

Redescription of a Poorly-known Marine Cyrtophorid Ciliate, *Dysteria pusilla* (Claparède et Lachmann, 1859) (Protozoa: Ciliophora: Cyrtophorida) from Qingdao, China

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Summary. The living morphology, infraciliature and silverline system of a poorly known marine cyrtophorous ciliate *Dysteria pusilla* (Claparède et Lachmann, 1859), isolated from a fish-culturing tank off Qingdao, China, have been investigated. An improved diagnosis for *Dysteria pusilla* is supplied: small marine *Dysteria*, body rectangular in outline when viewed from side, 15-30 x 10-20 μm *in vivo*; with one right ventral and 2 frontoventral kineties in right field; 7 short fragments of kineties in left equatorial field; oral structure simplified; three contractile vacuoles, two ventrally located, and one positioned dorso-frontally; macronucleus sausage-like.

Key words: Cyrtophorida, *Dysteria pusilla*, marine ciliate, morphology and infraciliature.

INTRODUCTION

The species-rich cyrtophorid ciliates *Dysteria* have been found worldwide in marine and freshwater benthic biotopes and frequently described (Dujardin 1841; Huxley 1857; Claparède and Lachmann 1859; Kent 1882; Möbius 1888; Calkins 1902; Schouteden 1906; Hamburger and von Buddenbrock 1911; Lepsi 1927; Kahl 1931, 1935; Wang and Nie 1932; Wang 1934; Wailes 1943; Tucolesco 1962; Biernacka 1963; Dragesco 1966; Jankowski 1967; Wilbert 1971; Borrer 1972; Agamaliev 1974, 1983; Aladro-Lubel *et al.* 1990; Carey 1992;

AL-Rasheid 1997). These organisms are bilaterally compressed and crawling over the substrate on their ventral side, mostly feed on bacteria, diatoms and small flagellates. Although over 30 nominal species have been reported over the last century, only within last few decades have silver impregnation techniques been employed for their study (Fauré-Fremiet 1965; Deroux 1965, 1976; Dragesco and Dragesco-Kernéis 1986; Gong *et al.* 2002). These studies have demonstrated that the ventral ciliary structure is highly species-specific and is therefore a reliable character for species separation, whereas body size, shape and other morphological characters relating to the organism *in vivo* are either variable or observer-dependent rendering identification difficult.

Recently, during an ecological as well as taxonomical survey on ciliates in biofilm (microbiotecton) of mariculture environments, we isolated a poorly-described form,

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Dysteria pusilla, of which the infraciliature and statistical data remained unknown and are supplied here.

MATERIALS AND METHODS

Dysteria pusilla (Claparède et Lachmann, 1859) was collected from the water tanks for culturing abalone (*Haliotis discus*) in the Research Laboratory of Mariculture, Ocean University of Qingdao (Tsingtao), China (36°08'N, 120°43'E). Ten glass slides, fixed in a slide frame as artificial substrate, were immersed in the water till biofilm was formed. After being exposed for about 15 days, the slides were carefully taken out and transferred to Petri dishes with marine water from the sampling site. Isolated specimens were maintained in the laboratory for about 1 week as raw cultures (water temperature 17 °C, salinity *ca* 30‰) in Petri dishes for observation and further studies.

Living cells were observed by differential interference microscopy. The infraciliature was revealed using the protargol impregnation method according to Wilbert (1975). The Chatton-Lwoff silver nitrate method was used to demonstrate the silverline system (Song and Wilbert 1995). Living individuals were examined and measured at 1000x magnification; drawings of stained specimens were performed at 1250x with the aid of a camera lucida. Terminology is mainly according to Corliss (1979) and Petz *et al.* (1995).

A few terms concerning the infraciliature of *Dysteria* are here defined.

Frontoventral kineties (FvK): somatic kineties, that extend over almost the entire length of the body; the anterior ends extend beyond the level of the cytostome and almost reach the apical groove; positioned between RvK and Re.

Right ventral kineties (RvK): ventral kineties of variable length, the anterior ends of which terminate at or below the level of cytostome; positioned between LK and FvK.

Left equatorial kineties (LK): short, densely packed ventral kineties, positioned equatorially, to the left of the RvK.

Right equatorial kinety (Re): the rightmost ventral kinety, positioned equatorially and to the right of the FvK; length depends on individuals, but usually short.

