Two Anaerobic Ciliates (Ciliophora, Armophorea) from China: Morphology and SSU rDNA Sequence, with Report of a New Species, *Metopus paravestitus* nov. spec.

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Anaerobe; armophorean; diversity; Metopidae; phylogeny.

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ABSTRACT
The morphology and phylogeny of two metopid ciliates, collected from anaerobic habitats in China, were investigated using live observation, protargol staining method, and SSU rDNA sequencing. The new species *Metopus paravestitus* nov. spec. can be distinguished by a combination of the following features: oblong cell with densely arranged ectobiotic prokaryotes perpendicular to cell surface, filiform intracytoplasmic structures packed in the anterior portion of the cell. Our work also demonstrates the wide geographical distribution of *Metopus es* (Mueller, 1776) Lauterborn, 1916. The order Metopida is consistently depicted as a paraphylum in SSU rDNA phylogeny. *Metopus paravestitus* nov. spec. is closely related to its marine congeners than to freshwater forms. The present study confirms once again the non-monophyly of the genus *Metopus* and genus Metopidae.

RECENTLY, there has been an increasing interest in the study of eukaryotic microorganisms in extreme environments, including anoxic and hypoxic habitats (Beaudoin et al. 2012; Bernhard et al. 2000; Fenchel 2012; Hu 2014; Kahl 1927; Silva-Neto et al. 2016; Vandaen and Foissner 2018; Wang et al. 2019a; Zhao et al. 2012). In ciliated protists, most free-living anaerobic or microaerobic organisms belong to the classes Plagiopylea, Armophorea, or Odontostomatea (Bourland et al. 2014, Bourland et al. 2017a, Bourland et al. 2017b, Bourland et al. 2018a, Bourland et al. 2018b, Fernandes et al. 2018, Foissner 2016a, Hu, 2019; Li et al. 2017; Lynn 2008; Paiva et al. 2017; Rotterová et al. 2018; Vd’aený and Foissner 2017a, Vd’aený and Foissner 2017b, Vd’aený and Foissner 2019; Vd’aený et al. 2019a, Wang et al. 2019a), and some other species are members of the genera *Cyclidium*, *Loxodes*, and *Caria-cothrix* within classes Oligohymenophorea, Karyorelictea, and Cariacotrichea, respectively (Esteban et al. 1995; Foissner 2016b; Hu 2014; Orsi et al. 2012; Xu et al. 2015). According to recent systematic classification, the class Armophorea comprises three orders, viz., Armophorida, Clevelandellida, and Metopidae (Bourland et al. 2018b; Janowski 1980). The representatives of this class often bear symbiotic prokaryotes and contain hydrogenosomes instead of classical mitochondria as an adaptation to the anaerobic environment (Fenchel et al. 1977). Among these three orders, metopids present highly variable body shapes (Lynn 2008).

*Metopus Claparède and Lachmann, 1858,* distributed in various anaerobic environments worldwide (Bourland et al. 2014, 2017a; Esteban et al. 1995; Foissner and Agatha 1999), is the most species-rich genus in the Metopida (Kahl 1932; Lynn 2008; Vd’aený and Foissner 2017a). Its research history can be traced to the description of *Metopus es* syn. *Trichoda es* by Müller (1776). Up to now, more than 80 species have been described (Omar et al. 2017; Roskov et al. 2016; Vd’aený and Foissner 2019), but from taxonomic and morphological viewpoints, metopids
are still insufficiently studied since many *Metopus* species descriptions still lack details on ciliature and/or molecular data (Foissner and Agatha 1999; Vd’ačný and Foissner 2017a). Recent research indicates that the variation of cell size and shape could be broad in the individual species as environmental conditions change (Bourland et al. 2017a; Esteban et al. 1995). Therefore, more detailed morphological, molecular, and ecological data on various populations from geographically distinct sites are required to clarify the systematics and biogeography of this group of ciliates.

*Metopus es* (Müller, 1776) Lauterborn, 1916, the type species of the genus, presents wide geographic distribution (Bourland et al. 2017a; Jankowski 1964; Kahl 1932). Bourland et al. (2017a) showed relatively high variability in both body shape and ciliary patterns among populations. In this study, we have isolated and investigated the morphology and ciliary pattern of a new metopid species and one Chinese population of *M. es*. We have compared these species to other *Metopus* species and analyzed their SSU rDNA sequences in order to make an integrative effort to combine morphological and molecular data to circumscribe these morphospecies.

