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ORIGINAL ARTICLE

Morphological and Ribosomal DNA-based Characterization of Six Antarctic Ciliate Morphospecies from the Amundsen Sea with Phylogenetic Analyses

Sun Young Kim[a,b], Joong Ki Choi[a], John R. Dolan[c], Hyoung Chul Shin[b], SangHoon Lee[b] & Eun Jin Yang[b]

a Department of Oceanography, Inha University, Incheon, 402-751, Korea
b Korea Polar Research Institute, KIOST, 213-3 Sondo-dong, Yeonsu-gu, Incheon, 405-840, Korea
c UMR 7093, Laboratoire d'Océanographie de Villefranche, Marine Microbial Ecology, Université Pierre et Marie Curie and Centre National de la Recherche Scientifique (CNRS), Station Zoologique, B.P. 28, 06230, Villefranche-sur-Mer, France

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18S; 28S; Antarctica; marine ciliates; planktonic ciliates; taxonomy; Tintinnida.

Correspondence
E.J. Yang, Korea Polar Research Institute, KIOST, 213-3 Sondo-dong, Yeonsu-gu, Incheon, 405-840, Korea
Telephone number: +82 32 760 5334;
FAX number: +82 32 760 5399;
e-mail: ejyang@kopri.re.kr

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ABSTRACT

We characterized six tintinnid ciliates from Antarctic waters using molecular markers and morphological traits: *Amphorellopsis quinquealata*, *Codonellopsis gaussi*, *Cymatocylis convallaria*, *Cy. calyciformis*, *Cy. drygalskii,* and *Laackmanniella prolongata.* The 100% similarity in SSU-ITS1-5.8S rDNA-ITS2-partial LSU rDNA sequences among *Cy. convallaria*, *Cy. calyciformis,* and *Cy. drygalskii* is supportive of synonymy. *Codonellopsis gaussi* and *L. prolongata* also showed high levels of similarity in SSU rDNA (99.83%) and the D2 domain of LSU rDNA (95.77%), suggesting that they are closely related. Phylogenetic analysis placed *Cymatocylis* in the Rhabdonellidae, *Amphorellopsis* in the Tintinnidae and *L. prolongata/Co. gaussi* within the Dictyocystidae.

THE Southern Ocean is known to harbor endemic tintinnid species (Petz et al. 2007; Pierce and Turner 1993). Among these, *Codonellopsis gaussi*, *Cymatocylis* spp., and *Laackmanniella prolongata* have been found frequently in Antarctic and Subantarctic waters due to both significant biomass and distinctive lorica morphologies (e.g., Alder and Boltovskoy 1991a,b; Boltovskoy and Alder 1992; Boltovskoy et al. 1989; Dolan et al. 2012; Heinbokel and Coats 1984, 1986; Monti and Fonda Umani 1995; Petz et al. 1995; Thompson 2004; Thompson et al. 1999; Williams et al. 1994). Like other tintinnid ciliates, these Antarctic forms have been identified based on lorica morphology. The possibility of synonymy among Antarctic species due to plasticity in lorica morphology has been repeatedly raised and discussed extensively (Alder and Boltovsky 1991b; Boltovsky and Alder 1992; Boltovskoy et al. 1990; Petz et al. 1995; Williams et al. 1994). Indeed, *Cymatocylis affinis* and *Cy. convallaria* have identical infraciliature patterns (Petz et al. 1995; Wasik and Mikołajczyk 1992). Consequently, this form was termed *Cy. affinis/convallaria*, and is now called *Cy. convallaria* (Petz et al. 1995). However, possible synonymy among other species of *Cymatocylis*, *Laackmanniella,* and *Codonellopsis* remains unclear because there is no clear documentation of variable lorica morphology (Alder and Boltovskoy 1991b; Boltovskoy and Alder 1992; Boltovskoy et al. 1990; Petz et al. 1995; Wasik and Mikołajczyk 1992). Although many studies have investigated Southern Ocean tintinnids, lack of accuracy and confidence in the species identities has complicated ecological and biogeographical studies of Antarctic tintinnid ciliates (Alder and Boltovskoy 1991b; Boltovskoy and Alder 1992; Boltovskoy et al. 1990; Dolan et al. 2012; Williams et al. 1994).

Since 2002 (Snoeyenbos-West et al. 2002), tintinnid ciliates have been investigated using molecular approaches based on small subunit ribosomal DNA (SSU rDNA) (Agatha and Strüder-Kypke 2007, 2012; Bachy et al. 2012, 2013; Gao et al. 2009; Kim et al. 2010; Li et al. 2009; Saccà et al. 2012; Santoferrara et al. 2012, 2013; Strüder-Kypke and Lynn 2003, 2008; Xu et al. 2012). Consequently, tintinnids are now well-represented among the choreotrich ciliates in phylogenetic trees. However, because SSU rDNA is highly conserved, even hypervariable regions do not contain species-specific markers (Santoferrara et al. 2013). Recently, the internal transcribed spacer (ITS) regions of the rDNA, which are less conserved than the SSU rDNA, have been used to study tintinnid phylogeny (Bachy et al. 2012, 2013; Snoeyenbos-West et al. 2002). The ITS region has also been used to study gene flow and distinguish cryptic species of oligotrichs (Katz et al. 2005; McManus et al. 2010). In a recent study, the phylogeny of tintinnids based on ITS and 5.8S rDNA regions corresponded well with SSU rDNA-based phylogeny, except for the placement of the genus *Tintinnidum* (Bachy et al. 2012). In studies of other groups of ciliates, large subunit ribosomal DNA (LSU rDNA) is commonly used for phylogenetic analysis as well as SSU rDNA (Gong et al. 2007; Hewitt et al. 2003; Nanney et al. 1998). Nanney et al. (1998) suggested that the divergent 2 (D2) domain of LSU rDNA can be used to identify cryptic species in *Tetrahymena*, *Paramecium,* and *Colpoda*. More recently, Santoferrara et al. (2013) supported the use of LSU rDNA as a genetic maker for tintinnid ciliates.

In this study, we employed a single-cell PCR method and obtained SSU, 5.8S, partial LSU including the D2 domain and the ITS region of ribosomal DNA sequences from six Antarctic forms described previously as individual species. Protargol staining and measurement of lorica shapes were also conducted for morphological characterization. The synonymy and phylogeny of these species are discussed based on both the morphological and molecular data.

MATERIALS AND METHODS

Sample collection

Samples were collected using a 20-μm plankton net from the Amundsen Sea (65°68′S, 111°27′W) on board the *RV Araon* in December 2010. Sea water temperature and salinity ranged from −1.68 °C to 1.09 °C (average −0.81 ± 0.85 °C) and 33.13–34.20 psu (average 33.80 ± 0.30 psu), respectively. On board, part of the sample was fixed in 80% ethanol at −80 °C until further processing for molecular analysis. The remainder of the sample was fixed in 6% Bouin's solution for subsequent morphological studies.

Cell isolation, PCR amplification, and sequencing

Preserved material in ethanol was transferred to autoclaved distilled water (DW). Individual cells were then selected under a dissecting microscope and deposited onto a slide using a Pasteur pipette. Each cell was isolated with a new pipette to prevent contamination. Each cell was rinsed at least five times with autoclaved DW to remove other organisms. Images of isolated cells were obtained using a microscope equipped with a digital camera to record lorica morphology. After imaging, cells were transferred to individual PCR tubes containing 20 μl of DW. Without use of a DNA extraction step, the PCR mixture was transferred to the PCR tube with the isolated cells, resulting in a 50-μl total volume. The TaKaRa LA Taq polymerase was used according to the manufacturer's instructions with EuKA and ReV2 primers (Table S1). PCR amplifications for the SSU-partial LSU rDNA gene were modified from Jung et al. (2011) with the following conditions: denaturation for 2 min at 94 °C, followed by 37 cycles of denaturation for 30 s at 95 °C, annealing for 40 s at 50 °C, extension for 4 min at 72 °C, and a final extension at 72 °C for 10 min. The PCR product was purified using the Gel Extraction Kit (QIAGene, Chatsworth, CA) and sequenced on an ABI 3700 sequencer (Applied Biosystems, Foster City, CA). Four or five additional internal primers were used to assist in sequencing the SSU-partial LSU rDNA sequences (Table S1).

DNA sequence comparisons

Sequence similarities were determined by comparison with DNA sequences of Antarctic species from this study. Sequences were aligned using CLUSTAL X v. 1.81 (Jeanmougin et al. 1998). Intra- and inter-specific similarities were investigated by comparing the DNA similarities of SSU rDNA, partial LSU rDNA, the D2 domain of LSU rDNA and ITS 1 using the Phydit program v. 3.1 (Chun 2001). The D2 domain of LSU rDNA was identified following the guidelines of Engberg et al. (1990).

