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Morphology and phylogeny of four marine or brackish water spirotrich ciliates (Protozoa, Ciliophora) from China, with descriptions of two new species

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Abstract

In this study, we investigated the morphology and molecular phylogeny of four marine or brackish spirotrichean ciliates found in China, namely: *Caryotricha sinica* sp. nov., *Prodiscocephalus orientalis* sp. nov., *P.* cf. *borrori*, and *Certesia quadrinucleata*. *Caryotricha sinica* is characterized by its small size, seven cirral rows extending posteriorly to about 65% of the cell length, and four transverse cirri. *Prodiscocephalus orientalis* differs from its congeners mainly by the number of cirri in the "head" region and on the ventral side. The SSU rDNA sequence of *P.* cf. *borrori* differs from that of other population of *P. borrori* by ca. 40 bp. Consequently, the nominal species *P. borrori* is considered to be a species-complex. New data are provided for *Certesia quadrinucleata.* The Chinese population of *C. quadrinucleata,* for example, has fewer left marginal cirri than the other populations for which such data are available. Phylogenetic analyses based on SSU rDNA sequence data show that the genus *Caryotricha* is monophyletic. All typical "discocephalids" with a discoid "head" form a strongly supported clade that is sister to the unstable uronychiids + pseudoamphisiellids clade within the Euplotia. The genus *Certesia* forms a sister group to the *Euplotes* clade, also within the Euplotia assemblage.

Keywords: Discocephalida; Euplotia; Hypotrichia; Phylogeny; Taxonomy

Introduction

Spirotrich ciliates are one of the most diverse groups in the phylum Ciliophora and are widely distributed in marine, freshwater, and terrestrial biotopes (e.g., Berger 2011; Dragesco and Dragesco-Kernéis 1986; Foissner 2016;

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Lu et al. 2018; Luo et al. 2018; Lyu et al. 2018a,b; Song et al. 2009). Many nominal species are, however, poorly known and their systematic positions remain uncertain due to the lack of information concerning their infraciliature, morphogenesis, and gene sequences. Since the routine application of silver staining and gene sequencing for documenting the diversity of spirotricheans, this situation has greatly improved (Abraham et al. 2019; Chen L et al. 2018; Chen X et al. 2018; Hu et al. 2019; Lyu et al. 2018c; Park et al. 2019; Santoferrara et al. 2016; Shin and Kim 1995; Wang et al. 2019; Yan et al. 2018; Zhao et al. 2018). Recently, several new species of spirotricheans have been established based on a combination of morphological, morphogenetic, and molecular data (Shao et al. 2018; Syberg-Olsen et al. 2016; Zhang et al. 2018). To explore the species diversity of this group, we surveyed Chinese coastal waters of the South China Sea and Yellow Sea and isolated four species belonging to three genera, i.e., *Caryotricha*, *Prodiscocephalus*, and *Certesia*.

Kiitrichidae Nozawa, 1941 is a poorly known family of the subclass Hypotrichia that is characterized by an ancestral infraciliature pattern, i.e., ciliated dikinetids on the dorsal side and uniform ventral cirri with no differentiation of marginal cirral rows, and has been widely considered as an early branching group within the hypotrichous ciliates sensu lato (Borror 1972; Lynn 2008; Shi 1999; Song and Wilbert 1997). According to Shi (1999), there are only three genera in the family Kiitrichidae, i.e., *Transitella* Gellert, 1950, *Kiitricha* Nozawa, 1941, and *Caryotricha* Kahl, 1932. Lynn (2008) also recognizes three genera of kiitrichids, i.e., *Kiitricha*, *Caryotricha* and *Musajevella* Alekperov, 1984, *Transitella* being regarded as a possible junior synonym of *Balantidioides* Penard in Kahl, 1930. *Caryotricha* is recognized by the following combination of characters: (1) the simple, ancestral pattern of the dorsal dikinetids (both basal bodies of each dikinetid bearing one short cilium); (2) uniform distribution of ventral cirri; and (3) the presence of a short, postoral row of cirri called the migratory cirral row. To date, only three species of *Caryotricha* have been reported: *C. convexa* Kahl, 1932 (type species), *C. minuta* (Xu et al., 2008) Miao et al., 2009, and *C. rariseta* Jiang et al., 2013. Here, we describe a new *Caryotricha* species that is characterized by its distinctive cirral rows and transverse cirri.

Discocephalids are rarely reported and their taxonomy is poorly understood (Curds and Wu 1983; Ehrenberg 1831; Jankowski 1978; Jiang et al. 2013; Kahl 1932; Lin et al. 2004; Song and Shao 2017; Wicklow 1982). *Prodiscocephalus* Jankowski, 1979 is a genus of marine *Discocephalus*-like ciliates. It can be distinguished from other discocephalids by the presence of a right marginal cirral row and sparsely distributed ventral cirri. There are two known species: *P. minimus* (Dragesco, 1968) Jankowski, 1979 (type species) and *P. borrori* (Wicklow, 1982) Lin et al., 2004 (type species of *Psammocephalus* Wicklow, 1982, which is now regarded as a junior synonym of *Prodiscocephalus*). *Prodiscocephauls borrori* was redescribed in detail by Lin et al. (2004) and Shao et al. (2008). Here, we provide morphological and molecular data for two populations of *Prodiscocephalus,* one of which represents a new species and the other belongs to the *P. borrori*-complex.

Fabre-Domergue (1885) discovered a *Euplotes*-like ciliate with a left marginal row for which he created the genus *Certesia,* with *C. quadrinucleata* the type species by monotypy. This species has been redescribed several times (Cui et al. 2009; Kahl 1932; Lin and Song 2004; Sauerbrey 1928; Wicklow 1983). Chen et al. (2010) provided details of its morphogenesis. Here, we provide the results of an integrative phylogenetic and systematic study based on a new population of *C. quadrinucleata*.

The main aim of this study is to provide detailed information on the taxonomy and molecular phylogeny of these four spirotrichean ciliates isolated from Chinese coastal waters.

Material and methods

Sampling and morphological methods (Fig. 1A–D)

Caryotricha sinica sp. n. was found on 11 April 2018 in a brackish water pond that formerly was connected to the sea, near Kuipeng Road (22°35′15.76″N, 114°26′10.28″E), Huizhou, Guangdong Province, China, where the water temperature was 25.5 °C and the salinity was 19‰. *Prodiscocephalus orientalis* sp. n. was isolated on 14 May 2018 from a reef off Xiao Lajia (22°36′48.84″N, 114°37′38.88″E), Huizhou, Guangdong Province, China, where the water temperature was 25.5 °C and the salinity was 33‰. *Prodiscocephalus* cf. *borrori* was isolated from samples collected on 12 October 2018 from a reef off Yinshatan (35°55′22.84″N, 120°12′17.44″E), Qingdao, Shandong Province, China, where the water temperature was 23 °C and the salinity was 30‰. *Certesia quadrinucleata* was collected on 16 October 2018 from sandy littoral sediments near Wanggezhuang (36°16′34.06″N, 120°40′16.65″E), Qingdao, Shandong Province, China, where the water temperature was 22 °C and the salinity was 30‰ (Fig. 1). Cells were maintained in habitat water for further studies. Although clonal cultures were not established, no similar morphotypes were present in the protargol preparations, indicating that morphological and molecular studies of each isolate dealt with a single species.

