



鳃基碘泡虫中国新记录及组织病理

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摘要: 为了鉴定感染鲤鳃的病原并分析其鳃部严重受损的病因, 本研究运用形态计量学、分子生物学、系统发育学和组织病理学方法, 对其进行了详细研究。结果显示, 该致病黏孢子虫寄生于鲤鳃弓, 孢子壳面观呈椭圆形或近圆形, 缝面观呈柠檬形, 顶面观呈橄榄形; 孢子长 9.2~11.8 μm , 宽 8.3~10.5 μm , 厚 4.4 μm ; 拥有 2 个大小不等的水滴形极囊, 2 个极囊前端略微靠拢, 大极囊长 3.5~4.7 μm 、宽 2.4~3.1 μm , 小极囊长 2.8~3.8 μm 、宽 1.8~2.6 μm , 大、小极囊极丝分别缠绕 5 圈和 3 圈; 部分孢子后端可以见“V”形褶皱, 但未观察到囊间突起、黏液被膜和嗜碘泡。18S rDNA 与已报道的鳃基碘泡虫相似度达 99.69%。因此, 该寄生虫的形态学与分子生物学数据以及宿主信息表明, 该种为鳃基碘泡虫, 属中国新记录。孢囊发生在病鲤鳃部传入和传出动脉之间。由于孢囊体积增大, 传入和传出动脉受到压迫而变形、堵塞; 鳃丝基部也因此发生机械性断裂; 部分孢囊周围结缔组织受到破坏。孢囊破裂后, 孢子浸润到周围组织。研究表明, 鳃基碘泡虫的孢囊压迫主血管, 破坏鳃丝, 致使鳃丝血流不畅、供血不足, 进而导致鲤呼吸功能丧失, 是病鲤致死的主要原因。本研究提供的鳃基碘泡虫的形态、分子和病理数据可为水产养殖中鳃基碘泡虫病的防控提供理论依据。

关键词: 鳃基碘泡虫; 中国新记录; 鲤; 组织病理

中图分类号: Q 179; S 941.5

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碘泡虫属 (*Myxobolus*) 是黏孢子虫中种类最多的属, 目前已达 979 种^[1]。绝大多数碘泡虫寄生于鱼类的鳃、咽、肠道、肝胰腺、肾脏、胆囊、体表和鳍等多种组织器官^[2-4]。一些碘泡虫可使宿主致病, 引起鱼类呼吸受阻、神经紊乱、消化功能失调及泌尿系统不畅等疾病, 最终使水产养殖业受到不同程度的经济损失^[5-7]。例如吴李碘泡虫 (*M. wulii*) 寄生于异育银鲫 (*Carassius auratus gibelio*) 的肝胰腺, 引起异育银鲫肝孢子虫病, 病鱼腹部膨大、行动缓慢、食欲速减, 最终死亡, 解剖后发现病鱼肝胰腺液化^[8]; 饼形

碘泡虫 (*M. artus*) 寄生于草鱼 (*Ctenopharyngodon idella*) 的前肠绒毛层, 在肠壁组织形成孢囊, 引起草鱼肠道发生病变, 病鱼食欲不振, 衰弱死亡, 死亡率达 90% 以上, 造成严重的经济损失^[9]; 洪湖碘泡虫 (*M. honghuensis*) 寄生于异育银鲫的咽部, 引起异育银鲫的“喉孢病”, 病鲫的咽部肿大、充血, 病鱼几乎不摄食, 形体消瘦, 行动迟缓, 体色变黑, 体表黏液增多, 眼球突出, 最终因呼吸困难和无法摄食而死亡^[10]。鉴于此, 随着高密度养殖化的发展和人们对鱼肉品质的高标准需求, 作为养殖鱼类常见病的碘泡虫病