Phylogenetic analysis

Sequences of SSU and LSU rRNA were used for phylogenetic tree construction. To represent the phylogeny of six Antarctic species, 81 SSU rRNA gene sequences of tintinnid ciliates were retrieved from the NCBI database (Fig. 40). Most tintinnid sequences were included, but partial sequences shorter than 1,429 bp were excluded. To compare the phylogenetic trees based on SSU rDNA and partial LSU rDNA, 29 LSU rRNA gene sequences of tintinnid ciliates were retrieved from the NCBI database following the method of Santoferrara et al. (2012). Each data set was aligned using SILVA Incremental Aligner (SINA) v. 1.2.11 (Pruesse et al. 2012) and refined manually using the Bioedit program v. 7.1 (Hall 1999). Hypervariable regions that could not be aligned unambiguously were removed. After the ends of the alignments were trimmed, separate phylogenetic analyses were performed for SSU rRNA (1,359 bp) and LSU rRNA (506 bp). Six species from the subclasses Choreotrichia (order Choreotrichida), Oligotrichia, and Stichotrichia were used as outgroups. The program MrModeltest v. 2 (Nylander 2004) selected the GTR + I + G as the best model using Akaike information criterion, which was used for both Bayesian and maximum-likelihood (ML) inference. The Bayesian tree was constructed from an output of 6,000 trees generated by MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003) with 6,000,000 cycles for the Markov Chain Monte Carlo algorithm and sampling every 1,000th generation. Stationary likelihood scores were determined by plotting the –lnL against the generation. The first 1,500 trees below the observed stationary level were discarded as burn-in. A ML tree was constructed with the PhyML v. 2.4.4 program (Guindon and Gascuel 2003). Reliability of internal branches was assessed using the non-parametric bootstrap method with 1,000 pseudoreplicates. TreeView v. 1.6.6 (Page 1996) and MEGA v. 4.0 (Tamura et al. 2007) were used to visualize tree topology.

Morphological observation

Lorica morphology was examined using light microscopy. Cells were picked randomly from fixed samples for morphological examination of their lorica using a Sedgwick–Rafter chamber. Observations and drawings of stained specimens were performed at 640X and 1,600X with a camera lucida. We followed the terminology proposed by Agatha and Riedel-Lorjé (2006). Protargol staining was conducted following the quantitative protargol staining method of Montagnes and Lynn (1987). Unfortunately, in our protargol preparations, it was not possible to clearly determine the somatic ciliary patterns in *Cymatocylis* spp. and *Amphorellopsis quinquealata*. For species identification, the original descriptions of Laackmann (1907, 1910), and the commonly employed monographs of Kofoid and Campbell (1929, 1939) and Petz et al. (1995), were used.

RESULTS

Description of six Antarctic species

Morphometric analyses of the loricae for the six taxa are presented in Table 1. We were only partially successful in

Table 1. Morphometric data of *Cymatocylis convallaria* (1st line), *Cy. calyciformis* (2nd line), *Cy. drygalskii* (3rd line), *Codonellopsis gaussi* (4th line), *Laackmanniella prolongata* (5th line), and *Amphorellopsis quinquealata* (6th line)

	x	*M*	SD	SE	CV	Max	Min	*n*
Lorica, total length	145	145	4.7	1.5	3	150	140	10
	242	240	27.4	5.7	11	310	215	23
	365	360	43.3	9.0	12	460	250	23
	166	170	16.8	3.5	10	195	130	23
	174	175	17.1	3.6	10	214	145	23
	178	175	18.2	3.8	10	210	140	23
Lorica, opening diameter	92	92	2.4	0.7	3	95	90	10
	95	95	5.2	1.1	5	100	80	23
	93	95	5.8	1.2	6	100	80	23
	38	38	2.3	0.5	6	43	35	23
	38 (18[a])	40 (18[a])	2.7 (4.8[a])	0.6 (1.0[a])	7 (27)	40 (30[a])	32 (12[a])	23 (23[a])
	45	45	1.8	0.4	4	48	40	23
Lorica, collar length[b]	4	4	0.5	0.2	12	5	4	10
	4	4	0.7	0.2	18	5	2	23
	3	3	0.6	0.1	19	4	2	23
	62	65	13.1	2.7	20	80	30	23
	77	75	18.5	3.9	25	120	50	23
	–	–	–	–	–	–	–	–
Lorica, bowl length	138	140	10.9	3.4	8	150	120	10
	162	160	17.0	3.6	11	210	135	23
	305	300	36.8	7.7	12	420	210	23
	104	105	8.3	1.7	8	120	90	23
	97	95	14.2	3.0	15	127	65	23
	45	45	1.8	0.4	4	48	40	23
Lorica, maximum bowl width	93	90	8.6	2.7	10	110	85	10
	94	90	7.2	1.9	8	110	90	15
	98	95	7.8	2.1	8	115	90	14
	64	63	8.2	1.7	13	85	48	23
	33	33	2.5	0.5	8	40	30	23
	–	–	–	–	–	–	–	–
Lorica, process length	15	15	5.8	2.9	38	20	10	4
	78	70	22.7	4.7	32	145	55	23
	56	60	22.7	4.7	38	95	30	23
	22	22	7.0	1.5	32	35	10	23
	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–

Measurements in μm.

M = median; Max = maximum; Min = minimum; *n* = number of individuals investigated; CV = coefficient of variation; SD = standard deviation; *x* = arithmetic mean; SE = standard error of arithmetic mean.

[a] The posterior opening diameter of the lorica in *Laackmanniella prolongata.*

[b] Length of inner collar in *Cymatocylis* spp.

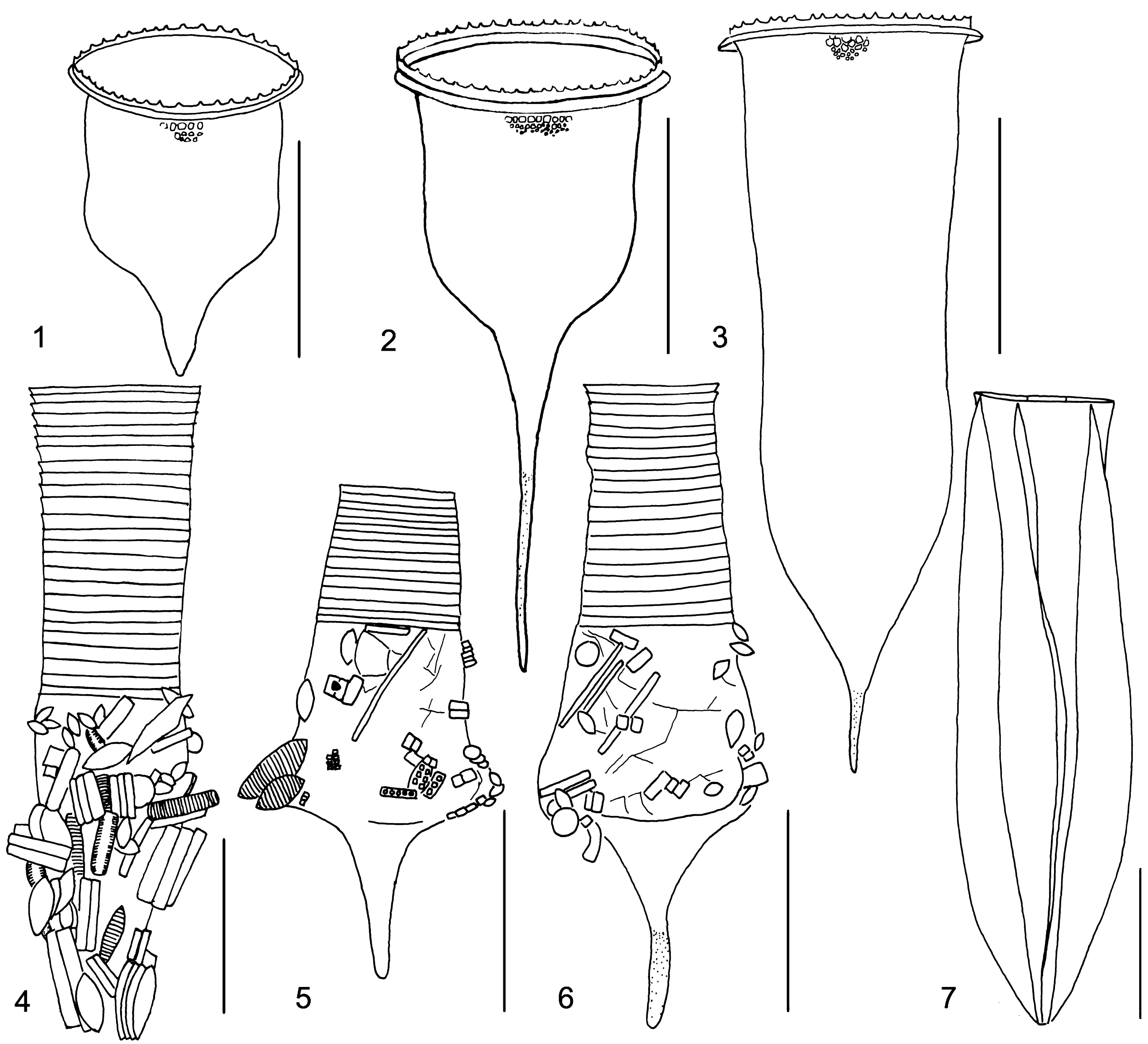

Figure 1–7 Lorica morphology of Antarctic species. **1**. *Cymatocylis convallaria*. **2**. *Cymatocylis calyciformis*. **3**. *Cymatocylis drygalskii*.**4**. *Laackmanniella prolongata*. **5, 6**. *Codonellopsis gaussi*. **7**. *Amphorellopsis quinquealata*. Scale bars: 100 μm (Fig. 1–3) and 50 μm (Fig. 4–7).

using protargol staining to characterize the infraciliature; complete mapping of the ciliature was not possible with our preparations. We were able to enumerate oral membranelles and somatic ciliature for *Laackmanniella prolongata* and *Codonellopsis gaussi*.