Cells were observed in vivo using bright field and differential interference contrast microscopy at 100–1000× magnification. The infraciliature and nuclear apparatus were revealed by protargol staining (Pan et al. 2014; Wilbert 1975). Counts and measurements were performed at magnifications of 1000×. Drawings of stained specimens were made with the help of a drawing attachment and photomicrographs. Taxon-

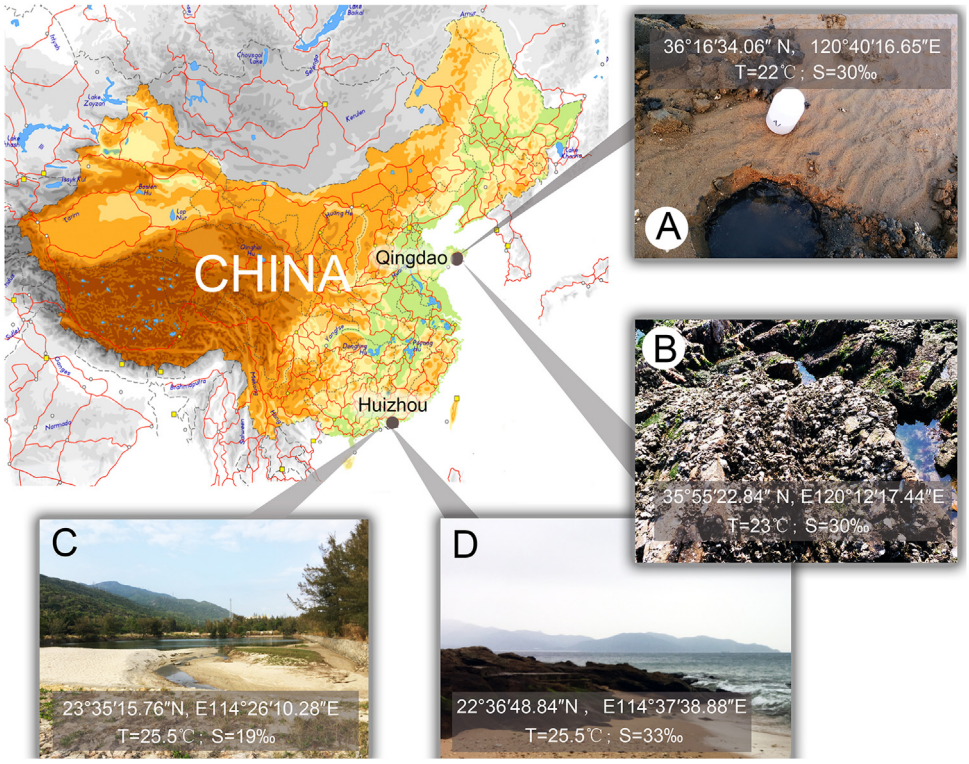

Fig. 1. A–D. Map of China showing the sampling locations and photographs showing the sampling sites. (A) A hole in the intertidal area at Wanggezhuang, Qingdao, from which *Certesia quadrinucleata* was collected. (B) A reef area with temporary ponds in Yinshatan, Qingdao, from which *Prodiscocephalus* cf. *borrori* was collected. (C) Type locality of *Caryotricha sinica* sp. n., brackish water pond near Huizhou. (D) Type locality of *Prodiscocephalus orientalis* sp. n., a reef off Xiao Lajia, Huizhou.

omy and systematics are according to Wicklow (1982), Lynn (2008), Gao et al. (2016), and Adl et al. (2019).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from cleaned cells using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, modified according to Sheng et al. (2018) and Huang et al. (2014). The SSU rDNA sequences were amplified by PCR using the forward primers 18SF (5'-AAC CTG GTT GAT CCT GCC AGT-3') or 82 F (5'-GAA ACT GCG AAT GGC TC-3') and the reverse primer 18SR (5'-TGA TCC TTC TGC AGG TTC ACC TAC-3') (Jerome et al. 1996; Medlin et al. 1988). The PCR amplifications were performed using Q5® Hot Start High-Fidelity 2× Master Mix DNA Polymerase (NEB, Ipswich, MA) with the following protocol: one cycle of initial denaturation at 98 °C for 30 s, followed by 18 cycles of amplification (98 °C, 10 s; 69–52 °C touch down, 30 s; 72 °C, 1 min) and another 18 cycles (98 °C, 10 s; 51 °C, 30 s; 72 °C, 1 min), with a final extension of 72 °C for 5 min. The amplicons were detected using agarose gel and sent for commercial sequencing (Tsingke Biological Technology Company, Qingdao, China).

Phylogenetic analyses

The SSU rDNA sequences of the four species were aligned with those of 50 other ciliates obtained from the GenBank database (see Fig. 6 for accession numbers). All sequences were aligned and edited using the GUIDANCE2 server (http://guidance.tau.ac.il/ver2/). The litostomateans *Loxophyllum utriculariae* (L26448), *Spathidium stammeri* (KY922825), and *Amylovorax dehorityi* (AF298817) were chosen as outgroup taxa. There were 1948 positions in the final alignment. Maximum likelihood (ML) analysis based on the above-mentioned alignment with 1,000 bootstrap replicates was performed using RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway with the GTRGAMMA model (Miller et al. 2010). Bayesian inference (BI) was applied to the same alignments using MrBayes 3.2.6 on XSEDE (Ronquist et al. 2012) under the best-fit model GTR + I + G selected by Akaike Information Criterion (AIC) in MrModeltest 2.2 (Nylander 2004). Markov chain Monte Carlo (MCMC) simulations were run for 1,000,000 generations with sampling every 100 generations and a burn-in of 1000 trees. The remaining trees were used to calculate a majority rule consensus tree and the posterior probabilities (PP). Tree topologies were displayed with MEGA X (Kumar et al. 2018).

The statistical reliability of alternative phylogenetic hypotheses was evaluated using the approximately unbiased (AU) test (Shimodaira 2002). The constrained ML tree was generated by limiting the monophyly of the target group with unspecified internal relationships. The site-wise log likelihood values for all ML topologies were calculated using PAUP 4.0 (Swofford 2002). The P values were calculated with CONSEL (Shimodaira and Hasegawa 2001).

ZooBank registration

The ZooBank registration number of the present work is: urn:lsid:zoobank.org:pub:707B3C1E-F470-4329-BBF6-6BBD557F5A1D.

Results

Kiitrichidae Nozawa, 1941

Caryotricha Kahl, 1932

Caryotricha sinica sp. n. (Fig. 2A–C, E, I–K, Table 1)

Diagnosis: Body in vivo 40–50 μm × 25–40 μm, oval in outline; cortical granules densely arranged, rod-shaped; adoral zone occupies about 65% of body length, with about 17 membranelles; invariably seven cirral rows on ventral and dorsal sides, middle row with about 13 cirri; four slightly enlarged transverse cirri located to right of posterior end of buccal cavity; one migratory cirral row with about five cirri located behind cytostome; five dorsal kineties; one globular macronucleus.

Type locality: Brackish water pond near Kuipeng Road (23°35′15.76″N, 114°26′10.28″E), Huizhou, Guangdong Province, China.

Type specimens: The protargol slide with the holotype specimen (Fig. 2B, C, I, J) circled in ink is deposited in the Laboratory of Protozoology, Ocean University of China (OUC), Qingdao, China, with registration number LCY2018041109-1. A protargol slide with paratype specimens is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2019.12.16.1.

Etymology: The species-group name *sinica* refers to the country (China) from which the species was first isolated.

Zoobank registration of *Caryotricha sinica* sp. n.: urn:lsid:zoobank.org:act:8762E16C-055E-44A5-A5E2-11D6E10D1F84.

Morphological description (Fig. 2A–C, E, I–K, Table 1)

Body 40–50 μm × 25–40 μm in vivo, inflexible, generally oval in outline, dorsoventrally flattened, left and right margins convex, anterior end slightly pointed, posterior end widely rounded (Fig. 2A, K). Oral apparatus conspicuous, approximately 65% of body length (Fig. 2A, B, I). Cytoplasm usually with numerous densely packed dark-grey to black inclusions (1–3 μm across) that render cell nearly opaque except central part, i.e., location of macronucleus (Fig. 2A, K). Contractile vacuole not observed, probably absent.

Pellicle rigid, with rod-shaped cortical granules, ellipsoidal in lateral view, about 1 μm long, circular when viewed from apical aspect, about 0.2 μm in diameter, located beneath the pellicle, oriented orthogonal to cell membrane, densely distributed between cirral rows and in other glabrous areas of cell (Fig. 2E). Macronucleus globular, about 17 μm across. Micronucleus not detected (Fig. 2C, I, J).

Locomotion by slowly crawling on substrate interspersed with long periods of inactivity; when disturbed, cells swim fast in a straight line while rotating about main body axis.

Adoral zone slightly curved, occupies about 65% of body length, consists of 14–21 membranelles with cilia up to 10 μm long in vivo; each membranelle composed of four basal body rows, first one very short, other three of almost equal length (Fig. 2A, B, I). Paroral and endoral of equal length, extending along right side of buccal cavity; paroral multiple-rowed, endoral single-rowed, both slightly curved, optically intersect anteriorly (Fig. 2B, I).

