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盾圆双眉虫与伪寡毛双眉虫的形态学重描述(原生动物, 纤毛门)

许 媛[1,2] 李继秋[1] 胡晓钟[2]

(1. 华南师范大学生命科学学院, 广东省高等学校生态与环境科学重点实验室, 原生动物学研究室, 广州 510631;
2. 中国海洋大学海洋生物多样性与进化研究所, 原生动物学研究室, 青岛 266003)

摘要: 研究对采自青岛沿海的两种海洋纤毛虫-盾圆双眉虫与伪寡毛双眉虫做了形态学重描述。盾圆双眉虫与前人所报道的种群具有十分相似的纤毛图式, 但在额-腹棘毛分布、大核片段、小膜及背触毛数目等方面表现出细微的变异性。此外, 该青岛种群个体较小。统计学比较还表明, 迄今缺乏研究的一海洋种, 泥生双眉虫极可能为盾圆双眉虫(*Diophrys scutum*)的同物异名。伪寡毛双眉虫(*Diophrys apoligothrix*)为一新近报道的罕见种, 研究基于新采集种群对其进行了补充性观察和描述。

关键词: 原生动物; 海洋纤毛虫; 双眉虫属; 纤毛图式.
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双眉虫是一类典型的海洋自由生纤毛虫, 在分类上隶属于游仆目、游仆虫科[1]。对于该属的研究已有许多报道, 但种类鉴定仍存在较多问题[2, 3]。其原因主要为, 在前人的报道中对于新种的描述基本是依据活体形态观察给出, 而缺乏必要的统计学数据和纤毛图式信息。因此, 依据现代分类学技术和观念对该类群进行补充描述是非常必要的。本文重描述了盾圆双眉虫(*Diophrys scutum*)和伪寡毛双眉虫(*Diophrys apoligothrix*)的两个沙栖种群, 并将其与相近种(群)进行了比较, 补充了活体及银染照片。

1 材料与方法

本文所涉及的两个种均于 2008 年 10 月采自青岛潮间带沙滩, 水温约 18℃, 盐度约 18‰。分离、培养、观察、染色制片等研究方法及所用名词术语主要参照 Corliss 和 Song, *et al*.[4, 5]。

2 结 果

2.1 盾圆双眉虫 *Diophrys scutum* (Dujardin, 1841) Kahl, 1932(图版Ⅰ; 表 1)

活体大小(80—100) μm×(35—60) μm; 虫体呈盾圆形 (图版Ⅰ-A-C、L); 背部隆起, 腹面平直, 厚腹比约 1∶2.5; 活体呈浅灰色。口区发达, 约占体长的 2/3 (图版Ⅰ-C); 口围带由 43—59 片小膜组成, 远端小膜绕过虫体前端背面后止于体右前 2/5 处(图版Ⅰ-F); 前端小膜长约 25 μm。波动膜发达, 位于口区右侧, 由口侧膜和口内膜构成; 口侧膜由许多短列的毛基粒组成, 每列含 2—3 个毛基粒, 口内膜则为单列毛基粒。额棘毛多为 5 根(极少 4 根), 长约 30 μm (图版Ⅰ-G, 箭头; 图版Ⅰ-M); 腹棘毛为 2—3 根, 长约 20 μm (图版Ⅰ-J, 单箭头; 图版Ⅰ-K, 箭头; 图版Ⅰ-M); 左缘棘毛恒为 2 根, 长约 30 μm (图版

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作者简介: 许媛(1986—), 女, 山东滨州人; 硕士研究生; 研究方向为原生动物学。E-mail: hdxy8626@163.com
通讯作者: 李继秋, E-mail: jqli@scnu.edu.cn

Ⅰ-J, 箭头; 图版Ⅰ-M); 3 根粗大的尾棘毛由体后右侧的凹陷处伸出, 长约 50 μm (图版Ⅰ-J, 双箭头; 图版Ⅰ-M); 横棘毛亦粗大, 恒为 5 根, 长约 50 μm (图版Ⅰ-K, 单箭头; 图版ⅠM)。背触毛短茬状, 长约 10 μm, 在体侧面和背面呈 6—7 列排布 (图版Ⅰ-H、I, 单箭头; 图版Ⅰ-N); 染色后显示其毛基粒对排列较密且连续。虫体背部皮层表面有均匀分布的圆形或卵圆形皮层突起, 直径约 0.5 μm, 无色 (图版Ⅰ-E, 箭头)。大核多由 2 枚(极少 1 枚)腊肠状片段组成 (图版Ⅰ-F、N); 小核在活体和染色标本均不可见。