Fragment of external kinety (Fe): short kinety formed by several basal bodies, antero-dorsally positioned at the end of the FvK.

RESULTS

Morphology and infraciliature of *Dysteria pusilla* (Claparède et Lachmann, 1859) (Figs 1-5, 7-18; Tables 1-3)

Improved diagnosis of *Dysteria pusilla*: small marine *Dysteria*, body rectangular in outline when viewed from side, 15-30 x 10-20 µm *in vivo*; with one right ventral and 2 frontoventral kineties in right field; 7 short fragments of kineties in left equatorial field; oral struc-

ture simplified; three contractile vacuoles, two ventrally located, and one positioned dorso-frontally; macronucleus sausage-like.

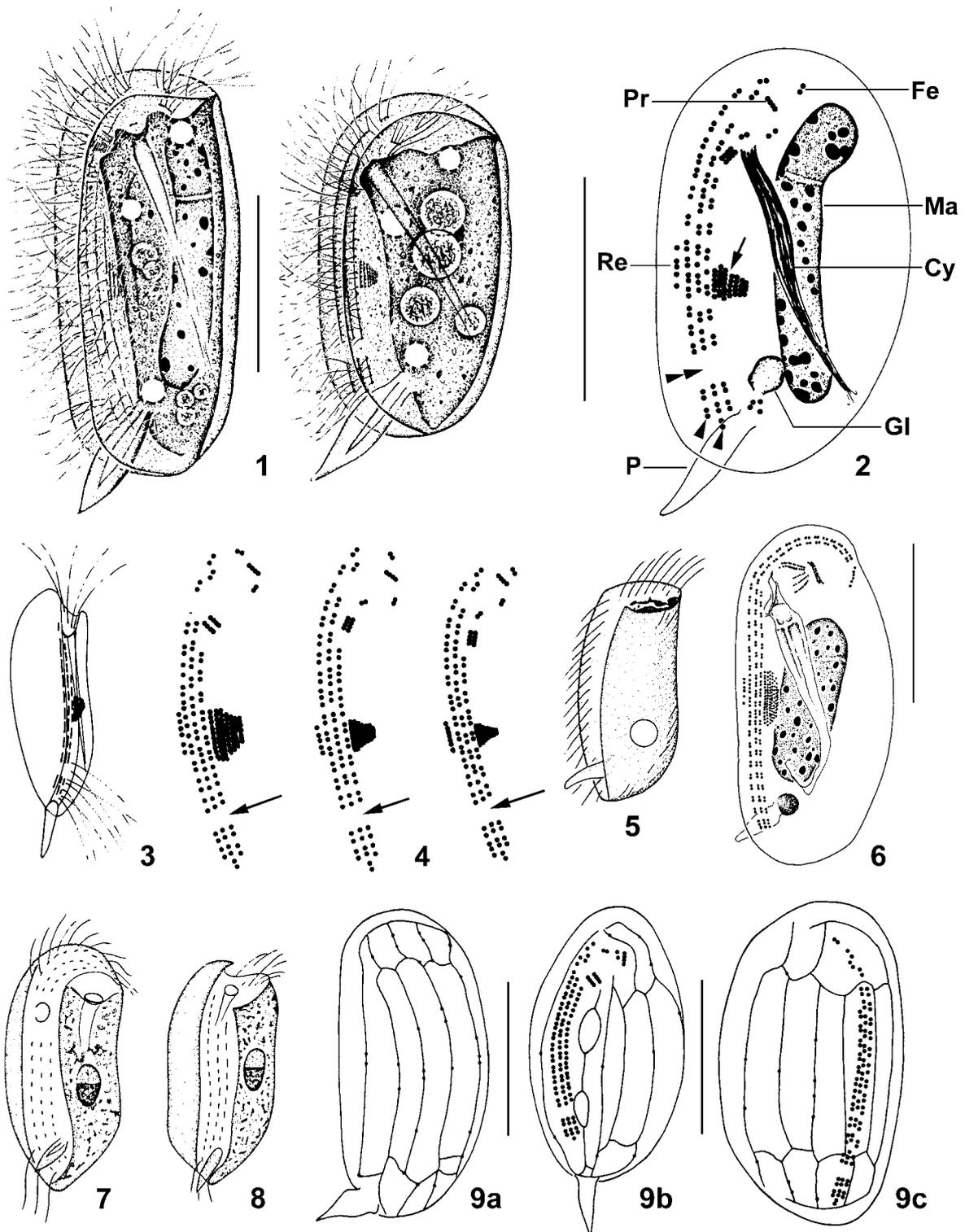
Neotype specimens: one neotype slide of protargol impregnated specimens is deposited in the Natural History Museum, UK with registration number 2003.3.26.1. Two paraneotypes are deposited in the Laboratory of Protozoology, Ocean University of China, P. R. China (number LF2001-12-02).