**MATERIALS AND METHODS**

**Collection and ciliate cultivation**

*Metopus paravestitus* nov. spec. was discovered in a sandy sample with seaweeds in an abandoned glass bottle recovered from the bottom of a marine farm near Shenzhen (22°64′39″N, 114°51′44″E), China in March 2016 (Fig. S1A, B, D). The sand and water (salinity 30‰) in the bottle were poured out into Petri dishes for isolation of ciliates. *Metopus es* is an occasional species found in anaerobic sediments, and it was collected from a freshwater pond with abundant reeds in Qingdao (36°06′36″N, 120°34′52″E), China in May 2016 (Fig. S1A–C). The pond was surrounded by woods and the fallen leaves contributed to an increased content of organic matter in the water.

**Morphological observations**

Living organisms were observed under a light microscope equipped with differential interference contrast illumination. Protargol was prepared through the protocol provided by Pan et al. (2013). Protargol staining was then carried out following the method of Wilbert (1975) to reveal the ciliature and nuclear apparatus. Measurements and counts were performed at 1,000X magnification. Drawing of specimens was done with the aid of a drawing microscope attachment.

**DNA extraction and gene sequencing**

Several cells of each species were picked out with glass micropipettes, washed five times using sterile water collected from the same habitat, and were then used for DNA extraction. Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. TaKaRa ExTaq polymerase (TaKaRa Biomedicals, Shiga, Japan) was used to amplify the SSU rDNA with the universal eukaryotic primers, EukA and EukB, (Medlin et al. 1988). PCR amplification, cloning, and sequencing were performed according to Song et al. (2018).

**Phylogenetic analyses**

A data set containing SSU rDNA sequences was created for phylogenetic analyses. It consisted of the two newly obtained sequences, 82 sequences of other amphoreans, and 10 sequences of Litostomatea, Spirotrichea, Odontostomatea, Rimateae binucleatus, Lacrymaria marina, Amphilopterus aescultae, and Balantium coli serving as outgroup. Sequences were aligned using the GUIDANCE algorithm with the default parameters via the GUIDANCE web server (Penn et al. 2010). Ambiguously aligned regions were excluded manually using the program BioEdit 7.0.5.2 (Hall 1999), resulting in a matrix of 1,460 characters. Maximum-likelihood (ML) analyses were carried out on CIPRES Science Gateway using RAxML–HPC2 version 8.2.10 (Stanatakis et al. 2008) with the GTR + I + G nucleotide substitution model. Support for the best ML tree resulted from 1,000 bootstrap replicates. A Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway server. The GTR + I + G model was selected by MrModeltest v.2.0 (Nylander 2004) as the best substitution model. Markov chain Monte Carlo simulations were run with two sets of four chains for 1,000,000 generations with a sample frequency of every 100th generation. The first 25% of constructed trees were discarded as burn-in. The remaining trees were used to calculate posterior probabilities using a majority rule consensus.

**RESULTS**

**Description of *Metopus paravestitus* nov. spec.**

Body size 90–120 x 25–60 µm in vivo and 71–113 x 22–55 µm after protargol staining (n = 24). Length: width ratio including preoral dome 2.8:1 on average. Cell yellowish brown or colorless under low magnification (Fig. 2A). Preoral dome flattened, distinctly twisted anteriorly, and widest at equator of cell (Fig. 1A, 2E, F). Cells dorsoventrally flattened about 3:2 (Fig. 2A, B). The anterior body area is wider than the posterior when viewed from left lateral (Fig. 2A), and the posterior end is blunt round to acute (Fig. 1E, 2E–G). Preoral dome flattened, distinctly twisted to left, occupying about 50% of body length in right view (Fig. 1A, B). Cell surface covered by a coat of ectobiotic prokaryotes that are perpendicular to cortex, each bar-shaped and more than 2 µm long (Fig. 1A, D, 2C–F). These ectobionts detach quickly when hosts die (Fig. 2D). Cytoplasm colorless, filled with spherical particles (0.5–
Description of Qingdao population of *Metopus es* (Müller, 1776) Lauterborn, 1916