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一直倍受关注^[5]。

鳃基碘泡虫 (*M. basilamellaris*) 最初从匈牙利霍尔托巴吉地区的鲤 (*Cyprinus carpio*) 鳃部检获, 对其进行了形态学和寄生部位的描述, 然而无病症和病理记录分析, 亦无分子数据参考^[11]。随后, Molnár 等^[12-13] 先后再次报道了鳃基碘泡虫, 并基于组织病理学简单描述了孢囊发生的确切位置, 但并没有表明其对宿主的影响。Eszterbauer^[14] 于 2004 年在匈牙利再次检获该虫种并补充了 18S rDNA 分子数据。本研究对检获自中国贵州铜仁地区鲤鳃部的鳃基碘泡虫进行了形态和分子特征、组织病理的研究, 并对其孢囊发生的精确部位和对宿主组织器官的影响进行了详细描述和研究, 以期对鳃基碘泡虫的致病性及该病的防治提供资料。

1 材料与方法

1.1 标本采集与物种鉴定

3 尾被感染的鲤于 2020 年 8 月购自中国贵州省铜仁市碧江区金色码头农贸市场, 带回室内后窒息而死并解剖。各组织器官进行肉眼观察和镜检, 仅在鳃部发现孢囊。将孢囊转移至离心管, 针头戳破获得游离孢子。新鲜孢子用超纯水冲洗 3 遍, 2 000 r/min 离心沉淀, 备用。虫种鉴定和样本处理均遵循 Lom 等^[15] 和赵元著等^[16] 报道的方法。

1.2 基因组 DNA 提取、目的基因扩增和测序

将无水乙醇固定后的孢子转移至 500 μ L 细胞裂解液中, 56 $^{\circ}$ C 金属浴过夜。基因组的提取严格按照 DNeasyTissue Kit (QIAGEN, Düsseldorf, Germany) 说明书执行。扩增 18S rDNA 的正/反向引物分别为 18E 5'-CTGGTTGATCCTGCCAGT-3'^[17] 和 18R 5'-CTACGGAAACCTTGTTACG-3'^[18]。PCR 体系 20 μ L, 包括 Mix 5 μ L, 18S rDNA 正/反向引物各 0.5 μ L, 基因组 DNA 5.8 μ L 和双蒸水 8.2 μ L。PCR 程序: 94 $^{\circ}$ C 预变性 90 s, 然后执行 35 个循环, 包括 94 $^{\circ}$ C 变性 20 s, 58 $^{\circ}$ C 退火 20 s, 72 $^{\circ}$ C 延伸 2 min, 接着 72 $^{\circ}$ C 再延伸 5 min, 最后 12 $^{\circ}$ C 保温。PCR 产物通过 1.0% 琼脂糖电泳和 DNA 琼脂糖胶试剂盒 (Omega Bio-Tek 和 Norcross City, GA, 美国) 分离和纯化。PCR 产物送至北京擎科生物技术有限公司测序。

1.3 序列分析和系统发育

以本研究获得的 18S rDNA 序列为基序, 在 NCBI (<https://www.ncbi.nlm.nih.gov>) 中经 BLASTn 同源性比对。选取同源性较高的 22 条碘泡虫序列, 以四角库道虫 (*Kudoa quadricomis*) (GenBank 登录号: FJ792721) 和蒜芥库道虫 (*K. alliaria*) (GenBank 登录号: DQ182561) 为外群。利用 CIPRES (http://www.phylo.org/sub_sections/portal/) 构建最大似然树 (ML tree)^[19], MrBayes 3.1.2 软件构建贝叶斯树 (BI tree), 运行 1 000 000 代^[20]。最后利用 FigTree v1.4.2 和 Adobe illustrator CS4 软件完成系统树的绘制^[21]。