Description: size 15-30 x 10-20 µm *in vivo*, slender form length: width about 2:1, while in plump specimens only 3:2 (Figs 1, 10). Body shape rectangular in outline when viewed from side: ventrally straight, dorsal side slightly convex. Both anterior and posterior margin blunt rounded (Figs 1, 10). Cells bilaterally flattened (*ca* 2:3); right plate more arched and slightly larger than left (about 24 x 14 vs. 21 x 11 µm) (Figs 1, 3). No conspicuous grooves or ridges on lateral sides. Cilia about 8 µm long *in vivo*. As in its congeners, ciliary rows mostly restricted to the ventral groove between two plates (Fig. 3). Podite (P) about 8 µm in length, distal end pointed, emerges subcaudally from left posterior ventral side. Cytoplasm colourless, usually containing several small food vacuoles, 2-4 µm in diameter. Cytostome in anterior 1/5-1/6 of cell length and ventrally located. Cytopharynx typical, diagonally oriented (Figs 2, 12, 13, 15).

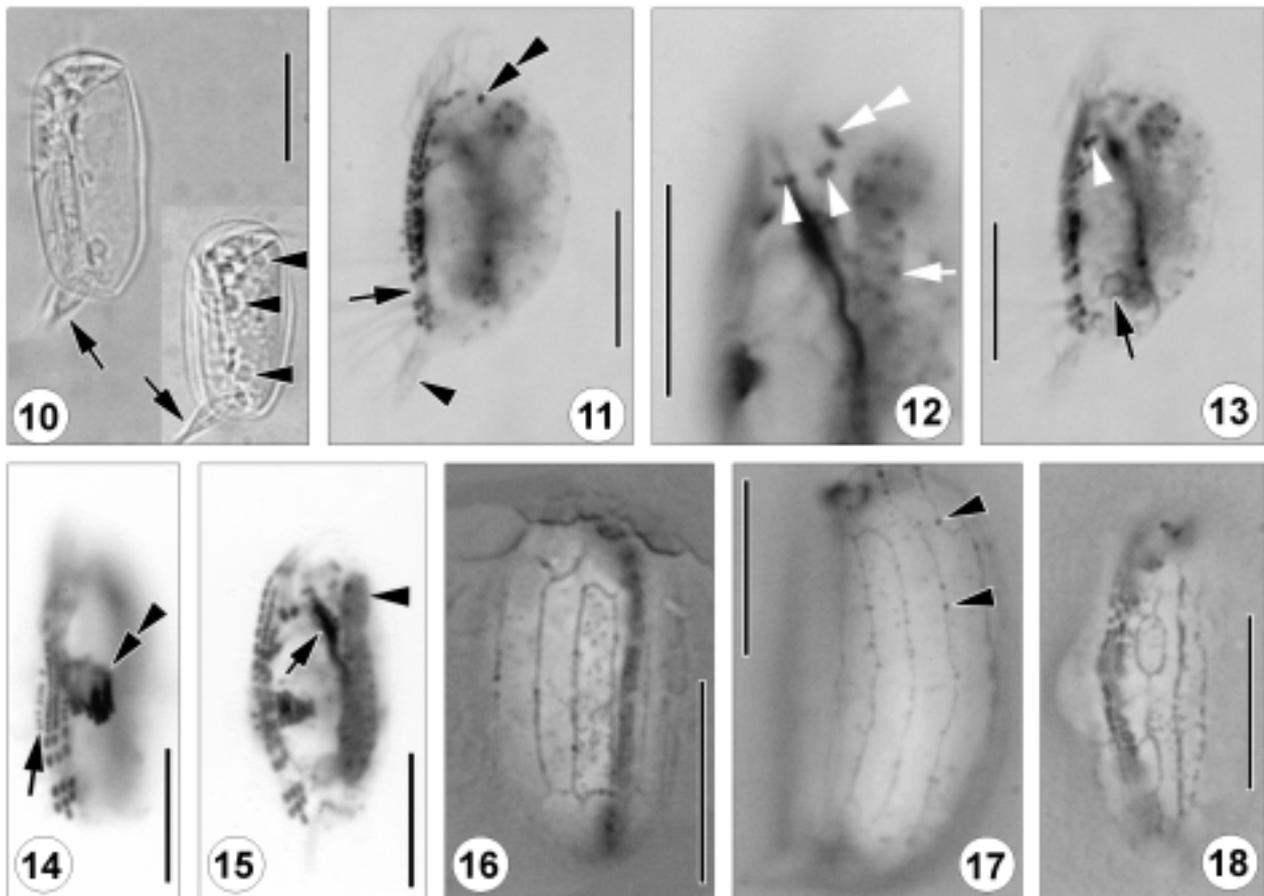
Three contractile vacuoles, 2-4 µm in diameter, of which one is located antero-dorsally in anterior 1/5-1/6 of cell length, and the other two are ventrally positioned respectively (Figs 1, 10).