Cirri arranged in seven longitudinal cirral rows (CR), usually with five rows on ventral side and two rows on dorsolateral side, extending up to 65% of cell length, with cilia ca. 10 μm long in vivo (Fig. 2A–C, I, J). Number of cirri in each row progressively increases from CR1 to CR7. CR1 located right of undulating membranes, composed of three to five cirri (buccal cirri?). Cirri generally undifferentiated, with exception of those in CR6 and CR7: the last two or three cirri in CR6 slightly smaller than others, cirri in CR7 distinctly smaller and more narrowly spaced than others (Fig. 2B, C, I, J). Three or four transverse cirri arranged in a pseudo-row, located to right of posterior end of buccal cavity, with cilia about 20 μm in length; bases of transverse cirri conspicuously longer than those of other cirri, thus clearly recognizable (Fig. 2A, B, I). Short migratory cirral row composed of three to eight close-set cirri, positioned posterior to oral apparatus, widely separated from other cirri (Fig. 2A, B, I, Table 1).

Five dorsal kineties, rightmost one slightly shortened anteriorly. Both basal bodies of each dorsal dikinetid bear a short cilium, 3 μm in length (Fig. 2C, J).

Euplotia Jankowski, 1979

Discocephalida Wicklow, 1982

Discocephalidae Jankowski, 1979

Prodiscocephalus Jankowski, 1979

Prodiscocephalus orientalis sp. n. (Fig. 3A–I, Table 2)

Diagnosis: Body in vivo 85–140 μm × 25–35 μm, elongate-elliptical in shape with conspicuous discoid "head" region; adoral zone bipartite, distal part with about six membranelles, proximal part with about 17 membranelles; usually seven cirri in "head" region; six to nine ventral cirri; 10–19 cirri in right marginal row; left marginal row divided into anterior and posterolateral parts with 15–21 and 5–13 cirri, respectively, last one or two posterolateral marginal cirri conspicuously smaller than others; six to eight very large transverse cirri; six to eight caudal cirri arranged in two dorsolateral rows; seven dorsal kineties; 21–37 macronuclear nodules.

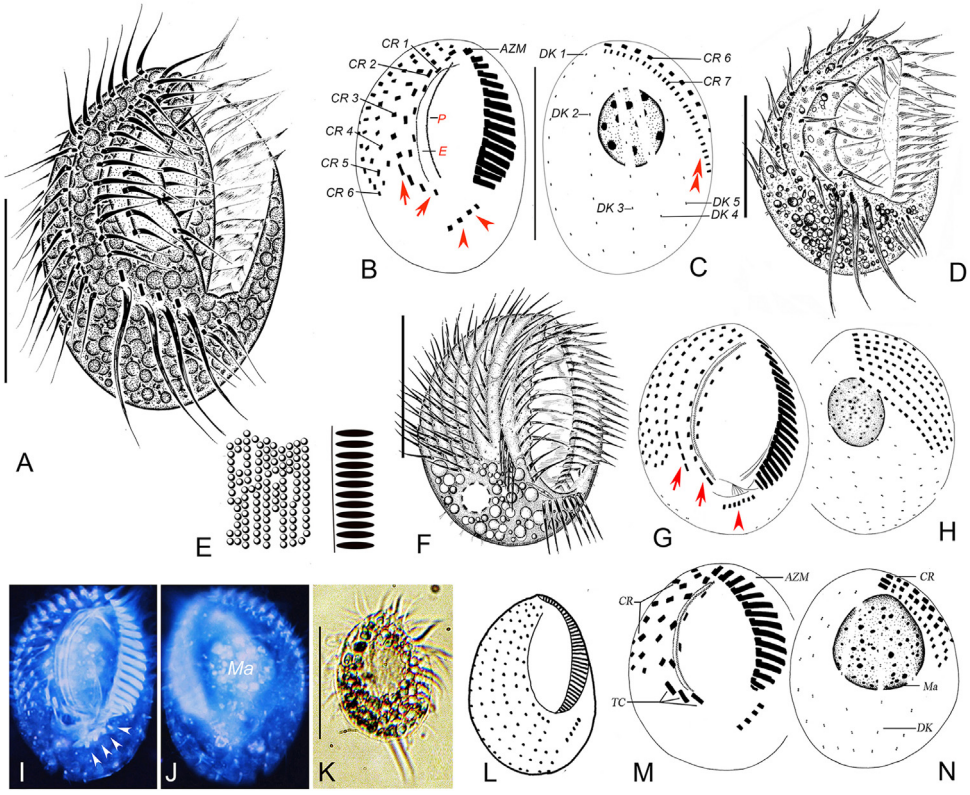

Fig. 2. A–N. *Caryotricha sinica* sp. n. (A–C, E, I–K), *C. convexa* (L, from Borror 1972), *C. minuta* (F–H, from Miao et al. 2009) and *C. rariseta* (D, M, N, from Jiang et al. 2013). (A, D–F, K, in vivo; B, C, G–J, L–N, after protargol staining; I, J are false-colored). (A, D, F) Ventral views of representative specimens. (B, C, G–J, L–N) Ventral (B, G, I, L, M) and dorsal (C, H, J, N) views of the holotype specimens, showing infraciliature and nuclear apparatus, arrowheads mark migratory cirral row, arrows indicate transverse cirri, double-arrowhead indicates smaller cirri in CR7. (E) Top and lateral views of rod-shaped cortical granules. (K) Ventral view in vivo. AZM, adoral zone of membranelles; CR 1–7, cirral row 1–7; DK, dorsal kineties; E, endoral; Ma, macronucleus; P, paroral; TC, transverse cirri. Scale bars: 20 μm.

Type locality: Reef off Xiao Lajia (22°36′48.84″N, 114°37′38.88″E), Huizhou, Guangdong Province, China.

Type specimens: The protargol slide (No. LCY2018051401-1) with the holotype specimen (Fig. 3D, E, F, G) circled in ink, and four paratype slides (No. LCY2018051401-2; LCY2018051401-3; LCY2018051401-4; LCY2018051401-5) with protargol-stained specimens, are deposited in the Laboratory of Protozoology, Ocean University of China (OUC). One paratype slide with protargol-stained specimens is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2019.12.16.2

Etymology: The species-group name *orientalis* recalls the fact that this species was first described from oriental (Chinese) coastal waters.

ZooBank registration of *Prodiscocephalus orientalis* sp. n.: urn:lsid:zoobank.org:act:AD9505FA-DBC9-4950-A899-0222B6B5FE51.

Morphological description (Fig. 3A–I, Table 2)

Body 85–140 μm × 25–35 μm in vivo, usually elongate-elliptical in outline, anterior portion constricted forming a small, discoid "head" region, posterior end broadly rounded, right margin straight, left margin more or less convex (Fig. 3A, H, I). Dorsal side slightly convex, ventral side flat with two grooves along marginal cirral rows and a cavity that extends ahead of transverse cirral row. Body rigid and non-contractile (Fig. 3B). Pellicle fragile. Neither cortical granules nor contractile vacuole were observed.

Cytoplasm colourless in "head" region and light grey in main body which is packed with droplets, food vacuoles, and ingested diatom frustules and sand grains (1–2 μm across) (Fig. 3A, H, I). Twenty-one to 37 globular macronuclear nodules, about 3–5 μm across in vivo, scattered throughout body apart from "head" region (Fig. 3E–G). Locomotion generally by crawling slowly on substrate, with quick backward jumps when changing direction or when being disturbed; sometimes swimming while rotating about main body axis.

