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水霉菌的形态及 ITS 区分子鉴定

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摘要: 研究利用形态学和分子生物学相结合的方法, 对从患病黄颡鱼病灶处分离的水霉菌株(HSY)的分类地位进行了研究。结果显示, 该菌在 18—23℃均能形成游动孢子囊, 但只在 20℃才大量释放游动孢子; 在 15℃培养约 3 周出现大量的藏卵器, 多数顶生, 形态与 *Saprolegnia litoralis* 和 *Saprolegnia ferax* 的均非常相似。序列比对分析显示, HSY 菌株与 *S. litoralis*、*S. bulbosa*、*S. oliviae*、*S. longicaulis*、*S. ferax*、*S. mixta* 和 *S. anomalies* 的 ITS 区(包括 5.8S rDNA)序列相似性均高于 99%。系统发育分析显示, 所选的真菌分为 3 个群, HSY 落于第一个群, MP 树显示 HSY 菌株与 *S. litoralis*、*S. bulbosa*、*S. oliviae*、*S. longicaulis*、*S. ferax* 以及 *S. mixta* 枝系均互相平行, 而 NJ 树显示其与 *S. ferax* 和 *S. mixta* 更近。综合形态学、ITS 区序列及系统发育分析, 将 HSY 菌株暂定为多子水霉(*Saprolegnia ferax*)。

关键词: 卵菌纲; 水霉; 分类; ITS rDNA; 系统发育
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水霉属(*Saprolegnia*)隶属于卵菌纲(*Oomycetes*)(现已归于不等双鞭毛界), 为常见的水生真菌, 极易感染淡水鱼和鱼卵, 通常称此感染病为水霉病(Saprolegniasis)[1,2]。暴发水霉病对天然或人工养殖的鱼体或鱼卵, 均能造成重大经济损失。但关于水霉病的研究, 至今仍然存在很多问题, 尤其关于病原菌的鉴定。依据传统的分类学标准, 水霉属的鉴定主要依据第一代游动孢子的释放模式, 种的鉴定则要求对有性器官作详细的描述, 包括藏卵器、雄器、以及卵孢子分化位置及数目等[3−6]。然而, 很多菌株特别是从鱼体病灶处分离的水霉, 在实验室条件下通常不能或很难发育形成有性器官[7−9], 而且有些种的有性器官在不同的生活环境中形状不稳定[5,7]。因此应用传统的分类学标准很难甚至无法将病灶处水霉鉴定到种的水平。

目前, 随着分子生物学的快速发展, 许多分子生物学手段, 如 DNA 指纹(DNA fingerprinting)[10]、核糖体 rDNA 内部转录间隔区(Internal Transcribed Spacer, ITS)[7,13]、扩增多态性 DNA(random amplification of polymorphic DNA, RAPD)[11,12]等已有效的用于水霉的分类学研究中。由于在进化上 ITS 区域高度保守而在种间甚至种内则高度变异, 且 ITS 区域具有片段小, 易于分析等优点, 近年来真菌分类学家越来越倾向利用 ITS 序列分析技术作为水霉种间分析和种的鉴定的分子标记[7,13]。

早在 20 世纪 20 至 30 年代国外就开展了水霉菌的分类研究工作[14,15], 国内起步相对较晚, 除 20 世纪 80 年代倪达书[16]初步描述了水霉外部形态外, 至今未见相关系统详细的报道。2007 年 12 月, 我们从患病的黄颡鱼病灶处分离了一株水霉菌。本文以该菌株为研究对象, 开展形态学的研究, 同时以 ITS 区域为分子标记, 进行分子生物学鉴定, 并利用分子发育信息详细地阐述其系统发育地位, 以期弥补国内水霉分类研究的不足, 并为鱼类水霉病的病原鉴定及后期药物防治的研究奠定基础。

