INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Wang *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:2515–2530
DOI 10.1099/ijsem.0.004065

MICROBIOLOGY SOCIETY

Morphological redescriptions and neotypification of two poorly known tintinnine ciliates (Alveolata, Ciliophora, Tintinnina), with a phylogenetic investigation based on SSU rRNA gene sequences

Rui Wang[1], Wen Song[2], Yang Bai[1], Alan Warren[3], Lifang Li[2,*] and Xiaozhong Hu[1,*]

Abstract

Two poorly known tintinnine ciliates collected from the coastal waters of PR China, viz., *Codonellopsis mobilis* Wang, 1936 and *Tintinnopsis chinglanensis* Nie & Ch'eng, 1947, were redescribed and neotypified using live observation, protargol staining and SSU rRNA gene sequencing. Ciliature information and SSU rRNA gene sequence data of both species were revealed for the first time and improved diagnoses were given based on the original descriptions and data from the present study. Further phylogenetic analyses inferred from SSU rRNA gene sequences and morphological data suggested that the genus *Tintinnopsis* is polyphyletic and that the genus *Codonellopsis* is non-monophyletic. The approximately unbiased test, however, does not reject the possibility that *Codonellopsis* is monophyletic.

INTRODUCTION

Planktonic ciliates are important components of the microbial loop in the neritic and oceanic pelagial and are major contributors to the energy flux through pelagic marine ecosystems [1–3]. Therefore, during the past three decades there has been increasing interest in studying their taxonomy using the state-of-art techniques [4–11]. Characterized by their highly specialized lorica, tintinnine ciliates have been extensively investigated since the classical oceanographic expeditions of the late nineteenth and early twentieth centuries. To date, about 1300 species and 75 genera of tintinnines have been identified almost exclusively based on features of their lorica [12–16]. However, polymorphism of the lorica has been observed in cultures of species belonging to different families, e.g., *Favella ehrenbergii* (Claparède & Lachmann, 1858) Jörgensen, 1924 [17], *Helicostomella subulata* (Ehrenberg, 1833) Jörgensen, 1924, *Parafavella denticulata* (Ehrenberg, 1840) Kofoid & Campbell, 1929 and *Tintinnopsis campanula* (Ehrenberg, 1840) Daday, 1887 [18], indicating that lorica morphology can be affected by environmental conditions and life cycle [17, 19, 20]. Furthermore, recent researches have revealed that a combination of both molecular and classic morphological data is indispensable for studying the diversity, taxonomy, phylogeny and biogeography of tintinnines [21–28]. Unfortunately, SSU rRNA gene sequences and cytological data, including details of the ciliature, are available for only 10 and 5 % of nominal morphospecies, respectively [18, 29–43].

Members of the genus *Codonellopsis* Jörgensen, 1924 are characterized by having a broadly obovate bowl with densely agglomerated particles and a cylindrical, hyaline collar that may be either spiraled or annular. According to Zhang *et al.* [44], this genus includes about 60 species. Among these, only *Codonellopsis glacialis* (Laackmann, 1907) Kofoid & Campbell, 1929 and *Codonellopsis gaussi* (Laackmann, 1909) Kofoid & Campbell, 1929 are known in terms of their ciliature [34, 45]. SSU rRNA gene sequence data are available for only five *Codonellopsis* species, the identification of which, with the exception of *C. gaussi*, were based on lorica structures alone.

The genus *Tintinnopsis* Stein 1867 can be recognized by its hard and entirely agglomerated lorica. To date, more than 160 nominal species of *Tintinnopsis* have been described

Author affiliations: [1]Institute of Evolution and Marine Biodiversity; Key Laboratory of Mariculture, Ministry of Education, Ocean University of China (OUC), Qingdao 266003, PR China; [2]Marine College, Shandong University, Weihai 264209, PR China; [3]Department of Life Sciences, Natural History Museum, London SW7 5BD, UK.
***Correspondence:** Lifang Li, qd_liliy@sina.com; Xiaozhong Hu, xiaozhonghu@ouc.edu.cn
Keywords: ciliates; lorica; SSU rRNA gene; systematics; taxonomy; Tintinnina.
Abbreviations: BM, buccal membranelle; CM, collar membranelles; DK, dorsal kinety; LA, lateral ciliary field; LF, left ciliary field; PK, posterior kinety; RF, right ciliary field.
The GenBank accession numbers for the SSU rRNA gene sequences of *Codonellopsis mobilis* and *Tintinnopsis chinglanensis* are MK799838 and MK799839, respectively. This article is registered in ZooBank under: urn:lsid:zoobank.org:pub:9F6D628B-4C3C-4910-820D-C1E2485E6D27.
One supplementary table is available with the online version of this article.

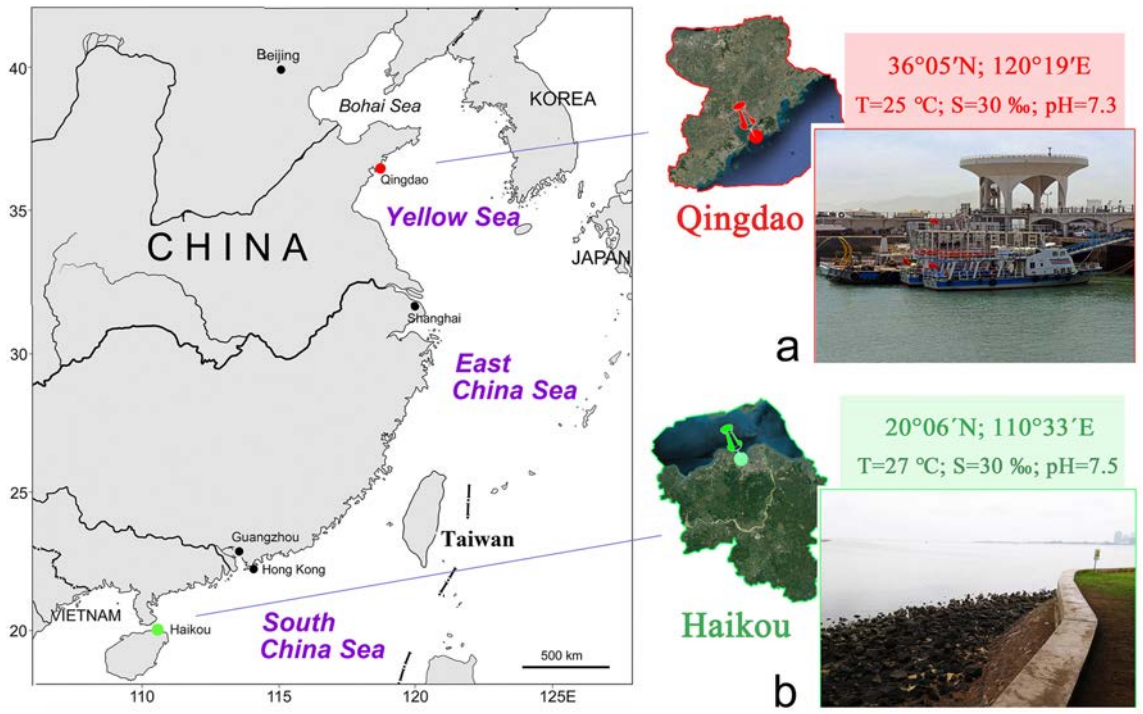

Fig. 1. (a–b) Map of China coast showing the sampling locations and photographs of the sampling sites. (a) Location and surrounding views of Zhanqiao pier, Qingdao, from which *Codonellopsis mobilis* was collected. (b) Location and landscape of sampling site of *Tintinnopsis chinglanensis*.

worldwide but cell features have been studied for only 14 of these, e.g., [28, 30, 32, 33, 36, 38]. Although genetic data are rapidly increasing in databases (e.g., NCBI), identifications of most environmental sequences lack morphological data or vouchered specimens [25].

In the present study, *Codonellopsis mobilis* and *Tintinnopsis chinglanensis* were collected from coastal waters of PR China. The lorica and cellular structures of each were investigated in live and silver-stained specimens. Meanwhile, their SSU rRNA gene sequences were characterized and analysed in order to determine their phylogenetic positions within the suborder Tintinnina. As the original descriptions lack cytological features, and no type specimens are known to be deposited in recognized scientific or educational institutions, we designate neotypes for *Codonellopsis mobilis* and *Tintinnopsis chinglanensis* from our specimens following the recommendations of Foissner [46].

METHODS

Collection

Surface water samples were collected by a plankton net with a mesh size of 25 µm (Fig. 1a–b). *Codonellopsis mobilis* was collected from coastal waters of Qingdao, northern PR China (36° 05′ 1.03″ N, 120° 19′ 23″ E), on 8 May 2012. *Tintinnopsis chinglanensis* was collected from coastal waters of Haikou, southern PR China (20° 06′ 2.56″ N, 110° 33′ 3.89″ E), on 17 May 2017.

Taxonomic studies

The water samples were transferred into Petri dishes for further study. Living cells were immediately isolated under a stereomicroscope (Guiguang XTL-200) at 45× magnification for further microscopic observation using bright field and differential interference contrast microscopy (Olympus BX 51) at 40–1000× magnifications. *In vivo* measurements were performed at magnifications of 200–1000×. The ciliature and nuclear apparatus were revealed using the protargol-staining method according to Wilbert [47]. The protargol powder was manually synthesized following the method described by Pan *et al.* [48]. The cells were stained after the manual removal of their lorica. Counts and measurements of stained specimens were conducted at a magnification of 1000×. Drawings of live cells were based on photomicrographs and hand-sketches, those of silver-stained cells were made with the help of a camera lucida at a magnification of ×1000.

Terminology follows Agatha and Riedl-Lorjé [18] and classification mainly follows Santoferrara *et al.* [24] and Adl *et al.* [49].

DNA extraction, PCR amplification, and sequencing

Although clonal cultures were not established, the two tintinnine species investigated here can be easily recognized by their lorica features. Furthermore, no other tintinnine morphotypes were present in the protargol preparations, indicating that morphological and molecular studies of each

isolate dealt with a single species. Genomic DNA extraction was performed according to the following method: for each species, two cells were isolated from the raw culture by inspection *in vivo* at 400× magnification, washed five times with 0.22 µm filtered habitat water to remove potential contamination and transferred into a 1.5 ml microfuge tube with a minimum volume of water. Genomic DNA was extracted from the cleaned cells using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol, but modified by using 1/4 of the suggested volume for each solution. The primers 82F (5′-GAAACTGCGAATGGCTC-3′) and 18 S-R (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) were used for SSU rRNA gene amplification [50, 51]. To minimize the possibility of PCR amplification errors, Q5 Hot Start High-Fidelity 2x Master Mix DNA Polymerase (New England BioLabs) was used [52]. PCR amplifications were performed according to the following protocol: 98 °C for 30 s, followed by 18 cycles of 98 °C for 10 s, 69 °C for 40 s with the remaining cycles decreasing in temperature by 1 °C for each cycle; then 72 °C for 90 s and 18 cycles at 98 °C for 10 s, 51 °C for 40 s, 72 °C for 90 s; and a final extension at 72 °C for 4 min. The PCR products were sequenced bidirectionally by the Tsingke Biological Technology Company (Beijing, PR China).

