.) *The Microbial Quality of Water*: 113–125. Freshwater Biological Association, Ambleside.

Finlay B.J. & Esteban G.F. 1998. Freshwater protozoa: biodiversity and ecological function. *Biodiversity and Conservation* 7: 1163–1186. <u>http://dx.doi.org/10.1023/A:1008879616066</u>

Foissner W. & Wilbert N. 1981. A comparative study of the infraciliature and silverline system of the freshwater scuticociliates *Pseudocohnilembus putrinus* (Kahl, 1928) nov. comb., *P. pusillus* (Quennerstedt, 1869) nov. comb., and the marine forms *P. marinus* Thompson, 1966. *Journal of Protozoology* 28: 291–297. <u>http://dx.doi.org/10.1111/j.1550-7408.1981.tb02853.x</u>

Foissner W., Berger H. & Kohmann F. 1994. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band III: Hymenostomata, Prostomatida, Nassulida. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft 1/94. Wasserwirtschaftsamt Deggendorf, Deggendorf.

Foissner W., Kusuoka Y. & Shimano S. 2009. Morphological and molecular characterization of *Histiobalantium natans viridis* Kahl, 1931 (Ciliophora, Scuticociliatia). *European Journal of Protistology* 45: 193–204.

Foissner W., Jung J.H., Filker S., Rudolph J. & Stoeck T. 2014. Morphology, ontogenesis and molecular phylogeny of *Platynematum salinarum* nov. spec., a new scuticociliate (Ciliophora, Scuticociliatia) from a solar saltern. *European Journal of Protistology* 50: 174–184. <u>http://dx.doi.org/10.1016/j.ejop.2013.10.001</u>

Gangloff M.M, Williams J.D. & Feminella J.W. (2006) A new species of freshwater mussel (Bivalvia: Unionidae), *Pleurobema athearni*, from the Coosa river drainage of Alabama, USA. *Zootaxa* 1118: 43–56.

Gao F. & Katz L. A. 2014. Phylogenomic analyses support the bifurcation of ciliates into two major clades that differ in properties of nuclear division. *Molecular Phylogenetics and Evolution* 70: 240–243. http://dx.doi.org/10.1016/j.ympev.2013.10.001

Gao F., Fan X., Yi Z., Strüder-Kypke M. & Song W. 2010. Phylogenetic consideration of two scuticociliate genera, *Philasterides* and *Boveria* (Protozoa, Ciliophora) based on 18S rRNA gene sequences. *Parasitology International* 59: 549–555. <u>http://dx.doi.org/10.1016/j.parint.2010.07.002</u>

Gao F., Katz L.A. & Song W. 2012a. Insights into the phylogenetic and taxonomy of philasterid ciliates (Protozoa, Ciliophora, Scuticociliatia) based on analyses of multiple molecular markers. *Molecular Phylogenetics and Evolution* 64: 308–317. <u>http://dx.doi.org/10.1016/j.ympev.2012.04.008</u>

Gao F., Strüder-Kypke M., Yi Z., Miao M., Al-Farraj S.A. & Song W. 2012b. Phylogenetic analysis and taxonomic distinction of six genera of pathogenic scuticociliates (Protozoa, Ciliophora) inferred from small-subunit rRNA gene sequences. *International Journal of Systematic and Evolutionary Microbiology* 62: 246–256. http://dx.doi.org/10.1099/ijs.0.028464-0

Gao F., Katz L.A. & Song W. 2013. Multigene-based analyses on evolutionary phylogeny of two controversial ciliate orders: Pleuronematida and Loxocephalida (Protista, Ciliophora, Oligohymenophorea). *Molecular Phylogenetics and Evolution* 68: 55–63. <u>http://dx.doi.org/10.1016/j.</u> <u>ympev.2013.03.018</u>

