The Journal of Eukaryotic Microbiology

ISOP Published by the International Society of Protistologists

Journal of Eukaryotic Microbiology ISSN 1066-5234

ORIGINAL ARTICLE

Pseudonotohymena antarctica n. g., n. sp. (Ciliophora, Hypotricha), a New Species from Antarctic Soil

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Keywords
18S phylogeny; cell division; ciliary pattern; Dorsomarginalia; morphology; taxonomy.

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Received: 17 December 2015; revised 20 October 2016; accepted October 25, 2016. Early View publication November 21, 2016

doi:10.1111/jeu.12381

ABSTRACT

A new soil ciliate, *Pseudonotohymena antarctica* n. g., n. sp., from King George Island, Antarctica, is described based on live observation, protargol impregnation, and its 18S rRNA gene. The new genus *Pseudonotohymena* is morphologically similar to the genus *Notohymena* Blatterer and Foissner 1988 in the following characteristics: 18 fronto-ventral-transverse cirri, a flexible body, undulating membranes, dorsomarginal kineties, and the number of cirri in the marginal rows. However, *Pseudonotohymena* differs from *Notohymena* particularly in the dorsal ciliature, that is, in possessing a nonfragmented dorsal kinety (vs. fragmented). In addition, the molecular phylogenetic relationship of the new species differs from that of *Notohymena* species. On the basis of the morphological features, the genetic data, and morphogenesis, we establish *P. antarctica* n. g., n. sp. In addition, the cyst morphology of this species is described.

BERGER (2008, 2011) noted that the 18-cirri pattern is not an apomorphy of the oxytrichids, but represents a plesiomorphy within the Hypotricha. Therefore, Berger (2008) reported four partly paraphyletic groups with this pattern reflecting the uncertainty in the classification of Hypotricha: Stylonychinae, Nonstylonychine Oxytrichidae, Nonoxytrichid Dorsomarginalia, and Nondorsomarginalian Hypotricha.

Berger (2006) established the Dorsomarginalia to include the hypotrichs with dorsomarginal kineties. Fragmentation of at least one kinety is characteristic of oxytrichids and a few other taxa (Berger 2006, 2008, 2011; Singh and Kamra 2013).

Urosomoida, *Urosoma*, and some other 18-cirri hypotrichs previously classified in the oxytrichids (Berger 1999) are assigned to the nonoxytrichid Dorsomarginalia because they lack the fragmentation of dorsal kinety 3 (Berger 2008, 2011; Kumar et al. 2014; Singh and Kamra 2013). Likewise, *Uroleptus* is a nonoxytrichid Dorsomarginalia genus based on morphogenetic data (Berger 2008, 2011), but does not group together with the previous taxa in the 18S phylogenies, suggesting a nonmonophyly of the nonoxytrichid Dorsomarginalia.

In this study, we report a new soil ciliate, *Pseudonotohymena antarctica* n. g., n. sp., collected from King George Island, Antarctica. The species is described based on live observations, protargol-impregnated specimens, and its 18S rRNA gene sequence.

MATERIALS AND METHODS

Sample collection and identification

Pseudonotohymena antarctica n. g., n. sp. was isolated from a soil sample collected near the King Sejong Station in the southwestern part of King George Island, Antarctica (62°14′28.56″S, 58°44′52.88″W), in January 2013. The sample was dried at 60 °C for 24 h and then stored at 4 °C until further processing. The ciliates were reactivated, using the nonflooded Petri dish method (Foissner et al. 2002), and incubated at 4 °C. We initially attempted to set up cultures, using single cells, but failed. Thus, all data are based on specimens from a raw culture.

Living specimens were examined with an inverted microscope (Zeiss Axio Vert.A1; Carl Zeiss, Oberkochen, Germany) at 50X–400X magnification and with a light

microscope (Zeiss Axio Imager 2; Carl Zeiss) at 50X–1,000X magnification, using both bright field and differential interference contrast optics. Protargol impregnation following "Procedure A" of Foissner was performed to reveal the infraciliature (Foissner 1991). The sizes of the stained specimens were calculated, using an image analyzer (AxioVision SE64 Rel. 4.9.1; Zeiss Co.). Drawings of live specimens were based on free-hand sketches, whereas those from protargol-impregnated specimens were made with a drawing device, using Illustrator. In the diagrams of the morphogenetic stages, parental structures are shown as contours and newly formed structures are colored black.

Terminology and classification generally follow Berger (1999, 2008, 2011).

Polymerase chain reaction amplification and sequencing

Single specimens were individually washed with distilled water, and genomic DNA was extracted, using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO). The optimized conditions for PCR were as follows: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 4 min, and a final extension at 72 °C for 7 min. The New EukA forward primer (5′-CTG GTT GAT YCT GCC AGT-3′), modified from Medlin et al. (1988), and the LSU rev2 reverse primer (5′-ACG ATC GAT TTG CAC GTC AG-3′) (Sonnenberg et al. 2007) were used to amplify almost the entire 18S rRNA gene. The PCR products were purified, using the QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany). Two internal primers (18S+810 [5′-GCC GGA ATA CAT TAG CAT GG-3′] and 18S-300 [5′-CAT GGT AGT CCA ATA CAC TAC-3′]) were used for sequencing (Jung et al. 2011). DNA sequencing was performed on the ABI 3700 platform (Applied Biosystems, Foster city, CA).

