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论著

扫描电镜观察猴体内寄生的蛇舌状虫属若虫及基于其18S rRNA基因的种系发育关系分析

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Ultrastructural Observation on Nymphal *Armillifer* sp. by Scanning Electron Microscopy and Phylogenetic Analysis Based on 18S rRNA

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摘要 目的 运用扫描电镜观察食蟹猴（*Macaca fascicularis*）体内分离的蛇舌状虫属若虫虫体表面的超微结构，并基于其18S rRNA序列分析其分子种系发育关系。 方法 将从食蟹猴分离的蛇舌状虫属若虫用戊二醛与锇酸双固定法，置于扫描电镜下观察其体表超微结构。PCR扩增若虫18S rRNA基因并测序；运用序列比对软件ClustalX 1.83将测序结果与GenBank上所有已登录的孔头舌虫目（Porocephalida）内部虫种序列进行多重比对分析，并使用MEGA 4.0采用邻位相连法构建种系发育树进行分析。 结果 扫描电镜示，若虫呈圆柱状，前段略粗，末端变细。腹环由前向后逐渐增宽，至12～13腹环时趋于等宽，腹环与腹环之间，在前半段连接紧密，在后半段有一定间隔。头部腹面正中为口，呈圆形，口稍上方两侧各见一对钩，钩几乎在同一平行线上。两外侧钩的正下方，头胸部最后一节胸环上各有一个对称的大型感觉乳突，紧接的第一节腹环靠中线处有一对呈对称的大型感觉乳突，腹环数目由此算起共29个腹环（末端有2个腹面未连接的不完整腹环不计算在内）。若虫全身布满类圆形的感觉乳突，但在头部背面与末节腹面未见有此类乳突出现。末端腹面有一块状隆起上可见肛门开口。参照文献，暂将该若虫定为串珠蛇舌状虫（*Armillifer moniliformis*）的若虫。PCR测序后获得18S rRNA部分基因序列，长度为1 836 bp，提交GenBank后获得登录号为HM048870。种系发育树显示其与尖吻蝮蛇舌状虫（*A. agkistrodontis*）和腕带蛇舌状虫（*A. armillatus*）构成自展值为95%的分支。前两者又构成一个独立的自展值为75%的分支。 结论 自食蟹猴体内分离的蛇舌状虫属若虫暂定为串珠蛇舌状虫若虫。

关键词： 串珠蛇舌状虫 若虫 超微结构 18S rRNA 种系发育树

Abstract： Objective To observe the ultrastructure of nymphal *Armillifer* sp. isolated from *Macaca fascicularis* by using scanning electron microscope (SEM), and analyze the phylogenetic relationships based on 18S rRNA gene sequences. Methods The parasite samples stored in 70% alcohol were fixed by glutaraldehyde and osmium peroxide. Ultrastructural characters of those samples were observed under SEM. Amplification and sequencing of the 18S rRNA gene were performed following the extraction of total genome DNA. Sequence analysis was performed based on multiple alignment using ClustalX1.83, while phylogenetic analysis was made by Neighbor-Joining method using MEGA4.0. Results The nymphs were in cylindrical shape, the body slightly claviform tapering to posterior end. Abdominal annuli were gradually widened from anterior to posterior parts, the 12th-13th abdominal annuli of which were similar in width. The annuli ranged closer in the front half body, whereas in the latter part there were certain gaps between them. The circular-shaped mouth located in the middle of head ventrally. Folds were seen in inner margin of the mouth with a pair of curved hooks on both sides above it which practically disposed in a straight line. Two pairs of large sensory papillae were observed symmetrically over the last thoracic annulus of cephalothoraxs lying below the outer hook, and the first abdominal annulus was near the median ventral line. The number of abdominal annuli was 29, not including 2 incomplete terminal annuli. Rounded sensory papillae were fully distributed on the body surface, except the dorsal side of head and the ventral part of the terminal annulus. Agglomerate-like anus opening was observed at the end of ventral abdominal annuli and distinctly sub-terminal. These morphological features demonstrated that the nymphs were highly similar with that of *Armillifer moniliformis* Diesing, 1835. A fragment of 18S rRNA gene (1 836 bp)sequences was obtained by PCR combined with sequencing, and was registered to the Gen-eBank database with an accession number HM048870. The phylogenetic tree indicated that *A. moniliformis*, *A. agkistrodontis* and *A. armillatus* were at the same clade with a bootstrap value at 95%, and *A. moniliformis* and *A. agkistrodontis* were solo at a clade with a bootstrap value of 75%. Conclusion The nymphs isolated from *Macaca fascicularis* are identified as *A. moniliformis* temporarily.

Keywords： Armillifer moniliformis Nymph Ultrastructure 18S rRNA Phylogenetic analysis

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