运动较迅速, 爬行于底质上。

2.2　伪寡毛双眉虫 *Diophrys apoligothrix* Song *et al.*, 2009 (图版Ⅱ; 表 1)

活体大小(35—75) μm× (25—40) μm。虫体呈卵圆形或长方形 (图版Ⅱ-A-C、J)。虫体厚腹比约 1∶2 (图版Ⅱ-D)。内质无色, 虫体无色或浅灰色。口区阔大, 约占体长的 3/5; 口围带由 24—31 片小膜组成, 口围带前端小膜长约 15 μm (图版Ⅱ-E、G, 单箭头; 图版Ⅱ-L)。波动膜发达, 位于口区右侧, 由口侧膜和口内膜构成; 口侧膜由许多短列的毛基粒组成, 每列含 2—3 个毛基粒, 口内膜为单列毛基粒。额棘毛 5 根, 长约 15—25 μm (图版Ⅱ-E,箭头; 图版 Ⅱ-K)。腹棘毛 2 根, 长约 15—20 μm (图版Ⅱ-H,单箭头; 图版Ⅱ-K)。左缘棘毛 1 根, 长约 15 μm (图版Ⅱ-H、K, 箭头)。尾棘毛 3 根, 长约 25—30 μm (图版Ⅱ-E, 单箭头; 图版Ⅱ-K)。横棘毛 5 根, 长约 35—40 μm (图版Ⅱ-G, 箭头; 图版Ⅱ-K)。5 列背触毛, 其中毛基粒对排列稀疏且连续, 背触毛长约 5—10 μm (图版Ⅱ-F, 单箭头; 图版Ⅱ-L)。虫体后部有内贮颗粒, 直径约 2.5—10 μm。大核多由 2 枚(极少 3 枚)长椭圆形片段组成 (图版Ⅱ-F, 箭头; 图版Ⅱ-I、J); 小核在活体和染色标本均不可见。

虫体运动较为缓慢, 时常在水中跳跃。

表 **1**　盾圆双眉虫与伪寡毛双眉虫的统计学特征
Tab. 1 Morphometric data on *Diophrys scutum* and *D. apoligothrix*

特征 Characters	种名 Species	最小值 Min	最大值 Max	平均值 Mean	标准差 SD	变异系数 *CV*	个体数 *n*
体长 Body length	盾圆双眉虫 *D. scutum*	65	125	105.4	2.3	12	29
	伪寡毛双眉虫 *D. apoligothrix*	48	75	59.8	7.2	12.1	26
体宽 Body width	盾圆双眉虫 *D. scutum*	42	77	61.9	1.5	13.1	29
	伪寡毛双眉虫 *D. apoligothrix*	30	55	41.1	6.7	16.4	26
围口小膜数 Number of adoral membranelles	盾圆双眉虫 *D. scutum*	43	59	50.6	3.5	6.9	29
	伪寡毛双眉虫 *D. apoligothrix*	24	31	27.1	1.5	5.6	26
额腹棘毛数 Number of frontoventral cirri	盾圆双眉虫 *D. scutum*	7	7	7	0	0	29
	伪寡毛双眉虫 *D. apoligothrix*	5	5	5	0	0	26
左缘棘毛数 Number of left marginal cirri	盾圆双眉虫 *D. scutum*	2	2	2	0	0	29
	伪寡毛双眉虫 *D. apoligothrix*	1	1	1	0	0	26
尾棘毛数 Number of caudal cirri	盾圆双眉虫 *D. scutum*	3	3	3	0	0	29
	伪寡毛双眉虫 *D. apoligothrix*	3	3	3	0	0	26

横棘毛数 Number of transverse cirri	盾圆双眉虫 *D. scutum*	5	5	5	0	0	29
	伪寡毛双眉虫 *D. apoligothrix*	5	5	5	0	0	26
中间一列背触毛的毛基体对数 Number of basal body pairs in middle dorsal kinety	盾圆双眉虫 *D. scutum*	6	11	9.1	0.3	16.6	23
	伪寡毛双眉虫 *D. apoligothrix*	5	6	5.3	0.5	9.4	9
背触毛列数 Number of dorsal kineties	盾圆双眉虫 *D. scutum*	6	7	6.1	0.3	5.1	29
	伪寡毛双眉虫 *D. apoligothrix*	5	5	5	0	0	26