Distal part of adoral zone of membranelles located on dorsolateral side of anterior body margin and comprises about six widely spaced membranelles; proximal part located on ventral side and comprises ca. 17 membranelles, each with four basal body rows, first row very short, second row slightly shorter than posterior two; cilia of membranelles up to 10 μm long in vivo. Paroral about 15 μm long, endoral about 7 μm

Table 1. Morphometric data for *Caryotricha sinica* sp. n.[a]

Character	Min	Max	Mean	M	SD	CV	n
Body length (μm)	26	45	34.6	34	4.9	14.2	25
Body width (μm)	20	33	25.4	25	3.7	14.4	25
Length of adoral zone (μm)	18	30	24.4	24	3.2	13.2	25
Adoral membranelles, number	14	21	16.5	16	1.6	9.6	25
Cirral rows, number	7	7	7.0	7	0.0	0.0	25
Cirri in CR1, number	3	5	3.5	3	0.6	17.3	21
Cirri in CR2, number	3	5	4.3	4	0.7	17.2	25
Cirri in CR3, number	8	11	9.0	9	0.9	10.1	25
Cirri in CR4, number	10	18	13.3	13	1.9	14.5	25
Cirri in CR5, number	13	20	16.0	16	1.8	11.2	25
Cirri in CR6, number	14	23	18.0	18	2.2	12.3	25
Cirri in CR7, number	17	30	23.4	23	3.1	13.1	25
CR1 length (μm)	12	18	13.9	13.5	2.0	14.2	10
CR2 length (μm)	11	18	15.4	16.0	2.2	14.1	10
CR3 length (μm)	12	20	15.5	15.0	2.5	15.9	10
CR4 length (μm)	13	21	16.5	16.5	2.3	14.1	10
CR5 length (μm)	15	23	19.2	20.0	2.5	12.9	10
CR6 length (μm)	17	26	21.0	21.0	2.7	12.9	10
CR7 length (μm)	14	26	20.5	19.5	3.5	17.1	10
Transverse cirri, number	3	4	4.0	4	0.2	5.3	23
Migratory cirri, number	3	8	4.8	4.5	1.2	24.1	24
Dorsal kineties, number	5	5	5.0	5.0	0.0	0.0	10
Dikinetids in DK1, number	9	12	10.3	10.0	1.2	11.3	10
Dikinetids in DK2, number	6	9	7.5	7.5	0.8	11.3	10
Dikinetids in DK3, number	7	9	7.6	7.0	0.7	9.6	9
Dikinetids in DK4, number	6	8	6.7	6.5	0.8	12.3	10
Dikinetids in DK5, number	3	7	5.0	4.5	1.4	28.3	10
Macronuclei, number	1	2	1.0	1	0.2	19.2	25

Abbreviations: CR1–7 = cirral row 1–7, CV = coefficient of variation in %, DK1–5 = dorsal kinety 1–5, Max = maximum, Mean = arithmetic mean, M = median, Min = minimum, n = number of cells measured, SD = standard deviation.

[a] All data are based on protargol-stained specimens.

long in vivo (length of cilia not measured); endoral slightly curved; paroral and endoral optically intersect at their anterior end (Fig. 3A, C, D, F).

Seven cirri in "head" region with cilia about 10 μm long in vivo; four arranged along right margin of buccal cavity of which anterior two (probably buccal cirri) are smaller (posterior one conspicuously so) and lie adjacent to paroral; posterior two of normal size and lie adjacent to endoral; two located in anterior portion of "head" region, slightly enlarged; one located near right margin above junction of "head" and main body (Fig. 3A, C, D, F). Usually eight ventral cirri, with cilia about 8 μm long in vivo, distributed in two groups: anterior six slightly enlarged, arranged almost in a line; posterior two (very likely pretransverse ventral cirri) located near transverse cirri. Six to eight conspicuously enlarged transverse cirri arranged along a curved pseudorow, located subcaudally, with cilia about 35 μm long in vivo and conspicuous anchoring fibres. Right marginal row composed of 10–19 cirri, commencing about 20% down length of cell. Left marginal row divided into two parts: anterior part with 15–21 widely spaced cirri; posterolateral part with 5–13 closely spaced cirri, located near posterior left dorsal margin, last one or two cirri of left marginal row conspicuously smaller than others (Fig. 3A, D–G, Table 2).

Seven dorsal kineties; dorsal cilia bristle-like, about 10 μm long in vivo. Six to eight conspicuous caudal cirri with cilia 10 μm long in vivo, arranged in two rows, located dorsolaterally near right-posterior cell margin (Fig. 3E, G).

Prodiscocephalus cf. ***borrori*** (Fig. 4A–K, Table 2)

Description of Qingdao population

Body in vivo 70–85 μm × 20–25 μm, elongate, ratio of length to width about 4:1, dorsoventrally flatted ca. 2:1 (Fig. 4A, D, E, G, H). Anterior portion constricted forming a small, conspicuous, discoid "head" region; posterior end broadly rounded; right margin straight, left margin slightly convex (Fig. 4A, D, E, G, H). Dorsal side more or less convex, ventral side flat with distinct groove along two marginal cirral rows and a cavity that extends from level of cytostome to transverse cirri (Fig. 4A, E). Body slightly flexible but non-contractile (Fig. 4E). Oral apparatus about 25% of body length. Pellicle fragile, cortical granules (about 0.5 μm in diameter) densely arranged in rows (Fig. 4F). Cytoplasm colourless or greyish, endoplasmic granules about 1–4 μm in size, moderately densely distributed within cytoplasm ren-

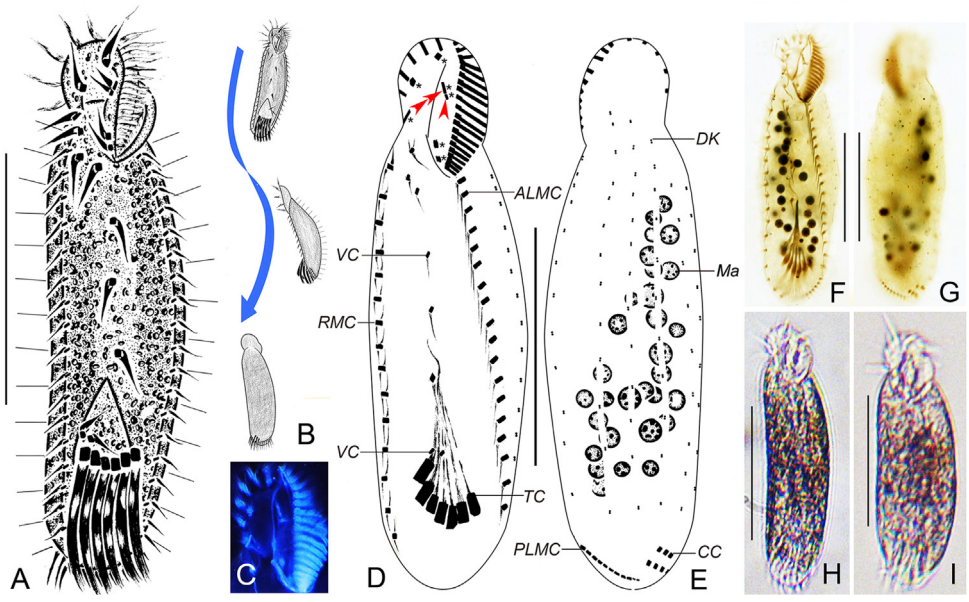

Fig. 3. A–I. Morphology and infraciliature of *Prodiscocephalus orientalis* sp. n. in vivo (A, B, H, I) and after protargol staining (C–G: note that C is false-colored). (A) Ventral view of a representative individual. (B) Pattern of locomotion when disturbed, arrow marks the direction of motion. (C) Ventral view of anterior portion. (D–G) Ventral (D, F) and dorsal (E, G) views of the holotype specimen showing the infraciliature and nuclear apparatus, arrowhead and double-arrowhead show two cirri in "head" region located near the paroral, "*" indicate the cirri in "head" region. (H, I) Ventral views, to show different body shapes in vivo. ALMC, anterior left marginal cirri; AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; Ma, macronuclear nodule; PLMC, posterolateral marginal cirri; RMC, right marginal cirri row; TC, transverse cirri; VC, ventral cirri. Scale bars: 50 μm.

dering main body opaque (Fig. 4A, D, E, G, H). Contractile vacuole not observed. 20–35 ovoidal macronuclear nodules, ca. 5 μm in diameter after protargol staining, scattered throughout cytoplasm, difficult to observe in vivo (Fig. 4C, J, K). Micronuclei not recognizable.

Locomotion usually by moderately rapid crawling on substrate with quick backward jumps when changing direction or on being disturbed, or swimming rapidly while turning about main body axis and then suddenly settling.

