灰盔累枝虫形态学及表膜下纤维系的研究

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摘要:利用活体观察及蛋白银染色技术对灰盔累枝虫(*Epistylis galea*)的形态学及表膜下纤维系进行了研究。该种主要鉴别特征为:活体个员的表膜柔软,虫体充分伸展时呈倒长锥形,体长 200—300μm,体宽 70—90μm;大核"J"字形,呈纵位。单一伸缩泡,直径 21—25μm,背位。纵向纤维细密。口围盘纤维呈网状,与纵向纤维直接相连,并在整体上呈兜网状。与本属其他相近种相比,本种第 3 咽膜(P_3)明显要长,其后 1/3 超出第 1 咽膜后继续向下呈弧形弯曲。本文对该种上述特征在分类学上的意义及其与相近种类之间的关系进行了讨论。

关键词:缘毛目;淡水纤毛虫;灰盔累枝虫;形态学;表膜下纤维系
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蛋白银染色之后所显示的嗜银结构能够清楚地揭示缘毛目纤毛虫表膜下纤维系的显微结构特征。利用该特征进行缘毛目纤毛虫的分类学研究可以有效地避免由于活体观察的局限性所引起的同物异名或异物同名现象[1]。目前,有关缘毛目纤毛虫表膜下纤维系显微结构的研究已取得了很大的成就[1—11],但有关灰盔累枝虫(*Epistylis galea* Ehrenberg,1831)表膜下纤维系结构的研究仍是空白。灰盔累枝虫隶属于原生动物纤毛门,寡膜纲,缘毛亚纲,缘毛目,累枝虫科,累枝虫属[12],是淡水水体中常见的大型缘毛类纤毛虫之一。该种自发表后曾有几位学者[13,14]对其形态学特征进行过研究,但这些研究大多仅限于光学显微镜下的活体观察。由于该累枝虫的表膜较软,活体状态下的外形变化较大,活体鉴定时容易与其他相近种类混淆,所以利用以往的文献很难对该种进行正确的鉴定。实际上,灰盔累枝虫表膜下纤维系的结构有其自身的特殊性,这些特征与累枝虫属的其他种类相比有着较大的区别,可作为分类上稳定而直观的科学依据。

本文利用蛋白银染色法结合活体观察对灰盔累枝虫的活体形态及其表膜下纤维系进行了详细的研究,重点放在该物种的表膜下纤毛系和口纤毛器上,以期为缘毛类纤毛虫的形态学和种属间的分类学工作提供科学依据。

1　材料与方法

灰盔累枝虫于 2002 年 4—10 月采自哈尔滨近郊的小王家屯附近松花江水域。样本采回后在实体解剖镜下将虫体与基质分离,然后放入过滤后的原池水中培养。具体方法见施心路等[1]。活体观察在明视场、暗视场和微分干涉差显微镜下完成。蛋白银制片法采用 Shi & Frankel。模式图及文中术语参照宋微波[9]、施心路[1]和 Corliss[17]。

2　结果

2.1　活体形态描述

灰盔累枝虫为群体淡水生活种,体柄二叉式分支,最后一级分支最短,蛋白银染色后明显可见各级分支的肌丝之间有分节现象(图版 I:7)。虫体柔软并呈半透明状,完全伸展时为倒置的长锥形,口围唇(PL)外展处

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最宽;口围盘口漏斗开口一端呈高度隆起;口前庭(Bc)较窄,伸缩泡(Cv)一个,较大,背位,21 —25μm(图 1:a;图版 Ⅰ:1 —2)。游泳体筒状(图 1:b)。