Cymatocylis convallaria Laackmann, 1910 (Table 1 and Fig. 1, 12, 13)

Cymatocylis convallaria Laackmann, 1910, Deutsch. Südpolar-Exp., 11:383, pl. 33, fig. 5, pl. 43, fig. 1–4.

Cymatocylis affinis Laackmann, 1910; Deutsch. Südpolar-Exp., 11:384, pl. 43, fig. 5–15.

Cymatocylis affinis/convallaria Boltovskoy, Alder & Spinelli, 1989, J. Plankton Res., 9:454.

Cymatocylis affinis/convallaria Boltovskoy, Dinofrio & Alder, 1990, J. Plankton Res., 12:403–412, table 1, fig. 2.

Cymatocylis affinis/convallaria Wasik & Mikołajczyk, 1994, Acta Protozool., 33:79–84, table 1, fig. 1–11.

Cymatocylis convallaria Petz, Song & Wilbert, 1995, Stapfia, 40:154–159, table 23, fig. 46.

The infraciliature structure of this species was revealed using protargol impregnation by Wasik and Mikołajczyk (1994) and Petz et al. (1995). *Cymatocylis affinis* and *Cy. convallaria* are now known to have identical patterns of infraciliature (Petz et al. 1995; Wasik and Mikołajczyk 1992), and were consequently termed *Cy. affinis/convallaria* and is now called *Cy. convallaria* by

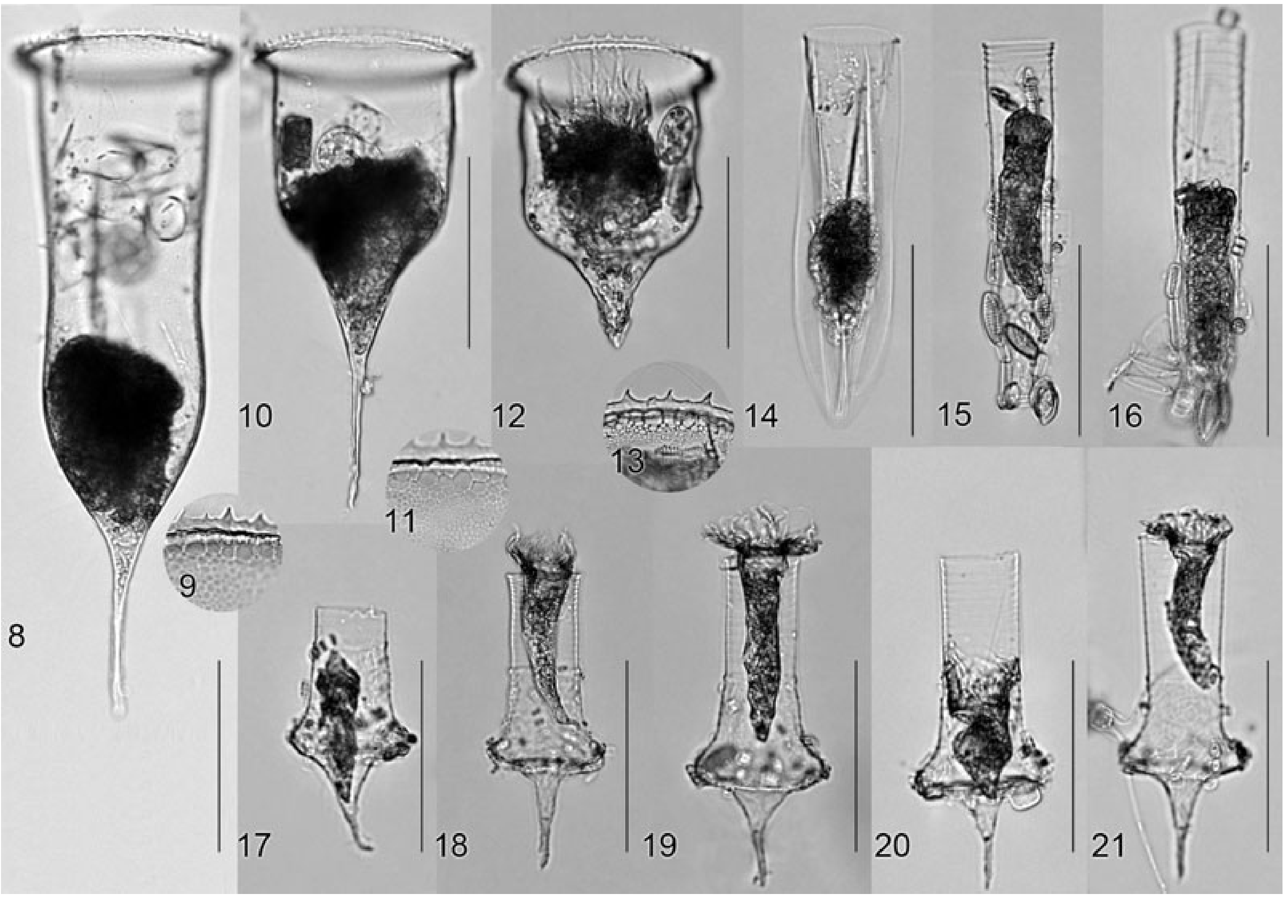

Figure 8–21 Lorica morphology of Antarctic species after Bouin's fixation. **8.** *Cymatocylis drygalskii*. **9.** Lorica wall with a polygonal structure of *Cymatocylis drygalskii*. **10.** *Cymatocylis calyciformis*. **11.** Lorica wall with a polygonal structure of *Cymatocylis calyciformis*. **12.** *Cymatocylis convallaria*. **13.** Lorica wall with a polygonal structure of *Cymatocylis convallaria*. **14.** *Amphorellopsis quinquealata*. **15, 16.** *Laackmanniella prolongata*. **17–21.** Showing variation in size of *Codonellopsis gaussi*. Scale bars 100 μm (Fig. 8, 10, 12, 14–21).

Petz et al. (1995). Additional synonyms have been introduced in Petz et al. (1995), and Petz (2005), but omitted in the present article because the synonyms are based only on lorica morphology or the explanation of synonymizations are absent.

Description of the Amundsen population

The lorica is hyaline and bell-shaped. The posterior end is pointed (Fig. 1, 12) or having a short process 10–20 μm in length (Table 1). The anterior end of the lorica possesses an inner collar with fine teeth (4–5 μm in length) and the outer collar is bent outward (Fig. 1, 13). The lorica wall has a polygonal structure with ~4–5 μm diagonals, which becomes finer (the diagonals become smaller in size) toward the posterior portion of the lorica (Fig. 13). The overall lorica size is 140–150 μm in length with a width of 85–110 μm (Table 1). The lorica opening diameter ranges from 90 to 95 μm (Table 1). Two globular macronuclei have been observed.

Comparison with original description

Laackmann (1910) described the lorica size of *Cy. convallaria* as 110–140 μm in length and 95–120 μm in width. The lorica opening diameter of *Cy. convallaria* was not provided in Laackmann (1910), but is estimated as ~85–100 μm based on illustrations. Laackmann (1910) reported the lorica size of *Cy. affinis* as 120–170 μm in length and 90–110 μm in width, which has been regarded as *Cy. convallaria* by Petz et al. (1995), and Wasik and Mikołajczyk (1994), by comparing the infraciliature structure. The lorica opening diameter of *Cy. affinis* is estimated to be 90–110 μm based on the illustrations of Laackmann (1910). Our specimens correspond well with the original descriptions of *Cy. affinis* and *Cy. convallaria*.

Comparison with similar species

Petz (2005) considered *Cymatocylis parva* as a synonym of *Cy. convallaria*. Laackmann (1907) described *Cy. parva* as having a lorica opening diameter of 47–67 μm, distinctly different from the original *Cy. convallaria* description and our population.

Cymatocylis calyciformis (Laackmann 1907) Laackmann, 1910 (Table 1 and Fig. 2, 10, 11)

Cyttarocylis calyciformis Laackmann, 1907, Zool. Anz., 31:236, fig. 3.

Cymatocylis calyciformis Laackmann, 1910, Deutsch. Südpolar-Exp., 11:391, pl. 36, fig. 4; pl. 42, fig. 12.

Cymatocylis calyciformis Petz, Song & Wilbert, 1995, Stapfia, 40:151–154, table 23 and fig. 45.

The infraciliatural structure of this species was reported using protargol impregnation (Petz et al. 1995). Additional synonyms have been introduced in Petz et al. (1995) and Petz (2005), but omitted in the present article because the synonymizations were not justified.

Description of the Amundsen population

The lorica is hyaline and cup-shaped with a 55–145 μm long posterior process (Table 1 and Fig. 2, 10). The anterior end of the lorica is formed of an inner collar with fine teeth 2–5 μm in length and an outer collar bent outward (Table 1 and Fig. 2, 11). The lorica wall has a polygonal structure with ~4–5 μm diagonals, which is finer toward the posterior portion of the lorica (Fig. 11). The overall lorica dimensions are 215–310 μm in length and 90–110 μm in width (Table 1). The lorica opening diameter ranges from 80 to 100 μm (average 95 μm, Table 1). Two globular macronuclei are also observed.