注: 自蛋白银制片; 单位: 微米

Note: Data based on protargol impregnated specimens and measurements in μm. Abbreviations: CV, coefficient of variation in %; Max, maximum; Min, minimum; Mean, arithmetic mean; n, number of specimens examined; SD, standard deviation

3 讨 论

3.1 盾圆双眉虫 *Diophrys scutum* (Dujardin, 1841) Kahl, 1932

盾圆双眉虫较为常见, 并有较详细的描述[6, 7]。然而该种由于形态特征上的高度变异性, 迄今已出现许多错误描述与同物异名[6, 8—12], 故需要详细比较。

与已知种群间的比较 Song & Packroff[7] 曾对该种做出详细报道, 所报道的纤毛图式与该种群的纤毛图式相近。但个体大小及围口小膜数有所不同: 前者活体体长为 140—200 μm, 围口小膜数为 61—73; 后者活体长度为 80—100 μm, 围口小膜数为 43—59。对于小膜数上的差别, 我们认为是由于个体大小差别导致, 属于种群间的差别。正是由于种群间或种群内在大小及形态上的变异, 盾圆双眉虫曾被多次错误鉴定, 因而出现诸多同物异名, 如 *Diophrys scutoides*、*D. quadricaudatus*、*D. magnus*、*D. tetramacronucleata* 和 *D. kasymovi* 均被认为是盾圆双眉虫的晚出异名[6, 8—12]。

基于本次观察结果, 综合前人所报道的其他种群特征(排除 *D. tetramacronucleata* 的数据, 因其可能属分裂间期而非正常时期), 这里给出盾圆双眉虫的新定义: 虫体呈盾圆形, 活体长度为 80—200 μm; 43—80 片围口小膜; 5—8 根额腹棘毛; 5—6 根横棘毛; 2—3 根左缘棘毛和 3—4 根尾棘毛; 5—9 列背触毛; 普遍 2 枚腊肠状的大核。本文所报道的种群是本种中体型较小的一个种群。

与属内相近种间的比较 (表 2、3) 自 Hill & Borror[17] 将广义的双眉虫属拆分成双眉虫属 *Diophrys*、拟双眉虫属 *Paradiophrys* 和类双眉虫属 *Diophryopsis* 以来, 双眉虫属迄今仅包括 9 个有效种, 即盾圆双眉虫 *Diophrys scutum*、悬游双眉虫 *D. appendiculata*、寡毛双眉虫 *D. oligothrix*、泥生双眉虫 *D. peloetes*、卡尔双眉虫 *D. kahli*、盐双眉虫 *D. salina*、多核双眉虫 *D. multinucleata*、日本双眉虫 *D. japonica* 和伪寡毛双眉虫 *D. apoligothrix*。与本种较相近的有寡毛双眉虫和泥生双眉虫。寡毛双眉虫

表 2 双眉虫属内几个相近种的形态学比较

Tab. 2 Morphological comparison of several closely related *Diophrys* species (Unite: μm)

种名 Species	活体长度 Body length *in vivo*	围口小膜数 Number of adoral membranelles	额腹棘毛数 Number of frontoventral cirri	横棘毛数 Number of transverse cirri	左缘棘毛数 Number of left marginal cirri	尾棘毛数 Number of caudal cirri	背触毛列数 Number of dorsal kineties	数据出处 Data source
盾圆双眉虫 *D. scutum*	80—100	43—59	7 或 8	5	2	3	6 或 7	present work
盾圆双眉虫 *D. scutum*	140—200	61—73	7	5	2	3	6 或 9	Song and Packroff [7]
盾圆双眉虫[1]	110—120*	75—80	5	5	2	3	5	Agamaliev [8]