Phylogenetic analyses and topology testing

The SSU rRNA gene sequences of *Codonellopsis mobilis*, *Tintinnopsis chinglanensis* and 76 other taxa downloaded from the NCBI genetic sequence database (GenBank) were used to reconstruct the phylogenetic trees (for accession numbers see Fig. 2 and S1 available in the online version of this article). The hypotrichs *Oxytricha granulifera*, *Gonostomum strenuum*, *Halteria grandinella*, *Laurentiella strenua*, *Steinia sphagnicola* and *Urostyla grandis* were selected as outgroup taxa. All sequences were aligned using the MUSCLE algorithm from the European Bioinformatics Institute (available at www.ebi.ac.uk/Tools/msa/muscle). The resulting alignment was manually edited, using the program BioEdit 7.0.5.2 [53] and both ends of the alignment were trimmed. The final length of the SSU rRNA gene alignment was 1664 positions. Maximum-likelihood (ML) analysis with 1000 bootstrap replicates was performed to estimate the reliability of internal branches using RAxML-HPC2 on XSEDE 8.2.8 [54] with the GTRGAMMA model provided on the online server CIPRES Science Gateway [55]. Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 on XSEDE 3.2.6 [56] on the CIPRES Science Gateway (available atwww.phylo.org/sub_sections/portal) with the best-fit model GTR+I+Γ selected by the Akaike information criterion (AIC) using the program MrModeltest 2 [57]. Markov chain Monte Carlo (MCMC) simulations were run with two sets of four chains for 4 000 000 generations at a sampling frequency of 100 and a burn-in of 10 000 trees (25 %). All remaining trees were used to calculate posterior probabilities (PPs) using a 50 % majority rule consensus. MEGA 4.0 [58] analyses were used to visualize the tree topologies.

To statistically test the monophyly of the genus *Codonellopsis* the approximately unbiased (AU) test [59] was performed. The constrained ML tree was generated based on SSU rRNA gene sequences by limiting the monophyly of all congeners with unspecific internal relationships within the constrained and the remaining taxa. The site-wise likelihoods for the resulting constrained and non-constrained ML topologies were calculated using PAUP and then analysed in CONSEL version 0.1 [60].

RESULTS

Class Oligotrichea Bütschli, 1887

Order Choreotrichida Small & Lynn, 1985

Suborder Tintinnina Kofoid & Campbell, 1929

Family Dictyocystidae Haeckel, 1873

Genus *Codonellopsis* Jörgensen, 1924

***Codonellopsis mobilis* Wang, 1936** (Figs 3a–f–5; Table 1)

Previous descriptions

1936 *Codonellopsis mobilis* n. sp. – Wang, Sinensia, 7 : 62

1952 *Codonellopsis mobilis* Wang, 1936 – Yin, J. Shandong Univ. (Phil. Soc. Sci.), 2 : 36–56

Improved diagnosis (based on original description and this study)

Lorica composed of a cylindrical collar with about five annulations and an obovoid bowl, ca. 20–50×60–70 µm and 90–110×80–95 µm, respectively. Cell proper inverted cone-shaped, 120–140×40–45 µm *in vivo* when fully extended. About 25 ellipsoidal macronuclear nodules. Ventral kinety composed of on average 93 monokinetids, commences anteriorly to the second kinety in right ciliary field. Dorsal kinety with about 84 dikinetids. On average 28 kineties in left ciliary field, the first three or four kineties conspicuously shortened; several kinetal fragments present below left ciliary field; ten kineties in right ciliary field, each basically composed of two or three dikinetids anteriorly and monokinetids posteriorly; the three rightmost kineties are conspicuously elongated. Lateral ciliary field with about 20 monokinetidal kineties. About 26 collar membranelles, seven of which extend into buccal cavity; one buccal membranelle.

Ecological features

Water temperature 25 °C, salinity 30 ‰ and pH 7.3.

Deposition of neotype and voucher specimens

A protargol slide (registration number: NHMUK 2020.1.23.1) with the neotype specimen (Figs 3b, c and 4m) was deposited in the Natural History Museum, London, UK. Another protargol slide with voucher specimens (registration number: SW2012050801–2) was deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, PR China.

Description of Qingdao population

Lorica on average 117 µm long and 87 µm wide, composed of a hyaline collar and an agglomerated bowl. Collar almost

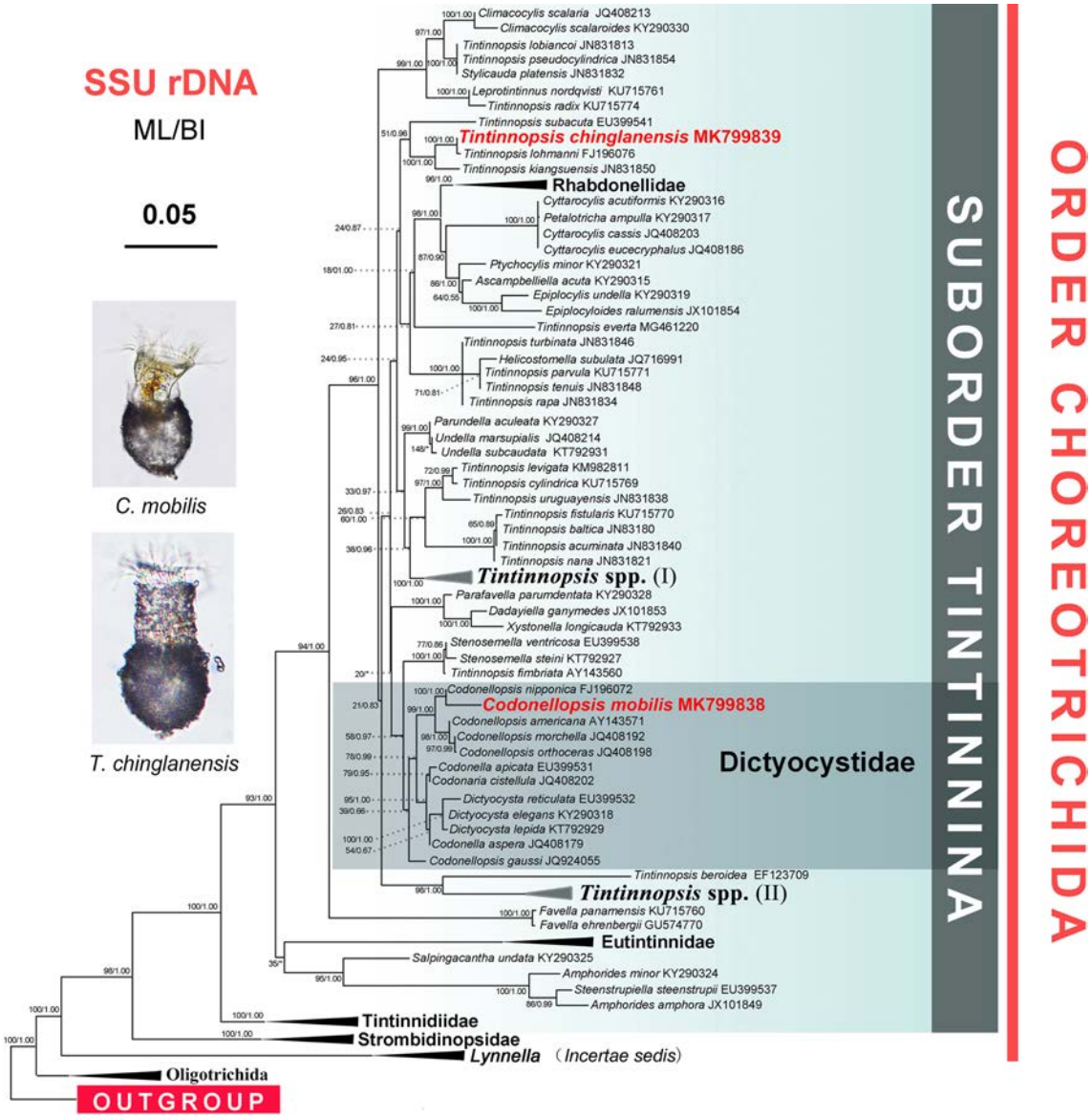

Fig. 2. Maximum-likelihood (ML) tree inferred from SSU rRNA gene sequences. Numbers near branch nodes denote ML bootstrap value/BI posterior probability. The scale bar corresponds to five substitutions per 100 nucleotide positions. All branches are drawn to scale. The newly sequenced *Codonellopsis mobilis* and *Tintinnopsis chinglanensis* in the present work were indicated in bold. *Tintinnopsis* species comprise *Tintinnopsis* species that are maximally supported; other maximally supported branches are also in bold font (for details, see Table S1).

cylindrical, occupying about 1/5 of lorica length, with a diameter of 60–65 μm and four to six annuli on surface *in vivo* which are invisible after fixation (Figs 3a and 4a, b, e). Bowl obovoid to almost globular, slightly pointed posteriorly, with densely agglomerated mineral particles (Figs 3a and 4a–d).

Cell proper elongate–obconical when fully extended, about 110–130×55–65 μm *in vivo*, posterior end narrowed forming a contractile peduncle, which is about 20 um long that is attached to the bottom of lorica (Figs 3a and 4a). Eighteen to thirty-six ellipsoidal macronuclear nodules scattered in cytoplasm, each about 10×7 μm in protargol preparations (Fig. 3b, Fig. 4k and Fig. 5f–g). Micronuclei not recognized, probably because they were insufficiently stained with protargol. Tentaculoids slender pin-shaped, about 15 μm long, located in outer portions of intermenmbranellar ridges (Figs 3a and 4a). No striae, accessory combs, contractile vacuole, cytopyge or extrusomes recognized. Cytoplasm colourless, with several food vacuoles up to 10 μm across containing yellow microalgae. Locomotion by swimming slowly while rotating about main cell axis (speed not measured), sometimes lying on substratum. When observed *in vivo*, cell proper projects through lorica aperture, retracting quickly into lorica when disturbed (Fig. 4a–c).