Movement typical by crawling over substrate or (occasionally) swimming very slowly in water for a short while.

Infraciliature as shown in Figs 2, 4, 11. Right field occupied by 0-1 (see Table 1) short right equatorial kinety (Re) comprising about 5 basal bodies and three long ventral kineties (two frontoventral and one right ventral kinety). All 3 ventral kineties composed of loosely arranged kinetosomes, which are, unlike in most other congeners, basically not fragmented except in the posterior part, where there is a conspicuous gap present (about 1.5 µm wide; double-arrowheads in Fig. 2; arrows in Fig. 4). Two frontoventral kineties (see Fig. 2, arrows) equal in length, each row consisting of 30-40 basal bodies (Table 1). One right ventral kinety (RvK) terminating subapically posterior to the level of cytostome, with about 20 basal bodies. The fragment of external kinety consisting of only one pair of basal bodies (Fig. 2,



Figs 1-9. *Dysteria pusilla* (1-4, 9, original; 5, from Claparède and Lachmann 1859; 7, 8 from Kahl 1935. 1, 3, 5, 7, 8, from life; 2, 4, protargol impregnation; 9, silver nitrate impregnation) and *D. cristata* (6, from Gong *et al.* 2002, protargol impregnation). **1** - left side views of two slender and wider individuals; **2** - left view of infraciliature, arrow indicates left equatorial kineties, arrowheads mark the posterior ends of two frontoventral kineties, double-arrowheads indicate the posterior gap of ventral kineties; **3** - ventral view; **4** - showing arrangement of the oral and somatic kineties from three individuals, arrows point to posterior gaps; note that that most oral structure is highly reduced into pairs of kinetosomes; **5** - left side view; **6** - left side view of infraciliature; **7, 8** - left side (**7**) and ventral (**8**) view; **9a-c** - left (**a**) and right side (**c**) as well as left-ventral view (**b**) of silverline system. Cy - cytopharynx, Fe - fragment of external kinety, Gl - glandule, Ma - macronucleus, P - podite; Pr - preoral kinety; Re - right equatorial kinety. Scale bars 12 μ m (1, 2, 9); 20 μ m (6).



Figs 10-18. Photomicrographs of *Dysteria pusilla* from life (10), after protargol (11-15) and silver nitrate impregnation (16-18). **10** - left view of two typical individuals; arrows indicate the podite, arrowheads mark the three contractile vacuoles; **11** - left view of infraciliature, arrow indicate the posterior gap in ventral kineties, arrowhead points to the podite, double-arrowheads mark the fragment of external kinety consisting of two kinetosomes; **12** - anterior portion, note the two pairs of kinetosomes (arrowheads) and the preoral kinety (double-arrowheads), arrow indicates the sausage-like macronucleus; **13** - left view, showing the argentophilic glandule (arrow) and preoral kinety (arrowhead); **14** - focusing on kinety level, to demonstrate the right equatorial kinety (arrow) and the densely-arranged left equatorial kineties (double-arrowheads); **15** - general view, note the cytopharynx (arrow) and the macronucleus (arrowhead); **16-18** - silverline system from right (**16**) and left side view (**17**), and left-ventral view (**18**), arrowheads indicate the tiny argentophilic granules in silverlines. Scale bars 10 μm .