Adoral zone of membranelles divided in two parts: distal part on dorsolateral side of anterior body margin, comprises about five widely spaced membranelles with cilia ca. 12 μm long; proximal part comprises about 16 membranelles, each with four basal body rows, first row very short, second row slightly shorter than third and fourth rows (Fig. 4A, B, I, J). Paroral long, single-rowed, commences near posterior end of adoral zone; endoral single-rowed and anteriorly located, very short, about one third of paroral in length; paroral and endoral do not optically intersect (Fig. 4B, I, J).

Invariably six cirri in "head" region with cilia about 8 μm long in vivo: two adjacent to posterior portion of adoral zone; three arranged along distal part of adoral zone; one (probably buccal cirrus) located near endoral (Fig. 4A, B, I, J). Invariably six ventral cirri with cilia about 8 μm long in vivo, divided into two groups: anterior four slightly enlarged, arranged almost in a line; posterior two smaller, located near transverse cirri. Seven large transverse cirri located subcaudally, with cilia about 25 μm long in vivo and conspicuous anchoring fibres (Fig. 4A, B, J), posteriormost cirrus located about 15 μm from posterior end of cell. Right marginal row with 6–8 cirri. Left marginal row bipartite: anterior part with 8–12 loosely arranged cirri; posterior part with 5–7 densely arranged cirri located dorsolaterally (Fig. 4A–C, J, K, Table 2).

Six dorsal kineties with bristle-like cilia about 10 μm in length some of which extend beyond cell margin when observed in vivo. Invariably seven caudal cirri distributed in two rows, located dorsolaterally near right cell margin (Fig. 4A, C, K).

Euplotia Jankowski, 1979

Euplotida Small and Lynn, 1985

Certesiidae Borror and Hill, 1995

Certesia Fabre-Domergue, 1885

Certesia quadrinucleata Fabre-Domergue, 1885 (Fig. 5A–L, Table 3)

Description of Qingdao population

Body in vivo 55–65 μm × 30–35 μm, oval in outline, dorsoventrally flatted, ratio of length to width about 2:1; anterior end blunt with a conspicuous projection on right side, posterior end rounded; dorsal side convex, ventral side flat; lateral margins straight (Fig. 5A, G–I, L). Body inflexible and non-contractile. Pellicle rigid, without granules. Cytoplasm colourless, usually with numerous crystals and food vacuoles rendering cell somewhat opaque in vivo (Fig. 5A, G–I, L). Contractile vacuole not observed. Four macronuclear nodules, two to the right and two to the left of cell mid-line, each pair connected by a funiculus, each nodule with numerous nucleoli (Fig. 5C, J, K). Micronucleus not observed.

Table 2. Morphometric characterization of *Prodiscocephalus orientalis* sp. n. (upper row) and *P.* cf. *borrori* (lower row).[a]

Character	Min	Max	Mean	M	SD	CV	n
Body length (μm)	75	153	115.8	115	15.8	13.6	25
	48	80	62.8	63	10.2	16.2	15
Body width (μm)	22	46	34.4	34	5.4	15.6	25
	16	37	25.5	24	5.7	22.4	15
Length of adoral zone (μm)	24	34	29.7	30	2.6	8.8	25
	15	23	19.1	20	2.2	11.5	15
Adoral membranelles, number	21	26	23.3	23	1.4	5.9	25
	19	24	21.1	21	1.2	5.9	15
DE-value	0.4	0.5	0.5	0.5	0.0	7.0	10
	0.4	0.7	0.5	0.6	0.1	18.4	10
Cirri in “head” region, number	6	7	7.0	7	0.2	2.9	25
	6	6	6.0	6.0	0.0	0.0	14
Ventral cirri, number	6	9	8.0	8	0.5	6.8	25
	6	6	6.0	6	0.0	0.0	11
Transverse cirri, number	6	8	7.0	7	0.4	5.8	25
	7	8	7.1	7.0	0.3	3.8	14
TC and rear end of cell, distance	11	21	14.8	14.0	3.4	22.9	10
	7	13	10.1	10.0	1.5	15.1	10
Right ventral cirri, number	10	19	14.8	15	1.7	11.5	25
	6	8	7.2	7	0.9	12.0	15
Anterior LMC, number	15	21	18.4	19	1.7	9.2	25
	8	12	10.4	10	1.2	11.9	15
Posterolateral LMC, number	5	13	11.0	11	1.6	14.9	25
	5	7	6.3	6.0	0.8	12.1	12
Caudal cirri, number	6	8	7.1	7	0.4	5.7	25
	7	7	7.0	7.0	0.0	0.0	12
Dorsal kineties, number	7	7	7.0	7	0.0	0.0	25
	6	6	6.0	6.0	0.0	0.0	12
Macronuclei, number	21	37	27.8	28	3.5	12.7	25
	20	35	28.9	28.0	4.3	14.9	12

Abbreviations: CV = coefficient of variation in %, DE-value = distance from anterior body end to distal end of adoral zone of membranelles divided by length of adoral zone of membranelles (for details, see Berger 2008), LMC = left marginal cirri, Max = maximum, Mean = arithmetic mean, M = median, Min = minimum, n = number of individuals examined, SD = standard deviation, TC = transverse cirri.

[a] All data are based on protargol-stained specimens (note that Posterolateral LMC of *P.* cf. *borrori* is mixture combination of caudal cirri and post-lateral right marginal cirri).

Table 3. Morphometric data of *Certesia quadrinucleata* Fabre-Domergue, 1885.[a]

Character	Min	Max	Mean	M	SD	CV	n
Body length (μm)	36	50	43.5	44	4.4	10.1	15
Body width (μm)	22	32	28.1	29	3.0	10.7	15
Length of adoral zone (μm)	15	25	19.7	19	2.7	13.6	15
Adoral membranelles, number	17	21	18.9	19	1.4	7.5	15
Frontoventral cirri, number	11	11	11.0	11.0	0.0	0.0	14
Transverse cirri, number	5	5	5.0	5.0	0.0	0.0	14
TC and rear end of cell, distance (μm)	7	16	12.6	13.0	2.8	22.5	10
Left marginal cirri, number	4	5	4.2	4	0.4	9.9	15
Macronuclei, number	4	4	4.0	4.0	0.0	0.0	8
Dorsal kineties, number	5	5	5.0	5	0.0	0.0	15
Dikinetids in DK1, number	11	20	16.5	18	2.5	15.1	15
Dikinetids in DK2, number	6	10	7.6	8	1.3	17.1	15
Dikinetids in DK3, number	6	9	7.1	7.0	1.0	14.1	14
Dikinetids in DK4, number	5	7	6.2	7	0.9	14.9	13
Dikinetids in DK5, number	6	8	6.9	7.0	0.9	12.6	14

[a] All data are based on protargol-stained specimens. CV = coefficient of variation in %, DK1–5 = dorsal kinety 1–5, Max = maximum, Mean = arithmetic mean, M = median, Min = minimum, n = number of individuals examined, SD = standard deviation, TC = transverse cirri.

