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Taxonomy and morphology of four "*ophrys*-related" scuticociliates (Protista, Ciliophora, Scuticociliatia), with the description of a new genus, *Paramesanophrys* gen. nov.

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Abstract. Generally, "*ophrys*-related" scuticociliates belong to a specialised group of ciliated protozoa that may act as commensals or pathogens of fishes and crustaceans. In the present study, four "*ophrys*-taxa" scuticociliates, i.e., *Paramesanophrys typica* gen. et sp. nov., *Mesanophrys carcini* (Grolière & Léglise, 1977) Small & Lynn in Aescht, 2001, *Metanophrys sinensis* Song & Wilbert, 2000, and *Metanophrys similis* Song *et al.*, 2002, were collected from Chinese coastal waters or mariculture ponds and investigated. *Paramesanophrys* gen. nov. is assigned to the family Orchitophryidae and differs from its other genera mainly by the position of the paroral membrane relative to membranelle 1–3, i.e., the membrane extends anteriorly to the posterior end of membranelle 3. The type species *P. typica* gen. et sp. nov., is defined by an elongated body with the posterior end depressed where the caudal cilium is located; 20 or 21 somatic kineties; double-rowed membranelle 1 with eight to ten basal bodies in each kinety; irregularly multi-rowed membranelle 2 and membranelle 3; scutica comprising *c.* seven or eight kinetosome pairs; a single macronuclear nodule; and marine habitat. The redescription of the three previously known species can be summarized as follows: 1) improved diagnosis is provided for *Metanophrys sinensis* Song & Wilbert, 2000 based on the original description and the present study; 2) some population-dependent characteristics of our new *Mesanophrys carcini* isolate are presented; 3) *Metanophrys similis*, collected from the South China Sea, resembles the original Qingdao population.

Keywords. Scuticociliates, *Paramesanophrys* gen. nov., *Paramesanophrys typica* gen. et sp. nov., *Mesanophrys*, *Metanophrys*.

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Introduction

Ciliates in the subclass Scuticociliatia, commonly found in ecosystems worldwide, exhibit a great biological diversity and play important roles in marine ecosystems (Thompson & Kaneshiro 1968; Foissner & Wilbert 1981; Cawthorn *et al.* 1996; Lynn & Strüder-Kypke 2005; Fan *et al.* 2011a, b, 2014; Pan *et al.* 2013a, b; Castro *et al.* 2014; Foissner *et al.* 2014; Zhan *et al.* 2014). Many of them are common pathogens of fishes and invertebrates and can cause severe disease or even death in economically important aquaculture animals (Pérez-Uz & Song 1995; Song & Wilbert 2002; Fan *et al.* 2009, 2010; Mallo *et al.* 2014; Ofelio *et al.* 2014). However, due to their small body size and a high degree of similarity in ciliature, the taxonomy of this group of organisms remains difficult and confusing (Thompson 1964; Agatha *et al.* 1993; Song 2000; Song & Wilbert 2000; Pan *et al.* 2010). Recent investigations in Chinese seas have shown a high diversity of scuticociliates, and the discovery of new

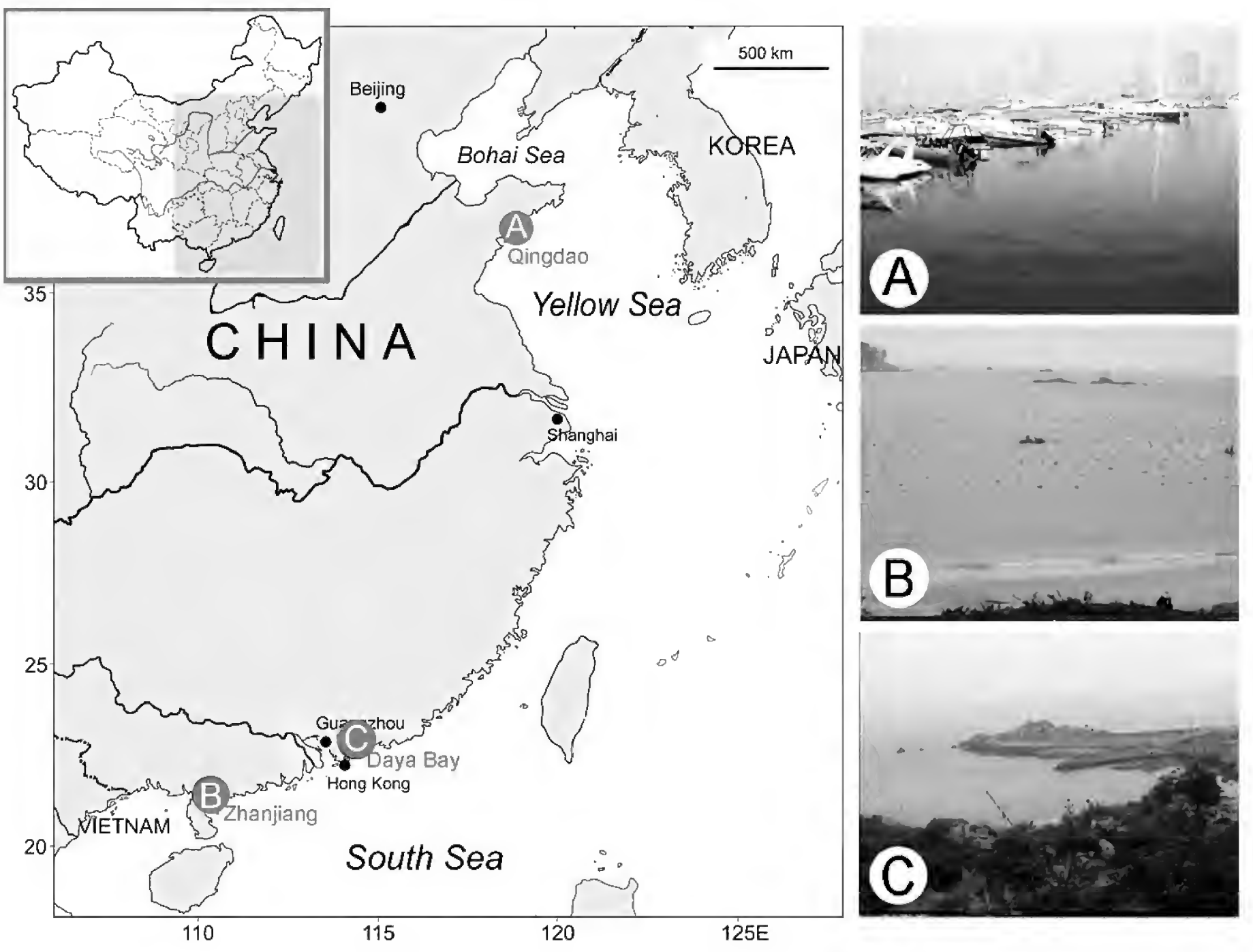

Fig. 1. Sampling sites. **A**. Coastal waters of the Yellow Sea at Qingdao, Shandong province. **B**. A coastal mariculture-region in Zhanjiang, Guangdong province. **C**. Coastal waters of Daya Bay, Guangdong province.

Table 1. Comparison of collection data for four species.