D. scutum								
盾圆双眉虫[2] *D. scutum*	160—200	56—60	7	5	2	3	6	Raikov and Kovaleva [11]
盾圆双眉虫[3] *D. scutum*	90—150*	54—62	8	6	2	3	6	Agamaliev [9]
盾圆双眉虫[4] *D. scutum*	55—65	36	7	5	2	3	5	Kattar [10]
盾圆双眉虫[5] *D. scutum*	100—110	50—60	7	5	3	4	6	Agamaliev [8]
寡毛双眉虫 *D. oligothrix*	50—100	26—35	7 或 8	5	2	3	4 或 5	Song and Packroff [7]
泥生双眉虫 *D. peloetes*	95—134	—	7	5	2	3	8	Borror [13]
泥生双眉虫 *D. peloetes*	45—65	60—65	7	5	2	3	8	Alekperov and Asadullayeva [14]
泥生双眉虫[6] *D. peloetes*	100—167	*ca.*50	7	5	2	3	8	Borror [15]

注: 1. 曾用名 *D. scutoides*; 2. 曾用名 *D. magnus*; 3. 曾用名 *D. kasymovi*; 4. 曾用名 *D. tetramacronucleata*; 5. 曾用名 *D. quadricaudatus*; 6. 曾用名 *D. scutum*; 1, 4, 5. 引自 Song[12]; 2, 3. 引自 Curds & Wu[6]; 6. 引自 Hu[16]

Note: 1. Formerly called *D. scutoides*; 2. formerly called *D. magnus*; 3. formerly called *D. kasymovi*; 4. formerly called *D. tetramacronucleata*; 5. formerly called *D. quadricaudatus*; 6. formerly called *D. scutum*; 1, 4, 5. synonyms suggested in Song[12]; 2, 3. synonyms suggested in Curds & Wu[6]; 6. synonyms suggested in Hu[16]; * after fixation, - no data available

表 3　盾圆双眉虫与泥生双眉虫每列背触毛的毛基体对数比较 (动基列从左到右编号)

Tab. 3　Number of basal body pairs per dorsal kinety in *Diophrys scutum* and the so-called *D. peloetes* (numbered from left to right)

背触毛列 Dorsal kinety	盾圆双眉虫[1] *D. scutum*	盾圆双眉虫[2] *D. scutum*	盾圆双眉虫[2] *D. scutum*	盾圆双眉虫[2] *D. scutum*	盾圆双眉虫[2] *D. scutum*	泥生双眉虫[2] *D. peloetes*
1	9—16	15—20	23—32	19	16—27	8—15
2	9—13	13—22	20—30	24	20—34	6—16
3	8—14	16—24	19—29	21	17—25	5—13
4	6—11	16—20	23—28	absent	18—24	5—13
5	5—12	11—14	16—22	6	12—18	6—17
6	5—15	—	—	—	—	7—16
7	8—15	—	—	—	—	6—10
8	—	—	—	—	—	6—12

注: 1. 数据自本工作; 2. 数据自 Borror, 1965 [13]

Note: 1. Data source- present; 2. Data source-B-orror, 1965 [13]

与本种的区别在于: 前者体型细长, 围口小膜数少, 口围带绕到虫体右侧的部分较后者明显短。

泥生双眉虫与本种非常相似, 迄今已报道过两个种群[13, 14]。另外, Borror[15] 报道的盾圆双眉虫据称是泥生双眉虫的一个种群[16]。Borror [11]认为该种与盾圆双眉虫的主要区别之一就在于前者的背触毛列数较多, 且每列背触毛的毛基体对数明显少于后者。表 3 给出了 5 个盾圆双眉虫种群与 1 个泥生双眉虫种群背触毛列的毛基体对数的比较。由此可见, 至少 2 个种群(尤其本次报道的种群)在背触毛的稀疏程度上与泥生双眉虫非常接近。另外, 考虑到盾圆双眉虫个体大小与形态统计学特征上的较大变异性, 以及这些种群在纤毛图式上的吻合性, 有理由认为泥生双眉虫与盾圆双眉虫在背触毛上的差别仅为种群间差异, 两者应为同种。

3.2　伪寡毛双眉虫 *Diophrys apoligothrix* Song *et al.*, 2009

与已知种群的比较　　本种较为罕见, 仅 Song, *et al*.[18]首次报道。本种群在形态与纤毛图式上与原始报道基本吻合, 但在个体大小及中间一列背触毛

的毛基体对数上有所不同: (1)前者活体大小为(35—75) μm× (25—40) μm, 而后者为 (60—103) μm× (40—70) μm; (2)前者中间一列背触毛列的毛基体对数目较稳定(5—6), 而后者变异较大(4—8)。因这些差异均可视为种群间差别, 故两者应为同种。