Phylogenetic analyses

Using Clustal X version 1.81 software (Jeanmougin et al. 1998), sequences from *P. antarctica* n. g., n. sp. and related species (for which data were retrieved from

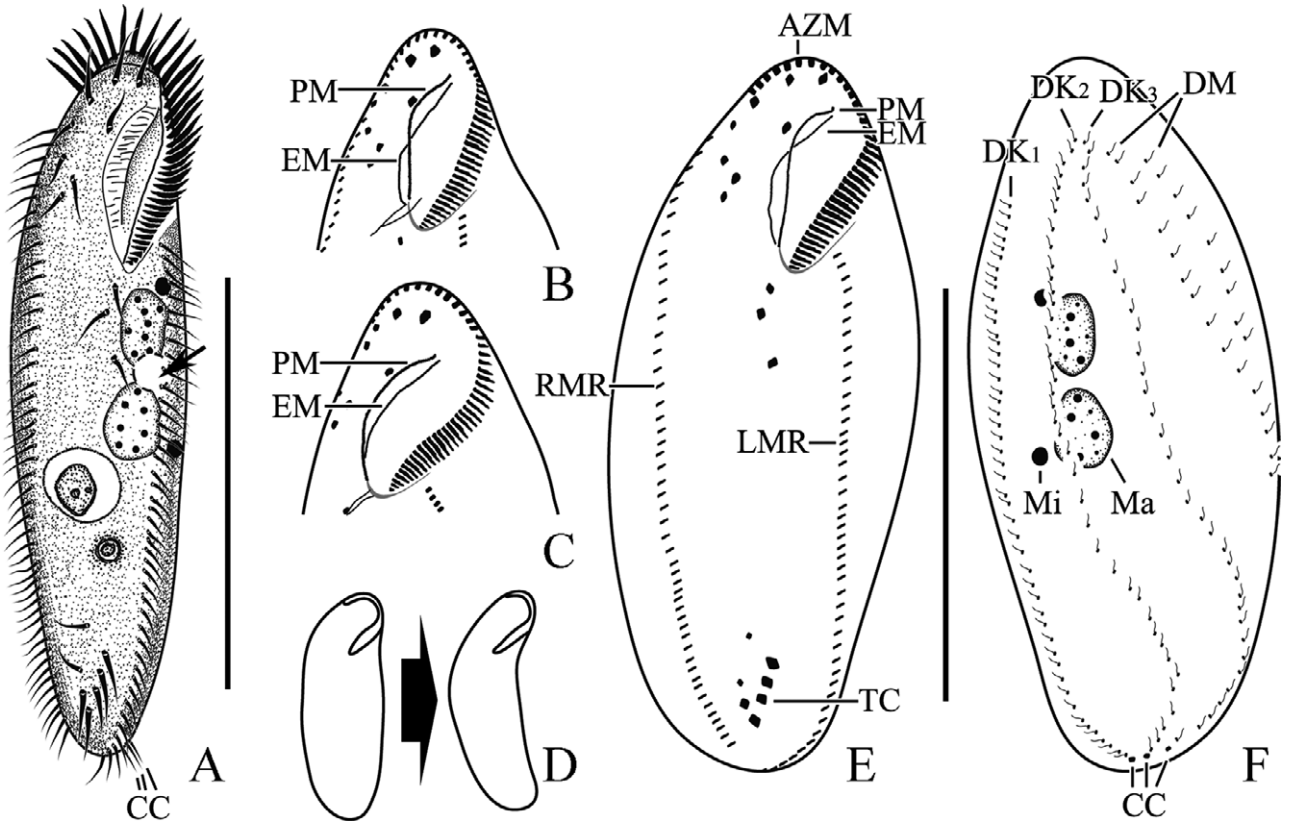

Figure 1 Morphology of *Pseudonotohymena antarctica* n. g., n. sp. alive (A, D) and after protargol staining (B, C, E, F). **A.** Ventral view of a representative individual; the arrow indicates the contractile vacuole. **B, C.** Anterior ventral views showing the pattern of the endoral and paroral membranes. **D.** Variation in body shape during locomotion. **E, F.** Ventral and dorsal views of holotype specimen showing the ciliary pattern and nuclear apparatus. AZM, adoral zone of membranelles; CC, caudal cirri; DK1-3, dorsal kineties1-3; DM, dorsomarginal kineties; EM, endoral membrane; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; RMR, right marginal row; TC, transverse cirri. Scale bars 100 μm.