该种虫体完全伸展时体长 200 —300μm,体宽 70 —90μm。口漏斗、伸缩泡、大小核及食物泡等细胞器均位于虫体上部 2/3 的区域内,下部约 1/3 的区域为一明显的亮区,呈透明状,表面隐约可见纵向细长的条纹(图 1:a)。大核呈"J"字形,纵位,起自于口围唇的近中部,横向弯曲后紧贴虫体外翻一侧的外缘下行,终止于反口纤毛环处或其稍下方。小核一枚,圆球形或椭球形。虽然部分虫体大核的末端可以延伸至反口纤毛环以下,但蛋白银制片后所有的大核均位于反口纤毛环以上部位。口漏斗区域较宽阔,胞咽(Cph)呈纺锤形,蛋白银制片后明显可见自口前庭后端一直延续到近反口纤毛环处。(图 1:a;图版 Ⅰ:10,13)

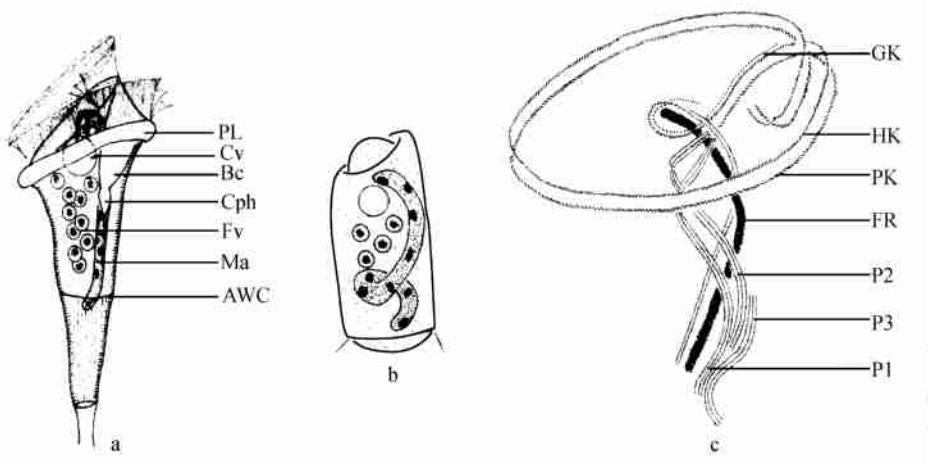

图 1　灰盔累枝虫

Fig. 1　*Epistylis galea*

a. 活体形态(General morphology of living zoid);b. 游泳体(Swimer);c. 口器的表膜下纤毛系(Buccal infraciliature);AWC:反口纤毛环(Aboral Wreath of Cilia);Bc:口腔(Buccal cavity);Cph:胞咽(Cytopharynx);Cv:伸缩泡(Contractel vacuole);FR:微丝网结构(Filamentous Reticulum);Fv:食物泡(Food vacuole);GK:生发毛基列(Germinal Kinety);HK:单毛基索(Haplokinety);Ma:大核(Macronucleus);P1 —P3:第 1 —3 咽膜(1st,2nd,&3 rd Peniculus);PL:口围唇(Peristomial lip);PK:多毛基索(Polykinety)

2.2　口纤毛器(图 1:c)

单毛基索(HK)和多毛基索(PK)均起自于细胞顶端口围唇的内侧,两者相伴而行,逆时针旋转约一圈半后彼此分离并相对而行。在单、多毛基索分行处,多毛基索向下延续为第 1 咽膜(P_1),此处也是第 2 咽膜(P_2)的上端点(图版 Ⅰ:8)。第 1 咽膜和第 2 咽膜分别由三列毛基索组成。共同逆时针沿口漏斗的内壁下旋,其中第 1 咽膜的下端一直延伸至胞口处(图版 Ⅰ:10)。第 2 咽膜在位于胞口较远处即终止,此处也是第 1 咽膜和第 3 咽膜(P_3)的交汇处(图版 Ⅰ:11)。第 3 咽膜最短,也由三列毛基索组成,与第 2 咽膜相邻,起自于近胞口处,也逆时针下旋少许就与第 1 咽膜汇合,此处是第 2 咽膜的下端点。第 3 咽膜和第 1 咽膜汇合后继续向下方的胞口处并行,其中第 1 咽膜在行至第 3 咽膜总长度下方的 2/3 处终止,第 3 咽膜则继续下行,胞口在入胞咽处的下部末端只有由三列毛基索组成的第 3 咽膜(图版 Ⅰ:10)。单毛基索位于多毛基索之外侧,长度约为多毛基索与第 1 咽膜的总和。单毛基索逆时针下旋至约与第 1、2 咽膜上端起始点的水平高度时即转到前庭内壁的对过,最终终止于胞口处(图版 Ⅰ:9)。微丝网结构(FR)清晰可见,着色较深,粗棒状,与单毛基索并行。生发毛基索(GK)位于单毛基索的上方,末端与单毛基索趋于分离(图版 Ⅰ:9,10,11)。