Gao F., Gao S., Wang P., Katz L. & Song W. 2014. Phylogenetic analyses of cyclidiids (Protista, Ciliophora, Scuticociliatia) based on multiple genes suggest their close relationship with thigmotrichids. *Molecular Phylogenetics and Evolution* 75: 219–226. <u>http://dx.doi.org/10.1016/j.ympev.2014.01.032</u>

Gao F., Warren A., Zhang Q., Gong J., Miao M., Sun P., Xu D., Huang J., Yi Z. & Song W. 2016. The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). *Scientific Reports* 6: 24874. <u>http://dx.doi.org/10.1038/srep24874</u>

Garner J.T., McGregor S.W., Tarpley T.A. & Buntin M.L. 2009. Freshwater mussels (Unionidae) in the headwaters of Chipola River, Houston County, Alabama. *Southeastern Naturalist* 8: 687–694. <u>http://dx.doi.org/10.1656/058.008.0410</u>

Grizzle J.M. & Brunner C.J. 2007. Assessment of Current Information Available for Detection, Sampling, Necropsy, and Diagnosis of Diseased Mussels. Prepared for the Alabama Department of Conservation and Natural Resources Wildlife and Freshwater Fisheries Division: 21–24. Montgomery, Alabama.

Grolière C.A. & Leglise M. 1977. *Paranophrys carcini* n. sp., Cilié Philasterina récolté dans l'hémolymphe du Crabe *Cancer pagurus* Linné. *Protistologica* 13: 503–507.

Haag W.R., Berg D.J., Garton D.W. & Farris J.L. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 13–19. <u>http://dx.doi.org/10.1139/f93-002</u>

Haggerty T.M., Garner J.T. & Rogers R.L. 2005. Reproductive phenology in *Megalonaias nervosa* (Bivalvia: Unionidae) in Wheeler Reservoir, Tennessee River, Alabama, USA. *Hydrobiologia* 539: 131–136. <u>http://dx.doi.org/10.1007/s10750-004-3915-2</u>

Hopkins S.H. 1934. The parasite inducing pearl formation in American freshwater Unionidae. *Science* 79: 385–386. <u>http://dx.doi.org/10.1126/science.79.2052.385-a</u>

Jones E.E. 1974. *The Protozoa of Mobile Bay, Alabama*. University of South Alabama Monograph 1. University of South Alabama, Mobile, Alabama.

Kahl A. 1931. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria). 2. Holosticha. Tierwelt Deutschlands 21: 181–398. Fischer Verlag, Jena.

Kaneshiro E. S. & Holz G.G. jr. 1976. Observations on the ultrastructure of *Uronema* spp., marine scuticociliates. *Journal of Protozoology* 23: 503–517.

Lobban C.S, Raymundo L.M. & Montagnes D.J.S. 2011. *Porpostoma guamensis* n. sp., a philasterine scuticociliate associated with brown band disease of corals. *Journal of Eukaryotic Microbiology* 58: 103–113. <u>http://dx.doi.org/10.1111/j.1550-7408.2010.00526.x</u>

Lynn D.H. 2008. *The Ciliated Protozoa. Characterization, Classification and Guide to the Literature*. Springer Verlag, Dordrecht.

Ma H., Choi J.K. & Song W. 2003. An improved silver carbonate impregnation for marine ciliated protozoa. *Acta Protozoologica* 42: 161–164.

Ma H., Song W., Gong J. & Warran A. 2004. Reconsideration of stomatogenesis in *Uronema marinum* Dujardin,1841 during asexual division (Protozoa: Ciliophora: Scuticociliatida). *Acta Zoologica Sinica* 50: 823–827.