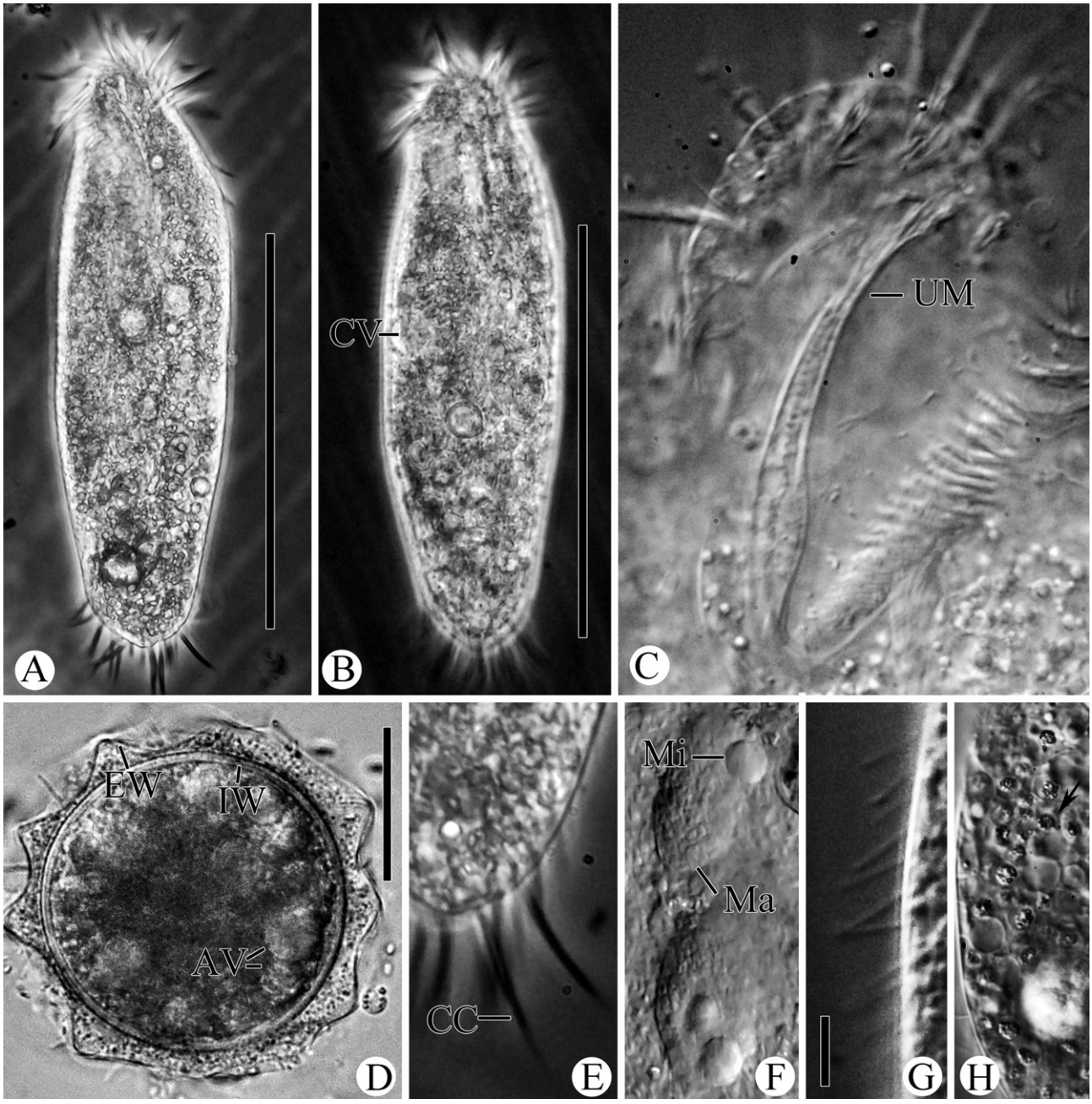

Figure 2 Photomicrographs of *Pseudonotohymena antarctica* n. g., n. sp. from live specimens using bright field and differential interference contrast optic methods. **A, B.** Dorsal views of typical specimens. **C.** Anterior ventral cell portion showing the undulating membranes. **D.** Optical section of resting cyst. **E.** Caudal cirri. **F.** Nuclear apparatus. **G.** The dorsal cilia at the left body margin. **H.** Crystal-like structures (arrow). AV, autophagous vacuoles; CC, caudal cirri; CV, contractile vacuole; EW, external cyst wall; IW, internal cyst wall; Ma, macronuclear nodules; Mi, micronuclei; UM, undulating membranes. Scale bars 100 μm (A, B), 30 μm (D), and 5 μm (G).

GenBank) were aligned and manually trimmed at both ends, using BioEdit version 7.1.11 (Hall 1999). The alignment was subsequently refined manually. Ambiguously aligned regions were excluded, using Gblocks version 0.91b (Talavera and Castresana 2007). The program jModelTest version 2.1.1 (Darriba et al. 2012) suggested the GTR + I (0.7100) + G (0.5840) model, based on the Akaike information criterion. MEGA version 5.2.2 (Tamura et al. 2011) was used to change the data format from FASTA to NEXUS or PHYLIP, permitting phylogenetic analysis, employing MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) and PhyML version 3.1 (Guindon et al. 2010), respectively. To perform Bayesian inference (BI) analysis, using MrBayes 3.1.2, a Markov chain Monte Carlo