Table 1. Morphometric characteristics of *Dysteria pusilla* (from protargol impregnated specimens). Abbreviations: Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of individuals examined, SD - standard deviation.

Characters	Min	Max	Mean	SD	n
Body length (μm)	17	24	21.3	2.0	36
Body width (μm)	8	16	12.1	2.1	36
Frontoventral kineties, number	2	2	2	0	36
Right ventral kineties, number	1	1	1	0	36
Left equatorial kineties, number	7	7	7	0	36
Basal bodies in one frontoventral kinety, number	30	41	32.8	7.7	15
Basal bodies in fragment of external kinety, number	2	2	2	0	16
Basal bodies in right equatorial kinety, number	0	7	3.8	2.1	16
Macronucleus, length (μm)	10	19	15.8	2.1	36
Macronucleus, width (μm)	2.5	4	2.8	0.4	36
Glandule, diameter (μm)	1	2.4	1.9	0.4	32

Table 2. Comparison of *Dysteria pusilla* with three closely-related marine *Dysteria* species.

Characters	<i>D. pusilla</i> (Claparède et Lachmann, 1859)	<i>D. cristata</i> (Gourret et Roeser, 1888) Kahl, 1931	<i>D. calkinsi</i> (Calkins, 1902) Kahl, 1931	<i>D. ovalis</i> (Gourret et Roeser, 1886) Kahl, 1931
Body size <i>in vivo</i> (µm)	15-30 x 10-20	40-50 x 25 -30	30-50 x 20-25	-
Body shape from side view	rectangular	wide oval	rectangular	oval
Contractile vacuoles, number	3	2	-	1*
Macronucleus, size (µm)	16 x 3	13 x 6	25 x 15	-
Right ventral kineties, number	1	1	3	2
Left equatorial kineties, number	7	5-7	ca 6	-
Frontoventral kineties, number	2	2	2	2
Basal bodies in one frontoventral kinety, number	30-41	72-90	-	-
Caudal spine	absent	absent	absent	absent
Argentophilic glandule, diameter (µm)	1- 2.4	2.5 -3	-	-
Podite, length (µm)	ca 8	ca 8	ca 15	-
Data source	Original	Gong <i>et al.</i> (2002)	Song and Wilbert (2002)	Fauré-Fremiet (1965)

- Data not available; * Data from Kahl (1931)

Table 3. Comparisons between the seven species of *Dysteria* for which the infraciliature is known.

Species	Body size <i>in vivo</i> (µm)	Right ventral kineties, No.	Left equatorial kineties, No.	Frontoventral kineties, No.	Data source
<i>D. pusilla</i> (Claparède et Lachmann, 1859)	15-30 x 10-20	1	7	2	Original
<i>D. cristata</i> (Gourret et Roeser, 1888) Kahl, 1931	40-50 x 25-30	1	5-7	2	Gong <i>et al.</i> (2002)
<i>D. armata</i> Huxley, 1857	-	2	-	4	Fauré-Fremiet (1965)
<i>D. monoxyla</i> (Ehrenberg, 1838) Kahl, 1931	80-110 x 30-40	3	6-9	2	Gong <i>et al.</i> (2002)
<i>D. brasiliensis</i> Faria, Cunha et Pinto, 1922	100-130 x 30-34	3	7-8	2	Song and Packroff (1997)
<i>D. ovalis</i> (Gourret et Roeser, 1886) Kahl, 1931	-	2	-	2	Fauré-Fremiet (1965)
<i>D. calkinsi</i> (Calkins, 1902) Kahl, 1931	30-50 x 20-25	3	ca 6	2	Song and Wilbert (2002)
<i>D. antarctica</i> Gong <i>et al.</i> 2002	145 x 65	2	ca 6	3	Petz <i>et al.</i> (1995)

- Data not available.

Fe). Seven left kineties as short rows of densely packed kinetosomes in mid-body area (Fig. 2, arrow; Fig. 12, double-arrowhead). Macronucleus long and sausage-like, about 16 x 3 μm after protargol impregnation longitudinally positioned in mid-body, characteristically heteromerous; micronucleus not observed. One argentophilic glandule (Fig. 2, G1; Fig. 13, arrow) subcaudally positioned, about 2 μm in diameter, often together with 3-4 unciliated basal bodies, always present near the base of podite.

Oral kineties simplified and difficult to define, but basically consisting of one short single-rowed and one double-rowed fragments, as well as 2 pairs of basal bodies (seldom one).