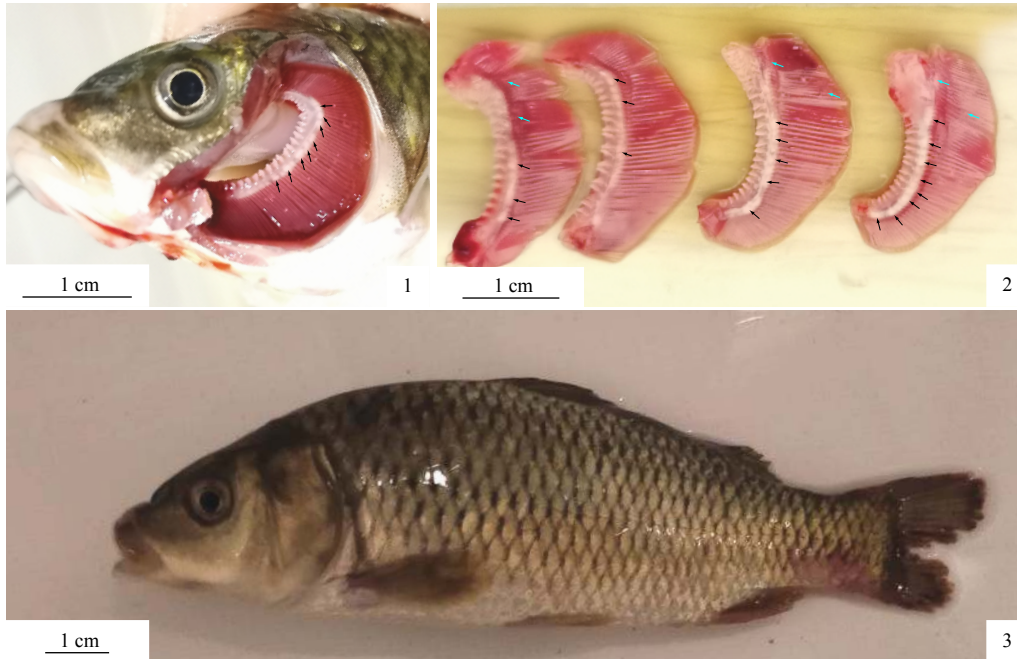
1.4 组织病理学观察

取病鲤剖检, 分别经肉眼和涂片检测各脏器组织。取病鲤鳃组织 0.5 cm^3 , Bouin 氏液固定 24 h, 自来水冲洗浸泡 12 h, 60% 乙醇脱水 0.5 h, 70% 乙醇脱水 0.5 h, 80% 乙醇脱水 1 h, 90% 乙醇脱水 1 h, 95% 乙醇脱水 2 h, 无水乙醇 I 脱水 0.5 h, 无水乙醇 II 脱水 0.5 h, 二甲苯透明 0.5 h, 56 $^{\circ}$ C 浸蜡 4 h 并包埋切片, 切片厚度为 5~6 μm 。二甲苯脱蜡 10 min, 无水乙醇 I 复水 5 min, 无水乙醇 II 复水 5 min, 95% 乙醇复水 5 min, 85% 乙醇复水 5 min, 自来水冲洗 1 min, 苏木素染色 15 min, 自来水漂洗 1 min, 1% 盐酸乙醇溶液分化 1 s, 自来水漂洗 10 min, 伊红复染 1 min, 蒸馏水漂洗 5 s, 95% 乙醇漂洗 1 min, 无水乙醇脱水 5 min, 二甲苯透明 10 min, 最后用中性树脂胶封片, Leica DM6000B 显微镜观察并拍照。

2 结果

2.1 鳃基碘泡虫的形态和分子特征及其系统发育分析

孢囊位于鳃丝基部的鳃弓处, 呈白色球状, 直径约 0.8~1.5 mm (图版 I -1, 2)。孢子壳面观呈椭圆形或近圆形, 缝面观呈柠檬形, 顶面观可观察到 2 个大小不等的极囊, 呈橄榄形。孢子长 9.2~11.8 μm , 宽 8.3~10.5 μm , 厚 4.4 μm , 孢子质丰富, 部分孢子后端可观察到“V”形褶皱, 未见囊间突起、黏液被膜和嗜碘泡。孢子拥有 2 个大小不等的水滴形极囊, 2 极囊的前端略微靠拢, 其中大极囊长 3.5~4.7 μm , 宽 2.4~3.1 μm , 极丝平均缠绕 5 圈, 小极囊长 2.8~3.8 μm , 宽 1.8~2.6