	Paramesanophrys typica gen. et sp. nov.	*Mesanophrys carcini*	*Metanophrys similis*	*Metanophrys sinensis*
Collecting site	Daya Bay, Guangdong	Qingdao, Shandong	Zhanjiang, Guangdong	Zhanjiang, Guangdong
Collecting date	21 Apr. 2011	26 Feb. 2010	6 Nov. 2011	6 Nov. 2011
Habitat	marine	marine	brackish water	brackish water
Temperature (°C)	19	11	21	20
Salinity (‰)	30	31	23	23
pH	7.5	7.3	6.7	6.8
Relative abundance	Low	Low	Low	High

taxa has highlighted the necessity to conduct further studies on this group (Wang *et al.* 2008a, b, 2009; Gao *et al.* 2010, 2012a, b, 2013; Pan *et al.* 2011, 2015a, b).

The "*ophrys*-taxa" scuticociliates include species of *Mesanophrys* Puytorac et al., 1974, *Metanophrys* Small & Lynn, 2001 and *Paranophrys* Thompson & Berger, 1965. Noticeably, they share many common morphological characteristics, e.g., body usually elongate, oval or cylindrical with a pointed anterior end but no apical plate; cytostome positioned at, or anterior to, the mid-body; buccal apparatus comprising a paroral membrane (PM) and three *Parauronema*-like membranelles, membranelle 1 (M1) and membranelle 2 (M2) each composed of two or more rows of kinetids. Most species are opportunistic parasites (Noland 1937; Borror 1963; Grolière & Léglise 1977; Strüder & Wilbert 1992; Song & Wilbert 2000; Song *et al.* 2002, 2003, 2009; Budiño *et al.* 2011). Among these genera, *Metanophrys* and *Mesanophrys* are the most closely related (body slender, with apical plate absent, cytostome in the anterior half of the body and three *Parauronema*-like membranelles), with their main difference being the position of the PM relative to M2 (PM extending anteriorly to the middle portion of M2 in *Metanophrys vs.* to the posterior end of M2 in *Mesanophrys*) (Small & Lynn 1985; Strüder & Wilbert 1992; Song & Wilbert 2000).

In the present study, a new genus, *Paramesanophrys* gen. nov., is established and detailed morphological information is provided for four scuticociliates, including one new species, *Paramesanophrys typica* gen. et sp. nov., and three nominal "*ophrys*" species.

Material and methods

Paramesanophrys typica gen. et sp. nov. was sampled on 21 Apr. 2011 from the coastal waters of Daya Bay near Huizhou (22°66'23" N, 114°65'09" E), China (Fig. 1C). *Mesanophrys carcini* was collected on 26 Feb. 2010 from the coastal waters off Olympic Sailing Center harbour of Qingdao (36°06'45" N, 120°39'78" E), China (Fig. 1A). *Metanophrys similis* and *M. sinensis* were collected on 6 Nov. 2010 from the surface water of a coastal shrimp-culturing pond off Zhanjiang (21°15'01" N, 110°44'04" E), China (Fig. 1B). Detailed collection information is given in Table 1. After isolation, cells were maintained in the laboratory as a uniprotistan culture (Pan *et al.* 2013a, b).

Cells were observed *in vivo* using an oil immersion objective with brightfield and Nomarski differential interference contrast optics. Mixtures of a saturated mercury dichloride solution and Bouin's fluid were used to fix samples. The protargol silver staining method (Wilbert & Song 2008; Pan *et al.* 2013a) was applied to reveal the infraciliature. Measurements were performed at magnifications of 100–1250×. Drawings were produced with the help of a camera lucida. Systematics and terminology are mainly used in accordance with Lynn (2008) and Small & Lynn (1985).

We failed to extract DNA from *Paramesanophrys typica* gen. et sp. nov. due to the low number of specimens of this species. If possible, we will try to acquire sequence data from it in the future.

Results

Subclass Scuticociliatia Small, 1967
Order Philasterida Small, 1967
Family Orchitophryidae Cépède, 1910

Genus ***Paramesanophrys*** gen. nov.
urn:lsid:zoobank.org:act:29EF1135-5A4C-4E60-8977-DAEB6EF21370

Diagnosis

Orchitophryidae with cytostome above mid-body; buccal apparatus consisting of three *Parauronema*-like membranelles; PM with zigzag structure, extending anteriorly to posterior end of M3; M1 composed of two rows of kinetids; scutica comprising basal body pairs arranged in a line parallel to somatic kineties; single caudal cilium.

Type species

Paramesanophrys typica gen. et sp. nov.

Etymology

The generic epithet, *Paramesanophrys*, refers to the similarity of the oral apparatus to that of the genus *Mesanophrys*.

Paramesanophrys typica gen. et sp. nov.
urn:lsid:zoobank.org:act:7B31578B-6C9A-4F80-BAA9-86C9ED9DC735
Figs 2–3, 4A; Table 2

Diagnosis

Size *in vivo* about 90–100 × 25–35 μm, elongate body, with pointed anterior end and narrowly rounded caudal end; posterior end distinctly depressed where caudal cilium located; buccal field approximately 40% of body length; 20 or 21 somatic kineties; M1 with 8–10 basal bodies in each kinety; M2 and M3 irregularly multi-rowed; scutica comprising *c.* seven kinetosome pairs; single macronuclear nodule; contractile vacuole caudally positioned; marine habitat.

Etymology

The epithet of this new species, *typica* (Greek, the type/typical, gender feminine), refers to the fact that it is the type of the new genus, *Paramesanophrys* gen. nov.

Type locality and ecological features

Coastal waters of Daya Bay (22°66'23" N, 114°65'09" E), Guangdong Province, China, with pH 8.0, salinity 31‰ and water temperature about 16 °C.

Type slides

A protargol slide with the holotype specimen encircled in black ink is deposited in the Laboratory of Protozoology, Ocean University of China (PXM-2011042101). A paratype slide is deposited in the Natural History Museum, London, UK (2016.3.10.1).

Description

Size 90–100 × 25–35 μm *in vivo*, body elongate, spindle-shaped, with pointed anterior end (Figs 2A–B, 3A–D). Posterior end narrowly rounded and distinctly depressed in middle of caudal margin at bottom of caudal cilium (Figs 2A, 3A, F). Buccal field approximately 40% of body length; shape of buccal cavity frequently changed from "falcate-shaped" to oval to circular, then conversed (Figs 2C, 3G, I–N). Pellicle slightly indented at bases of cilia (Figs 2F, 3E, H). Extrusomes spindle-shaped, *c.* 2–4 μm long (Fig. 2F). Cytoplasm colourless to greyish, containing several to many large (*c.* 5 μm across) food vacuoles filled with bacteria, often concentrated in anterior and posterior ends of body (Figs 2A, E, 3E, H). Single ellipsoid to spherical macronucleus, *c.* 15 μm across, no micronucleus observed (Fig. 3R). Contractile vacuole caudally located, approximately 8 μm across during diastole, pulsating at intervals of approximately 30 s (Figs 2A, 3F). Somatic cilia, approximately 10 μm long, densely arranged; single caudal cilium approximately 30 μm long (Figs 2A, 3E–F). Movement by swimming while rotating about long body axis without pause or by gliding on substrate (Fig. 2D).