Mallo N., Lamas J., Piazzon C. & Leiro J.M. 2014. Presence of a plant-like proton-translocating pyrophosphatase in a scuticociliate parasite and its role as a possible drug target. *Parasitology* 142: 449–462. <u>http://dx.doi.org/10.1017/S0031182014001267</u>

McElwain A. & Bullard S.A. 2014. Histological atlas of freshwater mussels (Bivalvia, Unionidae): *Villosa nebulosa* (Ambleminae: Lampsilini), *Fusconaia cerina* (Ambleminae: Pleurobemini) and *Strophitus connasaugaensis* (Unioninae: Anodontini). *Malacologia* 57: 99–239.

Miao M., Warren A., Song W., Wang S., Shang H. & Chen Z. 2008. Analysis of the internal transcribed spacer 2 (ITS2) region of scuticociliates and related taxa (Ciliophora, Oligohymenophorea) to infer their evolution and phylogeny. *Protistologica* 159: 519–533. <u>http://dx.doi.org/10.1016/j.protis.2008.05.002</u>

Miao M., Wang Y., Li L., Al-Rasheid K.A.S. & Song W. 2009. Molecular phylogeny of the scuticociliate, *Philiaster* (Protozoa, Ciliophora), with a description of a new species, *P. apodigitiformis* sp. nov. *Systematics and Biodiversity* 7: 381–388.

Noland L.E. 1925. Factors Influencing the Distribution of Fresh Water Ciliates. *Ecology* 6: 437–452. http://dx.doi.org/10.2307/1929108

Noland L.E. 1937. Observations on marine ciliates of the gulf coast of Florida. *Transactions of the American Microscopical Society* 56: 160–171. <u>http://dx.doi.org/10.2307/3222944</u>

Ofelio C., Blanco A., Roura Á., Pintado J., Pascual S. & Planas M. 2014. Isolation and molecular identification of the scuticociliate *Porpostoma notata* Moebius, 1888 from moribund reared *Hippocampus hippocampus* (L.) seahorses, by amplification of the SSU rRNA gene sequences. *Journal of Fish Diseases* 37: 1061–1065. <u>http://dx.doi.org/10.1111/jfd.12207</u>

Pan H., Huang J., Hu X., Fan X., Al-Rasheid K.A.S. & Song W. 2010. Morphology and SSU rRNA gene sequences of three marine ciliates from Yellow Sea, China, including one new species, *Uronema heteromarinum* nov. spec. (Ciliophora, Scuticociliatida). *Acta Protozoologica* 49: 45–59.

Pan H., Hu J., Jiang J., Wang L. & Hu X. 2016. Morphology and phylogeny of three *Pleuronema* species (Ciliophora, Scuticociliatia) from Hangzhou Bay, China, with description of two new species, *P. binucleatum* n. sp. and *P. parawiackowskii* n. sp. *Journal of Eukaryotic Microbiology* 63: 287–298. http://dx.doi.org/10.1111/jeu.12277

Pan X., Shao C., Ma H., Fan X., Al-Rasheid K.A.S., Al-Farraj S.A. & Hu X. 2011. Redescriptions of two marine scuticociliates from China, with notes on stomatogenesis in *Parauronema longum* (Ciliophora, Scuticociliatida). *Acta Protozoologica* 50: 301–310. <u>http://dx.doi.org/10.4467/16890027AP.11.027.0064</u>

Pan X., Zhu M., Ma H., Al-Rasheid K.A.S. & Hu X. 2013. Morphology and small-subunit rRNA gene sequences of two new marine ciliates, *Metanophrys orientalis* spec. nov. and *Uronemita sinensis* spec. nov. (Protista, Ciliophora, Scuticociliatia), with an improved diagnosis of the genus *Uronemita*. *International Journal of Systematic and Evolutionary Microbiology* 63: 3513–3523. <u>http://dx.doi.org/10.1099/ijs.0.053173-0</u>

Pan H., Hu J., Warren A., Wang L., Jiang J. & Hao R. 2015a. Morphology and molecular phylogeny of *Pleuronema orientale* spec. nov. and *Pleuronema paucisaetosum* spec. nov. (Ciliophora, Scuticociliata) from Hangzhou Bay, China. *International Journal of Systematic and Evolutionary Microbiology* 65 (12): 4800–4808. http://dx.doi.org/10.1099/ijsem.0.000651