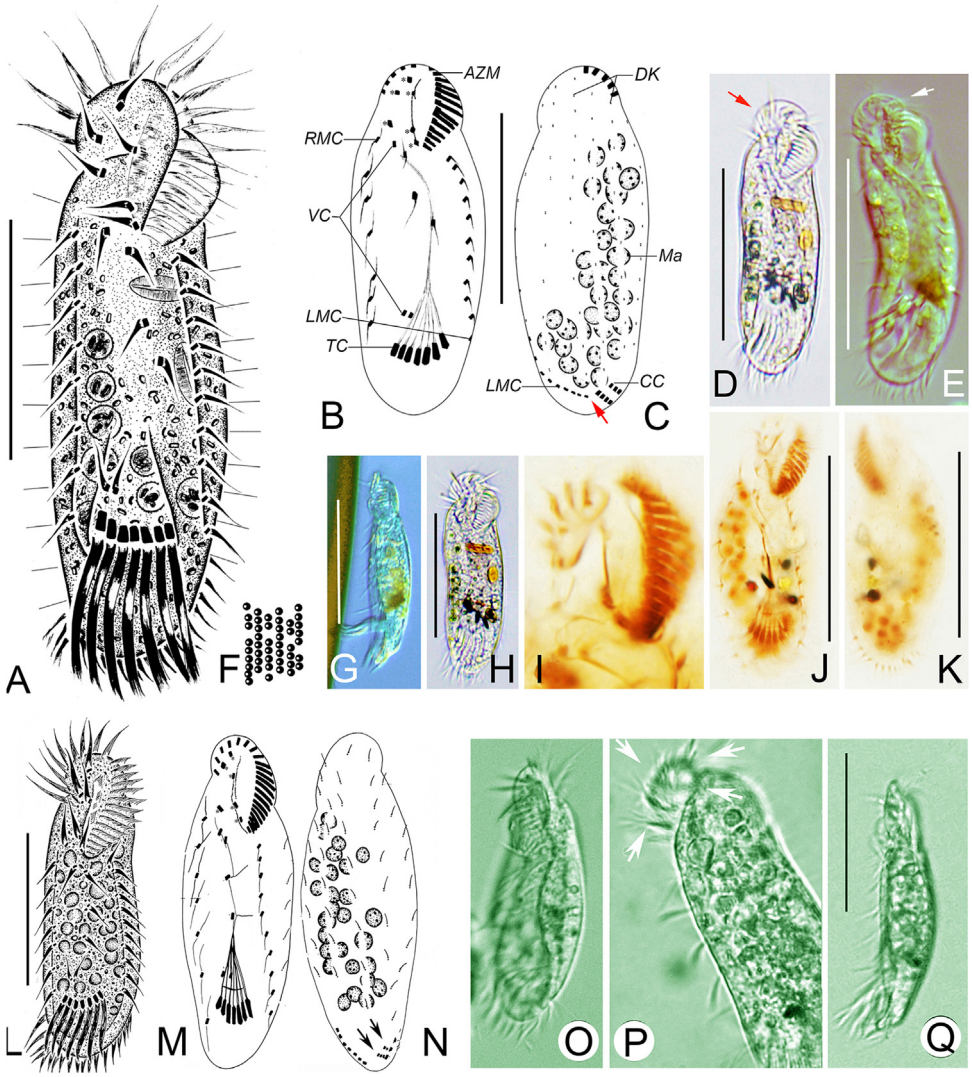

Fig. 4. A–Q. *Prodiscocephalus* cf. *borrori* (A–K) and *P. borrori* (L–Q, from Lin et al. 2004) (note that posterior part of LMC is a combination of caudal cirri and post-lateral right marginal cirri). A, D–H, L, O–Q, in vivo; B, C, I–K, M, N after protargol staining. (A, L) Ventral views of representative individuals. (B, C, I–K, M, N) Ventral (B, I, J, M) and dorsal (C, K, N) views showing the infraciliature and nuclear apparatus, arrow marks the posterolateral marginal cirri, "*" indicate the cirri in "head" region. (D, E, G, H, O–Q) Ventral views (D, E, H) and lateral view (G, O–Q) of cells in vivo, arrows in D, E show anterior parts of adoral zone, arrows in P indicate the constricted discoid "head". (F) Showing the cortical granules. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; Ma, macronucleus; LMC, left marginal cirri; RMC, right marginal cirri; TC, transverse cirri; VC, ventral cirri. Scale bars: 50 μm in D–P, 30 μm in A–C.

Locomotion typically by crawling on substrate, pausing frequently and then changing direction.

Adoral zone approximately 30–40% of cell length, composed of 17–21 membranelles, bases up to 20 μm long (Fig. 5A, B, J). Paroral broad and long, extending almost whole length of oral field, composed of many short rows of kineties (Fig. 5A, B, J).

Invariably 11 frontoventral cirri, about 15 μm in length, arranged mainly in two pseudo-rows along right-anterior margin of cell; five very strong transverse cirri with cilia about 35 μm long, rearmost one located about 13 μm from posterior end of cell, rightmost two with strong anchoring fibres that extend to anterior of cell, other three with relatively fine fibres; left marginal row composed of four or five cirri with cilia about 15 μm long in vivo (Fig. 5A, B, J).

Invariably five dorsal kineties that extend almost entire length of body. Leftmost kinety (DK1) curved left along posterior cell margin, composed of about 17 dikinetids; DK2–5 each composed of 5–10 dikinetids (Fig. 5C, K).

Phylogenetic analyses (Fig. 6)

All new SSU rDNA sequences have been deposited in GenBank with accession numbers, length and GC contents as follows: *Caryotricha sinica* sp. n. (MN543654, 1668 bp, 43.23%), *Prodiscocephalus orientalis* sp. n.

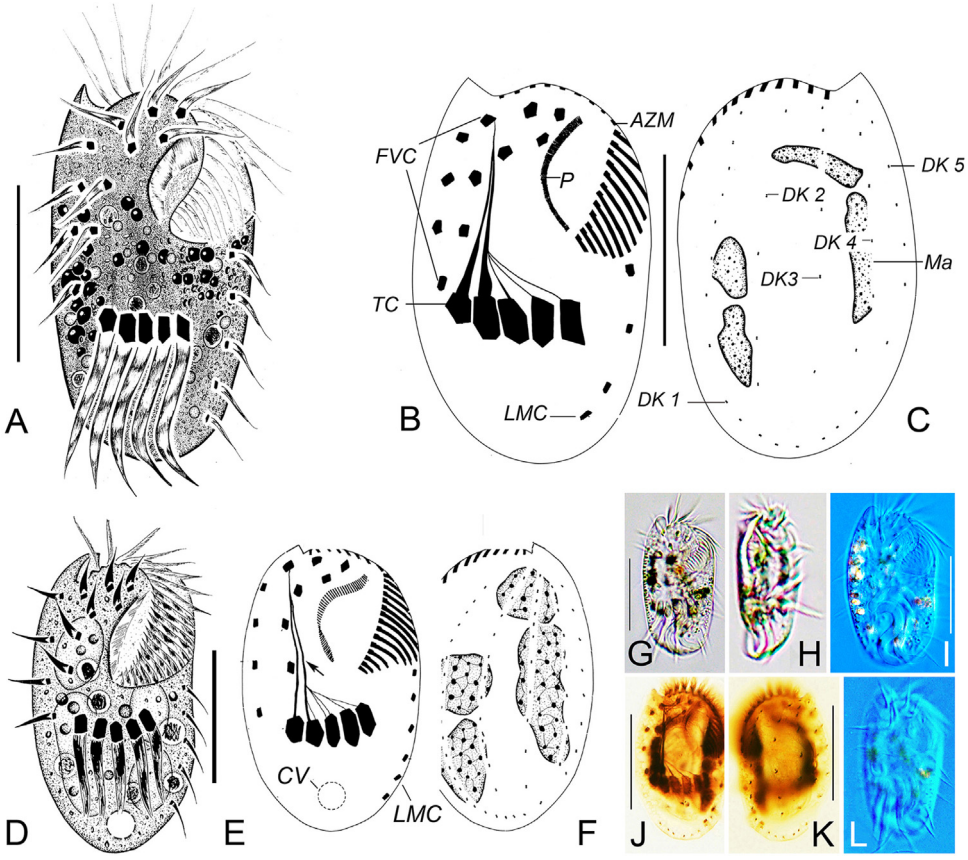

Fig. 5. A–L. Morphology and infraciliature of *Certesia quadrinucleata* from life (A, D, G–I, L) and after protargol staining (B, C, E, F, J, K). (A, D) Ventral views of typical individuals (D, from Lin and Song 2004). (B, C, E, F, J, K) Ventral (B, E, J) and dorsal (C, F, K) views showing the infraciliature and nuclear apparatus, arrow in E denotes two well-developed connecting fibers of the transverse cirri (E, F, from Lin and Song 2004). (G–I, L) Ventral (G, I, L) and lateral (H) views of cells in vivo. AZM, adoral zone of membranelles; CV, contractile vacuole; DK 1–5, dorsal kinety 1–5; FVC, frontoventral cirri; Ma, macronuclear nodules; LMC, left marginal cirri; P, paroral; TC, transverse cirri. Scale bars: 20 μm in D, 30 μm in A–C, G–L.

(MN543657, 1664 bp, 44.59%), *Prodiscocephalus* cf. *borrori* (MN543656, 1649 bp, 44.09%), *Certesia quadrinucleata* (MN543655, 1671 bp, 46.08%). The pairwise genetic distances among these new sequences and related species (such as members of Discocephalida and Kiitrichidae) are summarized in Table S1 and show that each group differs significantly from others. The phylogenetic relationships of each of these four species were determined by Bayesian inference (BI) and Maximum-Likelihood (ML) analyses of SSU rDNA sequences. The topologies of the BI and ML trees were almost identical, thus only the ML tree is presented here (Fig. 6).