Twenty or 21 somatic kineties, extending entire length of body and consisting of dikinetids in most of body and monokinetid in rest of body (Figs 2G–H, 3S). Buccal apparatus (Figs 2I, 3O–Q) consisting of PM and three *Parauronema*-like membranelles. M1 composed of two rows of kinetids with 8–10 basal bodies each (Figs 2I, 3Q). M2 and M3 irregularly multi-rowed. M3 much shorter than M2 (Fig. 3O–Q).

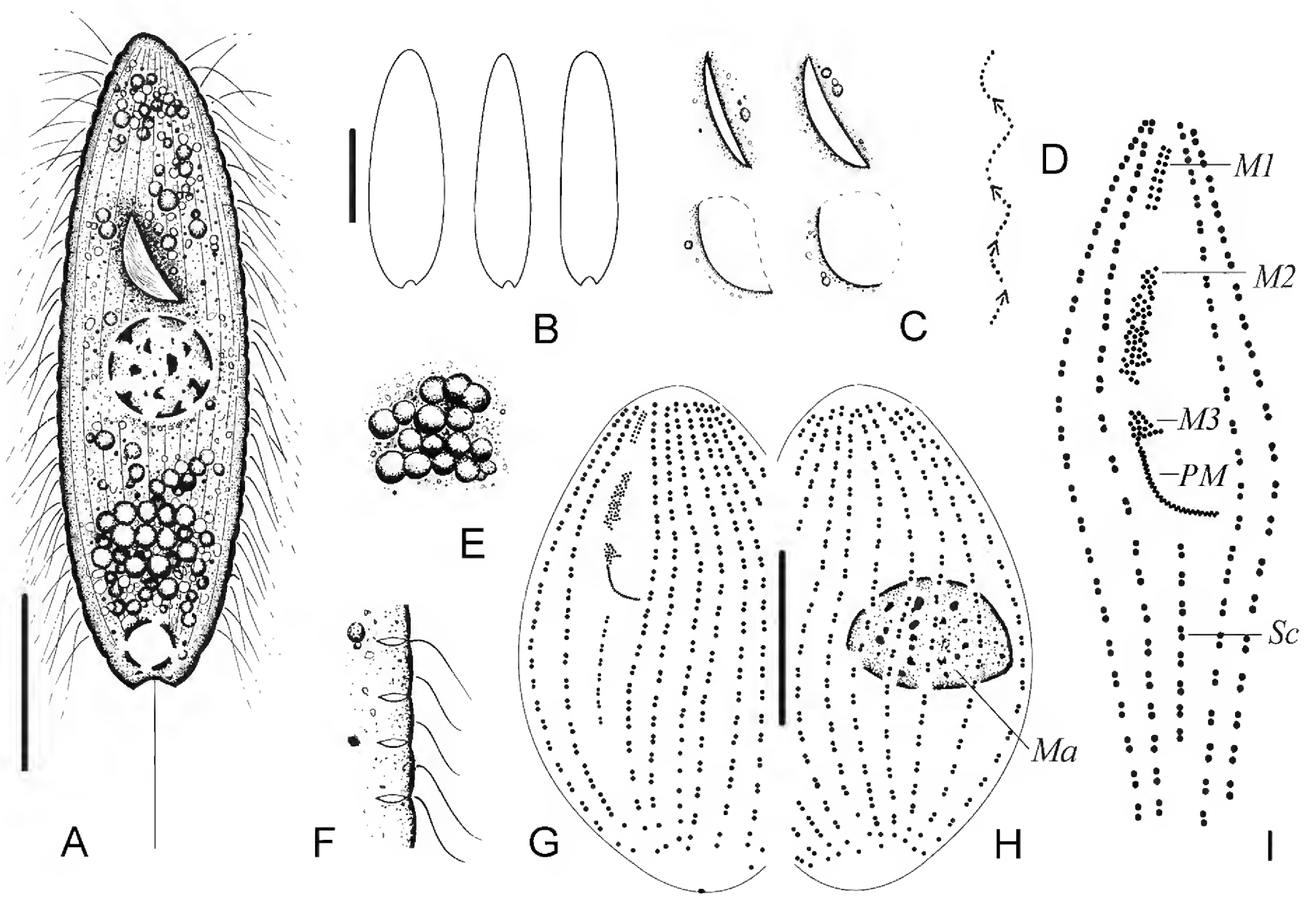

Fig. 2. *Paramesanophrys typica* gen. et sp. nov., from life (**A–F**) and after protargol staining (**G–I**). **A**. Ventral view of a representative individual. **B**. Different body shapes. **C**. Changing shapes of buccal field of the same individual. **D**. Movement trace. **E**. Food granules. **F**. Part of pellicle, to show extrusomes. **G–H**. Ventral (G) and dorsal (H) views of the same specimen (holotype), showing infraciliature and nuclear apparatus. **I**. Detailed structure of the buccal area. Abbreviations: M1–3 = membranelles 1, 2 and 3; Ma = macronucleus; PM = paroral membrane; Sc = scutica. Scale bars: A = 30 μm; B = 40 μm.

Table 2. Morphometric characterization of *Paramesanophrys typica* gen. et sp. nov. (Typ), *Mesanophrys carcini* (Grolière & Léglise, 1977) Small & Lynn in Aescht, 2001 (Car), *Metanophrys sinensis* Song & Wilbert, 2000 (Sin) and *Metanophrys similis* Song *et al.*, 2002 (Sim).

Character	Species	Min	Max	Mean	M	SD	CV	n
Body length (µm)	Typ	96	113	105.1	107	9.7	9.2	20
	Car	42	74	56.9	60	6.7	12.1	25
	Sin	33	59	43.6	41	14.4	27.1	23
	Sim	47	75	61.7	57	5.2	16.4	23
Body width (µm)	Typ	32	38	34.7	35	5.8	3.9	20
	Car	40	50	43.1	43	5.8	11.7	25
	Sin	16	24	19.9	20	1.9	9.9	23
	Sim	35	52	44.7	45	7.3	17.6	23
Number of somatic kineties	Typ	20	21	20.4	20	1.3	6.1	18
	Car	10	11	10.6	11	0.8	7.8	22
	Sin	10	11	10.1	10	0.6	5.9	20
	Sim	17	17	17	17	3.5	20.6	21
Length of buccal field (µm)	Typ	37	41	38.4	39	3.7	9.8	18
	Car	21	24	23.1	23	1.2	9.4	25
	Sin	15	26	19.6	19	1.6	8.7	23
	Sim	34	43	37.3	38	1.3	3.4	19
Macronucleus, length (µm)	Typ	13	16	14.7	15	2.8	10.5	18
	Car	10	14	12.8	12	1.5	12.6	24
	Sin	5	6	5.7	6	0.3	5.8	21
	Sim	8	11	9.5	9	1.9	21.6	22
Macronucleus, width (µm)	Typ	12	15	13.4	13	1.4	10.9	18
	Car	11	13	12.1	12	0.8	6.8	24
	Sin	6	7	6.3	6	1.3	20.4	21
	Sim	7	10	8.6	9	0.4	2.8	22
Number of basal bodies in somatic kinety 1*	Typ	22	24	23.2	23	1.1	4.8	17
	Car	35	40	37.4	37	4.6	9.9	24
	Sin	32	36	33.9	34	8.1	24.4	20
	Sim	23	26	24.1	24	4.9	2.1	22
Number of basal bodies in membranelle 1	Typ	8	10	8.6	9	1.2	12.4	14
	Car	7	9	8.0	8	0.4	5.0	19
	Sin	7	10	8.4	8	1.3	16.6	15
	Sim	6	6	6.0	6	0	0	14
Number of scutia pairs	Typ	7	8	7.4	7	0.6	8.5	14
	Car	4	4	4.0	4	0	0	19
	Sin	4	5	4.4	4	0.2	5.2	15
	Sim	5	7	6.1	6	0.3	5.1	14

Abbreviations: CV = coefficient of variation in %; M = median; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of individuals examined; SD = standard deviation.
* Basal body pairs counted as single units.