胞咽细长,制片后仍清晰可见,一直延伸到反口纤毛环处,并横向延伸几乎横贯胞体宽度的 2/3。胞咽壁经蛋白银染色后清晰可见(图版 Ⅰ:10,13)。

2.3　表膜下纤毛系

表膜下纤毛系主要包括纵长纤维和口围盘纤维,二者相互连接形成了一个连续的皮层网状纤维系(图版 Ⅰ:3 —6)。纵长纤维较细,纵贯细胞全长,均匀分布于整个虫体的外层,部分纵长纤维的始端在虫体细胞上端的单(多)毛基索处直接与口围盘纤维相连,相连部位为一系列呈"Y"形的纤维(图版 Ⅰ:4),向下延伸即为纵贯虫体全长的纵长纤维,各相邻的纵长纤维之间彼此相连形成一网状的纤维系。强

烈收缩的个体纵长纤维在行至反口纤毛环以下时逐渐趋于愈合并加粗,彼此之间形成致密的网状(图版Ⅰ:6),轻微收缩的个体纵长纤维并不明显加粗,整个虫体的纵长纤维形成一开口宽阔的漏斗状纤维网(图版Ⅰ:4)。本种的口围盘不明显,为致密的点文样网状结构,并不呈现典型的"盘"状,但在口前庭的入口处口围盘纤维较为稀疏(图版Ⅰ:3)。

3 讨论

累枝虫属是缘毛目中种类繁多的属,以往本属种间的分类通常根据活体观察的特征,如外部形状、大小、表膜环纹、群体枝型、口围唇的外部特征、大核形状、伸缩泡的位置和大小、游泳体的形状等[13]。缘毛目纤毛虫外部的形态特征可塑性很强,仅用活体观察的方法进行分类学研究具有一定的局限性,并且容易造成同物异名或异物同名现象。灰盔累枝虫细胞的结构特征在系统发育的研究中具有十分重要的地位。缪炜等[15]利用 18s 和 TTS1 序列对瓶累枝虫(*Epistylis urceolata*)、褶累枝虫(*E. plicatilis*)、金龟累枝虫(*E. hrysemydis*)和灰盔累枝虫(*E. galea*)等六种累枝虫的分子系统发育关系的研究表明,灰盔累枝虫与其他五种累枝虫分属于两个类群,可能较早地从祖先中分化出来。本文的研究结果从该种的细胞结构水平上证明,灰盔累枝虫表膜下纤维系的结构明显有别于其他种类。灰盔累枝虫的口围盘网状,与褶累枝虫的花盘状口围盘[10]和金龟累枝虫虫体上方由环状纤维形成的帽状结构[14]均有较大差异,后两者口围区的纤维趋向集中而灰盔累枝虫口围区的纤维呈现出松散态。该种类的口围盘与纵长纤维形成一整体上的网状结构更是其他种类所没有的现象。口围缘经蛋白银染色后着色较深且粗,呈现立体的圈状,是否应归入表膜下纤维系统仍需进一步地研究。金龟累枝虫、褶累枝虫、上村累枝虫[5]及螅状独缩虫[2]的第 3 咽膜末端均较平直,而灰盔累枝虫第 3 咽膜末端呈典型的弧形弯曲态(图Ⅰ:c),其结构本身就具有自身的特殊性。此外,灰盔累枝虫生发毛基索的末端与单毛基索趋于分离向外突出,在其他累枝虫上也未见报道。