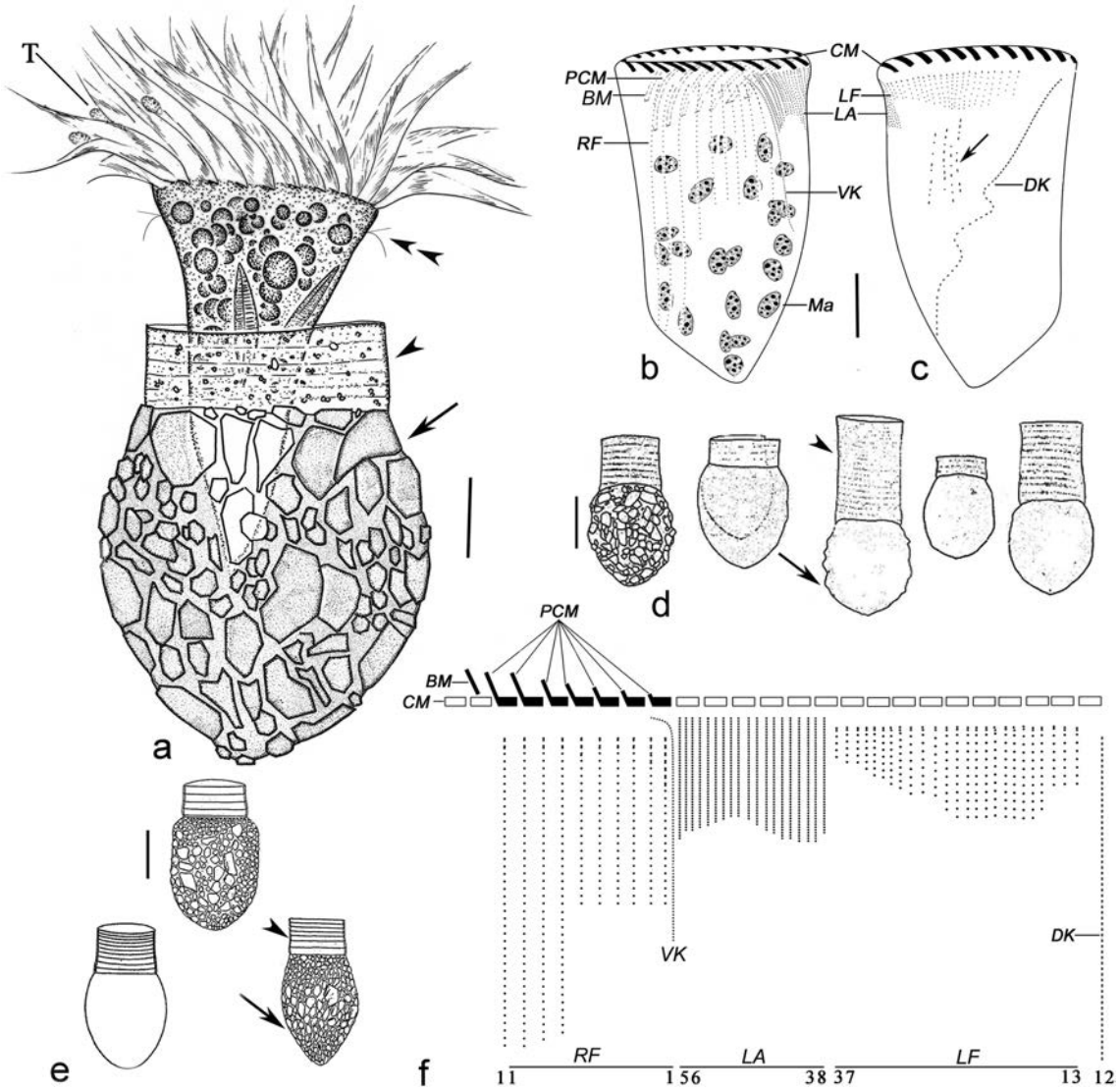

Fig. 3. *Codonellopsis mobilis* from life (a, d, e) and after staining with protargol (b, c, f). (a) Lateral view of a representative individual, arrowhead and arrow show collar portion and bowl portion, respectively; double arrowhead indicates the somatic cilia. (b, c) Ventral (b) and dorsal (c) side views of the neotype specimen, showing ciliary pattern; arrow in (c) indicates the configuration of kinetal fragments. (d, e) Lateral views of lorica of specimens sampled from Pe-Hai (d, from [63]) and Jiaozhou Bay (e, from [68]); arrowheads and arrows denote collar portion and bowl portion, respectively. (f) Kinetal map based on data from morphostatic specimens of the Qingdao population. BM, buccal membranelle; CM, collar membranelles; DK, dorsal kinety; LA, lateral ciliary field; LF, left ciliary field; Ma, macronuclear nodules; PCM, prolonged collar membranelles; T, tentaculoids; VK, ventral kinety. Scale bars=20 μm.

Somatic ciliary pattern comprising single ventral and dorsal kineties as well as right, left and lateral ciliary fields (Fig. 3b, c, f, Fig. 4f–m and Fig. 5a–g). Ventral kinety commences about 3 μm posteriorly to collar membranelles and anteriorly to second kinety of right ciliary field, anterior portion curves slightly leftwards parallel to kineties of lateral ciliary field, then extends longitudinally to postmedian of cell proper, about 48–62 μm long, composed of 80–102 densely spaced monokinetids with cilia 2 μm long after protargol staining (Fig. 3b, c, f, Fig. 4k). Right ciliary field about 7 μm away from collar membranelles, comprises 10–12 kineties about 5 μm apart; each kinety consists of two or three dikinetids anteriorly and monokinetids posteriorly, except for the two leftmost kineties, which have four to seven anterior dikinetids (Figs 3b and 4k); each basal body, both in dikinetids and monokinetids, bears a cilium that is 3 μm to 6 μm long (Fig. 4i); three or four rightmost kineties 90 μm long, i.e., about twice the length of the remaining kineties, terminate in posterior portion of cell proper (Figs 3b, f and 4k, l, m). Dorsal kinety commences about 7 μm posteriorly to collar membranelles, separated from right and left ciliary fields by conspicuously broad, unciliated stripes that are about 15 μm and 12 μm wide, respectively; extends with leftward curvature to ventral side of cell proper, terminating posteriorly below kineties of lateral and left ciliary fields; composed

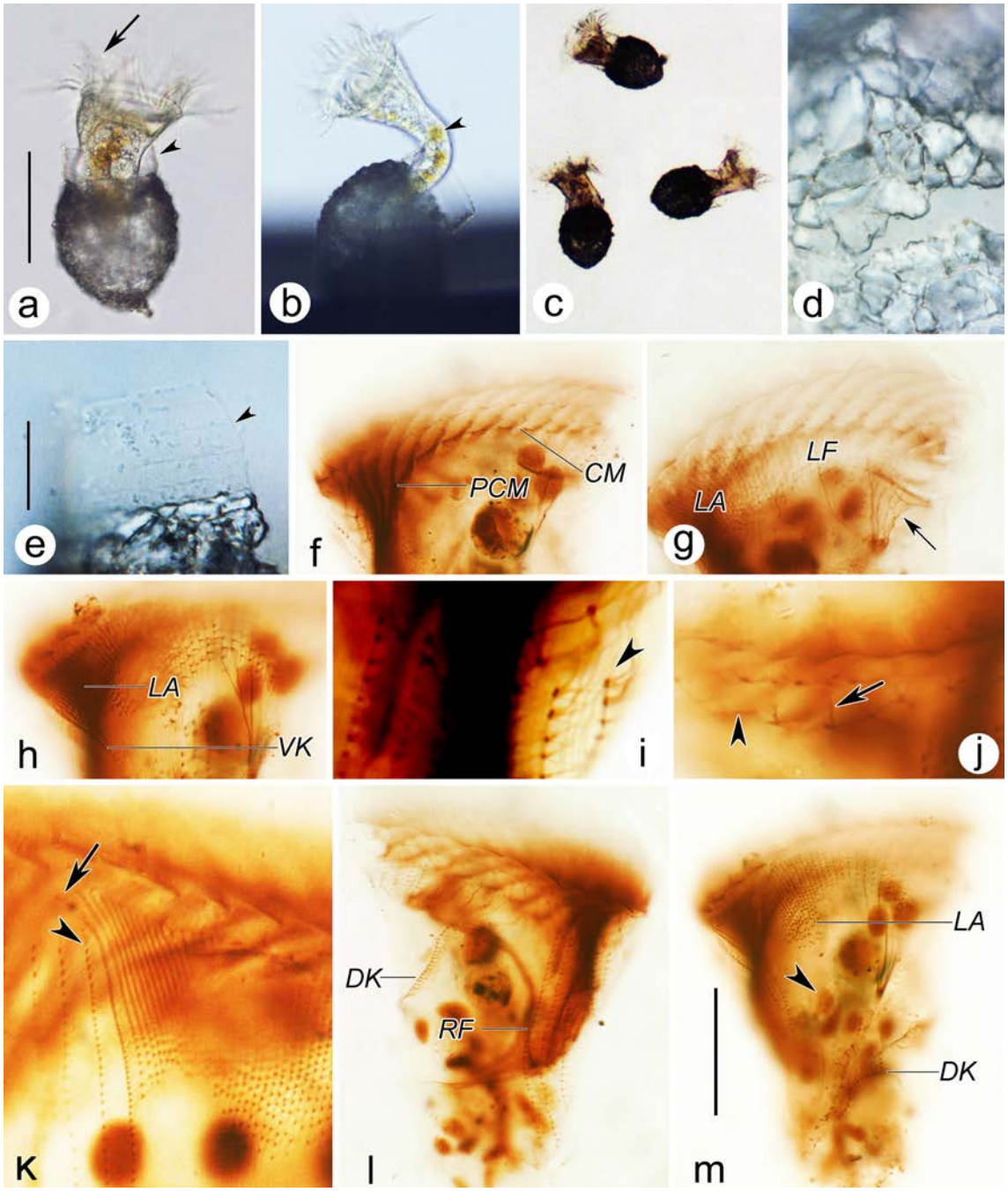

Fig. 4. *Codonellopsis mobilis* from life (a–e) and after staining with protargol (f–m). (a) A representative specimen from the neotype population; arrowhead shows the lucid collar portion, arrow denotes the pin-shaped tentaculoids. (b) A cell proper stretching out of the lorica, arrowhead indicates the microalgae in the cytoplasm. (c) Different individuals showing variations of body shape and size. (d) Lorica wall with numerous mineral particles. (e) Showing the annuli in the collar portion of lorica (arrowhead). (f) Details of adoral zone of membranelles. (g) Lateral view of specimen to show argyrophilic fibre bundles in the left ciliary field (arrow). (h) Partial details of anterior portion of the neotype, showing location and specific characters of the somatic kineties. (i) Anterior dikinetids in the right ciliary field (arrowhead). (j) Lateral view of the outer collar membranelles, showing the adoral ring (arrowhead) and fibres ring (arrow). (k–m) To show the arrangement the somatic kineties. (k) Ventral view with focal plane in the leftmost kinety of the right ciliary field (arrowhead) and ventral kinety (arrow). (l) Dorsal kinety and part of the right ciliary field. (m) Lateral view showing the lateral and left ciliary fields, arrowhead denotes macronuclear nodule. CM, collar membranelles; DK, dorsal kinety; LA, lateral ciliary field; LF, left ciliary field; PCM, prolonged collar membranelles; RF, right ciliary field; VK, ventral kinety. Bars, 40 µm (a, m), 20 µm (e).