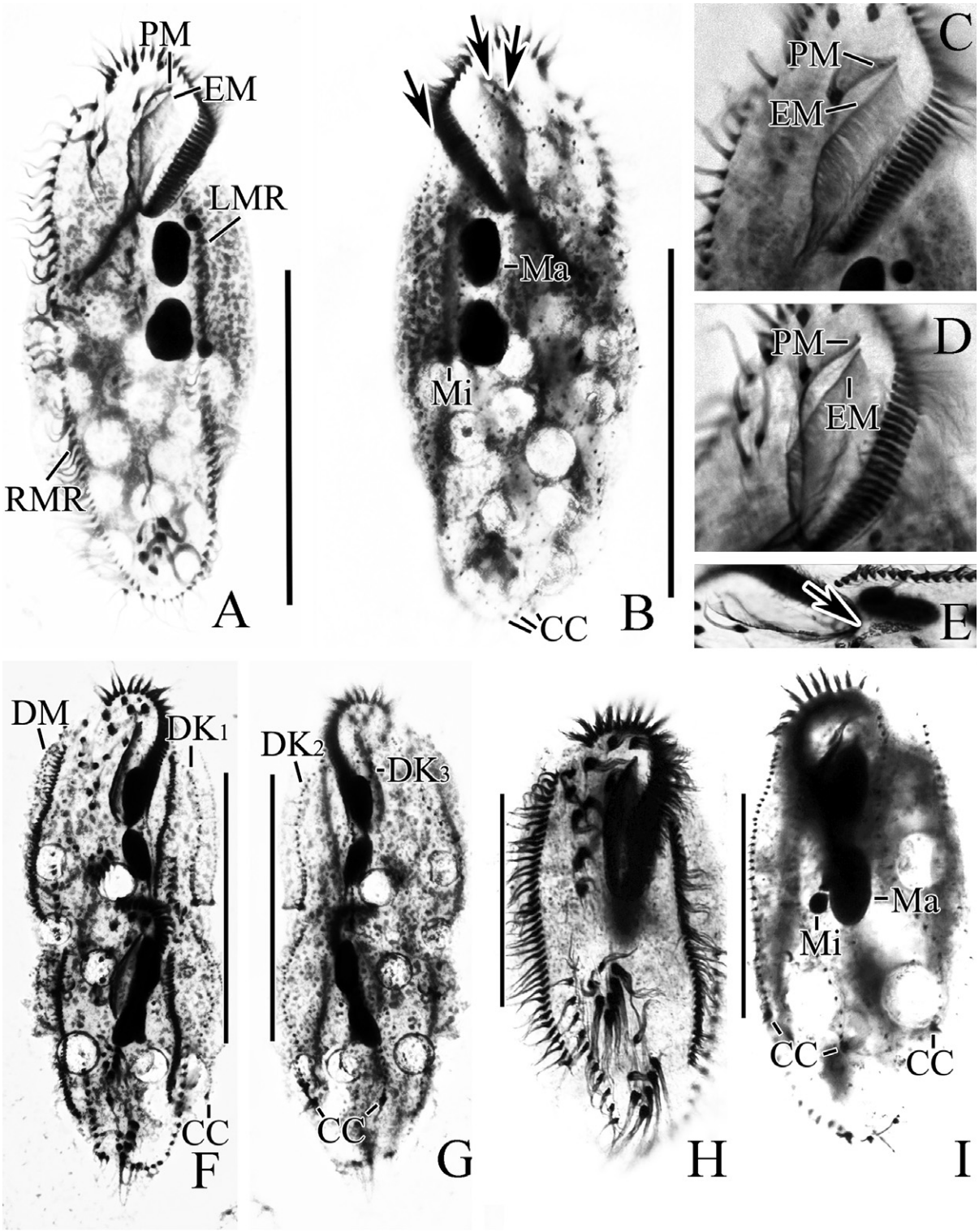

Figure 3 Photomicrographs of *Pseudonotohymena antarctica* n. g., n. sp. after protargol impregnation. **A, B.** Ventral and dorsal views of the holotype specimen. Arrows denote the dorsal kineties 1-3. **C, D.** Anterior ventral cell portions showing the undulating membranes in detail (see also Fig. 4). **E.** Ventral view of an early divider. Arrow marks the just originating oral primordium. **F, G.** Ventral and dorsal views of a late divider, showing the dividing macronucleus. **H, I.** Ventral and dorsal views of a posterior daughter cell. CC, caudal cirri; DK1-3, dorsal kineties1-3; DM, dorsomarginal kineties; EM, endoral membrane; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane; RMR, right marginal row. Scale bars 100 μm (A, B, F, G), and 50 μm (H, I).