灰盔累枝虫的纵长纤维和钟虫属的有相似性,但口围盘纤维网状,而钟虫属多为轮辐状。灰盔累枝虫的大核为"J"字型,这与钟虫属大幅度弯曲的肠状核和累枝虫属典型的横向的"C"字型核均不相同。

该种类的口围盘纤维和纵长纤维一一相连,这对于传导冲动和整个虫体的协同收缩有着重要的意义。咽膜结构具有其自身的特点,第 1 咽膜终止后第 3 咽膜向下大幅度弧形弯曲,生发毛基索终止后单毛基索分为两列与微丝网结构共同下旋且同时终止。这与钟虫属、螅状独缩虫、聚缩虫属均有差别。经蛋白银染色后胞咽壁清晰可见,咽头纤维向下延伸直到反口纤毛环处,且横向延伸几乎横贯整个虫体中部的 2/3,在累枝虫的其他种类上也未见报道。

综上所述灰盔累枝虫的鉴别特征如下:(1)群体生活,表膜柔软,体型较大,(200—300)μm ×(70—90)μm,大核为"J"字形。细胞核和细胞器均存于虫体上部 2/3 的细胞质中,虫体下部 1/3 的亮区内无任何细胞器。(2)纵长纤维纤细,口围盘纤维网状,二者直接相连,形成一整体上的兜网状结构。(3)三咽膜中,第 1 咽膜和第 3 咽膜相汇后即终止,第 3 咽膜继续向下呈弧形弯曲。(4)胞咽壁向下延伸直至反口纤毛环处,并横向弯曲约贯穿整个虫体中部的 2/3(制片标本)。

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STUDIES ON THE MORPHOLOGY AND INFRACILIATURE OF EPISTYLIS GALEA

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Abstract : *Epistylis galea* Ehrenberg ,1831 ,was collected from an eutrophic freshwater pond near Harbin ,China ,from April to October in 2002. By living and protargol-impregnated specimens ,the morphology ,infraciliature and myonemic system was studied. Living observations were carried out using both bright field and differential interference contrast microscopy. Protargol staining was done according to the method modified by Shi & Frankel (1990) to reveal the infraciliature and myonemic system of the organism. Drawings were made with the help of the camera lucida.

Epistylis galea ,a colony in fresh water which has a colonial stalk with two branches ,of which the last branch is shortest and is significant metamerism between each of myofilament after impregnated by protargol. Its whole body is relatively soft and highly transparency ;while looks like a cone when it uphold completely. As for life morphology zooids are reasonable stout and relatively constant in shape ,length in vivo is 200 —300μm ,width is 70 —90μm ,and the widest area are at the peristomial lip (PL) of the organism. The peristomial disc (PD) is significantly elevated when the cell is completely extending. Contractiel vacuole is only one , relatively big at top of the cell. Most of the cell organelles are located at the upper 2/3 of organism ,such as the vestibulum (infundibulum or buccal cavity) ,contractile vacuole (CV) ,macronucleus (Ma) ,micronucleus (Mi) and food vacuole (FV) ;the other 1/3 of cell ,however ,is spacious and rather highly transparency ,there are lots of longitudinal figure streaks. The macronucleus (Ma) is "J"-shape ,longitudinal originates the middle of peristomial lip (PL) ,transverse along the organism extend to aboral wreath of cilia (ACW) ;while macronucleus by protargol impregnated located at the upper of ACW ,the funnel is rather wide. Cytopharynx (Cph) ,which has been used protargol impregnated ,is spindle-shape ,originates cell 's fore head and reach constantly ACW.