图版 I 病鲤及受感染的鳃组织

1. 孢囊(黑色箭头)与受损鳃丝(蓝色箭头); 2. 附着在鳃上的孢囊; 3. 病鲤

Plate I Diseased *C. carpio* and its infected gills

1. cysts (black arrow) and the damaged gill filaments (blue arrow); 2. the cysts attached to the gills; 3. diseased *C. carpio*

μm, 极丝平均缠绕 3 圈(图 1, 表 1)。

本研究所获得的 18S rDNA 长度为 1 898 nt, 在 NCBI (<https://www.ncbi.nlm.nih.gov>) 的登录号为 MZ343303。经 NCBI 中 BLAST 比对结果显示, 其与已报道的鳃基碘泡虫 AF507971 相似度最高, 为 99.69%; BI 和 ML 系统发育树拓扑结构基本一致, 本研究种与鳃基碘泡虫 AF507971 独立聚支, 且具有很高的支持率 (BS=100, PP=1); 随后依次与寄生鲤的苍梧碘泡虫 (*M. tsangwuensis*) 和贝壳碘泡虫 (*M. musseliusae*) 分别聚支(图 2)。

2.2 病鲤临床症状与剖检

本研究获得的病鲤为幼鱼, 病鲤精神萎靡、行动缓慢, 对外界刺激反应迟钝(图版 I -3)。剖检结果显示, 鱼鳃呈淡红色, 部分鳃丝呈粉白色, 鳃表面黏液分泌明显较多, 鳃丝基部排列着许多大小不等的白色球状孢囊(图版 I -1, 2); 破损的尾鳍及其他组织并未发现寄生虫感染。

2.3 组织病理学观察

组织病理学结果显示, 大量成熟和未成熟孢

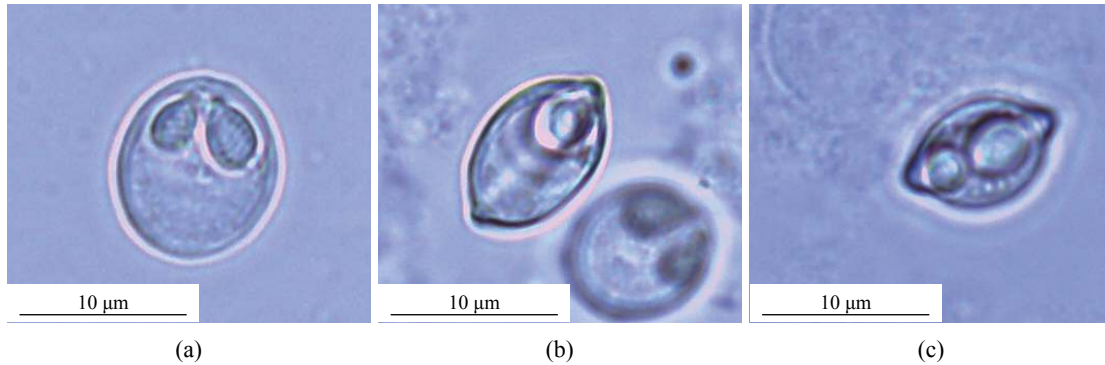


图 1 鳃基碘泡虫的成熟孢子

(a) 孢子壳面观; (b) 孢子缝面观; (c) 孢子顶面观

Fig. 1 Matured spores of *M. basilamellaris*

(a) mature spore in valvular view; (b) mature spore in sutural view; (c) mature spore in apical view