Size 120–170 × 30–55 μm in vivo with a ratio of length to width about 3.8:1. Shape elongate fusiform or slightly sigmoid and distinctly twisted anteriorly (Fig. 3A, H). Preoral dome lightly convex, overhanging left margin and slightly projecting from body, occupies about 40% of body length when viewed ventrally (Fig. 3L). Somatic ciliature composed of dikinetids, with both basal bodies ciliated, arranged in 30–42 meridional rows including dome kineties on preoral dome (Fig. 2I, J). Somatic cilia about 10 μm long in vivo with several elongated caudal cilia present, each 20–40 μm in length (Fig. 1A, 2F, H). Perizonal stripe extending approximately 45% of body length, invariably composed of five ciliary rows (Fig. 1B, G, 2I) with cilia paired about 15 μm long in vivo. Adoral zone of membranelles (AZM) conspicuously coarsely granular, 2.5 μm across, ellipsoidal and inconspicuous, attached to or near macronucleus (Fig. 1A–C). Obconical contractile vacuole terminally positioned (Fig. 1A, 2A, E), pulsating at intervals of more than 5 min. Swimming leisurely while rotating around long axis of cell (Table 1).

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**Table 1.** Morphometric characteristics of *Metopus paravestitus* nov. spec. (upper lines) and *Metopus es* (Müller, 1776) Lauterborn, 1916 (lower lines)

| Characteristicsa | Mean M | SD | CV | Min | Max | n |
|------------------|--------|----|----|-----|-----|---|
| Body, length     | 88.1   | 86.5 | 10.36 | 11.8 | 71 | 113 | 24 |
| Body, width      | 31.6   | 31.5 | 6.87 | 21.7 | 22 | 55 | 24 |
| Body length: body width ratio | 2.9 | 2.9 | 0.45 | 15.7 | 1 | 4 | 24 |
| Distance from anterior cell end to proximal end of adoral zone | 57.4 | 57.7 | 5.05 | 8.8 | 46 | 67 | 20 |
| Distance from anterior cell end to proximal end of adoral zone—body length, ratio in % | 51.9 | 52.0 | 3.52 | 6.8 | 44 | 60 | 24 |
| Macronucleus, length | 28.2 | 27.5 | 4.35 | 15.4 | 21 | 37 | 24 |
| Macronucleus, width | 11.8 | 12.0 | 2.25 | 19.0 | 7 | 16 | 24 |
| Micronucleus, number | 3.2 | 3.0 | 0.61 | 19.4 | 2 | 15 | 12 |
| Adoral membranelles, number | 32.7 | 33.0 | 2.15 | 6.6 | 27 | 36 | 21 |
| Somatic kinetics, number | 36.0 | 36.0 | 3.41 | 9.5 | 30 | 42 | 20 |
| Preoral dome kinetics, number | 23.4 | 23.0 | 3.27 | 14.0 | 18 | 31 | 19 |
| Perizonal ciliary stripe rows, number | 20.0 | 20.0 | 1.73 | 8.7 | 17 | 23 | 22 |
| Macronucleus, number | 10.3 | 10.0 | 1.34 | 12.9 | 8 | 13 | 19 |
| Micronucleus, number | 5.0 | 5.0 | 0.00 | 0.0 | 5 | 5 | 24 |
| Macronucleus, number | 5.0 | 5.0 | 0.00 | 0.0 | 5 | 5 | 20 |

CV = coefficient of variation in %; M = median; Max = maximum; Mean = arithmetic mean; Min = minimum; n = number of specimens measured; SD = standard deviation.

aData based on protargol-stained specimens. Measurements in μm.

SSU rDNA sequences comparison and the phylogeny of *Metopus* species

Length and GC content information of the two newly obtained SSU rDNA sequences are as follows: *M. paravestitus* nov. spec. 1,657 bp, 44.48% GC; *M. es* 1,587 bp, 42.15% GC. The sequence of the Qingdao population of *M. es* exhibited 97–99% similarities to those of thirteen conspecific populations (Table S1). The topologies of body length (Fig. 3H) and it is composed of five longitudinal rows with cilia about 12 μm long in vivo (Fig. 3L). Adoral zone comprising about 40 L-shaped membranelles (Fig. 3B, D, H).
of the phylogenetic trees from ML and BI analyses were almost identical, and thus only the ML tree topology is shown appended with support values derived from both analyses.