与属内相近种的比较 本属内与该种最为相近的是日本双眉虫 *Diophrys japonica*[16]。但二者存在明显差别: (1)本种具 5 列背触毛, 中间一列含 5—6 对毛基体; 日本双眉虫恒为 4 列背触毛, 中间一列含 10 多对毛基体。(2)本种具有较少的围口小膜数(24—31 *vs.* 30—46)。(3)日本双眉虫具有皮层颗粒, 而本种未观察到。(4)日本双眉虫在口围带下方有孤立的 2—3 对毛基体, 而本种无。

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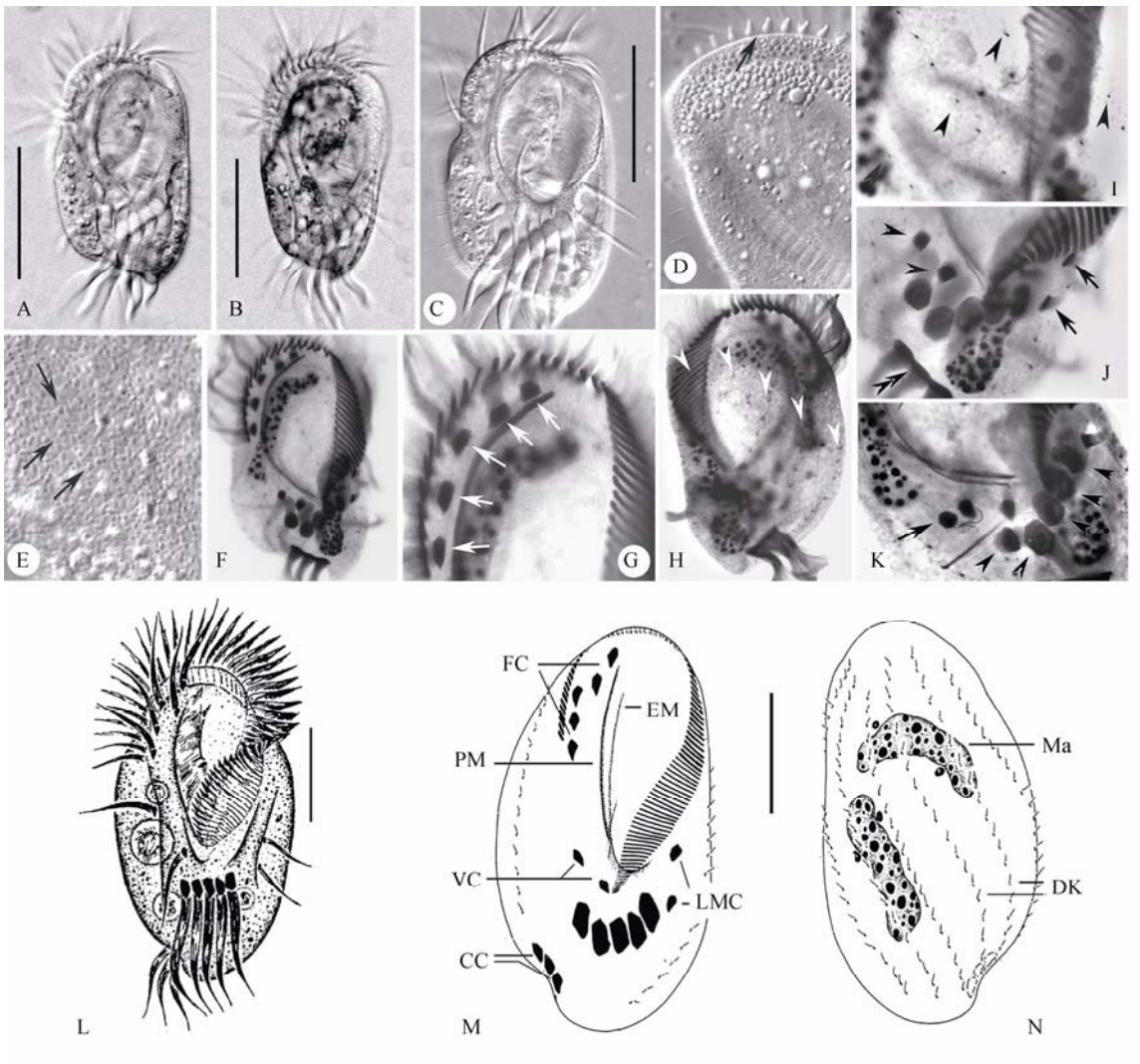
REDESCRIPTIONS OF TWO MARINE CILIATES, *DIOPHRYS SCUTUM* (DUJARDIN, 1841) KAHL, 1932 AND *DIOPHRYS APOLIGOTHRIX* SONG *ET AL.*, 2009 (PROTOZOA, CILIOPHORA)