Caryotricha sinica sp. n. was sister to the clade comprising *Caryotricha rariseta* (FJ876978) and *C. minuta* (EU275202). *Prodiscocephalus orientalis* sp. n. clustered with *Paradiscocephalus elongatus* (EU684746), forming a clade that was sister to the clade comprising *Prodiscocephalus* cf. *borrori* and *P. borrori* (DQ646880). *Prodiscocephalus* and *Paradiscocephalus* species clustered with *Discocephalus pararotatorius* and *D. ehrenbergi* within the Discocephalida assemblage (Fig. 6).

The fully statistically supported *Certesia quadrinucleata* clade, comprising our isolate and two other populations, was sister-group to representative species of the genus *Euplotes* (FJ998024, MF928801, MH795290). The *Euplotes-Certesia* group clustered tightly with two *Aspidisca* species (JX025168, MF928799).

Discussion

Caryotricha sinica sp. n

Comparison with congeners (Fig. 2D, F–H, L–N, Table 4)

Only three species of *Caryotricha* were previously recognized: *C. convexa* Kahl, 1932, *C. minuta* (Xu et al., 2008) Miao et al., 2009 and *C. rariseta* Jiang et al., 2013 (Fig. 2D, F–H, L–N; Table 4). *Caryotricha minuta* was first reported under the name *Kiitricha minuta* (Xu et al., 2008). It was transferred to *Caryotricha* by Miao et al. (2009) based on the presence of the migratory cirral row and the differentiated

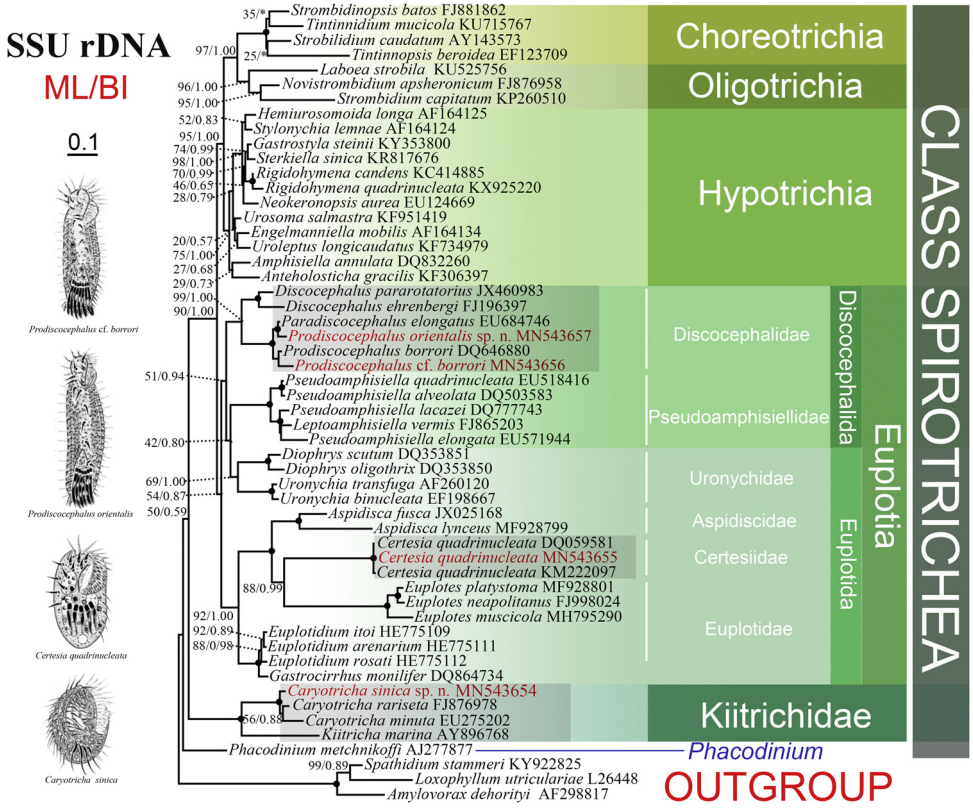

Fig. 6. The Maximum likelihood (ML) tree inferred from 18S rRNA gene sequences, showing the position of the *Caryotricha sinica* sp. n., *Prodiscocephalus orientalis* sp. n., *Prodiscocephalus* cf. *borrori*, and *Certesia quadrinucleata*. Numbers at nodes represent the bootstrap values of maximum likelihood analysis and the posterior probability of Bayesian inference analysis. Fully supported (1.00/100) branches are marked with solid circles. "*" indicates the disagreement between BI tree and the reference ML tree. All branches are drawn to scale. Scale bar corresponds to 5 substitutions per 100 nucleotide positions.

Table 4. Comparison of *Caryotricha sinica* sp. n. with congeners.[a]

Character	*C. minuta*	*C. minuta*	*C. rariseta*	*C. sinica*
Body size in vivo	40–80 × 30–60 μm	60–75 × 50–60 μm	35 × 20 μm	40–50 × 25–40 μm
No. of AM	22–27	22–25	13–15	14–21
No. of cirral row	9–12	11	7	7
No. of TC	6–7	2–3 in left row, 2–5 in right row	3	3–4
No. of MigC	6–9	6–8	3–5	3–8
No. of DK	7–9	7–8	4–6	5
No. of Ma	1	1	1	1–2
Locality	Inchon Harbour, Korea	Qingdao, China	Qingdao, China	Huizhou, China
Reference	Xu et al. (2008)	Miao et al. (2009)	Jiang et al. (2013)	Present study

[a] Abbreviations: AM = adoral membranelles, DK = dorsal kineties, Ma = macronuclei, MigC = migratory cirri, TC = transverse cirri.

transverse and dorsal ciliature. *Caryotricha minuta* differs from *C. sinica* sp. n. in having more adoral membranelles (22–27 vs. 14–21), cirral rows (9–12 vs. 7), cirri in cirral rows 1 and 2 (7, 11 according to the drawing vs. 3–5) and dorsal kineties (7–9 vs. 5), and in the number and arrangement of transverse cirri (6 or 7 arranged in two longitudinal rows vs. 3 or 4 in a single row) (Fig. 2F–H) (Miao et al. 2009; Xu et al. 2008). In addition, the molecular data clearly indicate that these two taxa are not conspecific (Fig. 6).

Jiang et al. (2013) isolated *C. rariseta* from coastal seawater off Qingdao, China. *Caryotricha rariseta* can be distinguished from *C. sinica* sp. n. by its smaller body size (30–40 μm × 15–25 μm vs. 40–50 μm × 25–40 μm in vivo), having fewer cirri in cirral rows 3–7 (mean 6, 8, 11, 12, 11 vs. 9, 13, 16, 18, 23, respectively), fewer transverse cirri (3 vs. usually 4) and fewer adoral membranelles (13–15 vs. 14–21) (Jiang et al. 2013).

No morphometric data are available for *C. convexa*. However, according to the descriptions by Kahl (1932) and Borror

(1972), the cirral rows of *C. convexa* extend to the posterior body end (vs. to about 2/3 down length of body in *C. sinica*), and therefore can be clearly distinguished from the new species (Fig. 2L).

Prodiscocephalus orientalis sp. n

Comparison with congeners (Table 5)

According to the generic revisions by Wicklow (1982) and Lin et al. (2004), only two species of *Prodiscocephalus* are recognised: *P. borrori* and *P. minimus* (Table 5).

Wicklow (1982) described *P. borrori* from sand at Foss Beach, USA. Compared to *P. borrori*, *P. orientalis* sp. n. can be distinguished by having more cirri in the "head" region (usually 7 vs. 6) and more dorsal kineties (7 vs. 6) (Lin et al. 2004; Wicklow 1982). The SSU rDNA sequence data also support the separation of these two species (Fig. 6) (Shao et al. 2008).

Prodiscocephalus minimus, the type species of the genus, was discovered by Dragesco (1968) from sand at Arcachon, France. It differs from *P. orientalis* sp. n. in having fewer ventral cirri (2 vs. 6–9), right marginal cirri (4–6 vs. 10–19) and anterior left marginal cirri (7–8 vs. 15–21) as well as in having no (vs. two) ventral cirri near the transverse cirri (Dragesco 1968).