Sequences from *M. paravestitus* nov. spec. and *M. contortus* cluster together with full support, being 90–97% similar. The newly sequenced *M. es* forms a fully supported clade with other populations of the same species, which then groups with the above mentioned clade (including *M. contortus* and *M. paravestitus*) but with low support. Other members of the genus *Metopus* fall into at least four separate clusters: (1) the fully supported clade of two *M. setosus* sequences (MH086824, MH086823); (2) the fully supported clade consisting of the sequences of *M. fuscus* and another two *M. setosus* sequences (KF607087, KY855536); (3) the fully supported clade comprised of *Metopus laminarius*, and *M. boletus*, merges with the monophyletic order Clevelandellida; (4) the moderately supported clade (e.g. *Metopus minor*, *Metopus hasei*, and *Metopus yantaiensis*), groups with the genera *Parametopidium* Aescht, 2001 and *Atopospira* (Kahl, 1927) Bourland and Wendell (2014).

Figure 1 *Metopus paravestitus* nov. spec. from life (A, D) and after protargol staining (B, C, E–G). (A) Ventral side of a representative specimen. (B, C) Ventral (B) and dorsal (C) views of the same specimen, showing ciliature and nuclear apparatus. (D) Intracytoplasmic structures (arrows) and epibiotic prokaryotes (arrowhead). (E) Different cell shape. (F) Structure of membranelles infraciliature from mid-portion of the adoral zone. (G) Anterior part of the cell, showing adoral zone of membranelles, perizonal ciliary stripe and paroral membrane (arrow). AZM = adoral zone of membranelles; CC = caudal cilia; CV = contractile vacuole; Ma = macronucleus; Mi = micronucleus; PD = preoral dome; PM = paroral membrane; PS = perizonal ciliary stripe. Scale bars: 30 μm (A–C), 10 μm (D), 50 μm (E).
Figure 2. *Metopus paravestitus* nov. spec. from life with bright-field (A–E), differential interference contrast (F–H) illuminations and after protargol staining (I, J). (A) Left view showing contractile vacuole. (B) Right view showing proximal margin of preoral dome (arrowhead). (C) Ventral view of anterior part of the cell, showing epibiotic prokaryotes (arrowheads) and needle-shaped intracytoplasmic structures (arrow). (D) Posterior part of the cell, showing leaving epibiotic prokaryotes (arrows) when the cell dies. (E–G) Ventral view of different cells showing shape variants, intracytoplasmic structures (arrows in F and G), epibiotic prokaryotes (arrowheads in F), contractile vacuole, and an elongated caudal cilium. (H) Dorsal view showing intracytoplasmic structures (arrow) and contractile vacuole (arrowhead). (I, J) Ventral (I) and dorsal (J) views of the same specimen, showing ciliature and macronucleus. AM = adoral membranelle; CV = contractile vacuole; Ma = macronucleus; PM = paroral membrane; PS = perizonal ciliary stripe. Scale bars: 40 µm (A, B, E–H), 10 µm (C, D), 30 µm (I, J).
Figure 3 *Metopus es* from life (A, E–G, J–M) and after protargol staining (B–D, H, I). (A) Ventral view of a representative specimen. (B, C) Ventral (B) and dorsal (C) views of the same specimen, showing ciliature and macronucleus. (D) Structure of membranelles infraciliature from the middle portion of the adoral zone. (E) Arrangement of extrusomes between adjacent kineties. (F) Macronucleus surrounded by innumerable granules (arrows). (G) Lateral view of ellipsoidal extrusomes. (H, I) Ventral (H) and dorsal (I) views of the same specimen, showing aggregate of dark spherical particles (arrowheads), perizonal ciliary stripe (arrow) and macronucleus (white asterisks). (J) Ventral view showing aggregate of dark spherical particles (double-arrowhead), posterior cortical fold (arrowheads), and proximal end of the adoral zone (arrow). (K) Right lateral view showing truncated distal end (arrow) and aggregate of dark spherical particles (double-arrowhead). (L) Ventral view showing cilia in dome kinety (arrow) and sausage-shaped macronucleus. (M) Macronucleus surrounded by innumerable irregular granules. AZM = adoral zone of membranelles; CV = contractile vacuole; Ma = macronucleus; PM = paroral membrane; PS = perizonal ciliary stripe. Scale bars: 40 µm (A–C), 3 µm (F), 60 µm (H–L).
DISCUSSION

Comparison of new species with related species

*M. paravestitus* nov. spec. belongs to the group of metopids which possess a five-rowed perizonal ciliary stripe and a leftward torsion of the anterior cell portion with a frontal lobe overhanging an obliquely situated adoral zone of membranelles without long setae arising over the posterior third of the body (Bourland et al. 2014, 2020; Esteban et al. 1995; Foissner 2016b; Jankowski 1964; Kahl 1927).