Comparison with the original description

The lorica of *Cy. calyciformis* was originally described as 440 μm in length and 106 μm in width, with a 133-μm lorica opening diameter and a bowl length of 266 μm (Laackmann 1907). Although the lorica shapes of our specimen and that described originally for *Cy. calyciformis* fit perfectly, the lorica opening diameter, generally considered a preserved character, does differ compared to that given by Lackmann, respectively, 133 μm and 95 μm. However, if the lorica width given by Laackmann (1910) was measured at the anterior lorica end, it possibly includes the outer collar of ~10 μm width, which is not considered in our measurements. Furthermore, there is considerable inconsistency among Laackmann's descriptions. Laackmann in 1910 reported the lorica size of *Cy. calyciformis* as 400–520 μm in length and 150 μm in width. He supplied two illustrations of *Cy. calyciformis*; the illustration of fig. 12 in plate 42 of Laackmann (1910) is ~120 μm, based on the magnification scale given, and the another one, fig. 5 in plate 36 is ~125 μm. Notably, other workers have also found specimens conforming to the general morphology of *Cy. calcyformis* to have an oral diameter of 95–102 μm (e.g. Fernandes 1999; Sassi and Melo 1993). Therefore, we identified our specimens as *Cy. calyciformis*.

Cymatocylis drygalskii (Laackmann, 1907) Laackmann, 1910 (Table 1 and Fig. 3, 8, 9)

Cyttarocylis drygalskii Laackmann, 1907, Zool. Anz., 31:236, fig. 2.

Cymatocylis drygalskii Laackmann, 1910, Deutsch. Südpolar-Exp., 11:376. pl. 36, fig. 3; pl. 41, fig. 1–8.

Additional synonyms have been introduced in Petz (2005), but omitted in the present article because information on synonymization is lacking.

Description of the Amundsen population

The lorica is hyaline and has cylindrical shape with a posterior process of 30–95 μm, tapering to a point (Table 1 and Fig. 3, 8). The anterior end of the lorica has an inner collar with fine teeth 2–4 μm in length and an outer collar bent outward (Table 1 and Fig. 3, 9). The lorica wall has a polygonal structure (~4–5 μm diagonal), which becomes finer toward the posterior portion of the lorica (Fig. 9). The overall lorica dimensions are 250–460 μm in length and 90–115 μm in width (Table 1). The lorica opening diameter ranges from 80 to 100 μm (Table 1). Two globular macronuclei are observed.

Comparison with the original description

Laackmann (1907) described the lorica of *Cy. drygalskii* as 249 μm in length, 81 μm in width, with a 103-μm lorica opening diameter. Later, Laackmann (1910) gave the lorica size of *Cy. drygalskii* as 160–275 μm in length, 80–100 μm in width, with a lorica opening diameter of 100–110 μm. Our specimens show considerable variability in lorica length compared to previous studies, which reflects the variability in length of the posterior process while the original description specifies only as "short".

Codonellopsis gaussi (Laackmann, 1907) Kofoid & Campbell, 1929 (Table 1 and Fig. 5, 6, 17–24, 27–33)

Codonella gaussi Laackmann, 1907, Zool. Anz., 31:239, fig. 12.

Leprotintinnus gaussi Laackmann, 1910, Deutsch. Südpolar-Exp., 11:407, pl. 47, fig. 1–4.

Codonellopsis gaussi Kofoid & Campbell, 1929, Univ. Calif. Publs Zool., 34:79, fig. 164.

Description of the Amundsen population

The lorica is bipartite and the anterior collar portion is tubular and hyaline with several spiral turns (Fig. 5, 6, 17–21). The posterior portion is formed by a bowl with a posterior swelling tapering to a closed posterior end, and is agglutinated with mineral particles or many diatoms (Fig. 5, 6, 17–21). The lorica is 130–195 μm in length and the lorica opening diameter is 35–43 μm (Table 1). The maximum width of the bowl ranges from 48 to 85 μm (Table 1). The bowl length of the lorica is 90–120 μm and the collar length is 30–80 μm (Table 1). Four macronuclei and two micronuclei are observed (Fig. 24, 32). The oral ciliature is composed of ~17 collar membranelles and one

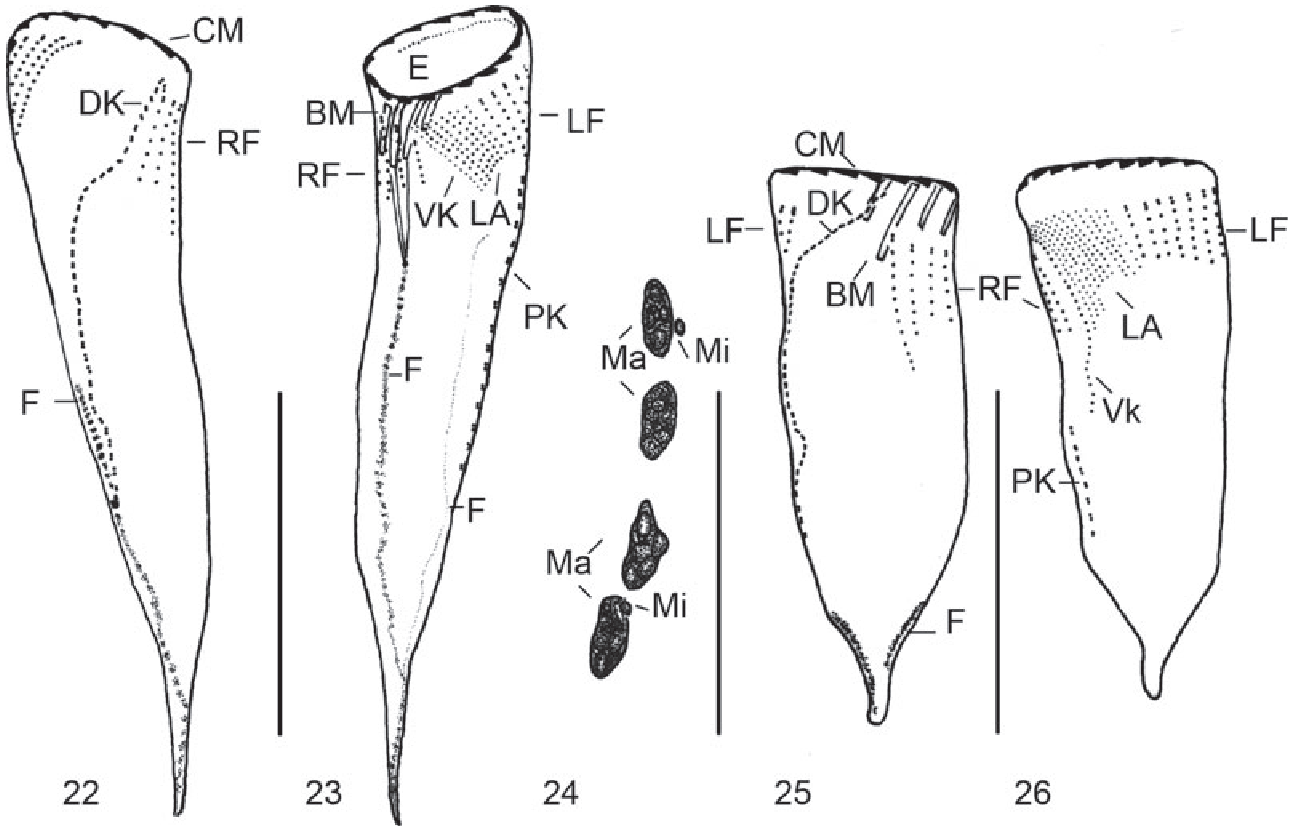

Figure 22–26 Morphology and infraciliature of *Codonellopsis gaussi* and *Laackmanniella prolongata* after protargol impregnation. **22, 23**. Dorsal and ventral views of *Codonellopsis gaussi*. **24.** Macronuclei and micronuclei of *Codonellopsis gaussi*. **25, 26.** Dorsal and ventral views of *Laackmanniella prolongata*. BM = buccal membranelle; CM = collar membranelles; DK = dorsal kinety; E = endoral membrane; F = fibers; LA = lateral ciliary field; LF = left ciliary field; Ma = macronuclei; Mi = mironuclei; PK = posterior kinety; RF = right ciliary field; VK = ventral kinety. Scale bars 50 μm.

buccal membranelle (Fig. 22, 23, 27). Complex ciliary patterns (Agatha and Strüder-Kypke 2012) are observed, which have one monokinetidal ventral, one dikinetidal dorsal, and one dikinetidal posterior kinety, as well as a right, left, and lateral ciliary field (Fig. 22, 23, 29, 30, 32, 33). One dorsal kinety originates near a first kinety of the right ciliary field, curves toward the left ciliary field and extends the posterior part. The posterior kinety was observed below the left ciliary field. We observed ~21–26 somatic kineties; ~9–11 in the lateral ciliary field, ~5–8 in the left ciliary field, and ~5–9 in the right ciliary field (Fig. 28, 29, 31). The lateral ciliary field is composed of densely spaced monokinetids. The kineties of right and left fields are composed of monokinetids and one anterior dikinetid.