PM with paired basal bodies organized in zigzag pattern, extending anteriorly to posterior end of M3 (Figs 2G, I, 3O, Q). Scutica located at posterior end of PM, comprising *c.* seven or eight kinetosome pairs aligned in line parallel to somatic kineties (Figs 2G, I).

Mesanophrys carcini (Grolière & Léglise, 1977) Small & Lynn in Aescht, 2001
Figs 4B, 5; Table 2

Small & Lynn in Aescht (2001) did not formally combine this species with *Mesanophrys* Small & Lynn in Aescht, 2001. However, since they fixed it as the type species, they automatically produced the combination.

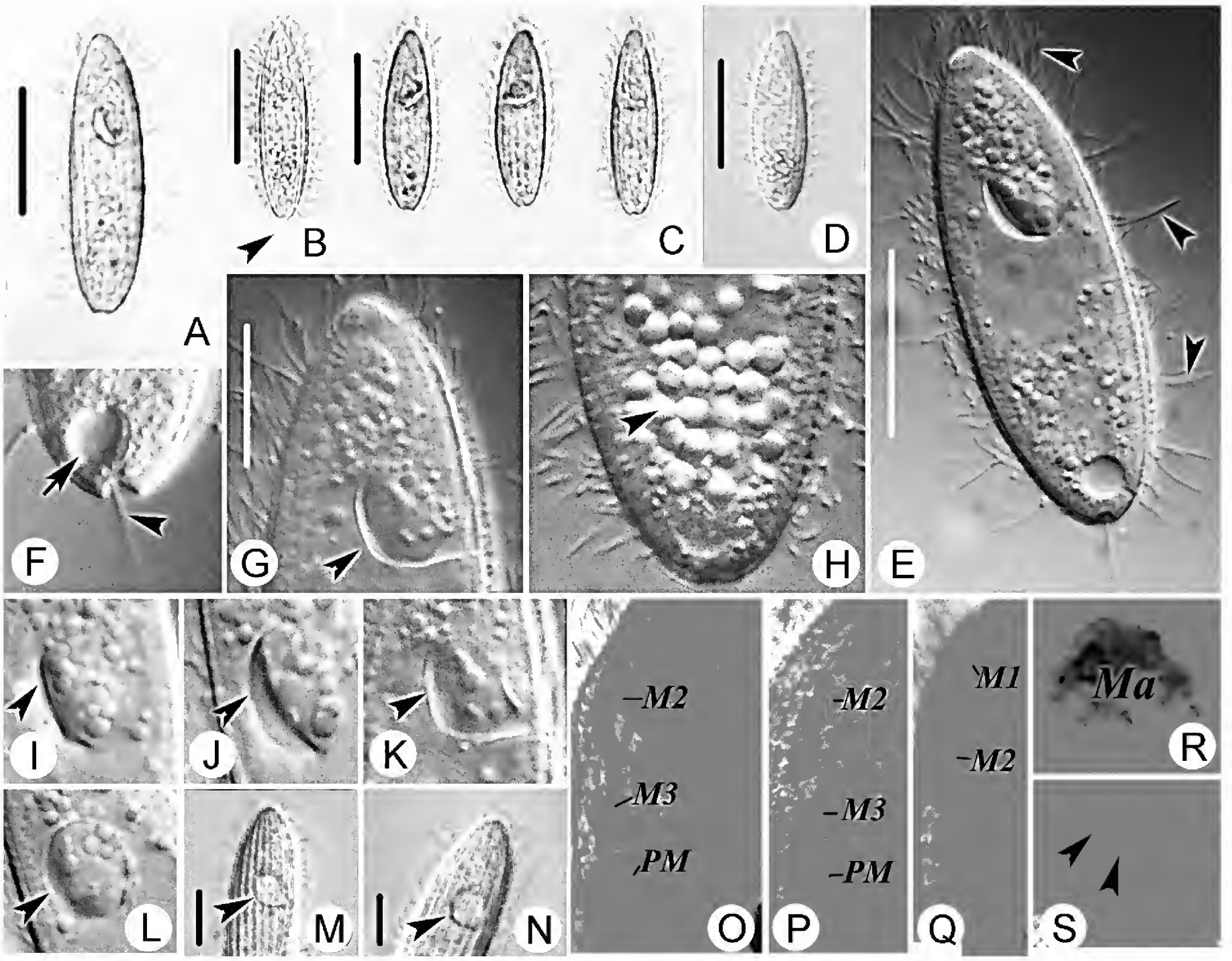

Fig. 3. *Paramesanophrys typica* gen. et sp. nov., from life (**A–N**) and after protargol staining (**O–S**). **A**. Ventral view of a representative individual. **B–E**. Different individuals; arrowhead in B shows caudal cilium, arrowheads in E mark somatic cilia. **F**. Posterior region of cell; arrow shows contractile vacuole and arrowhead marks caudal cilium. **G**. Anterior region of cell; arrowhead marks buccal region. **H**. Ventral view, showing food vacuoles (arrowhead). **I–N**. Ventral views, to show various shapes of buccal regions (arrowheads). **O–Q**. Detailed infraciliature of buccal area (P from holotype). **R**. Macronucleus. **S**. Posterior region; arrowheads show dikinetids of somatic kineties. Abbreviations: M1–3 = membranelles 1, 2 and 3; Ma = macronucleus; PM = paroral membrane. Scale bars: A, E = 40 μm; B–D = 70 μm; G, M–N = 10 μm.

Some characteristics, e.g., a larger body size and fewer somatic kineties, were found in the Qingdao population. Hence, a description of the Qingdao population as well as a comparison between different populations are supplied.