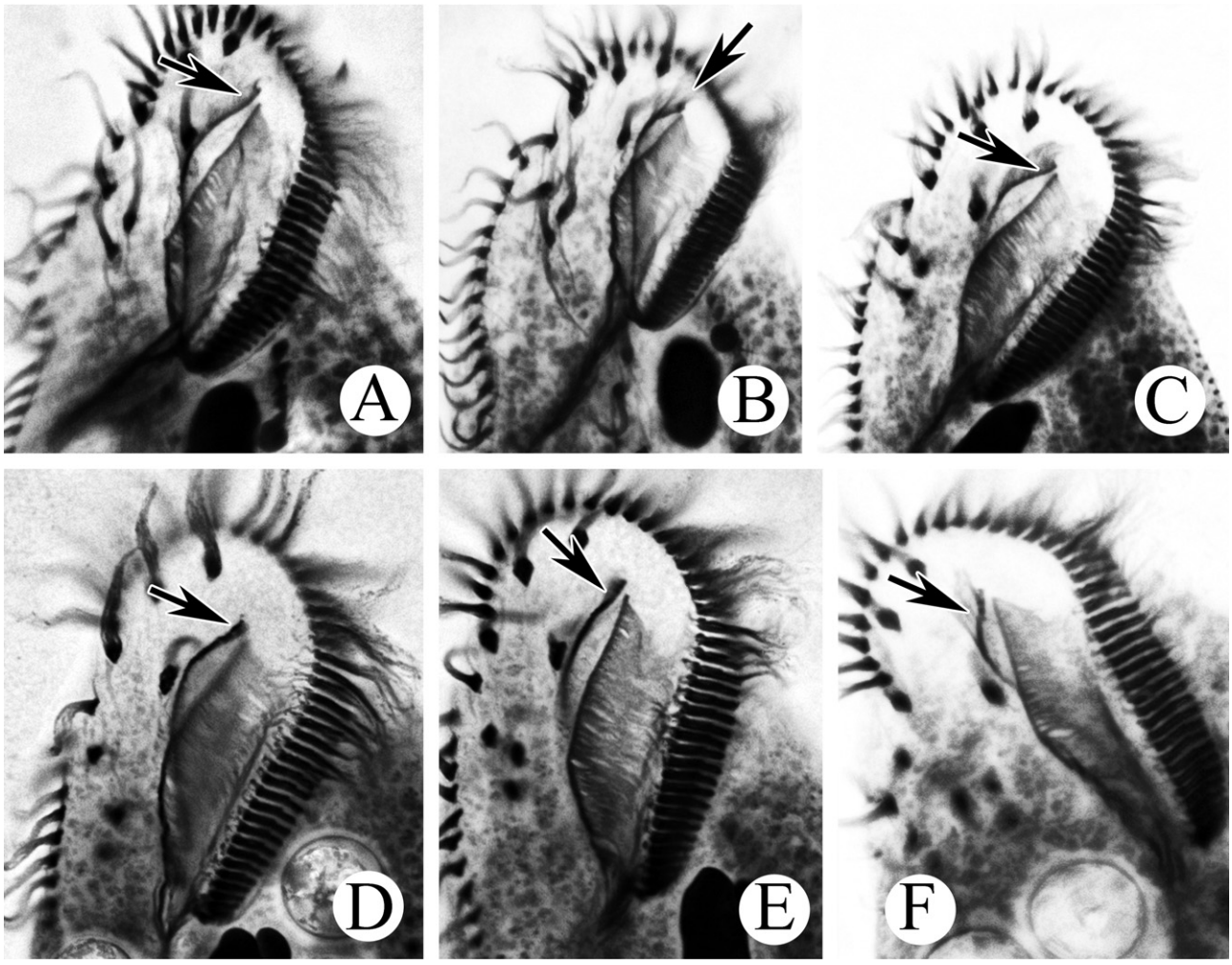

Figure 4 Photomicrographs of *Pseudonotohymena antarctica* n. g., n. sp. after protargol impregnation showing the anterior ventral cell portions with the endoral and paroral membranes.

algorithm (1,000,000 generations) was run. Trees were sampled at every 100 generations, and the first 300,000 generations served as the burn-in. Maximum likelihood (ML) trees were drawn, using the bootstrap procedure with 1,000 replications. Gene trees were drawn based on this 1,277 bp sequence. We retrieved 18S rRNA gene sequences from 70 species and six outgroup species (76 species in total) from the GenBank database (Table S1).

RESULTS

Description of *Pseudonotohymena antarctica* n. g., n. sp.

Description of morphostatic specimens

Cell dimensions 150–215 × 36–80 μm in vivo, usually approximately 155 × 60 μm in protargol preparations (Table 1), in which specimens are slightly inflated, that is, body width ranges from 41 to 89 μm. Length:width ratio in vivo approximately 2–3:1 in ventral and dorsal view, dorsoventrally flattened. Cell transparent and grayish at low magnification, flexible, ellipsoidal with rounded ends, posterior end broader than anterior one (Figs 1A, D–F, 2A, B, 3A, B; Table 1). Two macronuclear nodules, ellipsoidal, 17–28 × 7–14 μm in size in stained cells, in left cell half. Two micronuclei, spherical, 4–6 × 4–5 μm in protargol preparations, adjacent to macronuclear nodules (Fig. 1F, 2F, 3A, B; Table1). Contractile vacuole approximately 14 μm in diameter when fully extended, located in the middle of left cell half (Fig. 1A, 2B); collecting canals not recognizable. Crystal-like structures, reddish, approximately 2–3 μm in length, located principally in periphery of left and right cell sides (Fig. 2H). Cortical granules not recognizable. The ciliate feeds on greenish algae 8–10 μm in diameter and crawls on the bottom of the Petri dish by means of its cirri.

Frontal and transverse cirri approximately 20 μm long in vivo; remaining cirri about 15 μm long. Eighteen frontoventral-transverse (FVT) cirri: three frontal, one buccal, and

Table 1. Morphometric data on *Pseudonotohymena antarctica* n. g., n. sp.

Characteristics[a]	Mean	*M*	SD	CV	Min	Max	*n*
Body, length	156.1	155	15.2	9.8	137.5	187.5	19
Body, width	61.7	60	14.8	23.9	41	89	19
Anterior cell end to posterior end of adoral zone of membranelles, distance	50.7	51	2.7	5.3	47	55	19
Percentage of body length occupied by AZM	32.7	32.9	2.5	7.6	28.9	37.9	19
Adoral membranelles, number	41.2	41	1.8	4.5	37	44	19
Macronuclear nodules, number	2.0	2	0.0	0.0	2	2	19
Macronuclear nodule, length	20.8	19	3.4	16.2	17	28	19
Macronuclear nodule, width	9.4	9	1.5	16.3	7	14	19
Macronucleus nodules, distance in between	6.9	6	2.7	39.6	3.3	11.5	19
Micronuclei, number	2.0	2	0.0	0.0	2	2	19
Micronuclei, length	4.9	5	0.6	12.7	4	6	19
Micronuclei, width	4.2	4	0.4	9.1	4	5	19
Frontal cirri, number	3.0	3	0.0	0.0	3	3	19
Buccal cirrus, number	1.0	1	0.0	0.0	1	1	19
Frontoventral cirri, number	4.1	4	0.2	5.7	4	5	19
Postoral ventral cirri, number	3.0	3	0.0	0.0	3	3	19
Pretransverse ventral cirri, number	2.0	2	0.0	0.0	2	2	19
Transverse cirri, number	5.0	5	0.0	0.0	5	5	19
Right marginal cirri, number	43.0	43	2.6	6.0	40	49	19
Left marginal cirri, number	42.7	43	2.3	5.4	37	46	19
Caudal cirri, number	3.0	3	0.0	0.0	3	3	19
Dorsal kineties, number	3.0	3	0.0	0.0	3	3	19
Dorsal kinety 1, number of bristles	46.0	46	3.6	7.8	41	53	19
Dorsal kinety 2, number of bristles	38.2	39	2.8	7.2	32	42	19
Dorsal kinety 3, number of bristles	34.3	35	3.2	9.4	26	38	19
Dorsomarginal rows, number	2.3	2	–	–	2	3	19
Dorsomarginal row 1, number of bristles	18.7	19	2.2	11.6	16	24	19
Dorsomarginal row 2, number of bristles	7.9	8	2.1	26.0	4	12	19
Dorsomarginal row 3, number of bristles	2.0	1	–	–	1	4	5
Posterior body end to rearmost transverse cirrus, distance	6.2	6	1.3	21.1	4.5	9	19

CV, coefficient of variation (%); M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation.