XU Yuan[1,2], LI Ji-Qiu[1] and HU Xiao-Zhong[2]

(1. *Laboratory of Protozoology, Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education*, *South China Normal University*, *Guangzhou* 510631, *China*; 2. *Laboratory of Protozoology*, *Institute of Evolution & Marine Biodiversity*, *Ocean University of China*, *Qingdao* 266003, *China*)

Abstract: *Diophrys* species are a group of marine cosmopolitan free-living ciliates, therefore there are many reports concerning their morphology and diversity. However, species identification remains problematic owing to the fact that many previous descriptions were basically based on live observation and morphometric, and infraciliature data still remain lacking for some known species. The aims of this paper were to supply new information and to clarify confusions of two known *Diophrys* species, *D. scutum* (Dujardin, 1841) Kahl, 1932 and *D. apoligothrix* Song *et al*, 2009. Both organisms were collected from the upper layer of sandy sediments in the intertidal region in Qingdao, China, where the water temperature was about 18℃ and the salinity was 18‰. After isolation, pure cultures were kept, to which some rice grains were added to enrich bacterial food. Ciliates were examined *in vivo* using bright field and differential interference contrast microscopy. The infraciliature was revealed with protargol staining method. Counts and measurements of stained specimens were performed at a magnification of 1250 ×. Based on present and previous studies, an improved diagnosis was presented here for *D. scutum*: marine *Diophrys*, broadly oval body outline, *in vivo* 80—200 μm long; 43—80 adoral membranelles and the distal end of adoral zone was strongly curved; 5—8 frontoventral, 5—6 transverse, 2—3 left marginal and 3—4 caudal cirri; 5—9 dorsal kineties, which were always densely ciliated; usually two (rarely one) sausage-shaped macronuclear nodules. *D. apoligothrix* was a rare species, and the current population of it was morphologically identified by: (35—75) μm × (25—40) μm *in vivo*; body rigid with sculptured surface; adoral zone comprising about 24—31 membranelles; 5 frontal, 2 ventral, 5 transverse, 1 left marginal and 3 caudal cirri; 5 dorsal kineties and the middle kinety had about 5—6 basal body pairs, dorsal cilia about 5—10 μm; usually two ellipsoid macronuclear nodules; marine habitat. By now, nine morphospecies were included in the genus *Diophrys*, namely *D. scutum*, *D. appendiculata*, *D. oligothrix*, *D. peloetes*, *D. kahli*, *D. salina*, *D. multinucleata*, *D. japonica* and *D. apoligothrix*. Among these forms, *D. oligothrix* and *D. peloets* were very similar to *D. scutum*. However, *D. oligothrix* differed from *D. scutum* in: (1) slender body shape (*vs.* broadly oval body shape); (2) lower number of adoral membranelles (25—37 *vs*. 50—80); (3) the distal end of adoral zone was slightly curved (*vs*. strongly curved). According to former research, *D. peloetes* was separated from *D. scutum* by having 8 dorsal kineties and fewer cilia per kinety. However, based on data available and morphometric comparison, *D. peloetes* might be regarded as a junior synonym of *D. scutum*. Compared with other *Diophrys* species, *D. japonica* was closely related to *D. apoligothrix*. But the former could be distinguished from the later in having fewer dorsal kineties (4 *vs*. 5), more basal body pairs per kinety (10 *vs*. 5—6), higher number of adoral zone membranelles (30—46 *vs*. 24—31), cortical granules and 2—5 basal body pairs located close to the proximal membranelles.