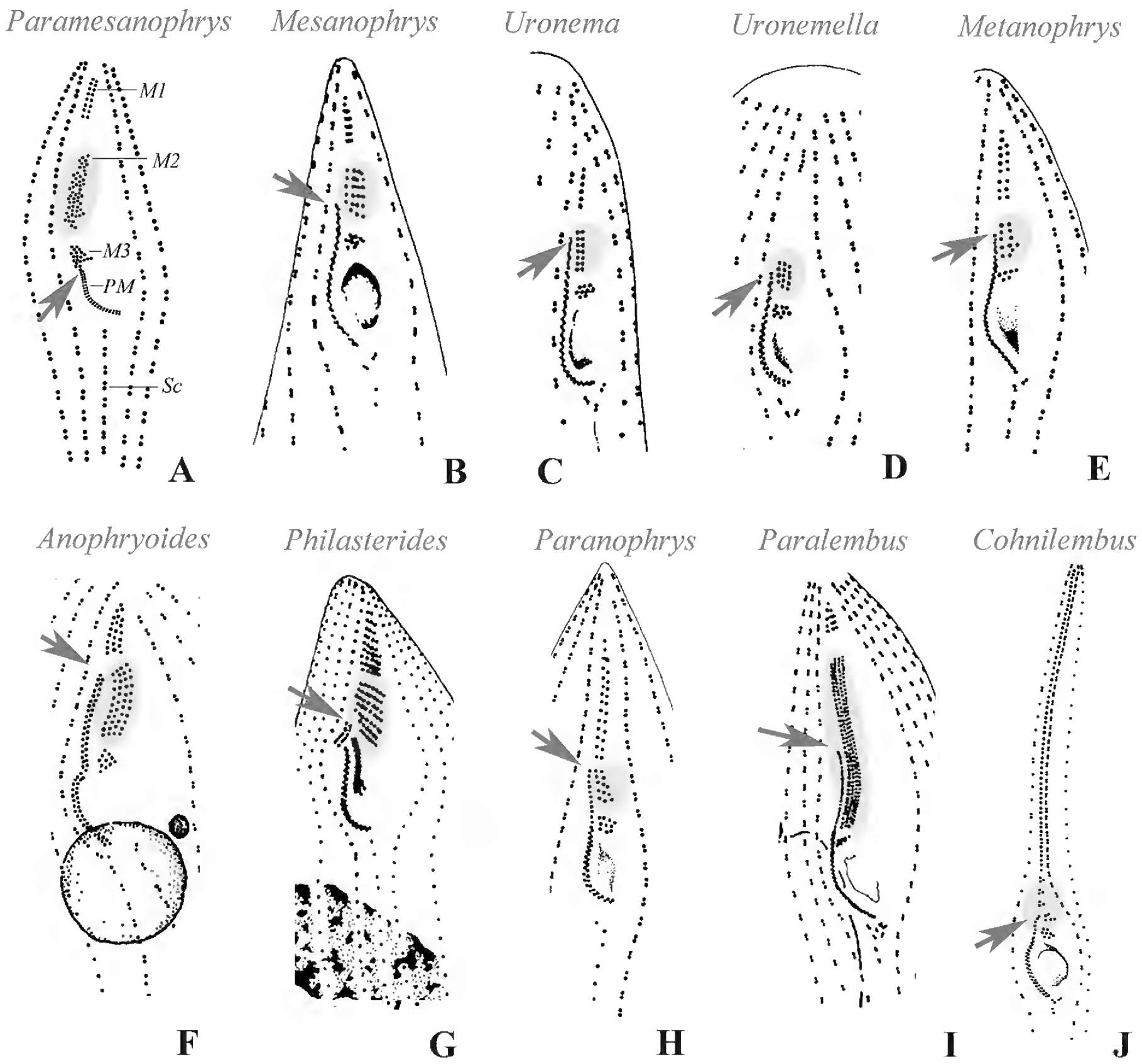

Fig. 4. Comparisons among different buccal apparatus patterns of *Paramesanophrys* gen. nov. and some related genera; arrows in A–J show different positions to which PM extends anteriorly and highlighted structures in A–J mark M2. **A**. *Paramesanophrys typica* gen. et sp. nov. (from the present work). **B**. *Mesanophrys carcini* (Grolière & Léglise, 1977) Small & Lynn in Aescht, 2001 (from Song & Wilbert 2000). **C**. *Uronema marinum* Dujardin, 1841 (from Song *et al.* 2009). **D**. *Uronemella filificum* (Kahl, 1931) Song & Wilbert, 2002 (from Song & Wilbert 2002). **E**. *Metanophrys sinensis* Song & Wilbert, 2000 (from Song & Wilbert 2000). **F**. *Anophryoides haemophila* Cawthorn *et al.*, 1996 (from Cawthorn *et al.* 1996). **G**. *Philasterides armatalis* Song, 2000 (from Song 2000). **H**. *Paranophrys marina* Thompson & Berger, 1965 (from Song *et al.* 2002). **I**. *Paralembus digitiformis* Kahl, 1931 (from Song & Wilbert 2000). **J**. *Cohnilembus verminus* (Müller, 1786) Kahl, 1933 (from Song 2000). Abbreviations: M1–3 = membranelles 1, 2 and 3; PM = paroral membrane; Sc = scutica.

Description of Qingdao population

Body size 45–65 × 15–25 μm *in vivo*, spindle-shaped to long fusiform, with pointed anterior end and narrowly rounded caudal end (Fig. 5A–C). Body shape variable, likely due to nutritional conditions or stage in life cycle: from slender, spindle-like to pyriform (Fig. 5C–D). Buccal field short and narrow, with length of about 30% of body (Fig. 5B). Somatic cilia densely arranged and about 6–8 μm long (Fig. 5B). Pellicle thin and smooth, with no distinguishable extrusomes. Cytoplasm colourless to slightly greyish, containing several to many differently-sized (3–5 μm) refringent granules (Fig. 5A, D). Single caudal cilium about 15 μm in length (Fig. 5B, arrow) and one large, spherical, centrally located macronucleus; one micronucleus closely associated with macronucleus. Contractile vacuole small (5 μm across), terminally positioned and pulsating at intervals of approximately 30 s (Fig. 5A, arrow). Movement by continuous swimming in water without pause or gliding slowly on substrate.

Ten or 11 somatic kineties, consisting of dikinetids in anterior two-thirds and monokinetid in posterior third of body (Fig. 5G, arrow). M1 slightly separated from apex, composed of two rows of kinetids with