Li et al. (2008) reported a novel discocephalid ciliate for which they established a new genus, i.e., *Paradiscocephalus elongatus*. Considering its similarity with *Prodiscocephalus orientalis* sp. n. when observed in vivo, the close proximity of their type localities and their relatedness in phylogenetic analyses, these two species should be compared. *Paradiscocephalus elongatus* can be distinguished from *Prodicocephalus orientalis* sp. n. by the number and arrangement of ventral cirri (11–16, in a zig-zag pattern, dispersed vs. 6–9 arranged in two groups) and the number of transverse cirri (9–11 vs. 6–8) (Li et al. 2008).

Prodiscocephalus cf. *borrori*

Comparison with congeners (Fig. 4L–Q)

Prodiscocephalus borrori was originally discovered in the USA and described as the type species of the genus *Psammocephalus* (Wicklow 1982). It was transferred to the genus *Prodiscocephalus* by Lin et al. (2004). Based on two Chinese populations, Lin et al. (2004) and Shao et al. (2008) provided redescriptions, details of its morphogenesis and analyses of its SSU rDNA-based molecular phylogeny (Fig. 4L–Q).

The present population of *P. borrori* closely matches previously described populations in terms of its general morphology and infraciliature. The only significant differences among them are the number of macronuclear nodules, i.e., up to 120 (Wicklow 1982), 23–34 (Lin et al. 2004) and 20–35 (present study), and the length of the bristles in dorsal kinety 3, i.e., 2 μm in the population described by Wicklow (1982); 6–8 μm in the population described by Lin et al. (2004); 10 μm in the present population.

Shao et al. (2008) reported a Qingdao population under the name *P. borrori*. They noted that: "The population studied here corresponds very closely with the population reported by Lin et al. (2004), hence a detailed description is unnecessary". In terms of its morphology, the population described by Shao et al. (2008) also corresponds closely with the present population. However, its SSU rDNA gene sequence (DQ646880) has a dissimilarity of 2.5% (40 bp) compared with the present population, so we doubt that the two are conspecific. These findings suggest that *P. borrori* might represent a species complex, although further studies are needed in order to verify this. Shao et al. (2008) proposed that, in their study of morphogenesis, Lin et al. (2004) confused the post-lateral right marginal cirri with caudal cirri and thus erroneously counted the caudal cirri. Because the two types of cirri can be recognized clearly during morphogenesis, but are impossible to distinguish in non-dividing cells, we tentatively regard both as caudal cirri in the present population.

Certesia quadrinucleata Fabre-Domergue, 1885

Comparison with congeners (Fig. 5D–F)

Certesia quadrinucleata has been isolated in several countries including China (Chen et al. 2010; Cui et al. 2009; Lin and Song 2004), France (Fabre-Domergue 1885), Germany (Kahl 1932; Sauerbrey 1928), and the USA (Wicklow 1983). Our population resembles previous populations except for the number of left marginal cirri, i.e., four or five in our population vs. five to seven in other Chinese populations (Chen et al. 2010; Lin and Song 2004), ca. seven in the USA population (Wicklow 1983) and 6–11 in populations from France and Germany (Fabre-Domergue 1885; Kahl 1932). In terms of other key characteristics, e.g., body size and shape, ciliary pattern and biotope, our population corresponds well with known populations of *C. quadrinucleata* (Fig. 5D–F).

Vacelet (1960) established the species *Certesia ovata* mainly on the basis of the arrangement, appearance and function of the transverse cirri, i.e., five transverse cirri divided into two groups, three on the right that are straight and active, and two on the left that are slightly curved and inactive. Lin and Song (2004) believed this to be insufficient to establish a new species and considered *C. ovata* to be a synonym of *C. quadrinucleata*. However, *C. ovata* has 13 frontoventral cirri (vs. 11 or 12 in *C. quadrinucleata*), making their conspecificity less convincing. More detailed investigations are therefore needed in order to determine the validity of *C. ovata*. In the meantime, we consider *C. ovata* to be a separate species.

SSU rDNA sequences and phylogenetic analyses

In the classification of Lynn (2008), the kiitrichids are assigned as a family (Kiitrichidae) within the order Kiitrichida Nozawa, 1941 of the class Spirotrichea. Subsequent studies based on morphological, morphogenetic and

Table 5. Comparison of *Prodiscocephalus orientalis* sp. n. with similar species.[a]

Character	*P. borrori*	*P. borrori*	*P. minimus*	*Paradiscocephalus elongatus*	*P. orientalis*
Body size in vivo	100 × 35 μm	40–110 × 10–40 μm	50–60 × 20–25[b] μm	110–180 × 35–65 μm	85–140 × 25–35 μm
No. of AM	20–24	17–27		22–28	21–26
No. of FC	ca. 6	6	7	6	6–7
No. of VC	ca. 6	6	2	11–16	6–9
No. of TC	5–9	7	7	9–11	6–8
No. of RMC	11–13	7–10	4–6	14–24	10–19
No. of ALMC	8–24	9–14	7–8	16–29	15–21
No. of PLMC	8–13	6–8		9–15	5–13
No. of CC	8	4–7		5–8	6–8
No. of DK	6	6		6–7	7
No. of Ma	up to 120	23–34	3–15	30–65	21–37
Locality	New Hampshire, USA	Qingdao, China	Arcachon, France	Qingdao, China	Huizhou, China
Reference	Wicklow (1982)	Lin et al. (2004)	Dragesco (1968)	Li et al. (2008)	Present study

[a] Abbreviations: ALMC = anterior left marginal cirri, AM = adoral membranelles, CC = caudal cirri, DK = dorsal kineties, FC = cirri on head field, Ma = macronuclei, No. = number, PLMC = posterolateral left marginal cirri, RMC = right marginal cirri, TC = transverse cirri, VC = ventral cirri.
[b] width estimated from illustration.

phylogenetic analyses support this hypothesis (Jiang et al. 2013; Li et al. 2009; Miao et al. 2009). In our study, both genetic distance and phylogenetic analyses show that the genera *Caryotricha* and *Kiitrichia* are clearly distinguishable from each other, forming two sister kiitrichid groups, which is consistent with Lynn's (2008) classification.

The molecular phylogeny of three discocephalid genera, namely *Discocephalus*, *Prodiscocephalus*, and *Paradiscocephalus*, has been the subject of several recent studies (Jiang et al. 2013; Li et al. 2008; Miao et al. 2011). Nevertheless, their evolutionary relationships remain unresolved due to the scarcity of genetic markers, and the gross undersampling of the group. Our phylogenetic analyses revealed the non-monophyly of the genus *Prodiscocephalus* with *P. orientalis* sp. n. included, although its monophyly was not rejected by the AU test ($p = 0.083$). Thus, the veracity of the molecular systematics of *Prodiscocephalus* and related genera might be increased by sequencing more gene markers and/or more taxa.

The unstable phylogeny of the pseudoamphisiellids and discocephalines is also reported by Miao et al. (2011). Even though the reliability of their cluster was refused by ML and NJ trees, the families Pseudoamphisiellidae and Discocephalidae grouped within the order Discocephalida in the BI tree. This assignment was accepted by Gao et al. (2016). However, in the SSU rDNA gene trees here and in Jiang et al. (2013), the close relationship between Discocephalidae and Pseudoamphisiellidae is not supported. Therefore, we conclude that more data are needed in order to resolve the phylogeny of these lineages.

There has been controversy about the systematic position of the genus *Certesia* since its first description (Borror and Hill 1995; Corliss 1979; Curds and Wu 1983; Fabre-Domergue 1885; Kahl 1932; Lin and Song 2004; Lynn 2008). Following a morphological redescription and analysis of its molecular phylogeny, it has been suggested that *Certesia* should be placed in the *Aspidisca–Euplotes* assemblage as the sister-group of *Euplotes* (Li and Song 2006; Lin and Song 2004). Our findings support this hypothesis.

Author contributions

Chunyu Lian: Resources, Investigation, Writing (original draft). Xiaotian Luo: Funding acquisition, Writing (review & editing). Alan Warren: Writing (review & editing). Yan Zhao: Writing (review & editing). Jiamei Jiang: Project administration, Funding acquisition, Writing (review & editing).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejop.2019.125663.

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