Key words: Protozoa; Marine ciliate; *Diophrys*; Infraciliature



图版Ⅰ 盾圆双眉虫的活体形态(图 A-E, L)及蛋白银染色后纤毛图示(图 F-K, M, N)

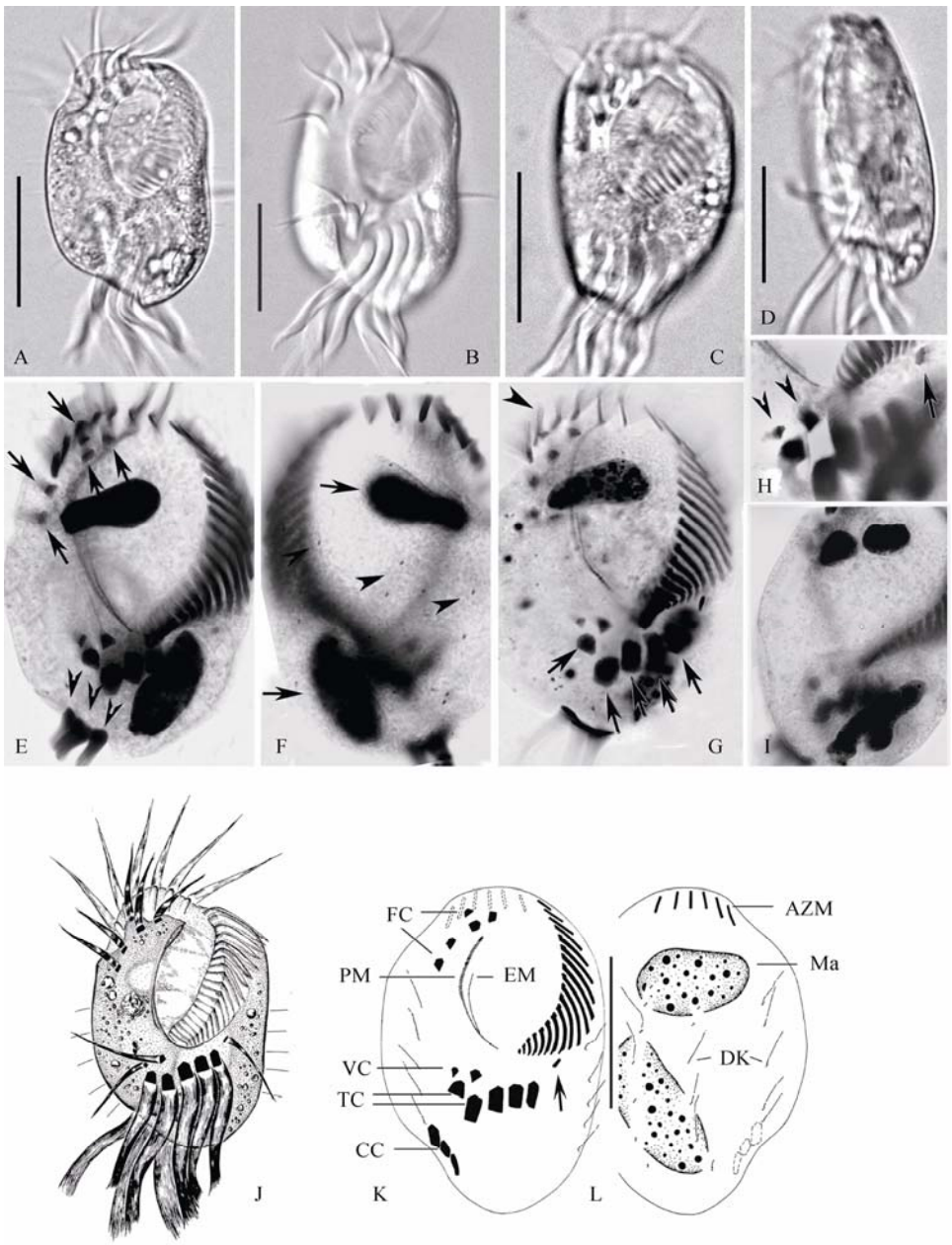
Plate Ⅰ Living morphology of *Diophrys scutum* from life (A-E, L) and after impregnation (F-K, M, N)