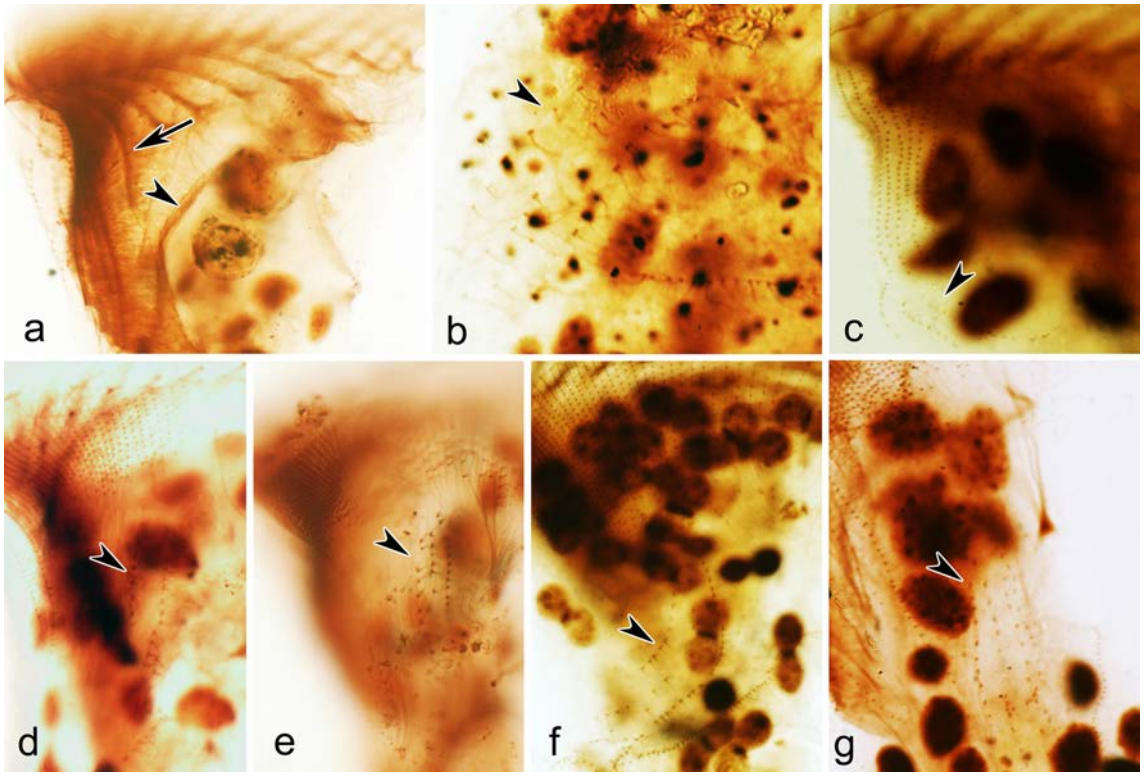

Fig. 5. *Codonellopsis mobilis*, specimens from Qingdao after protargol staining. (a) Lateral view of the buccal cavity to show the buccal membranelle (arrow) and endoral membrane (arrowhead). (b–g) Ventral views showing the highly variable kinetal fragments (arrowheads) in different specimens.

of 79–92 dikinetids, only posterior basal body of each kinetid bears a cilium that is about 3 μm long after protargol staining (Fig. 3b, c, f, Fig. 4i, m and Fig. 5d, f). Left ciliary field comprises 25–33 relatively densely spaced kineties, commences about 5 μm posteriorly to collar membranelles, left three and right ten of which are conspicuously shortened and progressively decrease in length in clockwise direction when viewed from apical aspect; all kineties composed of monokinetids and one anterior dikinetid; each basal body bears a 2 μm long cilium except the anterior basal body of the dikinetid which bears a 6 μm long cilium after protargol staining (Figs 3b, c, f and 4g, h, m). Several argyrophilic structures, probably fibrillar bundles, originate from collar polyketides and extend posteriorly to left ciliary field (Fig. 4f, g, m). Three to five kineties composed of tangled fragments posterior to left ciliary field, usually highly variable in length and position, comprising a mixture of monokinetids and dikinetids; usually only posterior basal body of each dikinetid bears a cilium that is 3 μm long, but sometimes both basal bodies are ciliated (Figs 3c and 5b–g). Lateral ciliary field commences about 3 μm posteriorly to collar membranelles and comprises 18–23 densely spaced kineties, most composed of about 50 densely spaced monokinetids except several middle kineties, which are slightly shortened and have fewer monokinetids; cilia in this field about 2 μm long (Figs 3b, c, f and 4h, k, m).

Oral apparatus forms a closed circle on anterior cell portion, orthogonal to main cell axis in contracted cells, composed of 18–20 collar membranelles with cilia up to 45 μm long and bases about 15 μm long; polykinetids of collar membranelles extend obliquely across peristomial rim, forming a contorted pattern, structure of polykinetids not recognizable (Figs 3a–c and 4). Two bundles of argyrophilic fibres associated with distal end of each collar membranelle, about 5 μm long, extend rightwards and leftwards and merge into neighbouring fibres underneath membranellar zone (Fig. 4j). Polykinetids of proximalmost seven collar membranelles extended 25–60 μm posteriorly in buccal cavity (Fig. 3b, f, Fig. 4f and Fig. 5a). Single buccal membranelle entirely within buccal cavity. Endoral membrane commences in dorsal portion of peristomial field and curves leftward for a long distance parallel to membranellar zone within buccal cavity (Fig. 5a).

The newly obtained SSU rRNA gene sequence was deposited in the GenBank database with length (bp), G+C content and accession number as follows: 1653, 47.1 mol%, MK799838.

Genus *Tintinnopsis* Stein 1867

Tintinnopsis chinglanensis **Nie & Ch'eng, 1947** (Figs 6a–e, 7a–k; Table 1)

Table 1. Morphometric characteristics of *Codonellopsis mobilis* (upper lines) and *Tintinnopsis chinglanensis* (lower lines).

Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; SE, standard error of arithmetic mean; CV, coefficient of variation in %; *n*, number of specimens examined.

Characteristic*	Min	Max	Mean	SD	SE	CV	*n*
Lorica, total length	112	121	117	3.1	0.82	2.6	25
	80	90	86	3.2	0.83	3.7	20
Lorica, collar length	20	25	23	4.3	0.88	18.5	25
	32	38	35	4.2	0.99	12.2	20
Lorica, bowl length	90	97	95	3.3	0.65	3.4	25
	48	54	52	3.5	0.75	6.7	20
Lorica, bowl width	85	92	87	4.6	0.97	5.2	22
	50	54	52	4.8	1.13	9.2	20
Lorica opening diameter, width	60	65	63	3.5	0.79	5.6	25
	33	36	35	2.5	0.52	7.1	20
Lorica, number of annulations in collar	4	6	5	0.4	0.12	8.0	25
	5	8	7	0.7	0.17	10.0	20
Cell proper, length	100	127	118	7.5	1.67	6.3	20
	35	45	40	2.7	0.70	6.8	18
Cell proper, width	65	75	69	3.8	0.82	5.5	20
	20	25	22	2.0	0.44	8.9	18
Macronuclear nodules, number	18	36	25	6.4	1.61	25.8	16
	2	2	2	0.0	0.00	0.0	15
Macronuclear nodule, length	6	12	9	2.0	0.46	19.9	16
	8	12	9	1.1	0.29	11.0	15
Macronuclear nodule, width	6	9	7	0.8	0.21	10.9	16
	4	6	5	0.5	0.13	10.6	15
Number of somatic kineties in total	55	63	60	1.7	0.89	2.8	15
	30	33	32	1.1	0.32	4.5	16
Ventral kinety, length	48	62	53	6.1	1.18	11.5	16
	19	28	24	1.9	0.58	7.9	15
Ventral kinety, distance to collar membranelles	2	5	3	0.4	0.19	13.3	16
	1	3	2	0.3	0.15	10.0	15
Ventral kinety, number of kinetids	80	102	93	7.3	2.31	7.8	16
	32	35	33	1.2	0.31	3.4	15
Posterior kinety, length†	15	18	16	1.2	0.38	7.3	10
Posterior kinety, number of dikinetids†	13	17	14	1.3	0.12	16.0	10
Posterior kinety, distance to end posterior of ventral kinety†	1	3	2	0.2	0.09	10.0	10
Right ciliary field, number of kineties	10	12	10	0.8	0.21	7.7	16
	9	10	9	0.5	0.12	5.4	15
Leftmost kinety in right ciliary field, distance to collar membranelles	4	6	5	0.4	0.45	8.0	10
	5	8	6	0.6	0.26	10	11
Dorsal kinety, length	85	96	90	3.8	0.98	4.1	18
	50	60	54	3.5	0.83	6.5	18
Dorsal kinety, number of kinetids	79	92	84	3.7	0.94	4.4	16
	24	30	26	1.8	0.85	7.0	15
Dorsal kinety, distance to collar membranelles	5	10	7	0.7	0.69	9.8	16
	4	9	6	0.8	0.75	13.3	15
Dorsal kinety, distance to first kinety of left ciliary field	9	16	12	1.5	0.92	12.5	16
	3	8	5	0.9	0.78	18	15

Continued

Table 1. Continued

Characteristic*	Min	Max	Mean	SD	SE	CV	*n*
Left ciliary field, number of kineties	25	33	28	2.8	0.71	9.7	15
	8	10	9	0.5	0.13	9.4	16
Lateral ciliary field, number of kineties	18	23	20	1.7	0.42	8.2	16
	13	16	14	1.0	0.22	6.6	20
Adoral zone of membranelles, diameter	62	71	68	2.8	0.75	4.1	12
	19	24	22	1.4	0.28	6.4	13
Collar membranelles, number	18	20	19	0.9	0.21	5.0	20
	18	22	20	1.5	0.33	7.4	20
Prolonged collar membranelles, number	7	7	7	0.0	0.00	0.0	20
	3	3	3	0.0	0.00	0.0	19
Buccal membranelles, number	1	1	1	0.0	0.00	0.0	17
	1	1	1	0.0	0.00	0.0	15

*Lorica data are based on living observations, and others are based protargol-stained specimens. The measurements were taken at 200–1000× magnifications in μm.
†Measurements were taken from specimens of *Tintinnopsis chinglanensis*.

Previous descriptions

1947 *Tintinnopsis chinglanensis* n. sp. – Nie and Ch'eng, Cont. Biol. Lab. Sci. Soc. China, 16 : 41–86

1952 *Tintinnopsis chinglanensis* Nie & Ch'eng, 1947 – Yin, J. Shandong Univ. (Phil. Soc. Sci.), 2 : 36–56

Improved diagnosis (based on original description and this study)

Lorica on average 80–120 μm long, with an opening diameter of 30–40 μm; composed of a cylindrical collar with about seven annuli and a subspherical bowl that is about 50–55 μm wide. Cell proper sub-obconical, on average 45×25 μm *in vivo*. Two ellipsoidal macronuclear nodules. On average 31 somatic kineties. Ventral kinety consists of about 33 monokinetids, commences anteriorly to first kinety of right ciliary field. Dorsal and posterior kineties with about 26 and 14 dikinetids, respectively. On average nine kineties in right ciliary field, nine in left ciliary field and 20 in lateral ciliary field. About 23 collar membranelles, three of which extend into buccal cavity; one buccal membranelle.