Comparison with the original description

Laackmann (1907) described the lorica of *Codonellopsis gaussi* as 155 μm in length, 54 μm in maximum width, with a 40-μm lorica opening diameter. Later Laackmann (1910) gave the dimensions as 140–180 μm in length, 40–60 μm in width, with a 30–40 μm opening diameter. Our specimens corresponded with the overall ranges reported by Laackmann (1907, 1910).

Comparison with similar species

Codonellopsis glacialis has been described as likely conspecific with *Co. gaussi* (Balech 1958, 1973). Petz et al. (1995) reported the infraciliature of a Weddell Sea population of *Co. glacialis* to consist of 18–19 collar membranelles and 25–29 somatic kineties. The dorsal and posterior kineties of *Co. glacialis* were incorrectly named as a ventral and dorsal kinety, respectively, in Petz et al. (1995), but these kineties were clearly shown in the illustration. The position of the dorsal and posterior kineties of *Co. glacialis* fit well with our *Co. gaussi.* Our Amundsen Sea population of *Co. gaussi* overlapped with the Weddell Sea population of *Co. glacialis* in infraciliature characteristics (~17 collar membranelles, ~21–26 somatic kineties and the ciliary pattern), supporting the synonymy of *Co. gaussi* and *Co. glacialis*. However, more information, especially molecular data for *Co. glacialis*, is required.

Laackmanniella prolongata (Laackmann, 1907) Kofoid & Campbell, 1929 (Table 1 and Fig. 4, 15, 16, 25, 26, 34–39)

Codonella prolongata Laackmann, 1907, Zool. Anz., 31:239, fig. 11.

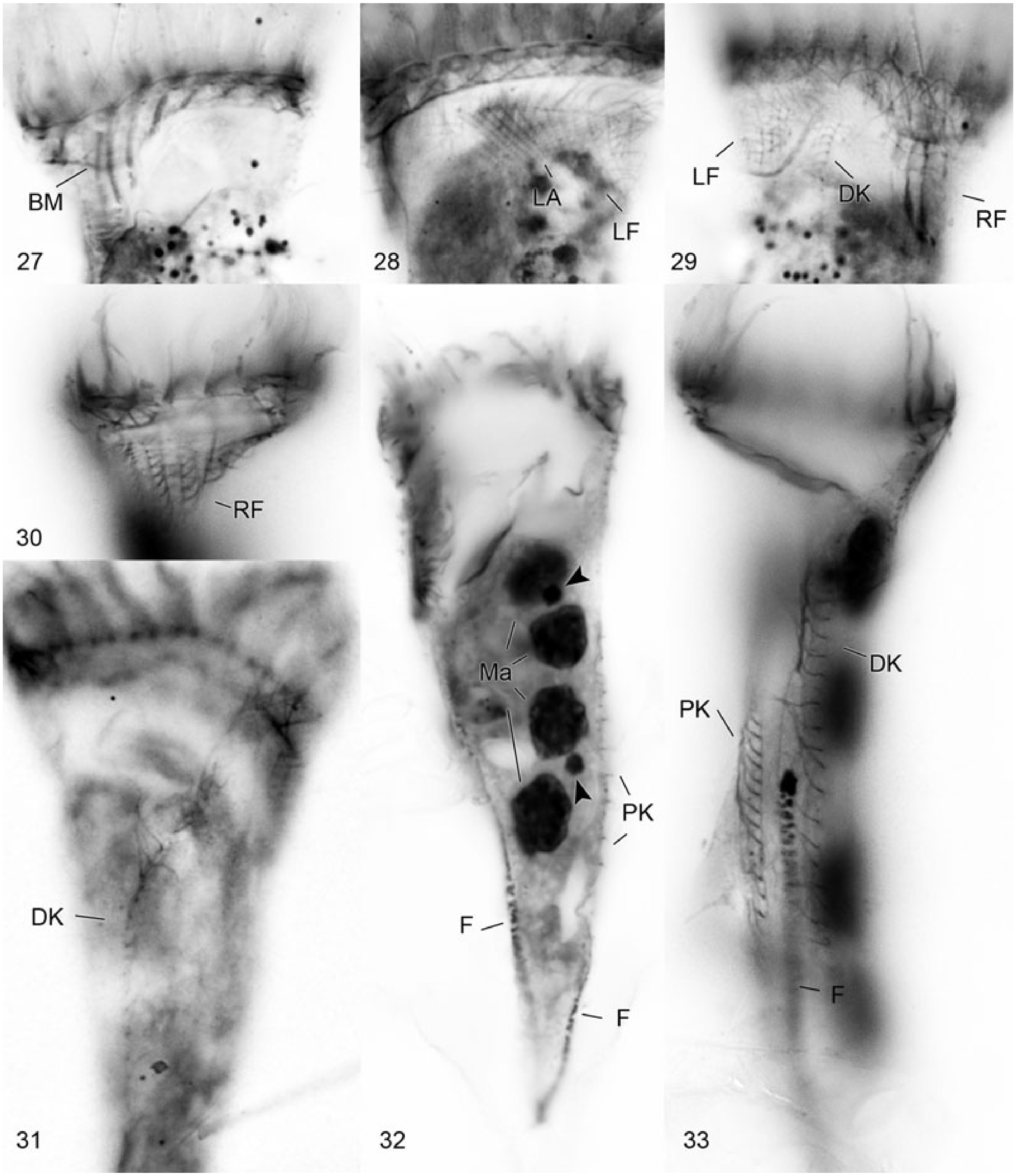

Figure 27–33 Micrographs of *Codonellopsis gaussi* after protargol impregnation. **27.** Ventral view showing buccal cavity. **28.** Lateral view showing lateral and left ciliary field. **29.** Dorsal view showing dorsal kinety, left and right ciliary field. **30.** Right ciliary field. **31.** Dorsal view showing dorsal kinety. **32.** Macronuclei. Ventral view. Arrow head marks the micronuclei. **33.** Dorsal view showing dorsal and posterior kinety.BM = buccal membranelles; DK = dorsal kinety; F, fibers; LA = lateral ciliary field; LF = left ciliary field; Ma = macronuclei; PK = posterior kinety; RF = right ciliary field. The micrographs have been modified manually using Adobe Photoshop program with burning and sharpness tool. The original images are included in Fig. S12–S18.