[a]All data based protargol-impregnated (Foissner 1991, Procedure A), randomly selected specimens. Measurements in (im. AZM, adoral zone of membranelles. [Table 1 was added on January 21, 2017 after original online publication.]

four (rarely five) frontoventral cirri; three postoral cirri near proximal end of adoral zone of membranelles; two pretransverse ventral and five transverse cirri. Pretransverse ventral cirri distinctly smaller than frontal and transverse cirri. One left marginal row with 37–46 cirri, commences near buccal vertex and extends along posterior cell margin; one right marginal row with 40–49 cirri, begins at level of buccal cirrus, extends along right cell margin, ending subterminally somewhat right of midline; posterior ends of marginal cirral rows thus clearly separated (Fig. 1A, E, 3A; Table 1).

Dorsal cilia approximately 5 μm long in vivo (Fig. 2G), arranged in three bipolar dorsal kineties, each with a caudal cirrus, and two posteriorly shortened dorsomarginal kineties extending slightly obliquely, terminating near right marginal row. Dorsomarginal kineties 1 and 2 composed of approximately 19 and 8 bristles, respectively; about one forth (five of 19) of specimens with a third dorsomarginal row composed of one cirrus on average.

Adoral zone composed of 37–44 membranelles, question mark-shaped, occupying approximately anterior third of ventral side in stained specimens. Oral apparatus with *Notohymena*-pattern (Berger 1999), that is, paroral membrane distinctly curved with hooked distal end and buccal cavity deep and wide. Paroral membrane commences about 15 μm posteriorly to anterior body end. Endoral membrane commences left of anterior end of paroral membrane and extends to buccal lip. Pharyngeal fibers about 22 μm in length after protargol impregnation (Fig. 1B, C, 3A, C, 4A–F; Table 1).

Resting cysts

Mature cysts approximately 53 μm in diameter, globular. Inner layer (endocyst) approximately 2 μm thick; outer layer (ectocyst) irregularly wrinkled (approximately 5–9 μm thick). Cytoplasm grayish, contains lipid droplets and autophagous vacuoles (Fig. 2D).

Morphogenesis

Several early and late dividers as well as postdividers were found. Early in division, an anarchic field of basal bodies develops left of the postoral ventral cirri (Fig. 3E, 5A). The anlage of dorsal kinety 3 is not fragmented neither in the available late dividers nor the postdividers.

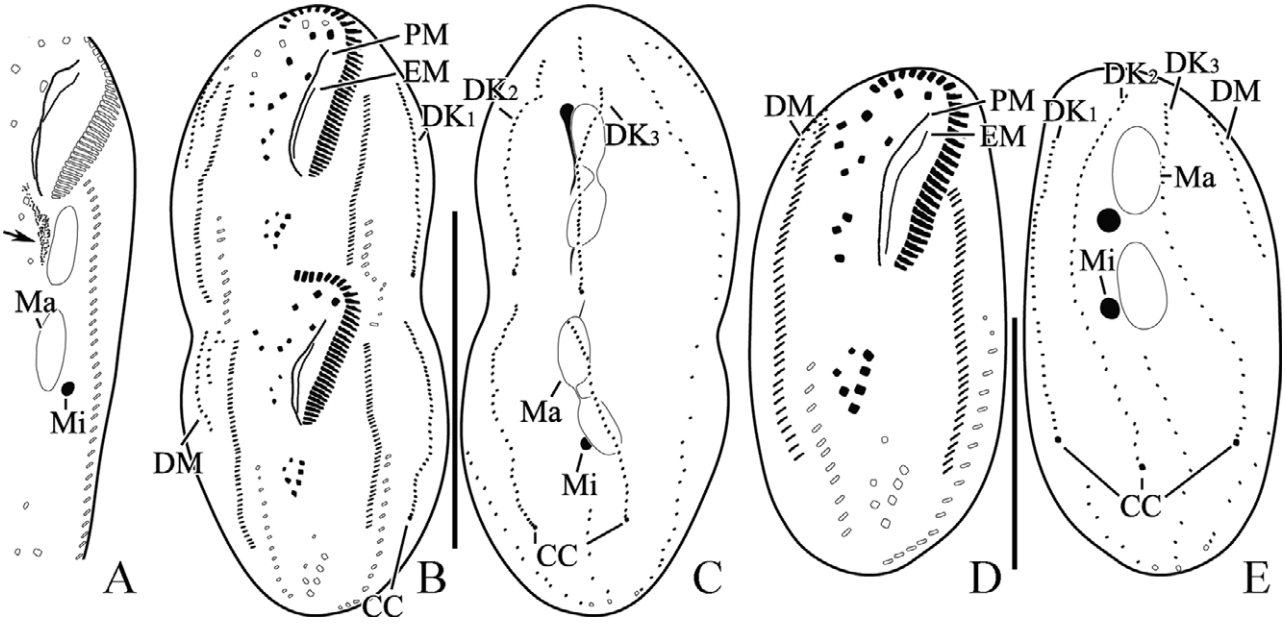

Figure 5 Dividers of *Pseudonotohymena antarctica* n. g., n. sp. after protargol impregnation. **A.** Left cell half of an early divider showing the oral primordium (arrow). **B, C.** Ventral and dorsal views of a late divider showing the migrating cirri and the nonfragmented dorsal kinety 3. **D, E.** Ventral and dorsal views of a posterior daughter cell. CC, caudal cirri; DK1-3, dorsal kineties1-3; DM, dorsomarginal kineties; EM, endoral membrane; Ma, macronuclear nodules; Mi, micronuclei; PM, paroral membrane. Scale bars 100 μm (B, C), and 50 μm (D, E).

The dorsal kineties are sigmoidal in postdividers, with one caudal cirrus each at the posterior ends of kineties 1–3 (Fig. 3G, I, 5C, E). In late dividers, the mass of fused macronuclear nodules starts to divide and two division products of the micronuclei are present (Fig. 3G, 5C). The dorsomarginal kineties very likely originate close to the anlage of the right marginal cirri (Fig. 3F, H, 5B, D). Two or three dorsomarginal kineties are evident at these stages. Parental cirri, dorsal kineties, and dorsomarginal kineties, which are not involved in the formation of the primordia are very likely resorbed. However, it remains unclear whether the parental adoral membranelles are retained or completely replaced by new membranelles.