The haplokinety (HK) and the polykinety (PK) are parallel with each other from the peak of cell ,making approximately about 1.5 turns around the PD in a counter clockwise direction ,then they separate. There are three infundibular polykineties (P_1 —P_3) , each of which consists of 3 rows of kinetosomes. P_1 and P_2 originate from the end of PK , P_2 converges with P_1 and P_3 at the abstomal terminal of P_3. After P_1 and P_3 confluenced , P_1 terminate at once ,but P_3 continue in bracklet-shaped bend ;after germinal row of kineties terminating ,haplokinety becomes two columns of kineties which parallel with filamentous reticulum ;both of them terminate at the same area. Compared with P_1 and P_2 , P_3 is significantly short. HK consists of two kinetosomes and lies on the side of the infundibulum opposite to the PK.

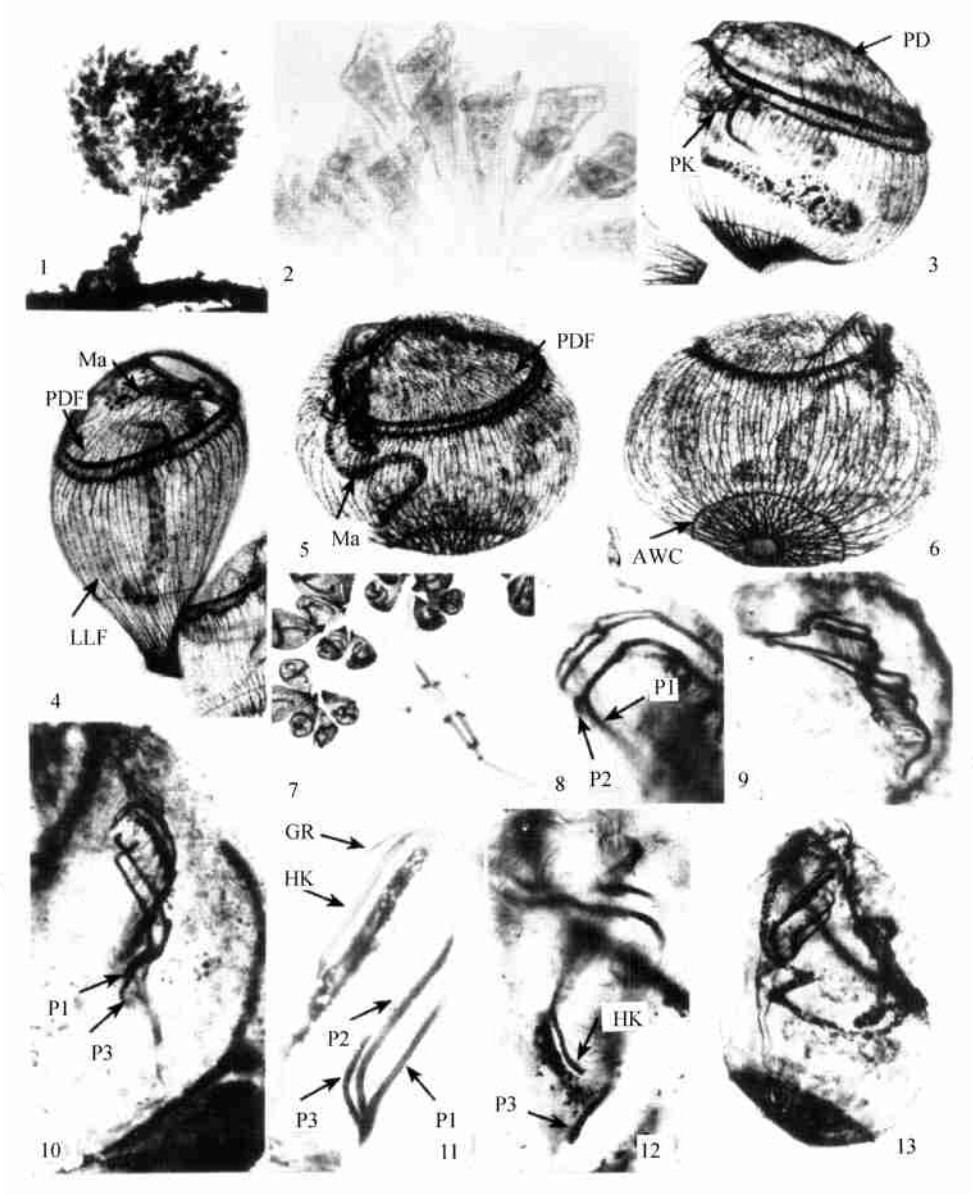
The filamentous reticulum (FR) ,paraller with HK ,is "S"-shape and considerable bright. Germinal row lies beyond the HK.