表 1 鳃基碘泡虫不同株系的形态学比较

Tab. 1 Morphological comparison of different strains of *M. basilemellaris*

鳃基碘泡虫 <i>M. basilemellaris</i>	本研究 this study	原始数据 ^[11] the original data ^[11]
孢子长×宽/μm spore length×width	(9.2~11.8)×(8.3~10.5)	(7.7~12.2)×(7.3~9.9)
孢子厚/μm spore thickness	4.4	4.5 (4.2~5)
大极囊长×宽/μm large polar capsule length×width	(3.5~4.7)×(2.4~3.1)	(3.2~5.4)×(2.2~3.3)
极丝圈数/圈(大极囊) polar filament number (large polar capsule)	5 (5~6)	5~7
小极囊长×宽/μm small polar capsule length×width	(2.8~3.8)×(1.8~2.6)	(2.5~4.4)×(1.8~3.3)
极丝圈数/圈(小极囊) polar filament number (small polar capsule)	3 (3~4)	3~4
宿主 host	鲤 <i>C. carpio</i>	鲤 <i>C. carpio</i>
寄生部位 infection sites	鳃弓 branchial arch	鳃弓 branchial arch
地理位置 locality	中国贵州 Guizhou, China	匈牙利霍尔托巴吉 Hortobágy, Hungary