Molecular analyses

The gene sequences from the single specimens analyzed were completely identical without any ambiguous bases. The 18S rRNA gene sequence is 1,800 bp in length and has a GC content of 45.8% (GenBank accession number KU821589).

Pseudonotohymena antarctica n. g., n. sp. is distinctly separated from *Notohymena apoaustralis*, although *Pseudonotohymena* possesses the *Notohymena*-pattern of the undulating membranes pattern. In the phylogenetic trees, *P. antarctica* n. g., n. sp. instead shows a relationship with the nonoxytrichid Dorsomarginalia (*Urosoma*, *Hemiurosoma*, *Heterourosomoida*), which is supported by the *Urosomoida*-pattern (nonfragmented dorsal kinety 3), except for *Urosomoida agilis*, the type species of the genus *Urosomoida*, does not cluster with the other nonoxytrichid Dorsomarginalia (Fig. 6).

DISCUSSION

Comparison of *Pseudonotohymena* n. g. with morphologically related genera

Pseudonotohymena antarctica n. g., n. sp. shows similarity in ciliature with *Notohymena* Blatterer and Foissner 1988; *Quadristicha* Foissner 2016; *Hemioxytricha* Foissner 2016; *Aponotohymena* Foissner 2016; *Urosomoida* Hemberger in Foissner 1982; *Heterourosomoida* Singh and Kamra 2015; *Hemiurosomoida* Singh and Kamra 2015; and *Rubrioxytricha* Berger 1999.

Pseudonotohymena is highly similar to *Notohymena* in the anteriorly hooked paroral membrane, the 18 frontoventral transverse cirri, and the presence of caudal cirri. However, both differ mainly by the fragmentation of dorsal kinety 3 (absent vs. present) (Blatterer and Foissner 1988).

Urosomoida can be separated from *Pseudonotohymena* by the presence of the *Oxytricha*-pattern of the paroral membrane (vs. *Notohymena*-pattern with hooked distal end curving both anteriorly and ventrally) and a reduced number of FVT cirri (especially, fewer pretransverse ventral and/or transverse cirri) (Hemberger in Foissner 1982).

Hemiurosomoida can be distinguished from *Pseudonotohymena* by the pattern of the undulating membranes (*Oxytricha*- vs. *Notohymena*-pattern) and the number of transverse (4 vs. 5) and caudal cirri (2 vs. 3) (Singh and Kamra 2015).

Heterourosomoida differs from *Pseudonotohymena* in the pattern of the undulating membranes (*Oxytricha*- vs. *Notohymena*-pattern) and the variability in the number of

Figure 6 Phylogenetic tree inferred from the 18S rRNA gene sequences showing the position of *Pseudonotohymena antarctica* n. g., n. sp. based on the Bayesian inference (BI) and maximum likelihood (ML) methods. GTR + I (0.7100) + G (0.5840) was selected as the best model from jModelTest version 2.1.1. Support values at the nodes represent the posterior probability of the BI model and the bootstrap values of maximum likelihood analysis. Asterisks denote bootstrap values < 50% or different topologies in BI and ML phylogenies.

frontoventral cirri (3–5 vs. usually 4) and transverse cirri (5 or 6 vs. 5) (Singh and Kamra 2015).

Aponotohymena is distinguished by a fragmented dorsal kinety 3 during cell division (vs. nonfragmented in *Pseudonotohymena*) and more than three caudal cirri (vs. invariably 3 in *Pseudonotohymena*) (Foissner 2016).

Quadristicha has two macronuclear nodules with one micronucleus in between them (vs. two adjacent to macronuclear nodules) and undulating membranes arranged in the *Oxytricha*-pattern (vs. *Notohymena*-pattern) (Foissner 2016).

Hemioxytricha is distinguishable from *Pseudonotohymena* by the pattern of the undulating membranes (*Oxytricha*- vs. *Notohymena*-pattern) and the number of caudal cirri (more than 3 vs. invariably 3) (Foissner 2016).

Rubrioxytricha differs from *Pseudonotohymena* in a reddish (vs. colorless) cytoplasm and one or two (vs. three) caudal cirri (Berger 1999). So, the Antarctic specimens do not match any known genus.

Morphogenesis

Since middle dividers were not available, we could not determine whether apokinetally formed clusters of kinetosomes merge with the oral primordium in a region between the transverse cirri and the postoral ventral cirri (IV/2, V/3, and V/4) or the origin of primordia I–VI of proter and opisthe between the undulating membranes and left transverse cirri.

The morphogenesis of *Pseudonotohymena* is similar to that of *Notohymena rubescens*, the type species of *Notohymena*, with the exception that the dorsal kineties do not fragment in the former. Furthermore, the position of the early oral primordium differs, that is, the oral primordium of *Notohymena rubescens* originates close to the uppermost transverse cirri (vs. near the postoral ventral cirri in *Pseudonotohymena*) (Voss 1991).

Phylogenetic analyses

The genus *Notohymena* contains six species, namely, *N. antarctica*, *N. pampasica*, *N. rubescens*, *N. saprai*, *N. selvatica*, and *N. apoaustralis* (Blatterer and Foissner 1988; Foissner 1996; Hemberger 1985; Kamra and Kumar 2010; Küppers et al. 2007; Lv et al. 2013), but only the latter had been sequenced. Despite a similar pattern of the paroral membrane, the species is not closely related to *Pseudonotohymena*, but to *Cyrtohymena*, *Paraurostyla*, *Pseudocyrtohymena*, *Ponturostyla*, and *Rubrioxytricha*, indicating that the pattern of the undulating membranes is a homoplasy.