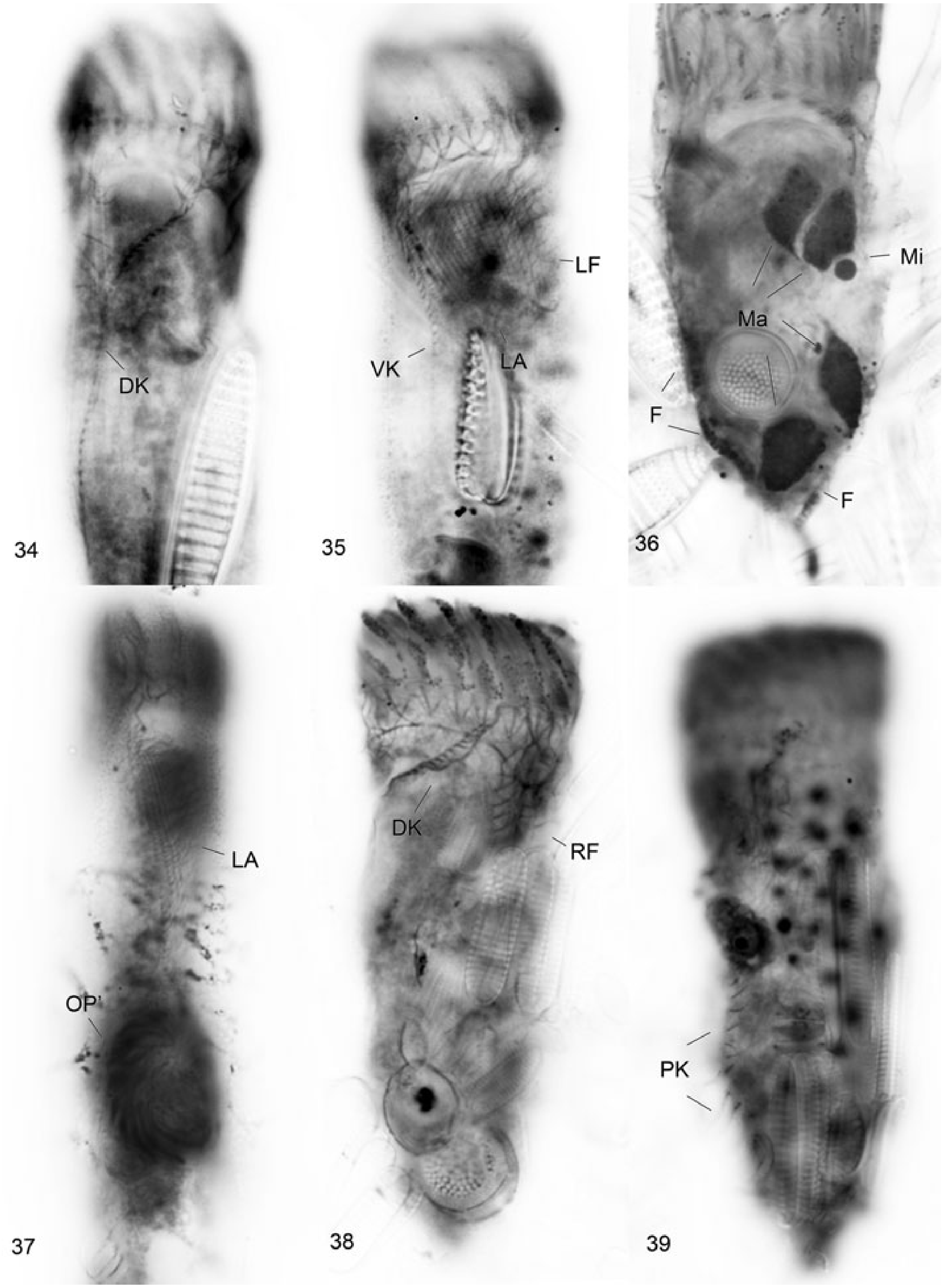

Figure 34–39 Micrographs of *Laackmanniella prolongata* after protargol impregnation. **34.** Dorsal view showing dorsal kinety. **35.** Ventral view showing ventral kinety, lateral, and left ciliary field. **36.** Macronuclei and micronuclei. **37.** Ventral view of oral primordium. **38.** Dorsal view of dorsal kinety and right ciliary filed. **39.** Ventral view showing posterior kinety. DK = dorsal kinety; F = fibers; LA = lateral ciliary field; LF = left ciliary field; Ma = macronuclei; Mi = micronuclei; PK = posterior kinety; RF = right ciliary field; VK = ventral kinety. The micrographs have been modified manually using Adobe Photoshop program with burning and sharpness tool. The original images are included in Fig. S19–S24.

Leprotintinnus prolongatus Laackmann, 1910, Deutsch. Südpolar-Exp., 11:403, pl. 46, fig. 10–12, pl. 48, fig. 5–7.

Laackmanniella prolongata Kofoid & Campbell, 1929, Univ. Calif. Publs Zool. 34:91, fig. 183.

This species has previously been placed in diverse genera (Kofoid and Campbell 1929; Laackmann 1907, 1910). Additional synonyms have been introduced in Petz (2005), but omitted in the present article because information on synonymization is lacking.

Description of the Amundsen population

The lorica is bipartite and the anterior collar portion is tubular and hyaline with several spiral turns (Fig. 4, 15, 16). A posterior portion, also cylindrical, is agglutinated with mineral particles or many diatoms and tapers to an open posterior end (Fig. 4, 15, 16). The lorica is 145–214 μm in length and 30–40 μm in width (Table 1). The collar length of the lorica is 50–120 μm (Table 1). The opening diameter of the lorica is 32–40 μm (Table 1). The bowl length of the lorica ranges from 65 to 127 μm (Table 1). The posterior opening diameter of the lorica is 12–30 μm (Table 1). Four macronuclei and two micronuclei are observed (Fig. 36). Approximately 17 collar membranelles and one buccal membranelle are observed (Fig. 25, 26). Most complex ciliary pattern (Agatha and Strüder-Kypke 2012) are observed, which have one monokinetidal ventral, one dikinetidal dorsal, and one dikinetidal posterior kinety, as well as a right, left, and lateral ciliary field (Fig. 25, 26, 34, 35, 38). The posterior kinety was observed below the right ciliary field near the ventral kinety and extends straight toward the posterior section (Fig. 39). In a dividing cell, we noted a ventral kinety which curves along the margin of the oral primordium (Fig. 37). There are ~27 somatic kineties; ~10 in the lateral ciliary field, ~11 in the left ciliary field and *circa* six in the right ciliary field. The lateral ciliary field is composed of densely spaced monokinetids. The kineties of right and left fields are composed of monokinetids and one anterior dikinetid.

Comparison with the original description

Laackmann (1907) described *Laackmanniella prolongata* as having a lorica 308 μm in length and with a 40-μm opening diameter. Later, Laackmann (1910) reported the lorica size to range from 175 to 310 μm in length and 30–50 μm in width. The length of our specimens is smaller than that mentioned by Laackmann (1907), but overlaps with the range given by Laackmann (1910; 145–214 μm vs. 175–310 μm). The bowl length of our specimen is also smaller than the original description, which is estimated from the illustration (65–127 μm vs. ca. 140 μm); however, the lorica opening diameter corresponds with the records of Laackmann (1907, 1910). Also, Laackmann (1910) reported a short lorica in *Leprotintinnus prolongata* forma *ventricosa* of 140–250 μm in length and 50–55 μm in width, which was collected between February and April. The specimen of this population is similar in length to those of our population, but had a wider opening diameter. The differences in the lorica length might represent different developmental stages, variable collar lengths, or (such as *Cymatocylis*) seasonal differences.

Comparison with similar species

There are only two species in *Laackmanniella*: *L. naviculaefera,* and *L. prolongata*. *L. naviculaefera* differs from *L. prolongata* in having a lorica shorter in overall length and showing a distinctive bulged bowl (Laackmann 1907). Although, *L. prolongata* is considered a synonym of *L. naviculaefera* by most workers (Alder 1999; Balech 1947, 1957; Hada 1970; Petz 2005), there is no infraciliature structure or DNA sequence of *L. naviculaefera* to compare with *L. prolongata.* In this article, our specimen is identified as *L. prolongata* based on the original description by Kofoid and Campbell (1929).

Amphorellopsis quinquealata (Laackmann, 1907) Balech, 1971 (Table 1 and Fig. 7, 14)

Tintinnus quinquealatus Laackmann, 1907, Zool. Anz., 31:236, fig. 1.

Tintinnus quinquealatus Laackmann, 1910, Deutsch. Südpolar-Exp., 11:412, pl. 47, fig. 13, 14; pl. 48, fig. 8.

Bursaopsis quinquealata Kofoid & Campbell, 1929, Univ. Calif. Publs Zool., 34:305, fig. 578.

Proamphorella quinquealata Kofoid & Campbell, 1939, Bull. Mus. Comp. Zool. Harv., 84:313.

Amphorellopsis quinquealata Balech, 1971, Hidrobiologia, 3:180, pl. 39, figs. 758, 759.

This species has been previously placed in diverse genera (Balech 1971; Kofoid and Campbell 1929, 1939; Laackmann 1907), and is identified by its current name, *A. quinquealata*, according to Petz (2005).

Description of the Amundsen population

The lorica is hyaline without any agglutinated particles (Fig. 7, 14). The overall form is an elongate chalice-shape with five well-developed wings, which spiral down from the anterior marginal edge to the posterior end. The bowl (excluding wings) is a conical. The lorica is 140–210 μm in length and the opening diameter is 40–48 μm (Table 1). In the oral ciliature, we observed ~19–21 collar membranelles. Two globular macronuclei are apparent (Table 1). Laackmann (1910) recorded two micronuclei in this species, but the micronuclei were not visible in our population.

Comparison with the original description

Laackmann (1907) described the lorica of *A. quinquealata* as 190–206 μm in length and 46–57 μm in width. Later, Laackmann (1910) reported the lorica of this species to range between 180 and 215 μm in length with a lorica opening diameter of 40–45 μm. Our specimens are slightly smaller and more variable in lorica length, but have a similar lorica opening diameter (40–48 vs. 46–57 μm or 40–45 μm; Laackmann 1907, 1910).

Table 2. Fourteen tintinnid individual cells sequenced: lengths of SSU, ITS1, 5.8S, ITS2, and partial LSU rDNA sequences

Species (isolate number)	Total length (bp)	SSU	ITS1-5.8S-ITS2	Partial LSU (D2 domain)	Accession number
Cymatocylis calyciformis (T99)	2,993	1,747	453	793 (189)	JQ924046
Cymatocylis calyciformis (T100)	2,993	1,747	453	793 (189)	JQ924047
Cymatocylis calyciformis (T105)	2,993	1,747	453	793 (189)	JQ924048
Cymatocylis calyciformis (T106)	2,993	1,747	453	793 (189)	JQ924049
Cymatocylis convallaria (T107)	2,993	1,747	453	793 (189)	JQ924050
Cymatocylis convallaria (T104)	2,993	1,747	453	793 (189)	JQ924051
Cymatocylis drygalskii (T101)	2,993	1,747	453	793 (189)	JQ924052
Codonellopsis gaussi (T103)	2,998	1,748	457	793 (189)	JQ924053
Codonellopsis gaussi (T96)	2,998	1,748	457	793 (189)	JQ924054
Codonellopsis gaussi (T95)	2,998	1,748	457	793 (189)	JQ924055
Laackmanniella prolongata (T97)	2,996	1,748	455	793 (189)	JQ924056
Laackmanniella prolongata (T98)	2,996	1,748	455	793 (189)	JQ924057
Amphorellopsis quinquealata (T102)	3007	1,754	452	801 (192)	JQ924058
Amphorellopsis quinquealata (T108)	3,007	1,754	452	801 (192)	JQ924059

Similarities of rDNA sequences from six Antarctic species

Table 2 summarizes the sequence data obtained for the 14 individual cells (Fig. S1–S11) deposited in GenBank under accession numbers JQ924046– JQ924059. Excluding *Cy. drygalskii*, two to four cells of each species were isolated and sequenced (Table 2). Table 3 shows the similarities of the SSU and partial LSU rDNA sequences among the cells. The total length of these sequences was ~3,000 bp, including the D2 domain, which is known to be a variable region of LSU rDNA. For a morphospecies, we found 100% similarity in both SSU and LSU rDNA sequences (Table 3). We also found no variation among the three *Cymatocylis* forms (*Cy. convallaria, Cy. calyciformis* and *Cy. drygalskii*) in SSU, ITS1, 5.8S, ITS2, and partial LSU rDNA sequences (Tables 3, 4; ITS2 data not shown). Except for the 100% similarity within the three *Cymatocylis* species, the minimum interspecific deviations in the SSU rDNA sequences were 0.17% for *Codonellopsis gaussi* and *Laackmanniella prolongata* (Table 3, 5) and 1.13% in the partial LSU rDNA sequences. Despite the distinct lorica morphologies, *Co. gaussi* and *L. prolongata* were very closely related in terms of the similarity of their SSU rDNA sequences. Notably, the dissimilarities of the ITS 1 sequences and the D2 domains of the LSU rDNA were 4.81% and 4.23% between *L. prolongata* and *Co. gaussi*, respectively (Table 5).