Ecological features

Water temperature 27 °C, salinity 30 ‰ and pH 7.5.

Deposition of neotype and voucher specimens

A protargol slide with the neotype specimen (Figs 6b, c, 7i, j) was deposited in the Natural History Museum, London, UK, with registration number NHMUK 2020.1.23.2. Another protargol slide (registration number: WR2017051701–2) with voucher specimens was deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, PR China.

Description of *Tintinnopsis chinglanensis*

Lorica about 80–90×50–55 μm, agglutinated, composed of a subspherical bowl and a cylindrical collar. Collar always shorter than bowl portion, occupies about 40 % of lorica length, with a diameter of 33–36 μm, not flaring, usually bulging out at the basal portion, with about five to eight spiral turns, slightly increasing in width towards the bowl. Bowl generally subspherical, posterior end rounded, rarely slightly pointed (Figs 6a, 7a–d).

Cell proper elongate sub-obconical, about 40–50×20–25 μm when fully extended, posterior end narrowed and always forming a peduncle, which is about 40 μm long, and attaches to bottom of lorica. Cell proper contractile, on average 35–45×20–25 μm after protargol staining (Fig. 7e). Two ellipsoidal, macronuclear nodules, about 8–12×4–6 μm, centrally located in cytoplasm (Fig. 7e); micronuclei not observed, probably due to being insufficiently stained with protargol. Contractile vacuole, cytopyge, striae, tentaculoids, and accessory combs not recognized in either live or preserved specimens. Cytoplasm colourless, with food vacuoles of various size (up to 20 μm) containing microalgae (Fig. 7a, b). Locomotion by rotating about main cell axis. Cell proper retracts into lorica by contractile peduncle when disturbed (Fig. 7c).

Somatic ciliary pattern comprises single ventral, dorsal and posterior kineties as well as right, left, and lateral ciliary fields (Figs 6b–e, 7e–k). Ventral kinety 19–28 μm long, commences 2 μm posteriorly to collar membranelles and about 2 μm anteriorly to first kinety of right ciliary field, extends posteriorly with a conspicuously rightward curve in last quarter, composed of 32–35 densely spaced monokinetids, each of which bears a cilium that is 1 μm long after protargol staining (Figs 6b, e, 7i). Most kineties of right ciliary field commence about 4–5 μm posteriorly to

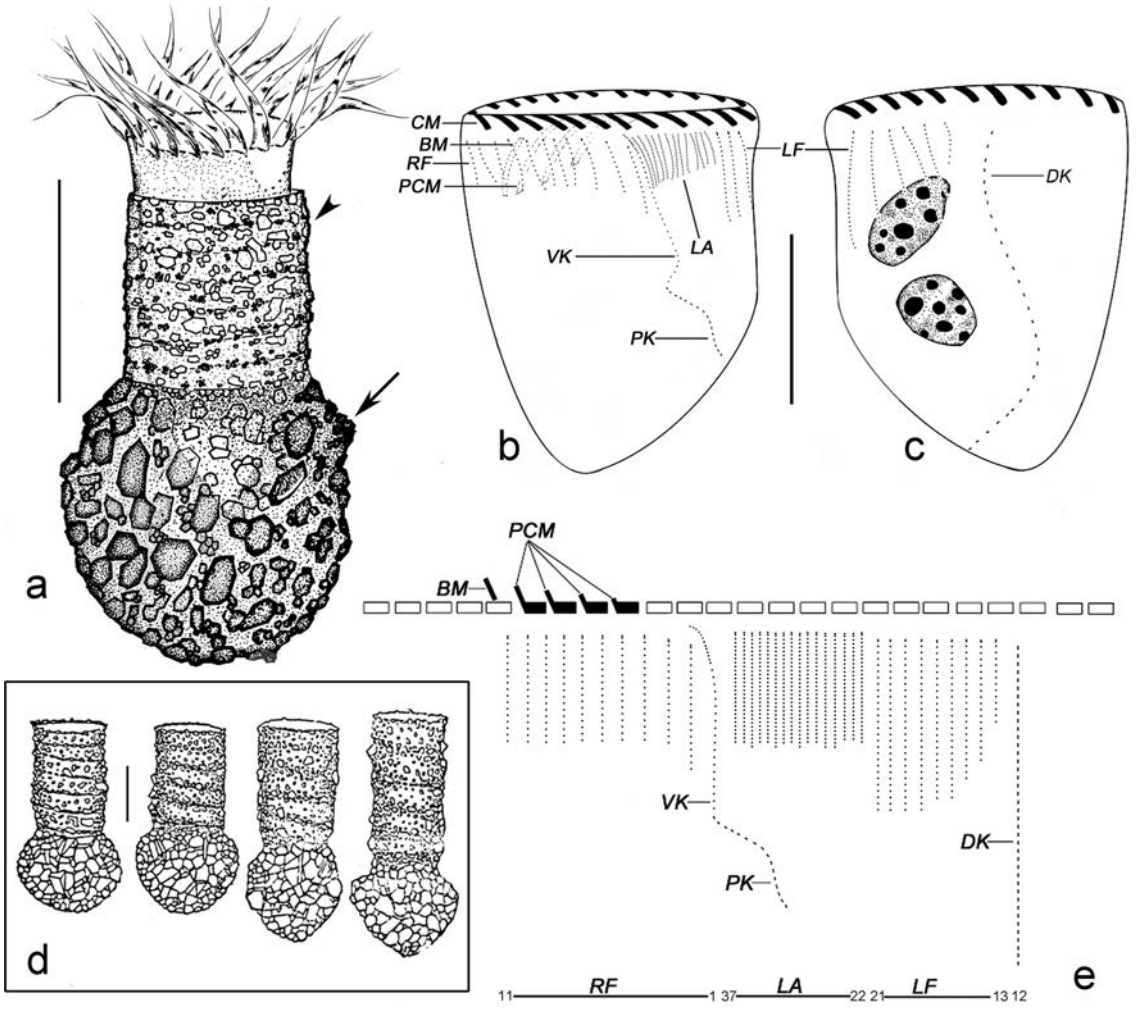

Fig. 6. *Tintinnopsis chinglanensis* from life (a, d) and after staining with protargol (b, c, e). (a) Lateral view of a representative individual, arrowhead and arrow show collar and bowl portion, respectively. (b, c) Ventral (b) and dorsal (c) side views of the neotype specimen, showing ciliary pattern and macronuclear nodules. (d) Variations of lorica of *Tintinnopsis chinglanensis* sampled from Ching-lan-kong (from [73]). (e) Kinetal map of a morphostatic specimen. BM, buccal membranelle; CM, collar membranelles; DK, dorsal kinety; LA, lateral ciliary field; LF, left ciliary field; PCM, prolonged collar membranelles; PK, posterior kinety; RF, right ciliary field; VK, ventral kinety. Bars, 40 µm (a), 20 µm (b, c), 25 µm (d).

collar membranelles except for the leftmost kinety, which commences 6 µm posteriorly to the collar membranelles; nine or ten widely spaced kineties, 12 µm long and 4–7 µm apart; each kinety composed of monokinetids and one anterior dikinetid; each kinetid has a cilium about 3 um long after protargol staining (Figs 6b, c, 7i). Dorsal kinety about 55 µm long, commences 6 µm posteriorly to collar membranelles, anterior portion extends rightwards, posterior portion extends leftwards; terminates at posterior end of cell proper, composed of 24–30 dikinetids, each kinetid bears a cilium about 2 µm long in protargol preparations (Figs 6c, e, 7j). Left ciliary field separated from collar membranelles by broad stripe, 4 µm wide, composed of eight to ten somatic kineties, each composed of monokinetids and one anterior dikinetid; each monokinetid and posterior basal body of each dikinetid bears a cilium that is about 2 µm long after protargol staining (Figs 6b, c, 7i, j). Lateral ciliary field composed of 13–16 densely spaced kineties, commences 3 µm posteriorly to collar membranelles, each kinety composed of about 20 densely spaced monokinetids (with 1 µm long cilia), and extends parallel to curved ventral kinety (Figs 6b, e, 7i, h). Posterior kinety commences 1 µm below posterior end of ventral kinety, extends leftward to posterior third of cell proper, composed of 13–17 dikinetids, only posterior basal body of each dikinetid bears a cilium that is about 2 µm long (Figs 6b, c, 7h).

Oral apparatus forms a closed circle at anterior cell portion, consisting of 21–24 collar membranelles of which three are significantly prolonged; cilia of membranelles about 25 µm long, structure of polykinetids non-recognizable; single