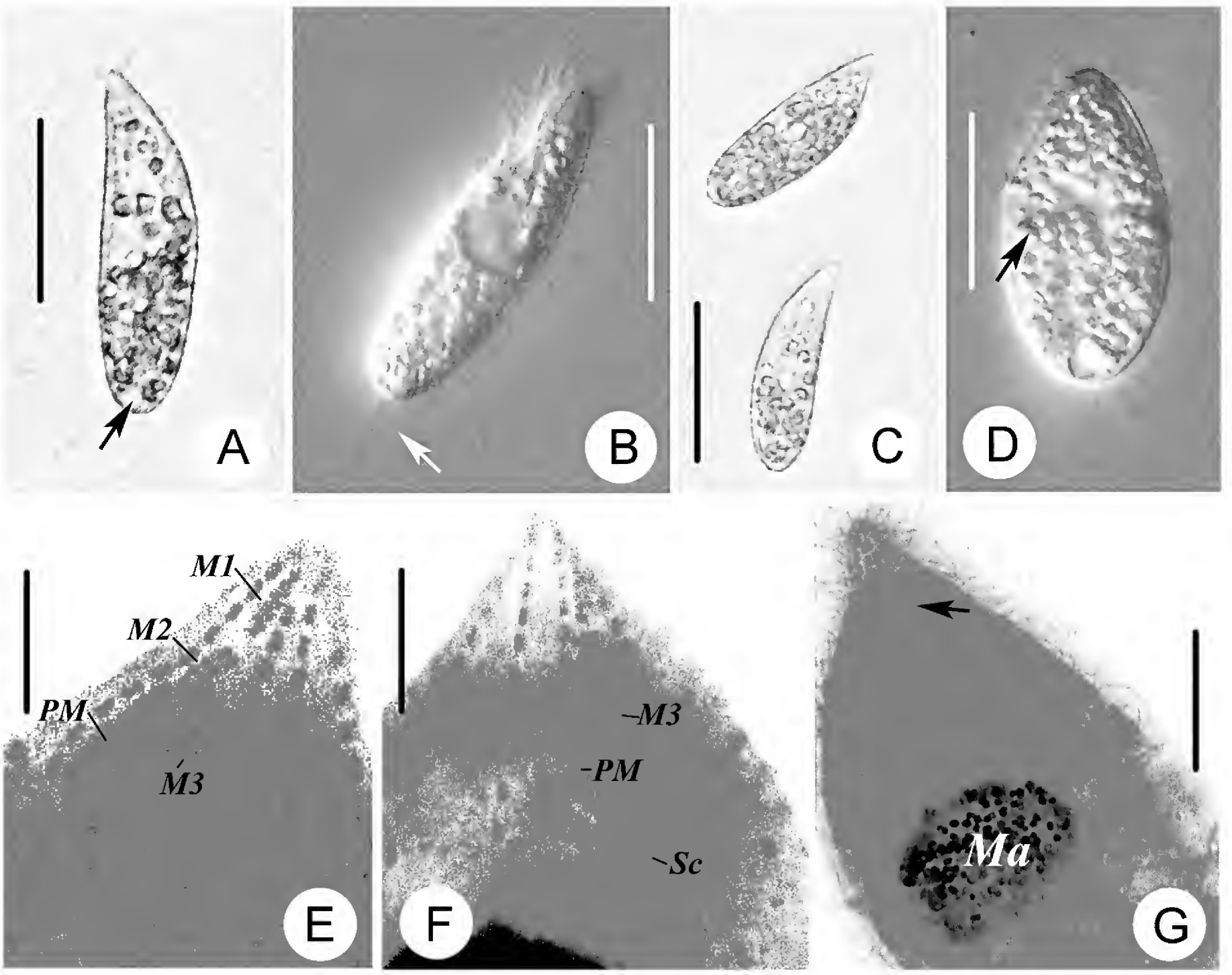

Fig. 5. *Mesanophrys carcini* Small & Lynn in Aescht, 2001, *in vivo* (**A–D**) and after protargol staining (**E–G**). **A**. Ventral view of a representative individual; arrow shows contractile vacuole. **B–D**. Ventral views of four individuals; arrow in B shows caudal cilium and arrow in D marks food vacuole. **E–F**. Ventral views, detailed structure of buccal area. **G**. Dorsal view; arrow indicates somatic kinety. Abbreviations: M1–3 = membranelles 1, 2 and 3; Ma = macronucleus; PM = paroral membrane; Sc = scutica. Scale bars: A–D = 30 μm; E–G = 5 μm.

7–9 basal bodies each (Fig. 5E–F). M2 composed of five or six longitudinal rows, each containing about 6–8 basal bodies (Fig. 5E–F). M3 located close to M2, much shorter than M2 and composed of three short, irregularly arranged rows of kinetosomes (Fig. 5E–F). PM extending anteriorly to posterior end of M2. Scutica Y-shaped, with *c.* four pairs of kinetosomes (Fig. 5E–F).

Ecological features

Salinity 32‰, pH 7.9 and water temperature about 11 °C.

Metanophrys sinensis Song & Wilbert, 2000
Figs 4E, 6; Table 2

This species was described by Song & Wilbert (2000) in detail based on their Qingdao population. In the current work, it is reported for the first time from South China Sea. An improved diagnosis is provided herein based on all these data; the improved parts are highlighted in bold.

Improved diagnosis

Slender to elongated oval body shape; *in vivo* about 25–50 × 10–20 μm with pointed anterior end; **buccal field about 30%–50% of body length**; mostly ten somatic kineties, of which somatic kinety 1 consists of *c.* 35 basal pairs; M1 composed of two rows, each with 7–10 kinetosomes, longer than M2; two-rowed M2; contractile vacuole pore located near posterior end of kinety 2; **extrusomes present**; marine habitat.

Description of Zhanjiang population

Body 25–30 × 10–15 μm *in vivo*, usually elongate-oval in outline, with anterior end distinctly pointed and posterior rounded (Fig. 6A–B). Body asymmetrical in outline when viewed ventrally, with anterior end slightly curved sideways (Fig. 6A–B). Ventral side almost straight, while dorsal side convex. Buccal field ⅖ to ½ of body length, with cytostome located anterior to equatorial plane of body (Fig. 6C). Cilia densely packed, about 7–8 μm long. Caudal cilium about 15 μm in length (Fig. 6B). Pellicle thin and slightly notched, with extrusomes about 2–3 μm long and dense beneath cortex (Fig. 6D). Endoplasm colourless to greyish, containing several food vacuoles and bar- or dumbbell-like crystals, which are usually 3 μm long and located in anterior and posterior regions of body (Fig. 6A–B, G). One large round to oval macronucleus approximately centrally located, with many small, irregularly shaped nucleoli on surface. Contractile vacuole about 5 μm in diameter and caudally positioned near ventral side (Fig. 6B). Movement with no special features, including swimming moderately fast, sometimes continuously swimming in water without pause.

Ten somatic kineties arranged longitudinally, and dikinetids about ¾ of length of each in anterior part (Fig. 6J). Buccal apparatus consists of three *Parauronema*-like membranelles (Fig. 6E–F). M1 slightly below apex and composed of two rows of kinetids with 7–10 basal bodies each, and longer than M2. M2 two-rowed, containing about five basal bodies in each row. M3 located close to M2, normally with three short, obliquely arranged rows of basal bodies. Scutica Y-shaped, with several pairs of kinetosomes (Fig. 6I). Silverline system in quadrangular mesh-pattern (Fig. 6H). Contractile vacuole pore located near posterior end of kinety 2.

Ecological features

Salinity 21‰, pH 7.3 and water temperature 26 °C.

Metanophrys similis Song *et al.*, 2002
Fig. 7; Table 2

This species is reported for the first time from the South China Sea. The morphological and behavioural characteristics of the Zhanjiang population closely resemble those of the Qingdao population; therefore, only the morphometric characterisation and important features are supplied.