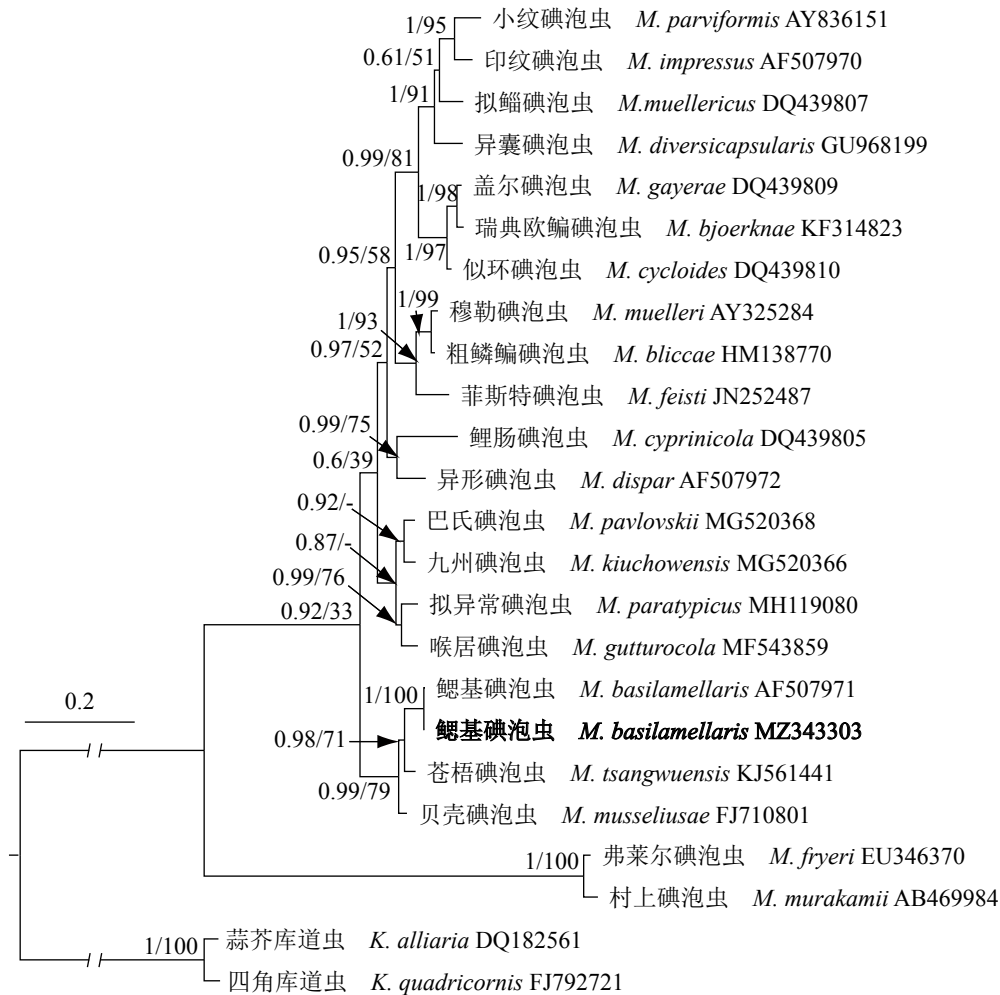


图 2 基于鳃基碘泡虫与相关碘泡虫的 18S rDNA 序列系统发育树

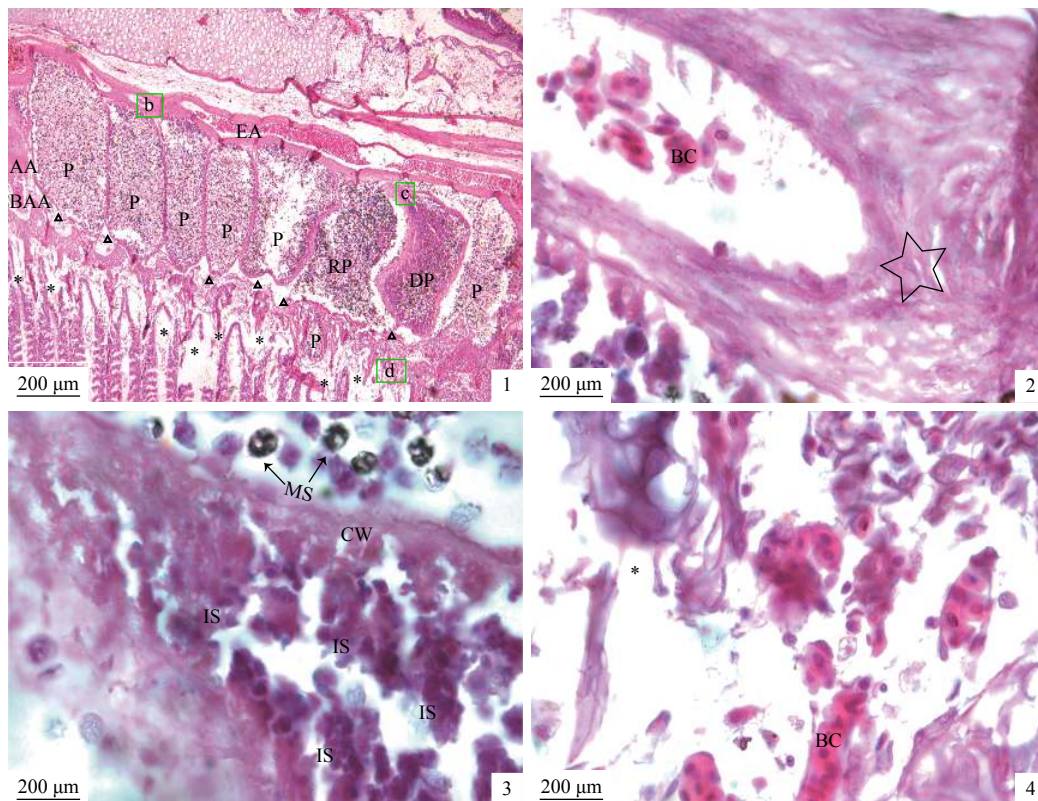
节点处分别为 BI/ML 树的靴攀值；以 BI 树为准，“-”表示 ML 树在此节点不一致或缺失；黑色加粗字体为本研究鳃基碘泡虫种群；“//”表示隐藏支长，为原始支长的三分之一