Silverline system on both left and right plate generally similar, composed of *ca* 10 longitudinal silverlines. Two transverse silverlines positioned subapically and subcaudally, respectively; silverline pattern of ventral field comprising two loops connected by a single line (Figs 9b, 18). Tiny argentophilic granules sparsely distributed on silverlines (Figs 9a-c, 16-18).

DISCUSSION

Comparison with related species

Dysteria pusilla was originally described by Claparède and Lachmann (1859) under the name *Aegyria pusilla*, which was very briefly characterized by “very small and without ribs”, no further information was given about the size, the number of contractile vacuoles and the shape of macronucleus in both original and the subsequent redescription (Claparède and Lachmann 1859, Kent 1882), Hamburger and von Buddenbrock (1911) reported a population isolated from the North Sea, Germany, with body size *ca* 32 x 24 μm . Later, this organism was simply mentioned again by Kahl (1931), and was illustrated possessing an elliptical macronucleus with body size of 25 μm in his subsequent document (Kahl 1935). Since then, no redescriptions have been made. In the absence of data concerning the infraciliature, we identified our organism mainly on the basis of its size, body shape, and the habitat. The only difference is the shape of macronucleus: according to Kahl’s (1935) redescription, *D. pusilla* has an oval shaped macronucleus, while, in Qingdao population, it is consistently sausage-like. Since the shape of macronucleus, as to the authors’ knowledge, is - in most cases - a population-dependent feature,

hence, we consider this difference as an intra-species variation.

As much as we know, 7 species have been described using modern methods (Table 3): *D. armata*, *D. monostyla*, *D. brasiliensis*, *D. antarctica*, *D. calkinsi*, *D. ovalis* and *D. cristata* (Deroux 1965, Fauré-Fremiet 1965, Dragesco and Dragesco-Kernéis 1986, Petz *et al.* 1995, Song and Packroff 1997, Gong *et al.* 2002, Song and Wilbert 2002). The former four species have considerable larger cell size (over 60 μm), and more ventral kineties (4 or more *vs.* 3), and thus can be clearly separated from *D. pusilla*.

Dysteria pusilla differs from *D. calkinsi* (Calkins, 1902) Kahl, 1931 in terms of: (1) the general appearance *in vivo* (rectangular *vs.* elongate with 2 conspicuous, longitudinally positioned ridges); (2) the infraciliature (3 *vs.* 5 ventral kineties) and (3) macronuclear size (16 x 3 *vs.* 25 x 15 μm) (Song and Wilbert 2002).

Although the cell size of *D. ovalis* (Gourret et Roeser, 1886) Kahl, 1931 remains unknown, it can be separated from *D. pusilla* by the body shape *in vivo* (oval *vs.* rectangular in *D. pusilla*) and the infraciliature (consistently 4 *vs.* 3 ventral kineties) (Fauré-Fremiet 1965).

Dysteria cristata (Gourret et Roeser, 1888) is perhaps the most similar organism to the Qingdao population of *D. pusilla*, *i.e.* both species have 3 ventral kineties (Figs 2, 6; Tables 2, 3). However, *D. pusilla* is identified by: (1) ventral kinety sparsely ciliated (30-40 basal bodies in each row *vs.* 72-90 in *D. cristata*); (2) the ventral kineties (FvK and RvK) in *D. pusilla* consistently exhibit a cilia-free gap at the posterior portion (*vs.* continuous in *D. cristata*); (3) highly simplified oral infraciliature in the former (see Figs 2, 6), and (4) the number of contractile vacuoles (3 *vs.* 2) (Gong *et al.* 2002).

Considering the small size, living body shape and habitat, at least two other incompletely described species (infraciliature unknown), *D. compressa* (Gourret et Roeser, 1888) and *D. navicula* Kahl, 1928, should be compared as well with *D. pusilla*.

Dysteria compressa differs from *D. pusilla* in the caudal spine (present *vs.* absent) and possibly also in the number of contractile vacuoles (2 *vs.* 3) (Table 2).

Dysteria navicula can be distinguished from *D. pusilla* by its larger body size (*in vivo* 35- 45 x 14- 18 *vs.* 15-30 x 10-20 μm), the body shape (slender and spindle-shaped *vs.* rectangular) and the number of contractile vacuoles (2 *vs.* 3) (Table 2) (Kahl 1931).

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