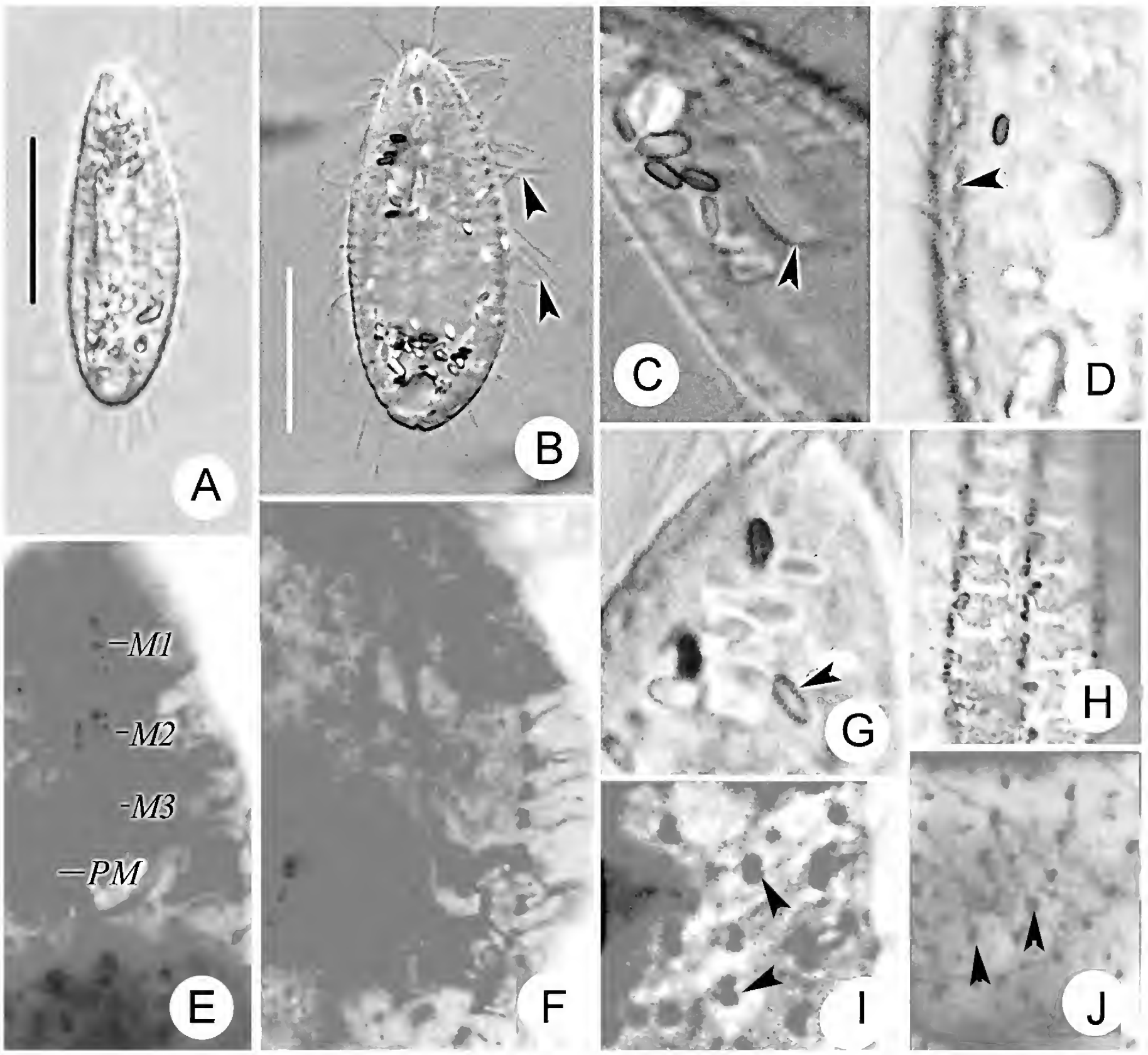

Fig. 6. *Metanophrys sinensis* Song & Wilbert, 2000, *in vivo* (**A–D, G**) and after protargol (**E–F**, **I–J**) or silver nitrate (**H**) staining. **A**. Ventral view of a typical individual. **B**. Ventral view of another individual; arrowheads mark somatic cilia. **C**. Ventral view; arrowhead exhibits buccal field. **D**. Notched pellicle (arrowhead). **E**. Detailed structure of buccal area. **F**. Individual in morphogenesis, to show buccal apparatus. **G**. Ventral view, showing bar-shaped crystal (arrowhead). **H**. Detail of somatic kinetids. **I**. **D**ikinetids of scutica (arrowheads). **J**. Posterior region; arrowheads show monokinetids of somatic kineties. Abbreviations: M1–3 = membranelles 1, 2 and 3; PM = paroral membrane. Scale bars: A–B = 15 μm.

Description of Zhanjiang population

Body *in vivo* about 35–40 × 20–25 μm, plump pyriform, tapering anteriorly and rounded posteriorly, and no apical plate formed (Fig. 7A–C). Ventral side almost straight, while dorsal side slightly convex (Fig. 7A). Length : width ratio approximately 2 : 1 (Fig. 7A–C). Buccal field occupies about 40% of total body length, with buccal cilia about 5–8 μm in length. Pellicle thick and strongly notched. Somatic cilia about 7–8 μm long and densely arranged (Fig. 7D, arrowheads). Single caudal cilium about 15 μm in length (Fig. 7F). Extrusomes, about 2 μm in length, arranged in rows between somatic kineties. Endoplasm colourless to greyish and contains abundant food vacuoles (Fig. 7E). One large, spherical to ovoid macronucleus centrally located (Fig. 7C, J). Contractile vacuole about 5 μm in diameter and caudally positioned near ventral side (Fig. 7A).