Pan X., Huang J., Gao F., Fan X., Ma H., AL-Rasheid K.A.S. & Miao M. 2015b. Morphology and phylogeny of four marine scuticociliates (Protista, Ciliophora), with descriptions of two new species: *Pleuronema elegans* spec. nov. and *Uronema orientalis* spec. nov. *Acta Protozoologica* 54: 31–43. http://dx.doi.org/10.4467/16890027AP.15.003.2190

Pan X., Yi Z., Li J., Ma H., Al-Farraj S.A. & Al-Rasheid K.A.S. 2015c. Biodiversity of marine scuticociliates (Protozoa, Ciliophora) from China: description of seven morphotypes including a new species, *Philaster sinensis* spec. nov. *European Journal of Protistology* 51: 142–157. <u>http://dx.doi.org/10.1016/j.ejop.2015.02.005</u>

Pan X., Fan X., Al-Farraj S.A., Gao S. & Chen Y. 2016. Taxonomy and morphology of four "ophrysrelated" scuticociliates (Protista, Ciliophora, Scuticociliatia), with description of a new genus, *Paramesanophrys* gen. nov. *European Journal of Taxonomy* 191: 1–18. <u>http://dx.doi.org/10.5852/</u> <u>ejt.2016.191</u>

Parker J.G. 1981. Ciliated protozoa of the polluted Tees estuary. *Estuarine*, *Coastal and Shelf Science* 24: 213–226. http://dx.doi.org/10.1016/S0302-3524(81)80129-6

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. *European Journal of Protistology* 32: 262–270. <u>http://dx.doi.org/10.1016/S0932-4739(96)80025-3</u>

Puytorac P. de, Grolière C., Roque M. & Detcheva R. 1974. A propos d'un cilié *Anophryoides salmacida* (Mugard, 1949) nov. gen. *Protistologica* 15: 223–230.

Song W. 1995. Morphological studies on the marine pathogenetic ciliate *Parauronema longum* nov. spec. (Ciliophora, Scuticociliata). *Journal of Ocean University of Qingdao* 25: 461–465.

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

Song W. & Wilbert N. 2002. Reinvestigations of three "well-known" marine scuticociliates: *Uronemita filificum* (Kahl, 1931) nov. gen., nov. comb., *Pseudocohnilembus hargisi* Evans & Thompson, 1964 and *Cyclidium citrullus* Cohn 1865, with description of the new genus *Uronemita* (Protozoa, Ciliophora, Scuticociliatida). *Zoologischer Anzeiger* 241: 317–331. <u>http://dx.doi.org/10.1078/0044-5231-00075</u>

Song J.Y., Kitamura S.I., Oh M.J., Kang H.S., Lee J.H., Tanaka S.J. & Jung S.J. 2009. Pathogenicity of *Miamiensis avidus* (syn.*Philasterides dicentrarchi*), *Pseudocohnilembus persalinus*, *Pseudocohnilembus hargisi* and *Uronema marinum* (Ciliophora, Scuticociliatida). *Diseases of aquatic organisms* 83: 133–143. <u>http://dx.doi.org/10.3354/dao02017</u>

Song W., Ma H., Wang M. & Zhu M. 2002a. Comparative studies on two closely-related marine ciliates: *Uronemita filificum* (Kahl, 1931) and *Uronema elegans* Maupas, 1883 with redescription of *Paranophrys marina* Thompson & Berger, 1965 (Protozoa, Ciliophora, Scuticociliatida) from China seas. *Acta Protozoologica* 41: 263–278.