Cytopharynx (Cph) is relatively figure and bright, extend approximately 2/3 of the length of the organisum.

The myonemal system is composed of two kinds of fibers: the longitudinal fibbers (LF) and peristomial disc fibbers (PDF). LF and PDF connect with each other and become one robust fiber net, which converged the whole organisum, comprising approximately 80 bands. Some LF branchs originate from HK and directly connect with PDF; while the others, which extend the whole length of LF, connect with each other, and then joining another LF's fibbers during extending. The PDF is unique. Parts of longitudinal fibber originate haplokinety, and directly connect with the peristomial disc (PD). The situation is "Y" shape fibber, the longitudinal fibber constantly extend the whole body and connect with each other and became one reticular fibber system, some shrink intensely longitudinal fibber more and more roughness and become a net-like. Slight shrinking, however, has no this phenomena, the total of longitudinal fiber became one fummel like network, the peristomal disc, like the shape of plate, is not spacious and stride network, yet at the entrance is rather few and scattered.

In brief, we conclude that its infraciliature and myonemic system reveals the following characteristics: (i) one contractal vacuole 21—25μm, in upset of mid-body region both the nuclear and the other oranelles situate in the cytoplasm which locates above the 2/3 part of the individual; (ii) LF and PDF connect directly with each other and become one robust fibre net. (iii) three infudibular polykineties (P_1—P_3) consist of 3 rows of kinetosomes; (iv) P_1 terminates near the 2/3 length of P_3, and P_3 enters into the beneath of cytopherynx where only observe three rows of P_3.

Key words: Peritrichida; *Epistylis galea*; Morphology; Infraciliature



图版 I

1 ,2 活体形态(1. 示群体生活状态;2. 示个体伸展状态) :3 —12 蛋白银制片(3. 口围盘纤维网 ,单毛基索 ,多毛基索和纵长纤维;4. 轻微收缩状态的纤维结构;5. 口围盘纤维和大核;6. 反口纤毛环和帚胚;7. 柄的构造;8. 第 1 咽膜、第 2 咽膜和多毛基索;9. 口器的整体结构;10. 第 1 咽膜和第 2 、3 咽膜的末端;11. 单毛基索 ,多毛基索 ,微丝网结构和三列咽膜;12. 单毛基索的末端和第 3 咽膜的末端;13. 单毛基索的末端和第 2 咽膜的末端)

1 ,2 *Epistylis galea* in living individuals ; 3 —12 individuals stained by protargol impregnated speciments (1. showing the general morphology of living colony. 2. showing typical cell in vivo. 3. showing the peristomial disc ,the haplokinety ,and the polykinety. 4. showing the infraciliature of slightly contracted individual. 5. showing the peristomial disc ,the macronucleus. 6. showing the aboral wreath of cilia ,the scopula. 7. showing the structure of the colonial stalk. 8. showing the peniculus1 , peniculus2 and the polykinety. 9. showing the parts of the infundibular infraciliature. 10. showing the peniculus1 and the peniculus3. 11. showing peniculus 1 —3 and the germinal row of kineties. 12. showing the haploykinety and the peniculus3. 13. showing the haploykinety and the peniculus2