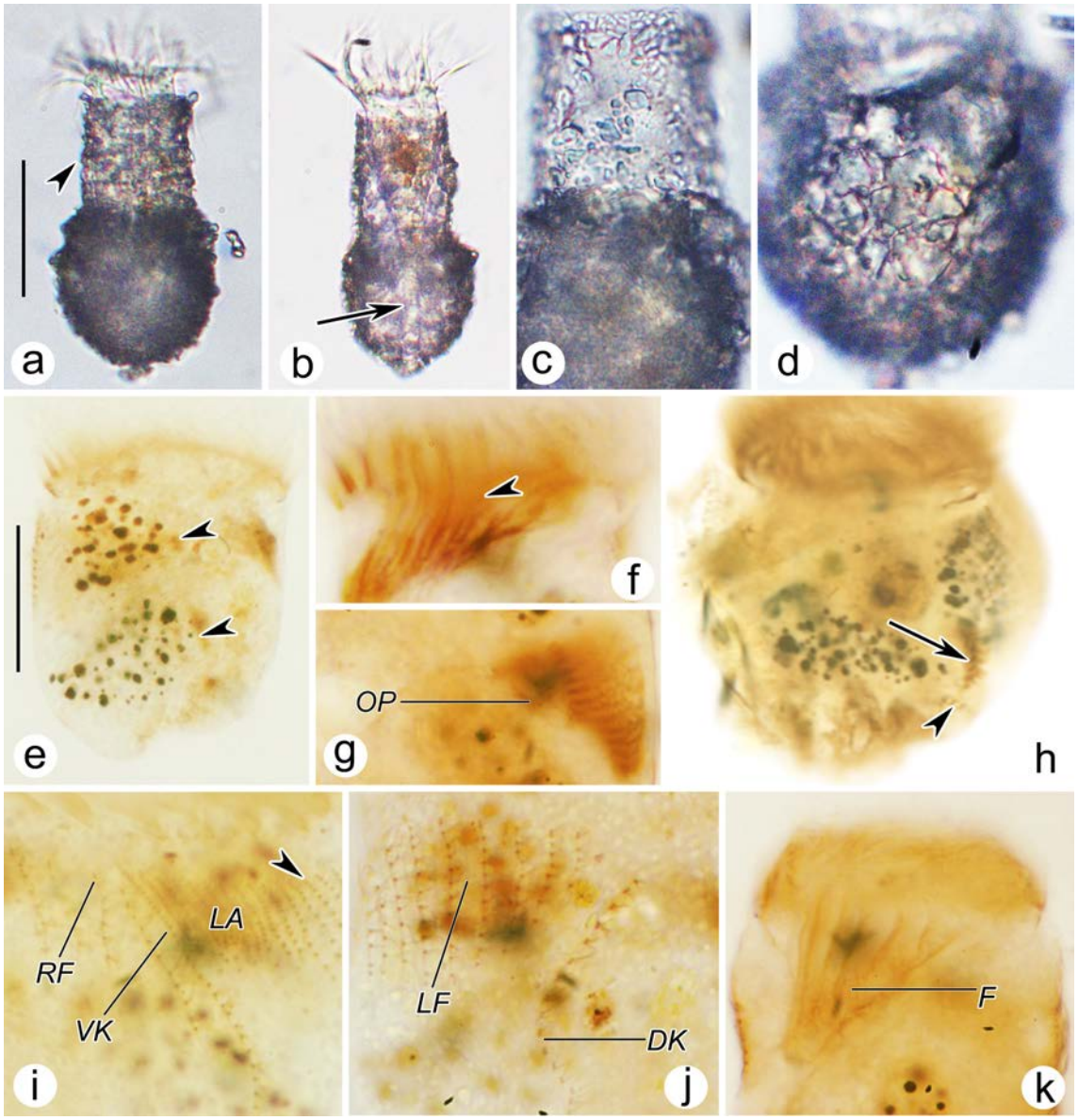

Fig. 7. *Tintinnopsis chinglanensis* from life (a–d) and after protargol staining (e–k). (a) A representative specimen, arrowhead denotes the visible annulation on surface of collar. (b) Lateral view showing the cell proper attached the bottom of lorica via the peduncle (arrow). (c) Details of the agglutinated collar of lorica. (d) Lorica wall with numerous mineral particles. (e) Lateral view to show macronuclear nodules (arrowheads). (f) Showing prolonged collar membranelles (arrowhead). (g) Details of oral primordium. (h) Oblique top view of an early divider, showing the location of oral primordium (arrow), arrowhead denotes the location of the commencement of the posterior kinety. (i, j) Ventral and dorsal views of the same specimen, showing the ciliary pattern, arrowhead denotes left ciliary field. (k) Details of fibrillar structures associated with collar membranelles. DK, dorsal kinety; F, fibrillar structures; LA, lateral ciliary field; LF, left ciliary field; OP, oral primordium; RF, right ciliary field; VK, ventral kinety. Bars, 40 μm (a), 20 μm (e).

buccal membranelle entirely within buccal cavity (Figs 6b, c, 7f). Endoral membrane not observed in protargol preparations. Several fibrillar bundles originate from proximal portion of collar polykinetids and extend posteriorly, terminating at centre of cell proper (Fig. 7k).

An early divider was observed in which the oral primordium is located posteriorly to the lateral ciliary field (Fig. 7g, h).

The newly obtained SSU rRNA gene sequence was deposited in the GenBank database with length (bp), G+C content and accession number as follows: 1643, 46.7 mol%, MK799839.

SSU rRNA gene sequence and phylogeny

We compared all available SSU rRNA gene sequences of the genus *Codonellopsis* (Figs 2 and 8). After deleting

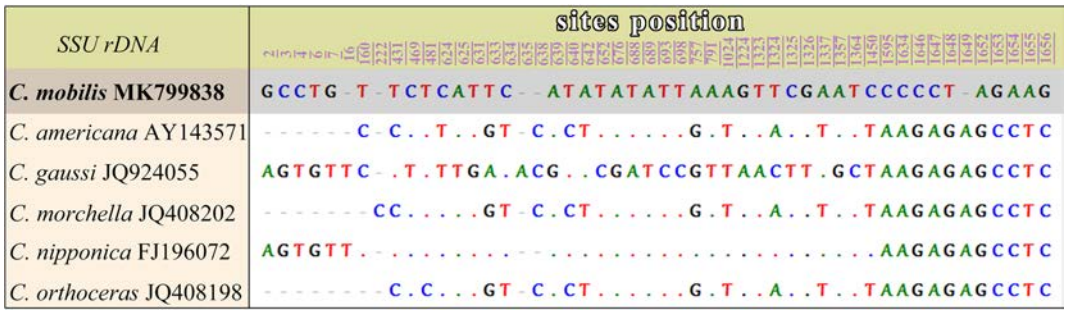

Fig. 8. Comparison of SSU rRNA gene sequences of six species of *Codonellopsis* showing the unmatched nucleotides between *Codonellopsis mobilis* and five congeners (with GenBank accession numbers). Nucleotide positions are given at the top of each column. Insertions and deletions are compensated by introducing alignment gaps (–). Matched sites are represented by dots (.).

both ends of the alignments (1656 bp), the numbers of unmatched sites and sequence similarities were calculated (Fig. 8), i.e., *C. americana*, *C. morchella*, and *C. orthoceras* have a close relationship that differing from each other by 1–3 nucleotides, and are distinct from sequences of other congeners; *C. mobilis* differs from *C. nipponica*, *C. gaussi* and *C. americana* in 17, 50 and 24 nucleotides, respectively; *C. gaussi* differs from its congeners by 25–50 nucleotides.

As the tree topologies inferred from the ML and BI algorithms are similar, only the ML tree is presented with support values from both methods at the branch nodes (Fig. 2). Phylogenetic analyses indicate but do not statistically support, the monophyly of the family Dictyocystidae. *Codonellopsis mobilis* clusters with *C. nipponica* with full support, forming a sister branch to the cluster of *C. americana*, *C. morchella*, and *C. orthoceras. Codonellopsis gaussi* branches far from them. However, the AU test (P=0.213>0.05) did not reject the monophyly of *Codonellopsis. Tintinnopsis chinglanensis* clusters with *T. lohmanni* in a clade that is sister to *T. kiangsuensis* with full support (100 % ml, 1.00 BI).

DISCUSSION

Remarks on *Codonellopsis*

Jörgensen [17] established the genus *Codonellopsis* for five *Codonella* species that share a hyaline, spiral collar, viz., *C. lusitanica*, *C. lagenula C. morchella*, *C. orthoceras* (the type species) and *C. tuberculata*. Based on the lorica characters, Kofoid & Campbell [13] revised the genus and assigned 18 species. In later studies, 41 additional species were reported from various places around the world, rendering *Codonellopsis* the largest genus in the family Dictyocystidae [61–70].

Identification of Qingdao population of *Codonellopsis mobilis*

This large *Codonellopsis* species was originally established by Wang [63] for a population isolated from the Gulf of Pe-Hai, but with only a short description based exclusively on lorica features (Fig. 3e). Our specimens from Qingdao correspond well with the original population in the main diagnostic features, i.e. lorica structure (a hyaline collar and an agglomerated bowl portion) and shape (a cylindrical collar and an obovoidal bowl), opening diameter of lorica (60–65 vs. 66–68 μm), lorica width (85–92 vs. 84–92 μm), and number of spiral turns (4–6 vs. 4–10). The Qingdao population does, however, differ from the original population in having: (1) a shorter collar (20–25 vs. 30–48 μm); (2) a shorter bowl (90–97 vs. 105–110 μm); and (3) a constant lorica shape (vs. lorica shape variable). In our opinion, both forms are conspecific since these differences could be considered as environment- and/or population-dependent.

Yin [68] recorded a Jiaozhou Bay population of *C. mobilis* which is similar to our specimens in terms of the lorica shape (see '(Fig. 3d), length of bowl portion (98–118 vs. 90–97 μm), and diameter of opening (62–86 vs. 60–65 μm). However, the former shows significant differences by having larger ranges of collar length (22–118 μm), number of anuli around the collar (3–22), and diameter of opening (62–86 μm), the latter two being key characters for identifying tintinnines at species level. Therefore, we suppose that Yin [68] very likely did not distinguish the present species from other forms.

Comments on Qingdao population of *Codonellopsis mobilis*

Since lorica shape and opening diameter are key characters for species discrimination among tintinnine ciliates, we focus on species that resemble *C. mobilis* in these respects. *Codonellopsis nipponica* Hada, 1964 is similar to *C. mobilis* in all main lorica features including: wide collar without windows; bowl surface roughened with agglomerated particles; length of bowl (88–100 vs. 90–97 μm); width of bowl (85–87 vs. 85–92 μm); diameter of opening (57–62 vs. 60–65 μm); and number of spiral turns (5–6 vs. 4–6). *Codonellopsis nipponica* can be morphologically separated from our specimens by inflating obviously in the posterior of collar. Significantly, the Qingdao population of *C. mobilis* clusters with *C. nipponica* (FJ196072) in the SSU rRNA gene tree, these having a sequence similarity of 99.82 % (1760 bp). However, based on the micrograph provided by Li *et al.* [71], the latter might be misidentified as it does not have an inflated collar, which is a key character of *C.*

nipponica. Therefore, the SSU rRNA gene sequence FJ196072 is probably not that of *C. nipponica*.

Codonellopsis mobilis has several kinetal fragments posterior to the left ciliary field, the structure of which is atypical for the genus *Codonellopsis*. It is notable that this unusual ciliary pattern has been reported in *Eutintinnus pectinis* and *Cymatocylis calyciformis* [39, 45]. However, the latter two species can be separated from *C. mobilis* by their distinct lorica features and genus-level SSU rRNA gene sequence divergence [72]. In addition, the high number of macronuclear nodules in *C. mobilis* and [13], which probably evolved independently from the oligotrichid family Tontoniidae [21] is unique among all tintinnines investigated so far, The structure of the kineties in the right ciliary field of our specimens also deviates from previously studied patterns by having two to seven anterior dikinetids (to be verified by SEM), whereas the recorded tintinnines always have one anterior dikinetid. These findings for *C. mobilis* suggest that further investigations are needed to determine its systematic assignment. Hitherto, cell features were known for only two *Codonellopsis* species, i.e., *C. glacialis* (Weddell Sea population) and *C. gaussi* (Amundsen Sea population), which may be synonymous based on their infraciliature data [39, 45]. Both can be separated from *C. mobilis* by lacking the special features mentioned above and in having fewer prolonged collar membranelles (3 vs. 7), macronuclear nodules (4 vs. ~25), and somatic kineties (~21–26 vs. 60). Furthermore, the SSU rRNA gene sequence of *C. gaussi* (JQ924055) has a dissimilarity of 6.1 % (101 bp) compared with our new sequence of *C. mobilis*, which represents a genus-level divergence in tintinnine ciliates according to Santoferrara *et al.* [72]. The difference in the morphology of *C. mobilis* and *C. gaussi* is consistent with the genetic variation.

In the SSU rRNA gene tree, *Codonellopsis gaussi* is only distantly related to *C. mobilis* clustering instead with the remaining three *Codonellopsis* species for which sequence data are available, viz., *C. americana, C. morchella,* and *C. orthoceras*, with high support. Unfortunately, ciliature data of the latter three are lacking, therefore a revision for *Codonellopsis* is currently impossible because cytological and molecular characters are known for less than 10 % of species in this genus.