Phylogenetic analyses

The Bayesian and ML trees based on SSU and partial LSU rDNA sequences showed that the six new sequences of *Cymatocylis*, *Laackmanniella*, *Codonellopsis,* and *Amphorellopsis* are well placed in the Tintinnida (Fig. 40).

Three *Cymatocylis* species were clustered together within the Rhabdonellidae in all phylogenetic analyses (Fig. 40). The placement of *Cymatocylis* within the Rhabdonellidae had high supporting values (95% and 89% in BI trees based on SSU and partial LSU rDNA sequences). *Laackmanniella prolongata* and *Codonellopsis gaussi* were clustered together in the phylogenetic tree within the cluster of the Dictyocystidae, *Stenosemella nivalis,* and *S. pacifica*. The placement of *L. prolongata* and *Co. gaussi* in this cluster had only 59% and 58% supporting values in BI and ML trees based on SSU rDNA, respectively. In the BI and ML trees based on the LSU rDNA, *L. prolongata* and *Co. gaussi* clustered with *Tintinnopsis parvula*, which might be due to the lack of LSU rDNA sequences available for Dictyocystidae. The sequence of *Amphorellopsis quinquealata* was placed in the Tintinnidae, but the sequences clustered with *Salpingella*, not with *Amphorellopsis acuta* in the phylogenetic tree (Fig. 40). To classify these groups, sequences of more species, as well as morphological studies of the lorica and somatic ciliary pattern, are required.

The trees based on partial LSU rDNA sequences were mostly in agreement with those based on SSU rDNA sequences, excluding the placement of *Tintinnopsis parvula* (Fig. 40). This is noteworthy because according to Santoferrara et al. (2012) a single *T. parvula* was the source of both the SSU and LSU rDNA sequences.

DISCUSSION

Morphological comparison of the *Codonellopsis gaussi* and *Laackmanniella prolongata*

Despite their distinct lorica morphologies, *Codonellopsis gaussi* and *Laackmanniella prolongata* are closely related to the terms of genetic similarity. Also, they share the most complex ciliary pattern (Agatha and Strüder-Kypke 2007). However, the location of the posterior kineties differs between the two species (below the left ciliary field vs. right ciliary field for *Co. gaussi* and *L. prolongata*, respectively). The taxonomic significance of differences in the position of the posterior kinety has not yet been considered (i.e., Agatha and Strüder-Kypke 2013). However, the infraciliature structure overall is well known to be a conserved character and the different positions of the

Table 3. Similarities of SSU and partial LSU rDNA sequences from six Antarctic species (%)

	Species	T99	T100	T105	T106	T107	T104	T101	T103	T96	T95	T97	T98	T102	T108
		SSU rDNA (1,758 bp)													
T99	*Cymatocylis calyciformis*	–	100.00	100.00	100.00	**100.00**	**100.00**	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T100	*Cymatocylis calyciformis*	100.00	–	100.00	100.00	**100.00**	**100.00**	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T105	*Cymatocylis calyciformis*	100.00	100.00	–	100.00	**100.00**	**100.00**	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T106	*Cymatocylis calyciformis*	100.00	100.00	100.00	–	**100.00**	**100.00**	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T107	*Cymatocylis convallaria*	**100.00**	**100.00**	**100.00**	**100.00**	–	100.00	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T104	*Cymatocylis convallaria*	**100.00**	**100.00**	**100.00**	**100.00**	100.00	–	**100.00**	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T101	*Cymatocylis drygalskii*	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	–	97.08	97.08	97.08	97.20	97.20	93.75	93.75
T103	*Codonellopsis gaussi*	91.05	91.05	91.05	91.05	91.05	91.05	91.05	–	100.00	100.00	**99.83**	**99.83**	94.50	94.50
T96	*Codonellopsis gaussi*	91.05	91.05	91.05	91.05	91.05	91.05	91.05	100.00	–	100.00	**99.83**	**99.83**	94.50	94.50
T95	*Codonellopsis gaussi*	91.05	91.05	91.05	91.05	91.05	91.05	91.05	100.00	100.00	–	**99.83**	**99.83**	94.50	94.50
T97	*Laackmanniella prolongata*	90.92	90.92	90.92	90.92	90.92	90.92	90.92	**98.87**	**98.87**	**98.87**	–	100.00	94.61	94.61
T98	*Laackmanniella prolongata*	90.92	90.92	90.92	90.92	90.92	90.92	90.92	**98.87**	**98.87**	**98.87**	100.00	–	94.61	94.61
T102	*Amphorellopsis quinquealata*	84.43	84.43	84.43	84.43	84.43	84.43	84.43	85.95	85.95	85.95	85.82	85.82	–	100.00
T108	*Amphorellopsis quinquealata*	84.43	84.43	84.43	84.43	84.43	84.43	84.43	85.95	85.95	85.95	85.82	85.82	100.00	–
		Partial LSU rDNA (804 bp)													

Shading is used to allow comparisons between individuals of the same species. Bold is used to highlight the similarity within morphospecies.

Table 4. Similarities of ITS1 and D2 domain of LSU rDNA sequences from six Antarctic species (%)

	Species	T99	T100	T105	T106	T107	T104	T101	T103	T96	T95	T97	T98	T102	T108
								ITS 1 (107 bp)							
T99	*Cymatocylis calyciformis*	–	100	100	100	**100**	**100**	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T100	*Cymatocylis calyciformis*	100	–	100	100	**100**	**100**	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T105	*Cymatocylis calyciformis*	100	100	–	100	**100**	**100**	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T106	*Cymatocylis calyciformis*	100	100	100	–	**100**	**100**	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T107	*Cymatocylis convallaria*	**100**	**100**	**100**	**100**	–	100	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T104	*Cymatocylis convallaria*	**100**	**100**	**100**	**100**	100	–	**100**	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T101	*Cymatocylis drygalskii*	**100**	**100**	**100**	**100**	**100**	**100**	–	89.11	89.11	89.11	91.09	91.09	76.24	76.24
T103	*Codonellopsis gaussi*	77.78	77.78	77.78	77.78	77.78	77.78	77.78	–	100	100	95.19	95.19	76.24	76.24
T96	*Codonellopsis gaussi*	77.78	77.78	77.78	77.78	77.78	77.78	77.78	100	–	100	95.19	95.19	76.24	76.24
T95	*Codonellopsis gaussi*	77.78	77.78	77.78	77.78	77.78	77.78	77.78	100	100	–	95.19	95.19	76.24	76.24
T97	*Laackmanniella prolongata*	77.78	77.78	77.78	77.78	77.78	77.78	77.78	95.77	95.77	95.77	–	100	78.22	78.22
T98	*Laackmanniella prolongata*	77.78	77.78	77.78	77.78	77.78	77.78	77.78	95.77	95.77	95.77	100	–	78.22	78.22
T102	*Amphorellopsis quinquealata*	72.34	72.34	72.34	72.34	72.34	72.34	72.34	76.06	76.06	76.06	75	75	–	100
T108	*Amphorellopsis quinquealata*	72.34	72.34	72.34	72.34	72.34	72.34	72.34	76.06	76.06	76.06	75	75	100	–
								D2 domain (193 bp)							

Shading is used to allow comparisons between individuals of the same species. Bold is used to highlight the similarity within morphospecies.

posterior kinety are considerable. Therefore, while these two species are closely related, genetically, they may be considered as separate species.

Phylogenetic analyses of tintinnid species

Our sequences were placed at positions that differed from the traditional, mostly lorica morphology-based, classifications (Fig. 40), which is similar to many recent studies (e.g., Bachy et al. 2012, 2013; Santoferrara et al. 2012, 2013). One of the disagreements between morphology and molecular phylogeny was resolved using the detailed morphological observation of *Codonella*, *Codonaria,* and *Dictyocysta* by Agatha (2010). The presence of a lorica sac in these three genera is suggestive of a close relationship, which was later supported by SSU rRNA phylogenies. Agatha and Strüder-Kypke (2012) united *Codonaria*, *Codonella,* and *Codonellopsis* in the family Dictyocystidae based on their morphological characteristics and molecular phylogeny.