Fig. 2 Phylogenetic tree of 18S rDNA sequences of *M. basilemellaris* with relative species

Numbers located at the branches nodes are bootstrap confidence values resulting from BI/ML; values of BI are taken as the criterion here,“-”represents that the values of ML are different from BI or lacking; bold black fonts represent *M. basilemellaris* population in this study; “//” means the length of hiding branches, one third of the original branches

囊紧密地排列在鳃丝基部, 位于传入和传出动脉血管之间, 个别孢囊位于鳃丝基底部。由于孢囊拥挤和体积增大, 有的孢囊已经溃破, 孢子弥撒在周围的结缔组织(图版 II -1)。孢囊的最外层被鳃丝上皮细胞或周围结缔组织包被, 孢囊周围仍可观察到结缔组织, 但发生不同程度的挤压破碎, 部分鳃丝组织变形或断裂(图版 II -1,

4)。孢囊一侧紧贴鳃的传出动脉, 持续发育并压迫传出动脉, 造成血管变形、黏连堵塞(图版 II -1, 2); 另一侧挤压传入动脉, 致使传入动脉和动脉分支的结构发生损坏(图版 II -1, 4)。未成熟的孢囊内营养体明显较多, 分布在孢囊壁周围, 少数成熟孢子分布于孢囊中央; 成熟孢囊内成熟孢子充满整个囊腔(图版 II -1)。



图版 II 病鲤鳃部组织病理

1. 鳃丝横切; 2~4. 依次为图 1 中 b、c 和 d 的放大; AA. 传入动脉, BAA. 传入动脉分支, BC. 血细胞; CW. 孢囊壁, EA. 传出动脉, DP. 发育中孢囊, IS. 未成熟孢子, MS. 成熟孢子, P. 孢囊, RP. 破裂孢囊; *代表断裂的鳃丝, △代表受到压迫的结缔组织, ☆代表血管发生黏连而堵塞的位置

Plate II Histopathological changes of the gill of diseased *C. carpio*

1. the cross section of gill filament; 2-4. the enlargement of b, c and d in figure 1, respectively; AA. afferent arteries, BAA. the branch of afferent arteries, BC. blood cell, CW. cyst wall, EA. efferent arteries, DP. developing plasmodium, IS. immature spores, MS. mature spores, P. plasmodia, RP. ruptured plasmodium; * represents the fractured gill filaments, △ represents the compressed connective tissues, ☆ represents the position where the blood vessel is stuck and blocked

3 讨论

鳃基碘泡虫具有严格的组织向性, 寄生于鲤鳃丝基部, 或鳃丝基部和鳃弓之间的管腔中^[13]。原始报道中的鳃基碘泡虫孢子壳面观呈圆形或椭圆形, 具有 2 个大小不等的极囊, 孢子后端的壳瓣上具有“V”形褶皱, 孢子前端无囊间突和嗜碘泡, 印度墨水染色显示部分孢子有黏液被膜^[11]。本研究检获的碘泡虫与鳃基碘泡虫原始报道在

孢子的形态特征、宿主及孢囊所在的部位均一致; 除孢子宽比原始种群稍宽外, 其余量度大小高度一致(表 1), 该量度差异应属于种内变化范围^[22]; 且其 18S rDNA 与鳃基碘泡虫 AF507971 具有 99.69% 的同源性, 亦属于种内范畴^[23]; 此外, 系统发育分析结果也表明二者应为同种(图 2)。故本研究获得的碘泡虫应为鳃基碘泡虫。这是该种在中国的首次报道。