Identification of Haikou population of *Tintinnopsis chinglanensis*

Tintinnopsis chinglanensis, originally described by Nie & Ch'eng [73] based on lorica characteristics, was sampled from Ching-lan-kong in Hainan Island during 1932–1933. The Haikou population described here was identified as *Tintinnopsis chinglanensis* as it is similar to the type population in the following features: (1) shape of lorica (tubular collar with several annulations and a bulging bowl); (2) length of lorica (81–112 vs. 80–90 μm); (3) diameter of opening (31–35 vs. 33–36 μm); (4) presence of irregular foreign particles on the wall of the lorica. Although there are some minor differences between the two populations, i.e., collar length (32–38 vs. 48–76 μm in the original population) and bowl size (50–54 vs. 30–40 μm in the original population), we believe that these are population- or individual-dependent differences, especially as Nie and Ch'eng mentioned that collar length varies significantly. Yin [68] made a redescription based on specimens from Jiaozhou Bay, which corresponds well with the original population. Since all key lorica characters of our specimens, i.e., size and shape *in vivo*, opening diameter, and number of spiral turns, are consistent with both the original description and redescription [68, 73], the identity of the present population is not in doubt.

Comments on Haikou population of *Tintinnopsis chinglanensis*

In terms of the lorica size and shape, our specimens resemble four *Tintinnopsis* species, viz., *T. lohmanni* Laackmann, 1906, *T. pistillum* Kofoid & Campbell, 1929, *T. spiralis* Kofoid & Campbell, 1929, and *T. subacuta* Jörgensen, 1899. *Tintinnopsis lohmanni* is distinguished from our specimens by having a much larger lorica opening (50–57 vs. 33–36 μm in diameter), which is a good feature for interspecific discrimination as the diameter of lorica opening shows intraspecific stability. The current phylogenetic analyses revealed that *T. chinglanensis* is very closely related to *T. lohmanni* (FJ196076) from which its SSU rRNA gene sequence differs only by two nucleotides. However, we believe that the latter was misidentified by Li *et al.* [68] and is likely a population of *T. chinglanensis* because it corresponds very well in the diameter of the lorica opening. *Tintinnopsis pistillum* differs from *T. chinglanensis* by having more annulations (15–17 vs. 7) and a conspicuously shorter bowl portion (20 vs. 48–54 μm) [13]. *Tintinnopsis spiralis* can be separated from *T. chinglanensis* by having distinct flaring below the rim of the suboral region of lorica, and more spiral turns (10–12 vs. 7) [13]. Although *Tintinnopsis subacuta* clusters closely with *T. chinglanensis* in the phylogenetic tree (Fig. 2), it can be distinguished from the latter by the presence of an aboral point, a marked aboral expansion and a 3 % divergence in its SSU rRNA gene sequence [72]. Based on the cytological features of *T. subacuta* reported by Pierce [74] in his unpublished Ph.D. thesis, further differences with *T. chinglanensis* include: (1) the presence (vs. absence) of an extraordinary long ciliary tuft that extends outside the lorica; (2) the position of the posterior kinety below the lateral ciliary field (vs. below the ventral kinety); (3) the number of macronuclear nodules (8 vs. 2).

The complex ciliary pattern of tintinnine ciliates, which is composed of a right, left and lateral ciliary fields as well as a ventral, dorsal, and posterior kinety, has been reveled in a wide variety of taxa at genus level, and the posterior kinety is generally located posteriorly to either the left or the lateral ciliary field. In our specimens, the posterior kinety commences posteriorly to the ventral kinety. This arrangement is so far known only in *Tintinnopsis tocantinensis* Kofoid & Campbell, 1929, which differs from our specimens in its distinctive lorica (i.e., lorica composed of a cylindrical portion, a bulbous part and a tapered portion vs. a subspherical bowl and cylindrical collar), and the position at which the ventral kinety commences (i.e., anterior to the third or fourth kinety of right ciliary field vs. anterior to the first kinety) [13].

Although *T. kiangsuensis* (JN831850) shows a closer relationship with *T. chinglanensis* in the SSU rRNA gene tree, this finding is likely dubious as *T. kiangsuensis* was originally described from a freshwater biotope whereas the sequenced specimens were collected from marine coastal waters of Argentina. Furthermore, the specimens identified as *T. kiangsuensis* can be separated from our specimens by its campanulate lorica. The non-monophyly of *Tintinnopsis* revealed here is consistent with previous studies [21, 22, 24]. Nevertheless, the genus cannot be revised currently as morphological features of the cell and genetical data are lacking for most species, including the type species.

Neotypification

The main justifications for neotypification of *Codonellopsis mobilis* and *Tintinnopsis chinglanensis* are the lack of type specimens of either species and the close proximities of the neotype localities to the original localities. *Codonellopsis mobilis* was originally isolated from the Gulf of Pe-Hai, now known as the Bohai Sea, which is the northwestern and innermost extension of the Yellow Sea [63]. The population described in the present study was isolated from coastal waters at Qingdao, which is on the Yellow Sea coast of northern PR China adjacent to the Bohai Sea. *Tintinnopsis chinglanensis* was originally isolated from Ching-lan-kong, Hainan Island [73]. The population described in the present study was isolated from coastal waters of Haikou, which is also on Hainan Island. Thus, in both cases, the neotype locality is in the same geographical region as the original thereby meeting a key qualifying condition for neotypification as stated in the International Code of Zoological Nomenclature, i.e., Article 75.3.6, 'that the neotype came as nearly as practicable from the original type locality' [75].

Funding information
This work was supported by the Natural Science Foundation of China (project numbers: 41776133, 31772431, 31801955).

Acknowledgements
Many thanks are due to Dr Peter Vďačný and two anonymous reviewers for their helpful and constructive comments that greatly improved our work. We thank Ms Tengteng Zhang (OUC) for sequencing *Tintinnopsis chinglanensis*. We should also like to thank Professor Weibo Song (OUC) for his constructive suggestion in drafting the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Fenchel T. The microbial loop – 25 years later. *J Exp Mar Bio Ecol* 2008;366:99–103.
2. Caron DA, Countway PD, Jones AC, Kim DY, Schnetzer A. Marine protistan diversity. *Ann Rev Mar Sci* 2012;4:467–493.
3. Worden AZ, Follows MJ, Giovannoni SJ, Wilken S, Zimmerman AE *et al*. Environmental science. rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science* 2015;347:1257594.
4. Gao F, Warren A, Zhang Q, Gong J, Miao M *et al*. The all-data-based evolutionary hypothesis of ciliated protists with a revised classification of the phylum Ciliophora (Eukaryota, Alveolata). *Sci Rep* 2016;6:24874.
5. Hu X, Lin X. *Song WB Ciliate atlas: Species found in the South China Sea*. Beijing: Science Press; 2019.
6. Liu W, Xu D, Ma H, Al-Farraj SA, Warren A *et al*. Taxonomy and molecular systematics of three oligotrich (s.l.) ciliates including descriptions of two new species, *Strombidium guangdongense* sp. nov. and *Strombidinopsis sinicum* sp. nov. (Protozoa, Ciliophora). *Systematics and Biodiversity* 2016;14:452–465.
7. Liu WW, Zhang KX, Chen CZ, JQ L, Tan YH *et al*. Overview of the biodiversity and geographic distribution of aloricate oligotrich ciliates (Protozoa, Ciliophora, Spirotrichea) in coastal waters of southern China. *Syst Biodivers* 2019;18:1–14.
8. Song W, Li J, Huang Y, Al-Rasheid KAS, Liu W *et al*. Morphology of three Aloricate Choreotrich ciliates, including description of a new species *Parastrombidinopsis costalis* sp. N. (Ciliophora, Choreotrichia), and phylogeny of the genus *Parastrombidinopsis*. *AP* 2018;57:153–167.
9. Song W, Xu D, Zhang Q, Liu W, Warren A *et al*. Taxonomy and phylogeny of two poorly studied genera of marine oligotrich ciliates including descriptions of two new species: *Cyrtostrombidium paraboreale* sp. n. and *Apostrombidium orientale* sp. n. (Ciliophora: Spirotrichea). *Eur J Protistol* 2019;70:1–16.
10. Song WB, Warren A, Hu X. *Free-living ciliates in the Bohai and Yellow Seas, China*. Beijing: Science Press; 2009.
11. Wang R, Song W, ZZ Y, Lin XF, Al-Rasheid KAS *et al*. Morphology and molecular phylogeny of two new species of *Spirostrombidium* (Ciliophora, Oligotrichia), with a key to species in the genus. *Syst Biodivers* 2018;16:743–756.
12. Jörgensen E. Mediterranean Tintinnidae: report on the Danish oceanographical expeditions 1908—1910 to the Mediterranean and adjacent seas. *Vol II Biology* 1924;3:1–110.
13. Kofoid CA, Campbell AS. A conspectus of the marine and freshwater Ciliata belonging to suborder Tintinnoinea, with descriptions of the new species, principally from the Agassiz expedition to the eastern tropical Pacific, 1904–1905. *Univ Calif Publs Zool* 1929;34:1–403.
14. Kofoid CA, Campbell AS. Reports on the scientific results of the expedition to the eastern tropical Pacific, in charge of Alexander Agassiz, by the U. S. Fish Commission Steamer "Albatross," from October, 1904, to March, 1905, Lieut.-Commander L. N. Garrett, U. S. N., Commanding. XXXVII. The Ciliata: The Tintinnoinea. *Bull Mus comp Zool Harv* 1939;84:1–473.
15. Hofker J. Studien uber Tintinnidea. *Arch Protistenk* 1931;3:315–402.
16. Laval-Peuto M, Gold K, Storm ER. The ultrastructure of *Tintinnopsis parva*. *Trans Am Microsc Soc* 1979;98:204–212.
17. Kim SY, Yang EJ, Gong J, Choi JK. Redescription of *Favella ehrenbergii* (Claparède and Lachmann, 1858) Jörgensen, 1924 (Ciliophora: Choreotrichia), with phylogenetic analyses based on small subunit rRNA gene sequences. *J Eukaryot Microbiol* 2010;57:460–467.
18. Agatha S, Riedel-Lorjé JC. Redescription of *Tintinnopsis cylindrica* Daday, 1887 (Ciliophora: Spirotricha) and unification of Tintinnid terminology. *Acta Protozool* 2006;45:137–151.
19. Santoferrara LF, Tian M, Alder VA, McManus GB. Discrimination of closely related species in tintinnid ciliates: new insights on crypticity and polymorphism in the genus *Helicostomella*. *Protist* 2015;166:78–92.
20. Laval-Peuto M, Brownlee DC. Identification and systematics of the Tintinnina (Ciliophora): evaluation and suggestions for improvement. *Annls Inst océanogr Paris* 1986;62:69–84.
21. Agatha S, Strüder-Kypke MC. Phylogeny of the order Choreotrichida (Ciliophora, Spirotricha, Oligotrichea) as inferred from morphology, ultrastructure, ontogenesis, and SSrRNA gene sequences. *Eur J Protistol* 2007;43:37–63.
22. Agatha S, Strüder-Kypke MC. Reconciling cladistic and genetic analyses in choreotrichid ciliates (Ciliophora, Spirotricha, Oligotrichea). *J Eukaryot Microbiol* 2012;59:325–350.
23. Santoferrara LF, Bachy C, Alder VA, Gong J, Kim Y-O *et al*. Updating biodiversity studies in loricate protists: the case of the

tintinnids (Alveolata, Ciliophora, Spirotrichea). *J Eukaryot Microbiol* 2016;63:651–656.