Instead, *Pseudonotohymena antarctica* n. g., n. sp. clusters with the nonoxytrichid Dorsomarginalia. According to Berger (2008), nonoxytrichid Dorsomarginalia consist of, inter alia, *Hemiurosoma*, *Nudiamphisiella*, *Parakahliella*, *Uroleptus*, *Urosoma*, *Urosomoida*, and *Vermioxytricha*. In the phylogenetic tree, the genera *Uroleptus* and *Urosomoida*, however, do not group with the remaining nonoxytrichid Dorsomarginalia (Fig. 6). Accordingly, the nonoxytrichid Dorsomarginalia seem to be nonmonophyletic and the nonfragmented dorsal kinety a homoplasy.

Urosomoida-pattern of dorsal morphogenesis in the nonoxytrichid Dorsomarginalia

In oxytrichids, morphogenesis is an important feature for classification. Although possessing 18 fronto-ventral-transverse cirri, hypotrichs that lack a fragmentation of dorsal kineties, namely, the genera *Gonostomum*, *Urosoma*, *Urosomoida*, and some *Oxytricha* species, are very likely misplaced in the oxytrichids as indicated by fragmentary molecular analyses (Berger 2008, 2011; Paiva et al. 2009; Schmidt et al. 2007). Such data suggest that the dorsal morphogenesis and the ventral cirral pattern are equally important for inferring relationships among hypotrichs (Berger 2008).

To date, six patterns of dorsal morphogenesis have been described in oxytrichids (Berger 1999; Shao et al. 2015): the *Oxytricha*-, *Urosomoida*-, *Gonostomum*-, *Tachysoma*-, *Coniculostomum*-, and *Hemigastrostyla*- patterns. The new species described here exhibits the *Urosomoida*-pattern.

Recently, Foissner (2016) established the family Urosomoididae for taxa with less than 18 fronto-ventral-transverse cirri, a flexible cell, one or two dorsomarginal kineties, and a nonfragmented dorsal kinety 3, namely for

the genera *Urosomoida*, *Lepidothrix*, *Oxytrichella*, *Hemiurosomoida*, *Paraurosomoida*, *Erimophrya*, *Hemioxytricha*, and *Quadristicha* (Foissner 2016; Foissner et al. 2002; Hemberger 1985 in Foissner 1982; Singh and Kamra 2013, 2015). Owing to its 18 fronto-ventral-transverse cirri, the new genus described here does not belong to this family.

Actually, the genus *Pseudonotohymena* belongs to the nonoxytrichid Dorsomarginalia, which is nonmonophyletic as indicated by molecular phylogenies. For instance, the genus *Uroleptus*, which lacks a fragmentation of dorsal kineties and thus belongs to the nonoxytrichid Dorsomarginalia, clusters with the Oxytrichidae. Consequently, the lack of fragmentation of the dorsal kineties requires a reassessment in phylogenetic analyses as this characters apparently represents a homoplasy.

TAXONOMIC SUMMARY

Pseudonotohymena n. g

Diagnosis. Nonoxytrichid Dorsomarginalia with *Notohymena*-like pattern of undulating membranes; 18 FVT cirri; flexible body with colorless cytoplasm; caudal cirri present; dorsal kinety 3 nonfragmented during cell division (*Urosomoida*-pattern).

Type species by monotypy. *Pseudonotohymena antarctica* n. sp.

Etymology. The name *Pseudonotohymena* is a composite of the Greek prefix *pseudo-* false and genus group name *Notohymena* Blatterer and Foissner 1988 indicating a similarity with *Notohymena.*

Zoobank registration. urn:lsid:zoobank.org:act:ADD9983A-BCE5-412C-9942-E4935D245BE1.

Pseudonotohymena antarctica n. sp

Diagnosis. Size on average 183 × 58 μm in vivo and 155 × 60 μm after in protargol-impregnation. Body ellipsoidal in ventral and dorsal view, with both ends rounded. Two macronuclear nodules and two micronuclei in left cell half. Cortical granules absent. Adoral zone with on average 41 membranelles. Right and left marginal rows with on average 43 cirri each. Three bipolar dorsal kineties, two (rarely three) dorsomarginal kineties. Three caudal cirri.

Type locality. Soil with moss from King George Island, Antarctica, 62°14′28″S, 58°44′52″W.

Type material. The holotype slide (ACNS000065) and three paratype slides (ACNS000066, ACNS000068, ACNS000071) with protargol-impregnated specimens (including some dividers) have been deposited in the Korea Polar Research Institute, Incheon, South Korea. One paratype slide is deposited in the Natural History Museum, London, UK, with the registration number NHMUK 2016.10.6.1. Each specimen is marked with circles or bars on the bottoms of the slides.

Etymology. Named after the region where the species was discovered: Antarctica.

Gene sequence. The 18S rRNA gene sequence of *Pseudonotohymena antarctica* n. g., n. sp. has been deposited in GenBank under the accession number KU821589.

Occurrence and ecology. As yet, found only at the type location. The soil parameters were the following: soil pH 6.7; temperature for maintaining cell culture, 4 °C. Soil texture: loamy sand.

Zoobank registration. urn:lsid:zoobank.org:act:B4F18C3B-AC7E-44C5-8D6F-DA97213AD67E.

ACKNOWLEDGMENTS

The authors wish to thank E. Potvin for assistance. This study was supported by the Korea Polar Research Institute (research grant PE16020 and PM15050). The authors also want to thank two anonymous reviewers and the associate editor for useful comments which improved the paper significantly.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. List of specimens, together with information regarding lengths and accession numbers of their SSU rRNA gene sequences.