鳃是复杂的器官,由鳃弓和许多梳齿状鳃丝构成,鳃弓呈拱形结构,为鳃丝提供物理支撑;每根鳃丝的两侧排列着许多鳃小片,称为二级鳃丝,主要由上皮细胞、黏液细胞和柱细胞构成,上面布满毛细血管,行使鳃的全部呼吸功能^[24-26]。碘泡虫可寄生于宿主鳃部的不同部位,如鳃小片、鳃丝末端、鳃丝软骨、鳃弓软骨等组织^[13],由宿主的结缔组织或上皮组织包裹形成孢囊,以隔离并防止其扩散到周围组织,是宿主对碘泡虫感染最常见的组织病理学反应^[27]。由于之前报道的鳃基碘泡虫样本中是成熟孢囊,而非如此密集且处于不同发育阶段的大小孢囊,所以本研究成熟与未成熟孢囊的比较结果表明,伴随孢囊体积逐渐增大,鳃基碘泡虫的孢子成熟是由孢囊中间向四周发展(图版II-1);与此同时,再次明确孢囊的发生部位为传入和传出动脉之间及鳃丝基部。此外,同本研究中的病鲤鳃组织相比,捷克和匈牙利的学者先后发现的鳃基碘泡虫对寄生部位组织破坏性并不严重,因此并没有记载该寄生虫对宿主及其组织的损伤^[11-13];此外,由本研究中病理组织学结果不难判断鳃基碘泡虫造成鳃丝缺血性坏死,最终致使病鲤因鳃功能衰竭而死亡;如若防治不当或不及时,可成为鲤严重致病种。病鲤鳃部黏液较多,推测由于鳃基碘泡虫感染、刺激鳃小片的黏液细胞,致使鳃黏液分泌增多。

综上,鳃基碘泡虫为中国新记录种。本研究补充了鳃基碘泡虫孢囊的精细定位与内部孢子成熟顺序以及病理特征的新数据,为鳃基碘泡虫的防控提供了基础资料。

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New record for *Myxobolus basilemellaris* in China with histopathological insights into gill infestation

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Abstract: *Myxobolus* including 979 species, is the largest genus in Myxozoa, which can parasitize different tissues in fish and bring about myxosporosis to result in various degrees of economic losses in the fishery. To identify the pathogen infecting the gill of common carp and analyze the pathogeny of its gill seriously damaged, those methods on morphometry, molecular biology, phylogeny and histopathology were employed in the research. The results showed that the myxospore of the pathogen was elliptic or nearly round in valvular view, lemon shape in sutural view, olive shaped in apical view. The myxospore was 9.2-11.8 μm long, 8.3-10.5 μm wide and 4.4 μm thick. Two water-drop polar capsules were unequal in size. The anterior ends of the two polar capsules were slightly close together. The larger polar capsule was 3.5-4.7 μm long and 2.4-3.1 μm wide, while the smaller one was 2.8-3.8 μm long and 1.8-2.6 μm wide. Polar filaments within the large and small polar capsules were coiled with 5 and 3 turns, respectively. V-shaped folds were obvious in the posteriors of some spores. However, intercapsular appendix as well as mucous envelope and aiodinophilous vacuole was not observed. The similarity of 18S rDNA between *M. basilemellaris* reported and the present species was 99.69%. Thus, morphological and molecular data of the parasite as well as host species proved that the present species is *M. basilemellaris*, and this is a new record in China. The cysts developed between the afferent and efferent arteries of the diseased carp's gills. Due to the increase of cyst volume, the afferent and efferent arteries were compressed to be deformed and blocked, and the bases of filaments were also mechanically fractured. Some connective tissues around the cysts were destroyed. Moreover, the myxospores infiltrated into the surrounding tissues after the rupture of the cysts. Therefore, the main cause of death for diseased carp is that the cysts of *M. basilemellaris* oppressed the main vessels and fractured the gills, which resulted in poor blood flow and insufficient blood supply, and finally led to the loss of respiratory function of gills. The morphological, molecular and pathological data on *M. basilemellaris* from this study can provide a theoretical basis for its prevention and control in aquaculture.

Key words: *Myxobolus basilemellaris*; new record in China; *Cyprinus carpio*; histopathology

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