The new species can be distinguished by a combination of the following characteristics: (i) ectobiotic prokaryotes

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**Figure 4** Maximum-likelihood (ML) tree based on SSU rDNA sequence data. The newly sequenced *Metopus paravestitus* nov. spec. and *Metopus es* are indicated in bold. Support values at the nodes are for BI and ML, respectively. Disagreements in ML and BI tree topologies are indicated by “*” All branches are drawn to scale. The scale bar corresponds to 10 substitutions per 100 nucleotide position. GenBank accession numbers are given for each species.
arranged perpendicular to the body surface, (ii) filiform intracytoplasmic structures densely packed in the anterior portion of the cell, (iii) seawater habitat, (iv) an in vivo size of 90–120 × 25–60 μm, and (v) 27–36 adoral membranelles and 30–42 somatic kineties. In terms of its medium cell size, an elongated body shape as well as the seawater biotope, the new species should be compared with *M. vestitius* Kahl, 1932, *M. halophila* sensu Esteban et al. (1995) and *M. nivaensis* Esteban et al., 1995. Among these, *Metopus paravestitus* nov. spec. resembles *M. vestitius* in having ectobiotic prokaryotes covering the cell surface and filiform intracytoplasmic structures in the anterior portion of the cell. However, it differs in possessing a larger cell size (90–120 μm long vs. about 70 μm long), a higher numbers of adoral membranelles (27–36, on average 33 vs. 20), a higher number of somatic kineties (30–42, on average 36 vs. about 25), and the lack of a distinctive, long, spine-like posterior body extension (Esteban et al. 1995; Kahl 1932). Compared with the new species, *M. halophila* sensu Esteban et al. (1995) lacks the filiform intracytoplasmic structures, it has smaller ectosymbionts (<1 μm long; illustrated in the figure 38 in the reference by Esteban et al. (1995) vs. more than 2 μm), fewer somatic kineties (about 20 vs. 30–42), and less adoral membranelles (12–15 vs. 27–36) (Esteban et al. 1995). *Metopus nivaensis* can be separated from the new species by a wider preoral dome, ectosymbionts (absent vs. present), and more somatic kineties (50 vs. 30–42) (Esteban et al. 1995).

Other species of *Metopus* that could be compared in terms of habitat are *Metopus verrucosus* (da Cunha, 1915) Kahl, 1932 and *M. halophilus* Kahl, 1925 also found in marine environments (Kahl 1932; Kirby 1934). However, both species differ from *M. paravestitus* in having a fusiform (vs. elliptical) body shape. Additionally, the former has tufts of ectobiotic prokaryotes (vs. densely arranged), and the latter is smaller (60–90 μm long vs. 90–120 μm long).

*Metopus contortus* (Quennerstedt, 1867) Kahl, 1932 is also commonly found in anaerobic marine habitats and has as well an ellipsoidal body shape; however, it lacks the filiform intracytoplasmic structures and the characteristically arranged ectosymbiotic prokaryotes as described in *M. paravestitus*, and it thus cannot be confused with this new species (Dai et al. 2008; Esteban et al. 1995; Kahl 1932).

Finally, *Tropidoactractus spinosus* (Kahl, 1927) Rotterová et al. 2018 (basionym: *Metopus spinosus* Kahl, 1927, *M. caudatus* sensu Jankowski (1964)) and another marine form, *Metopus caudatus* da Cunha, 1915 can be easily separated from the new species by having a characteristic spine-like tail (da Cunha 1916; Rotterová et al. 2018); in addition, the former has fewer adoral membranelles (11–13 vs. 27–36) and also less somatic kineties (12 or 13 vs. 30–42).

**Remarks on Metopus es**

Since the original report by Müller (1776), *Metopus es* has been recorded many times from various biotopes all over the world seemingly indicating its global distribution (Bourland et al. 2017a; Claparède and Lachmann 1858; Jankowski 1964; Wang and Nie 1935). Recently, Bourland et al. (2017a) made an authoritative redescription based on several populations from geographically distinct areas, and compared this species with similar species in detail. The present population corresponds well with those isolates of the species in the following features: (i) a slightly sigmoidal body shape and cell size (108–161 μm vs. 48–146 μm); (ii) ratio of distance between anterior cell end and proximal end of adoral zone of membranelles to body length (34–52% vs. 32–60%), (iii) number of somatic kineties (18–31 vs. 24–32) and adoral membranelles (28–48 vs. 24–47), (iv) inconspicuous caudal cilia, (v) presence of cortical granules, and (vi) conspicuous dark particle aggregation at the anterior body protion. The Qingdao population of this species matches as well the Nanking population in (1) length: width ratio of cells, (2) elongated fusiform body shape, and (3) the body size (120–170 × 30–55 μm vs. 120–150 × 40–45 μm) but differs on the proportion of the preoral dome to total body length (40% vs. 25–30%) (Wang and Nie 1935), which could be population-dependent characteristic (Bourland et al. 2017a, b). Therefore, we consider that all these forms are conspecific.