A-C. 低倍镜下腹面观不同体形; D. 背面观, 箭头示虫体口围带前端; E. 背面观, 箭头示虫体背部皮层颗粒; F. 腹面观, 示纤毛图式; G. 口区纤毛图式, 箭头示额棘毛; H. 背面观, 单箭头示触毛列; I. 高倍镜下背面观, 单箭头示背触毛列; J. 虫体后部, 箭头示左缘棘毛; 单箭头示腹棘毛; 双箭头示尾棘毛; K. 个别标本, 箭头示 3 根腹棘毛; 单箭头示横棘毛; L. 活体线条图, 引自 Song & Packroff[7]; M, N. 蛋白银染示纤毛图示, 引自 Song & Packroff[7]

A-C. ventral views under low magnification to show different body shapes; D. dorsal view, arrow marks the anterior ridge; E. dorsal view, arrows show the cortical granules; F. ventral view, to show the general infraciliature; G. buccal area, arrows mark the frontal cirri; H. dorsal view, arrowheads show the dorsal kineties; I. dorsal view at high magnification, arrowheads show the dorsal kineties; J. posterior portion of the cell, arrows mark the left marginal cirri; arrowheads show the ventral cirri; double arrowheads indicate the caudal cirri; K. one specimen, arrow marks three ventral cirri; arrowheads show the transverse cirri; L. living morphology of *D. scutum*, from Song & Packroff[7]; M, N. infraciliature after protargol impregnation of *D. scutum*, from Song & Packroff[7]

CC: 尾棘毛; DK: 背触毛列; EM: 口内膜; FC: 额棘毛; LMC: 左缘棘毛; Ma: 大核; PM: 口侧膜; VC: 腹棘毛. 比例尺: 50 μm (A-C); 60 μm (L-N)

CC: caudal cirri; DK: dorsal kineties; EM: endoral membrane; FC: frontal cirri; LMC: left marginal cirri; Ma: macronuclei; PM: paroral membrane; VC: ventral cirri. Scale bars: 50 μm (A-C); 60 μm (L-N)



图版Ⅱ 伪寡毛双眉虫的活体形态(图 A-D, J)和蛋白银染色后纤毛图示(图 E-I, K, L)
Plate Ⅱ Living morphology of *Diophrys apoligothrix* from life (A-D, J) and after impregnation (E-I, K, L)

A-C. 低倍镜下腹面观不同体形; D. 低倍镜下侧面观; E. 腹面观, 示虫体纤毛图式, 箭头示额棘毛, 单箭头示尾棘毛; F. 背面观, 箭头示大核, 单箭头示背触毛列; G. 腹面观, 箭头示横棘毛, 单箭头示口围带小膜; H. 虫体中部腹面观, 箭头示左缘棘毛, 单箭头示腹棘毛; I. 个别标本, 示 3 个大核; J. 活体线条图, 引自 Song, *et al.*[18]; K, L. 蛋白银染示纤毛图示, 引自 Song, *et al.*[18], 箭头示一根左缘棘毛
A-C. ventral views at low magnification to show different body shapes; D. lateral view at low magnification; E. ventral view, to show the general infraciliature. Arrows indicate the frontal cirri and arrowheads mark the caudal cirri; F. dorsal view, arrows mark the macronuclei and arrowheads show the dorsal kineties; G. ventral view, arrows show transverse cirri and arrowhead marks the adoral zone of membranelles; H. ventral view of the middle region, arrow shows the left marginal cirrus and arrowheads mark the ventral cirri; I. one specimen shows three macronuclei; J. living morphology of *D. apoligothrix*, from Song, *et al.*[18]; K, L. infraciliature after protargol impregnation of *D. apoligothrix*, from Song, *et al.*[18], arrow marks single left marginal cirrus
AZM: 口围带小膜; CC: 尾棘毛; DK: 背触毛列; EM: 口内膜; FC: 额棘毛; Ma: 大核; PM: 口侧膜; TC: 横棘毛; VC: 腹棘毛. 比例尺: 25 μm (A-D); 45 μm (J-L)
AZM: adoral zone of membranelles; CC: caudal cirri; DK: dorsal kineties; EM: endoral membrane; FC: frontal cirri; Ma: macronuclei; PM: paroral membrane; TC: transverse cirri; VC: ventral cirri. Scale bars: 25 μm (A-D); 45 μm (J-L)