24. Santoferrara LF, Alder VV, McManus GB. Phylogeny, classification and diversity of Choreotrichia and Oligotrichia (Ciliophora, Spirotrichea). *Mol Phylogenet Evol* 2017;112:12–22.
25. Santoferrara LF, Rubin E, Mcmanus GB. Global and local DNA (meta)barcoding reveal new biogeography patterns in tintinnid ciliates. *J Plankton Res* 2018;40:209–221.
26. Strüder-Kypke MC, Lynn DH. Morphological versus molecular data–phylogeny of tintinnid ciliates (Ciliophora, Choreotrichia) inferred from small subunit rRNA gene sequences. *Denisia* 2008;23:417–424.
27. Wang Y, Wang C, Jiang Y, Katz LA, Gao F *et al*. Further analyses of variation of ribosome DNA copy number and polymorphism in ciliates provide insights relevant to studies of both molecular ecology and phylogeny. *Sci China Life Sci* 2019;62:203–214.
28. Xu D, Sun P, Shin MK, Kim YO. Species boundaries in tintinnid ciliates: a case study--morphometric variability, molecular characterization, and temporal distribution of *Helicostomella* species (Ciliophora, Tintinnina). *J Eukaryot Microbiol* 2012;59:351–358.
29. Agatha S, Tsai S-F. Redescription of the tintinnid *Stenosemella pacifica* Kofoid and Campbell, 1929 (Ciliophora, Spirotricha) based on live observation, protargol impregnation, and scanning electron microscopy. *J Eukaryot Microbiol* 2008;55:75–85.
30. Agatha S. Redescription of the tintinnid ciliate *Tintinnopsis fimbriata* Meunier, 1919 (Spirotricha, Choreotrichida) from coastal waters of northern Germany. *Denisia* 2008;23:261–272.
31. Agatha S. A light and scanning electron microscopic study of the closing apparatus in tintinnid ciliates (Ciliophora, Spirotricha, Tintinnina): a forgotten synapomorphy. *J Eukaryot Microbiol* 2010;57:297–307.
32. Agatha S. Redescription of *Tintinnopsis parvula* Jörgensen, 1912 (Ciliophora: Spirotrichea: Tintinnina), including a novel Lorica matrix. *Acta Protozool* 2010;49:213–234.
33. Bai Y, Wang R, Liu W, Warren A, Zhao Y *et al*. Redescriptions of three tintinnine ciliates (Ciliophora: Tintinnina) from coastal waters in China based on lorica features, cell morphology, and rDNA sequence data. *Eur J Protistol* 2019;72:125659.
34. Choi JKI, Coats DW, Brownlee DC, Small EB. Morphology and Infraciliature of Three Species of *Eutintinnus* (Ciliophora; Tintinnina) with Guidelines for Interpreting Protargol-Stained Tintinnine Ciliates. *J Protozool* 1992;39:80–92.
35. Ganser MH, Agatha S. Redescription of *Antetintinnidium mucicola* (Claparède and Lachmann, 1858) nov. gen., nov. comb. (Alveolata, Ciliophora, Tintinnina). *J Eukaryot Microbiol* 2019;66:802–820.
36. Gruber MS, Strüder-Kypke M, Agatha S. Redescription of *Tintinnopsis everta* Kofoid and Campbell 1929 (Alveolata, Ciliophora, Tintinnina) based on taxonomic and genetic analyses–discovery of a new complex ciliary pattern. *J Eukaryot Microbiol* 2018;65:484–504.
37. Foissner W, Wilbert N. Morphologie, Infraciliatur und Ökologie der limnischen Tintinnina: *Tintinnidium fluviatile* Stein, *Tintinnidium pusillum* Entz, *Tintinnopsis cylindrata* Daday und *Codonella cratera* (Leidy) (Ciliophora, Polyhymenophora)*. *J Protozool* 1979;26:90–103.
38. Jiang Y, Yang J, Al-Farraj SA, Warren A, Lin X. Redescriptions of three tintinnid ciliates, *Tintinnopsis tocantinensis*, *T.* Radix, and *T. cylindrica* (Ciliophora, Spirotrichea), from coastal waters off China. *Eur J Protistol* 2012;48:314–325.
39. Kim SY, Choi JK, Dolan JR, Shin HC, Lee S *et al*. Morphological and ribosomal DNA-based characterization of six Antarctic ciliate morphospecies from the Amundsen sea with phylogenetic analyses. *J Eukaryot Microbiol* 2013;50:497–513.
40. Saccà A, Strüder-Kypke MC, Lynn DH. Redescription of *Rhizodomus tagatzi* (Ciliophora: Spirotrichea: Tintinnida), based on morphology and small subunit ribosomal RNA gene sequence. *J Eukaryot Microbiol* 2012;59:218–231.
41. Smith SA, Song W, Gavrilova NA, Kurilov AV, Liu WW *et al*. *Dartintinnus alderae* n. g., n. sp., a brackish water tintinnid (Ciliophora, Spirotrichea) with dual-ended lorica collapsibility. *J Eukaryot Microbiol* 2018;2018:400–411.
42. Sniezek JH, Capriulo GM, Small EB, Russo A. *Nolaclusilis hudsonicus* n. sp. (Nolaclusiliidae n. fam.) a bilaterally symmetrical tintinnine ciliate from the lower Hudson River estuary. *J Protozool* 1991;38:589–594.
43. Snyder RA, Brownlee DC. *Nolaclusilis bicornis* n. g., n. sp. (Tintinnina: Tintinnidiidae): a tintinnine ciliate with novel lorica and cell morphology from the Chesapeake Bay estuary. *J Protozool* 1991;38:583–589.
44. Zhang WC, Feng MP, Yu Y, Zhang CX, Xiao T. *An Illustrated Guide to Contemporary Tintinnids in the World*. Beijing: Science Press; 2012.
45. Petz W, Song WB, Wilbert N. Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia* 1995;40:1–223.
46. Foissner W. Neotypification of protists, especially ciliates (Protozoa, Ciliophora). *Bull zool Nomencl* 2002;59:165–169.
47. Wilbert N. Eine verbesserte Technik Der Protargolimprägnation für Ciliaten. *Mikrokosmos* 1975;64:171–179.
48. Pan X, Bourland WA, Song W. Protargol synthesis: an in-house protocol. *J Eukaryot Microbiol* 2013;60:609–614.
49. Adl SM, Bass D, Lane CE, Lukeš J, Schoch CL *et al*. Revisions to the classification, nomenclature, and diversity of eukaryotes. *J Eukaryot Microbiol* 2019;66:4–119.
50. Elwood HJ, Olsen GJ, Sogin ML. The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. *Mol Biol Evol* 1985;2:399–410.
51. Medlin L, Elwood HJ, Stickel S, Sogin ML. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 1988;71:491–499.
52. Wang C, Zhang T, Wang Y, Katz LA, Gao F *et al*. Disentangling sources of variation in SSU rDNA sequences from single cell analyses of ciliates: impact of copy number variation and experimental error. *Proc Biol Sci* 2017;284:20170425.
53. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95–98.
54. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
55. Miller MA, Pfeiffer W, Schwartz T. *Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov*. New Orleans, LA; 2010. pp. 1–8.
56. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003;19:1572–1574.
57. Nylander JAA. *MrModeltest version 2. Evolutionary Biology Centre*. Uppsala, Program distributed by the author: Uppsala University; 2004.
58. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24:1596–1599.
59. Shimodaira H. An approximately unbiased test of phylogenetic tree selection. *Syst Biol* 2002;51:492–508.
60. Shimodaira H, Hasegawa M. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 2001;17:1246–1247.
61. Hada Y. Report of the biological survey of Mutsu Bay. 24. The pelagic Ciliata, suborder Tintinnoinea. *Sci Rep* 1932;7:553–573.
62. Wang CC, Nie DS. A survey of the marine protozoa of Amoy. *Contr Biol Lab Sci Soc Chin, Zool Ser* 1932;8:285–385.
63. Wang CC. Notes on Tintinnoinea from the Gulf of Pe-Hai. *Sinensia* 1936;7:353–370.
64. Hada Y. Studies on the Tintinnoinea from the Western tropical Pacific. *J Fac Sci, Hokkaido Univ* 1938;6:87–190.

65. Campbell AS. *The Oceanic Tintinnoina of the Plankton Gathered During the Last Cruise of the Carnegie*. Washington, D.C: Carnegie Institution of Washington; 1942. pp. 1–163.
66. Balech E. Tintinnoinea de Atlantida (R. O. del Uruguay) (protozoa Ciliata Oligotr.). *Com Mus Argent Cienc Nat* 1948;7:1–23.
67. Hada Y. New species of the Tintinnida found from the inland sea. *Bull Suzugamine Women's Coll* 1964;11:1–4.
68. Yin GD. Preliminary investigation on ciliates of tintinnids in Jiaozhou Bay. *J Shandong Univ* 1952;2:36–56.
69. Komarovsky B. Contributions to the knowledge of the red sea: 14. The Tintinnina of the Gulf of Eylath (Aqaba). *Sea Fisheries Res Station. Haifa* 1959;21:1–40.
70. Alder VA. Tintinnoinea. In: Boltovskoy D (editor). *South Atlantic Zooplankton*. Leiden: Backhuys Publishers; 1999. pp. 321–384.
71. ZC L, ZZ Y, Jiang Y, Gong J, Clamp JC *et al*. Phylogenetic investigation on five genera of tintinnid ciliates (Ciliophora, Choreotrichia), based on the small subunit ribosomal RNA gene sequences. *Prog Nat Sci* 2009;19:1097–1101.
72. Santoferrara LF, McManus GB, Alder VA. Utility of genetic markers and morphology for species discrimination within the order Tintinnida (Ciliophora, Spirotrichea). *Protist* 2013;164:24–36.
73. Nie DS, Ch'eng PS. Tintinnoinea of the Hainan region. *Contr Biol Lab Sci Soc Chin* 1947;16:41–86.
74. Pierce RW. *Morphology and infraciliature of selected species of Tintinnina with a phylogenetic analysis of the Tintinnina based on infraciliature. Ph.D. Thesis*. USA: University of Rhode Island; 1997.
75. ICZN. *International Commission on Zoological Nomenclature). International Code of Zoological Nomenclature*. London: International Trust for Zoological Nomenclature; 1999.