**Phylogenetic analyses**

The SSU rDNA sequence-based phylogenetic analyses show the paraphyly of the family Metopidae. This is consistent with previous research (Bourland et al. 2017a; Omar et al. 2017; Silva-Neto et al. 2016). *Metopus* is a morphologically well-outlined genus; however, phylogenetically speaking, it seems not to be one “valid” genus as its members are non-monophyletic based on ribosomal gene markers. With new data added to the phylogenetic analyses, the close relationship between *Metopus es* (type of the genus) and *Brachonella contorta* (Bourland et al. 2017a, Bourland et al. 2018b; Rotterová et al. 2018) is challenged in the present work and two recent studies by Bourland et al. (2018a) and Vďačný et al. (2019). These results indicate that the interspecific relationship within the family and genus still remains unresolved. Multiple gene-based phylogenies and extensive taxa sampling will probably help to clarify this issue.

In the analysis, there is nearly no support of the relationship between *Metopus es* and the clad of *Metopus paravestitus* and *Metopus contortus*, but they still should be considered as members of the genus *Metopus* based on certain common morphological characteristic (e.g. anterior part of body uniquely twisted to left; holotrichous somatic ciliation; multiple oral polykinetids) and lack of the genus characters of *Brachonella* (a dominant preoral dome, extreme posteriorization of the cytostome and a highly sprialized adoral zone) and *Urostomides* (four-rowed perizonal ciliary stripes). Finally, the endosymbiotic order Cleve-landelida clearly forms a monophylum which always clusters deeply within the order Metopida with almost
maximum symbiotic support. This observation may help to infer how this symbiotic species evolved from free-living species, as it was hypothesized by Vďačný et al. (2019).

**TAXONOMIC SUMMARY**

Phylum Ciliophora Doflein, 1901  
Class Armophorea Lynn, 2004  
Order Metopida Jankowski, 1980  
Family Metopidae Kahl, 1927  
Genus Metopus Claparède and Lachmann, 1858

**Metopus paravestitus nov. spec**

**Diagnosis:** Body long elliptical in outline, in vivo about 90–120 × 25–60 μm. Cell surface covered by a coat of ectobiotic prokaryotes upright to cortex. Filiform intracytoplasmic structures mainly distributed in the anterior body portion. Macronucleus located in anterior body half, ellipsoidal to elongated reniform; micronucleus ellipsoidal. Contractile vacuole terminally located. On average, 36 somatic kineties; caudal cilia about 20–40 μm long. Perizonal stripe commonly composed of five rows. Adoral zone extends about 50% of body length, composed of an average of 33 membraneles. Marine habitat.

**Type locality:** Sandy sediment from a marine farm near Shenzhen, China (22°38′17″N, 114°05′52″E).

**Type materials:** One slide with protargol-stained specimens including the holotype (registration numbers: LS2016030101) and other slide with paratypes (registration number: LS2016030102) were deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China. The holotype (Fig. 2I, J) and relevant paratypes were marked by black ink circles on back side of the slides.

**Etymology:** The species-group name *paravestitus* is a composite of the Greek prefix *para-* (close to; similar to; resembling) and the species-group name *vestitus* (Latin word meaning a cover or covering), indicating the new species resembles or is similar to but different from the species *Metopus vestitus*.

**ZooBank LSID:** urn:lsid:zoobank.org:act:2C059056–3E1D–4A00–9D39–751DB4633EA6

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**AUTHORS’ CONTRIBUTIONS**

XH conceived and guided the study. SL and WZ conducted sampling and performed laboratory work. XH and SL identified the species. QZ did the phylogenetic analyses and the results interpretation. SL drafted the manuscript, and WZ, QZ, BP-U and XH made further revisions. BP-U also polished English. All authors read and approved the final version of manuscript.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Sample location.

**Table S1.** The GenBank accession numbers not shown in the phylogenetic tree.