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1 材料与方法

1.1 菌株来源及分离

菌株分离于成年黄颡鱼(*Pelteobagrus fulvidraco*)(2007 年 12 月购于武汉大学水利水电学院集贸市场)。取病鱼病原菌丝体或病灶组织于 70%酒精漂洗 3s, 用无菌水冲洗两次后, 接种于含有 100 μg/mL 双抗(Penicillin-streptomycin)的玉米琼脂培养基(CMA)(玉米粉 30 g, 琼脂 7.5—9 g, 蒸馏水 500 mL)。依据 Paul 所述方法[13]使用大麻子(Hemp seed)分离纯化。纯化菌株编号为 HSY, 常规保存, 定期传种。

1.2 无性繁殖(Asexual reproduction)

纯化 HSY 点种于 CMA 培养基, 并在接种点外缘 1—1.5 cm 处, 放置数颗灭菌的大麻子, 25℃培养。3—4d 后, 将大麻子菌落无菌接种于过滤的灭菌湖水培养基中, 18—23℃培养, 每隔 4—5h 于倒置显微镜(Zeiss Axiovert 200)下观察其孢子释放模式, 记录第一代游动孢子释放时间, 观察孢子囊形状及孢子发芽情况并测量其大小。

1.3 有性器官(Sexual reproduction)培育

纯化 HSY 大麻子菌落置于平板中, 分别水培于 7℃、15℃、18℃和 23℃, 每个温度做 3 个平行, 持续培养 4 周或直到有性器官出现, 期间利用倒置显微镜(Zeiss Axiovert 200)定期检查有性器官形成情况。

1.4 DNA 提取及 PCR 扩增

采用 CTAB 裂解法[17]提取基因组 DNA, 即取 10 μg 培养 3—4d 的菌丝体于 1.5 mL 的 EP 管内, 并向管中加入 20 μL TE buffer (10 mmol/L Tris-Hcl, pH 8.0; 10 mmol/L EDTA, pH 8.0), –70℃冰冻 30min。随后, 用与 EP 管配套的玻璃研磨棒室温研磨 3—5min。此步骤重复一次。之后向菌丝研磨液中加入 300—400 μL 双蒸水, 混匀, 取研磨稀释液 100 μL 并向其中加入 500 μL CTAB 裂解缓冲液[100 mmol/L Tris-Hcl, pH 8.0; 20 mmol/L EDTA, pH 8.0; 1.4 mol/L NaCl; 3% CTAB; 2% β-巯基乙醇 (用前加入); 4% (*w*/*v*) PVP], 65℃水浴 2—3h。加入等体积的酚∶氯仿∶异戊醇(25∶24∶1)混合溶液, 轻摇 10min, 8000 r/min 离心 5min, 抽提 2 次后, 加入等体积的氯仿∶异戊醇(24∶1)混合液, 后续步骤同常规抽提法。

引物 ITS1: 5′ TCCGTAGGTGAACCTGCGG 3′和 ITS4: 5′ TCCTCCGCTTATTGATATGC 3′用于扩增两个转录间隔区(ITS1 和 ITS2)包括 5.8S rDNA[17]。PCR 反应程序为: 94℃, 5min; 94℃, 30s, 58℃, 30s, 72℃, 1min, 35 个循环; 之后, 72℃延伸 5min。利用 1%的琼脂糖凝胶分离 PCR 产物并使用试剂盒[Gel Extraction Kit (OMEGA)]纯化目的片段。纯化的扩增片段克隆于 pMD-18T 载体[TaKaRa Biotechnology (Dalian)]中, 进行连接与转化, 挑出 3 个阳性克隆, 利用 M13 常规测序。

1.5 序列比对及系统进化分析

所得序列用 Sequencing Analysis 5.2 (Applied Biosystems)软件反复进行校对, 去除两端不确定的序列后, 在 GenBank 中进行 BLASTn 比较搜索, 同时下载同源性序列, 菌株 HSY ITS 序列提交 GenBank, 登录号为 EU925560, 以绵霉属菌(*Achlya conspicua*, AF218144)作为系统进化分析的外群。采用 Clustal X 1.83[18] 对所选序列(表 1)进行比对, 随后用 Seaview 软件进行手工校正。运用 MEGA3 computer package[19] 和 PAUP4.0b10[20] 分别构建 Neighbour-Joining (NJ)树和 Maximum-Parsimony (MP)树, 自举值(Bootstrap)分析均采用 1000 次重复抽样。