In the phylogenetic trees based on SSU rDNA, *Codonellopsis gaussi* clustered with *Laackmanniella prolongata* within Dictyocystidae (Fig. 40). Notably, we found no evidence of a lorica sac in either form. Because of the genetic similarities, we suggest that *Co. gaussi* and *L. prolongata* should be united in one genus. However, further studies are required using live observation or scanning electron microscopy, including a detailed lorica structure comparison of these two species with other *Codonellopsis* species to place *Co. gaussi* and *L. prolongata* appropriately.

The placement of *Cymatocylis* species in the phylogenetic tree suggests that the familial affiliation of this species is not correct (Fig. 40). Based on the phylogenetic analysis, *Cymatocylis* does not group with *Favella* of Ptychocylididae, but instead with *Schmidingerella* of Rhabdonellidae. *Schmidingerella* was recently established as a second cluster of *Favella* in the gene tree by Agatha and Strüder-Kypke (2012) based on morphological observations of the lorica ultrastructure and somatic ciliary pattern. *Cymatocylis* species are grouped with *Schmidingerella* and *Metacylis*; however, this is supported only by the BI tree based on SSU rDNA (Fig. 40). Laval-Peuto and Brownlee long ago argued that to correctly classify tintinnid species various approaches should be employed such as observation of cytology, and even non-morphological characteristics such as ecological and behavioral data (Laval-Peuto and Brwonlee 1986). Recently, several studies have discussed the usefulness of combined molecular

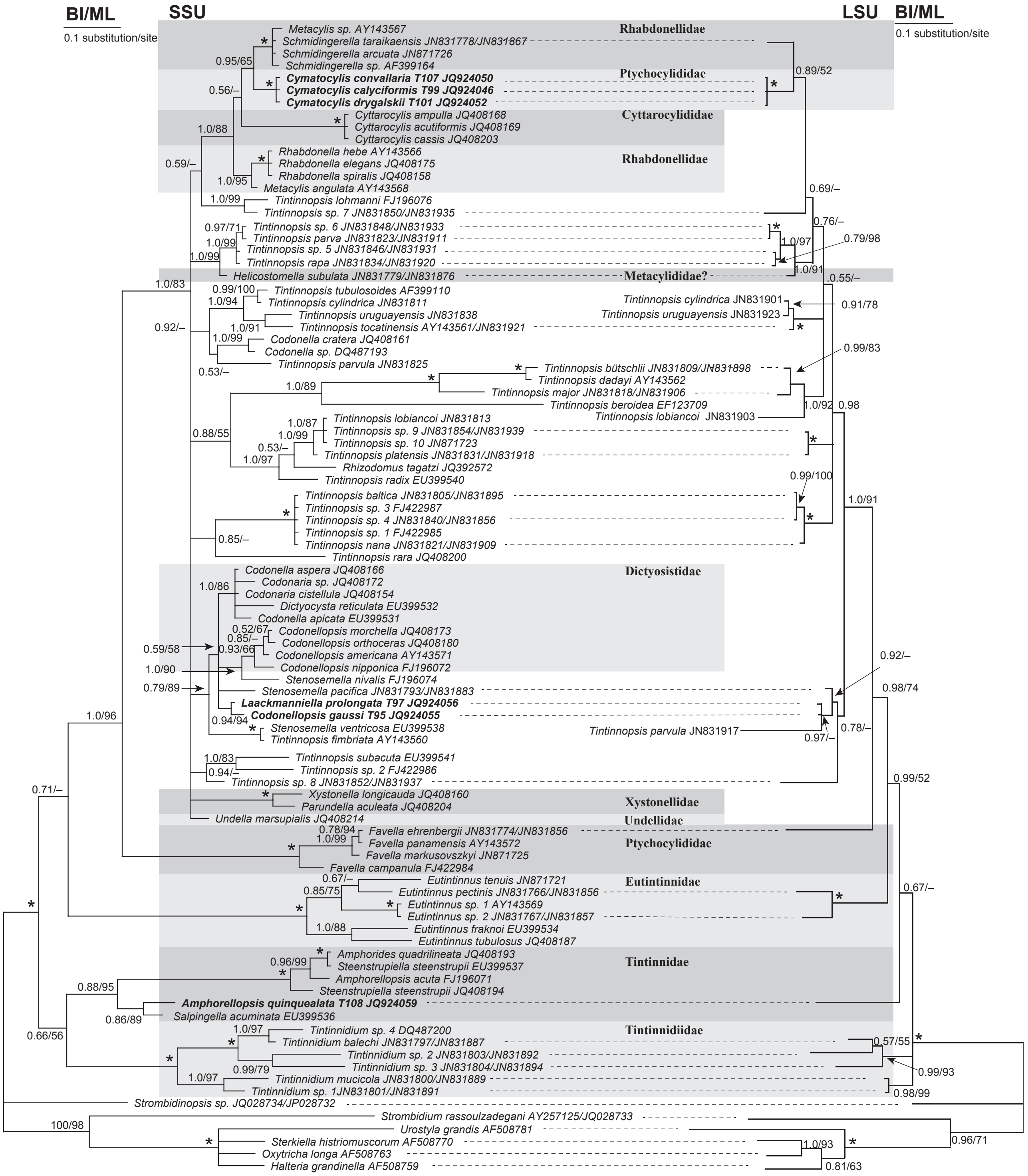

Figure 40 Bayesian trees based on small subunit rRNA gene (left) and large subunit rRNA gene (right) sequences showing the relationships between six Antarctic tintinnid species (bold) and other tintinnid ciliates. Numbers at the nodes represent support values in the following order: Bayesian posterior probabilities using the MrBayes algorithm (BI) and bootstrap values from maximum likelihood (ML) analyses as% of 1,000 replicates. Asterisk (*) denotes nodes with full bootstrap support in all algorithms. A hyphen (-) represents support values < 50% and disagreement between BI and ML at a given node.

Table 5. Dissimilarities of different segments between *Laackmanniella prolongata* and *Codonellopsis gaussi*

Region	Different/total nucleotide	Dissimilarity (%)
18S-ITS1-5.8S-ITS-partial of LSU rDNA	21/2,996	0.7
SSU rDNA	3/1,748	0.17
ITS1	5/104	4.81
5.8S	0/154	0.00
ITS2	4/197	2.03
Partial of LSU rDNA	9/793	1.13
D2 domain	8/189	4.23

analysis, cytological studies, and observation of lorica ultrastructure (Agatha 2010; Agatha and Strüder-Kypke 2012). All these lines of evidence will likely be needed to unambiguously place *Cymatocylis* among tintinnids.

In conclusion, molecular markers provide vital support in identifying tintinnids, but should continue to be combined with morphological observations. Disagreements between molecular and morphological phylogenies suggest a need to employ a variety of genetic markers (including perhaps ITS2 secondary structure) and morphological features beyond those of the loricae traditionally used for identification and classification. Even the biometric approaches for objective classification of lorica types (e.g. Williams et al. 1994) have proven inadequate indicating a need for the identification and use of additional morphological characteristics, those neglected in traditional classifications. Studies of morphology and ecology coupled with molecular establish more reliable hypothesis about tintinnid evolution and to circumscribe tintinnid species properly.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1–S11. Micrographs of specimens sequenced. **S1, S2**. *Codonellopsis gaussi* (T95 and T96). **S3, S4**. *Laackmanniella prolongata* (T97 and T98). **S5.** *Amphorellopsis quinquealata* (T108). **S6.** *Cymatocylis drygalskii* (T101). **S7–S9.** *Cymatocylis calyciformis* (T105, T99 and T106). **S10, S11.** *Cymatocylis convallaria* (T107 and T104). The micrographs of T100 (Cymatocylis calyciformis), T103 (*Codonellopsis gaussi*) and T102 (*Amphorellopsis quinquealata*) are excluded due to low resolution. All images were taken without a coverglass because of the next step (DNA extraction), and were distorted by water.

Fig. S12–S18. Original image of Fig. 27–33. Micrographs of *Codonellopsis gaussi* after protargol impregnation. **S12.** Ventral view showing buccal cavity. **S13.** Lateral view showing lateral and left ciliary field. **S14.** Dorsal view showing dorsal kinety, left and right ciliary field. **S15.** Right ciliary field. **S16.** Dorsal view showing dorsal kinety. **S17.** Macronuclei. Ventral view. Arrow head marks the micronuclei. **S18.** Dorsal view showing dorsal and posterior kinety. BM = buccal membranelles; DK = dorsal kinety; F = fibers; LA = lateral ciliary field; LF = left ciliary field; Ma = macronuclei; PK = posterior kinety; RF = right ciliary field.

Fig. S19–S24. Original image of Fig. 34–39. Micrographs of *Laackmanniella prolongata* after protargol impregnation. **S19.** Dorsal view showing dorsal kinety. **S20.** Ventral view showing ventral kinety, lateral and left ciliary field. **S21.** Macronuclei and micronuclei. **S22.** Ventral view of oral primordium. **S23.** Dorsal view of dorsal kinety and right ciliary filed. **S24.** Ventral view showing posterior kinety. DK = dorsal kinety; F = fibers; LA = lateral ciliary field; LF = left ciliary field; Ma = macronuclei; Mi = micronuclei; PK = posterior kinety; RF = right ciliary field; VK = ventral kinety.

Table S1. Primers used for DNA sequencing.