Song W., Shang H., Chen Z. & Ma H. 2002b. Comparison of some closely-related *Metanophrys*taxa with description of a new species *Metanophrys similis* nov. spec. (Ciliophora, Scuticociliatida). *European Journal of Protistology* 38: 45–53. http://dx.doi.org/10.1078/0932-4739-00848

Stokes A.C. 1888. A preliminary contribution toward a history of the fresh-water infusoria of the United States. *Journal of the Trenton Natural History Society* 1: 71–319.

Thompson J.C. 1964. A redescription of *Uronema marinum*, and a proposed new family Uronematidae. *Virginia Journal of Science* 15: 80–87.

Thompson J.C. 1966. *Glauconema trihymene* n. g. n. sp., a Hymenostome Ciliate from the Virginia Coast. *Journal of Protozoology* 13: 393–5.

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). *Journal of Protozoology* 12: 527–531.

Wang Y., Hu X., Long H., Al-Rasheid K.A.S., Al-Farraj S. & Song W. 2008a. Morphological studies indicate that *Pleuronema grolierei* nov. spec. and *P. coronatum* Kent, 1881 represent different sections of the genus *Pleuronema* (Ciliophora: Scuticociliatida). *European Journal of Protistology* 44: 131–140. http://dx.doi.org/10.1016/j.ejop.2007.08.008

Wang Y., Song W., Hu X., Warren A., Chen X. & Al-Rasheid K.A.S. 2008b. Descriptions of two new marine species of *Pleuronema*, *P. czapikae* sp. n. and *P. wiackowskii* sp. n. (Ciliophora: Scuticociliatida), from the Yellow Sea, North China. *Acta Protozoologica* 47: 35–45.

Wang Y., Song W., Warren A., Al-Rasheid K.S., Al-Quraishy S., Al-Farraj S.A., Hu X. & Pan H. 2009. Descriptions of two new marine scuticociliates, *Pleuronema sinica* n. sp. and *P. wilberti* n. sp. (Ciliophora: Scuticociliatida), from the Yellow Sea, China. *European Journal of Protistology* 45: 29–37. http://dx.doi.org/10.1016/j.ejop.2008.06.001

Wiackowski K., Hryniewiecka-Szyfter Z. & Babula A. 1999. How many species are in the genus *Mesanophrys* (protista, ciliophora, facultative parasites of marine crustaceans)? *European Journal of Protistology* 35: 379–389. <u>http://dx.doi.org/10.1016/S0932-4739(99)80047-9</u>

Wilbert N. & Song W. 2008. A further study on littoral ciliates (Protozoa, Ciliophora) near King George Island, Antarctica, with description of a new genus and seven new species. *Journal of Natural History* 42: 979–1012. http://dx.doi.org/10.1080/00222930701877540

Williams J.D., Bogan A.E. & Garner J.T. 2008. Freshwater mussels of Alabama and the Mobile Basin in Georgia, Mississippi and Tennessee. The University of Alabama Press, Mobile.

Zhan Z., Stoeck T., Dunthorn M. & Xu K. 2014. Identification of the pathogenic ciliate *Pseudocohnilembus persalinus* (Oligohymenophorea: Scuticociliatia) by fluorescence *in situ* hybridization. *European Journal of Protistology* 50: 16–24. <u>http://dx.doi.org/10.1016/j.ejop.2013.09.004</u>

Manuscript received: 27 March 2016 Manuscript accepted: 8 June 2016 Published on: 7 December 2016 Topic editor: Rudy Jocqué Desk editors: Jeroen Venderickx and Kristiaan Hoedemakers

Printed versions of all papers are also deposited in the libraries of the institutes that are members of the *EJT* consortium: Muséum national d'Histoire naturelle, Paris, France; Botanic Garden Meise, Belgium; Royal Museum for Central Africa, Tervuren, Belgium; Natural History Museum, London, United Kingdom; Royal Belgian Institute of Natural Sciences, Brussels, Belgium; Natural History Museum of Denmark, Copenhagen, Denmark; Naturalis Biodiversity Center, Leiden, the Netherlands.