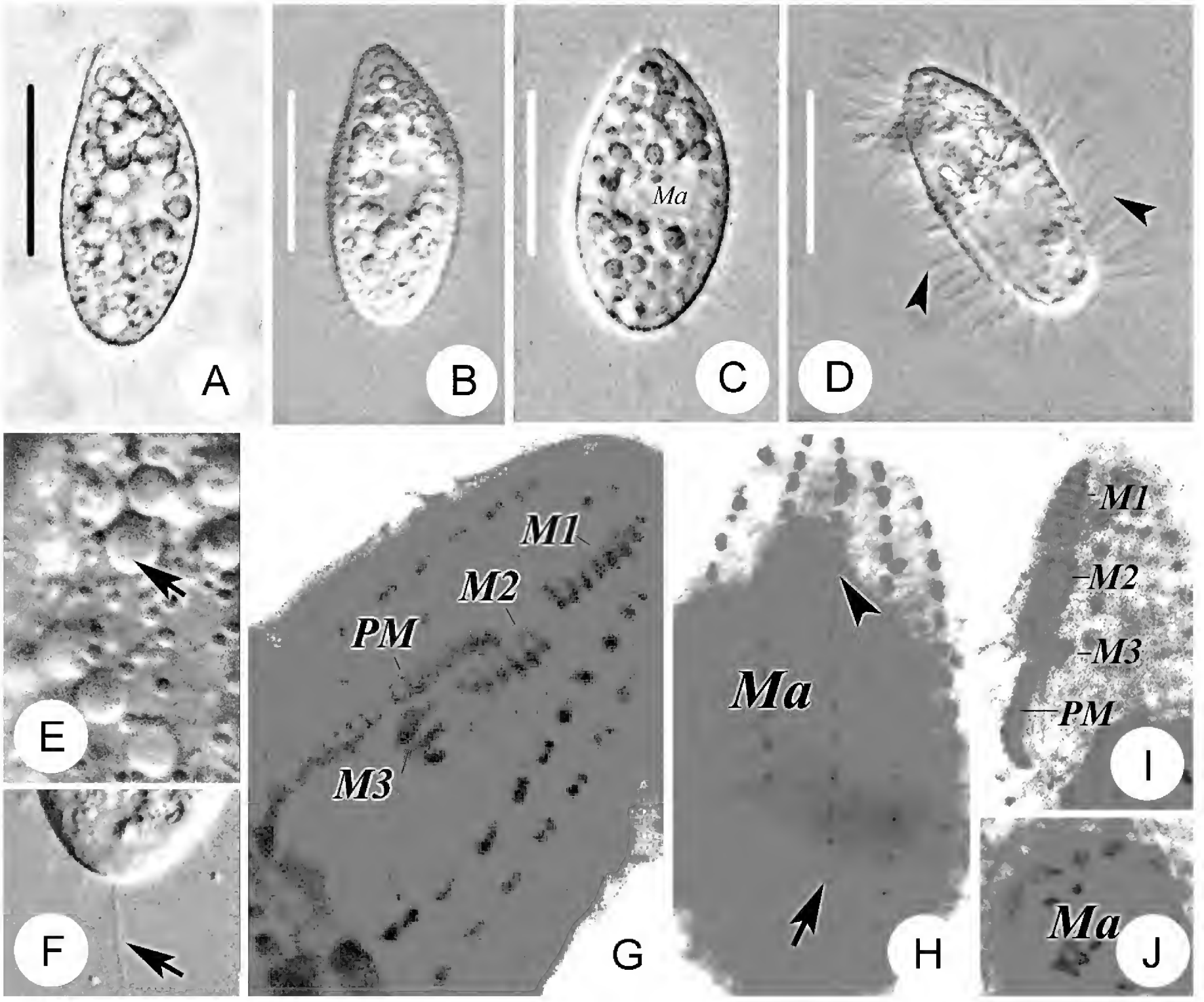

Fig. 7. *Metanophrys similis* Song *et al.*, 2002, *in vivo* (**A**–**F**) and after protargol staining (**G**–**J**). **A**. Ventral view of a typical individual. **B**–**D**. Ventral views of three individuals; arrowheads in D mark somatic cilia. **E**. Food vacuole (arrow). **F**. Posterior region; arrow shows caudal cilium. **G**, **I**. Ventral views, to show detailed structure of the buccal area. **H**. Dorsal view; arrow shows monokinetids, arrowhead marks dikinetids. **J**. Macronucleus. Abbreviations: M1–3 = membranelles 1, 2 and 3; Ma = macronucleus; PM = paroral membrane. Scale bars: A–D = 30 μm.

Locomotion by swimming moderately fast, sometimes continuously without pause, or by crawling on substrates.

Twelve somatic kineties with dikinetids arranged in approximately anterior half of each row and monokinetids positioned posteriorly (Fig. 7H). M1 positioned near apex and comprised of three longitudinal rows of kinetids with six basal bodies each (Fig. 7G, I). M2 three-rowed, as long as M1 and also composed of about six basal bodies in each longitudinal row (Fig. 7G, I). M3 located close to M2 and normally comprised of three short, parallel arranged rows of basal bodies (Fig. 7G, I). PM extends to about anterior third of body (Fig. 7G). Scutica, with about 5–7 basal bodies, arranged in long line.

Ecological features

Salinity 21‰, pH 7.3 and water temperature 26 °C.

Discussion

About *Paramesanophrys* gen. nov. and *P. typica* gen. et sp. nov.

The family Orchitophryidae is characterised as follows: small- to medium-sized body; ovoid-shaped; caudal cilium often present; oral region in anterior ⅓ to ½ of body; scutica aligned along midventral postoral region; bacterivorous and histophagous; marine habitats, always as facultative parasites of crustaceans, asteroids, fish and free-swimming (Lynn 2008). *Paramesanophrys* gen. nov. should be assigned to Orchitophyridae based on its morphological characters and habitat.

Hitherto, five genera have been assigned to Orchitophryidae according to Lynn (2008), namely *Anophryoides* de Puytorac & Grolière, 1979, *Mesanophrys* Small & Lynn in Aescht, 2001, *Metanophrys* Puytorac *et al.*, 1974, *Orchitophrya* Cépède, 1907 and *Paranophrys* Thompson & Berger, 1965. Compared with these related genera, *Paramesanophrys* gen. nov. has a unique oral apparatus, with the PM extending anteriorly to the posterior end of M3 (*vs.* PM extending anteriorly to the anterior end/ middle portion/posterior end of M2; Fig. 4) (Small & Lynn 1985; Strüder & Wilbert 1992; Cawthorn *et al.* 1996; Song & Wilbert 2000).

Besides having a unique *Paramesanophrys*-type PM, *Paramesanophrys typica* gen et sp. nov. also has the scutica comprising *c.* seven or eight kinetosome pairs aligned in a line parallel to the somatic kineties and a conspicuous pellicle depression in the middle of caudal margin. This combination of features clearly separates it from all known scuticociliates at the species level.

Mesanophrys carcini (Grolière & Léglise, 1977) Small & Lynn in Aescht, 2001

The main characteristics that aid in identifying this species are the slender body, short buccal field, oral apparatus and somatic infraciliature (Song & Wilbert 2000). The characteristics of the Qingdao population are different from those of the population reported by Song & Wilbert (2000) in having a larger body size (on average 55 × 20 μm *vs.* 40 × 12 μm) and a variable number of somatic kineties (10 or 11 *vs.* constantly 11; Table 3). These variations are considered population-dependent (Song & Wilbert 2000).

Metanophrys sinensis Song & Wilbert, 2000

Our population is virtually identical to the two Qingdao populations (Song & Wilbert 2000; Ma & Song 2003), that is, they agree in body size and shape, habitat, infraciliature, silverline system and marine habitat, except the proportion of buccal field length to body length (40–50% in the present study *vs.* ca. 30–40% in the previous studies) and the presence of extrusomes (*vs.* not observed in previous descriptions) (Song & Wilbert 2000; Ma & Song 2003; Table 3). Nevertheless, it is believed that they are conspecific because of their close similarity in other living characteristics and infraciliature.

Table 3. Morphometrical comparison of known *ophrys*-species populations. Abbreviations: QD, Qingdao or Qingdao population; ZJ, Zhanjiang or Zhanjiang population.

	Mesanophrys carcini	***Mesanophrys carcini* QD**	***Metanophrys similis***	***Metanophrys similis* ZJ**	***Metanophrys sinensis***	***Metanophrys sinensis* QD**	***Metanophrys sinensis* ZJ**
Sample location	shrimp culture pond, QD	coastal waters, QD	coastal waters, QD	maricultural waters, ZJ	molluscan culture pond, QD	molluscan culture pond, QD	mariculture waters, ZJ
Body size in vivo (μm)	30–55 × 10–15	45–65 × 15–25	30–45 × 10–12	35–40 × 20–25	30–50 × 10–20	30–50 × 10–20	25–30 × 10–15
Somatic kineties (no.)	11 or 12	10 or 11	11 or 12	12	10	10 or 11	10
Buccal length/ body length (%)	25–33	30	40–50	40	40	35–40	40–50
Extrusome	not observed	not observed	present	present	not observed	not observed	present
Data source	Song & Wilbert (2000)	present study	Song *et al.* (2002)	present study	Song & Wilbert (2000)	Ma & Song (2003)	present study

Metanophrys similis Song *et al.*, 2002

The Zhanjiang population is identical to the original description (Song *et al.* 2002) according to the body size, ciliature and habitat; hence, the identity of this species is not in doubt. Compared with the original description, the population described in this paper has a different body shape (plump pyriform *vs.* slender body shape in Song *et al.* 2002; Table 3), which may be due to different nutritional conditions (Song *et al.* 2002).

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