2 结 果

2.1 无性繁殖

在 18—23℃下, HSY 大麻子菌落生长茂盛, 直径可达 1.5—4.0 cm, 均有游动孢子囊的形成, 但只在 20℃时游动孢子才大量释放, 开始释放时间为 14—16h 后, 释放方式为典型的水霉属菌模式(图版 I-1、2)。外菌丝中等粗壮, 基部有稀疏分枝, 直径为 20—58 μm。动孢子囊很多, 一般比菌丝粗壮, 形状多样, 如纺锤形、棍棒形、船形, 呈直线或弯曲, 侧生或顶生于菌丝, 大小为(110—490) μm×(25—62) μm, 新的动孢子囊通常从中空的老囊中生出。成熟的动孢子从囊中逸出, 第一代游动孢子梨形, 两根鞭毛顶生; 第二代游动孢子肾形, 两根鞭毛侧生, 游动活泼, 为水霉属标准的两游现象, 孢子直径为 9—12 μm。

2.2 有性器官培育

HSY 在 15℃培养约 3 周发现大量的藏卵器(图版 I-3), 卵细胞亦分化良好。藏卵器球形或梨形(图版 I-4), 多数顶生, 少数侧生, 直径为 75—108 μm; 卵壁光滑。藏卵器主枝粗壮, 多数弯曲(图版 I-5)。

表 1　用于 ITS 序列分析的同源序列

Tab. 1　The homologous sequences used for ITS rDNA sequence analysis

登录号 Accession No.	种名 Species	来源 Sources	地区 Origins
EF064134	*Saprolegnia anomalies*[1]	土壤 Soil	Poland
AY270032	*Saprolegnia longicaulis*	河流 River	Argentina
AM228790	*Saprolegnia ferax*[1]	奥斯塔欧洲鳌虾 *Astacus astacus*	Finland
AM228788	*Saprolegnia ferax*[2]	水 Water	USA
AM228850	*Saprolegnia diclina*[1]	北澳海鳟 *Nematalosa erebi*	Australia
DQ322632	*Saprolegnia anomalies*[2]	水 Water	India
EU124763	*Saprolegnia ferax*[3]	两栖动物卵 Amphibian eggs	*
EF126339	*Saprolegnia mixta*[1]	土壤 Soil	Poland
AB219390	*Saprolegnia mixta*[2]	*	Japan
AM228845	*Saprolegnia ferax*[4]	水 Water	USA
AY310503	*Saprolegnia litoralis*	小龙虾 Crayfish	Germany
EF126324	*Saprolegnia bulbosa*[1]	土壤 Soil	Poland
AB219387	*Saprolegnia ferax*[5]	*	Japan
AY270031	*Saprolegnia oliviae*	河流 River	Argentina
AY267011	*Saprolegnia bulbosa*[2]	溪流 Stream	Australia
AM228817	*Saprolegnia australis*[1]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228811	*Saprolegnia diclina*[2]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228812	*Saprolegnia australis*[2]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228813	*Saprolegnia australis*[3]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228819	*Saprolegnia australis*[4]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228816	*Saprolegnia australis*[5]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228804	*Saprolegnia parasitica*[1]	褐鳟 *Salmo trutta*	Finland
AY455771	*Saprolegnia parasitica*[2]	溪红点鲑 Brook trout	Japan
AY455776	*Saprolegnia parasitica*[3]	红鲑 Sockeye salmon	Japan
AY647193	*Saprolegnia salmonis*[1]	红鲑 Sockeye salmon	Japan
EU551153	*Saprolegnia salmonis*[2]	红鲑 Sockeye salmon	Argentina
EU551152	*Saprolegnia salmonis*[3]	红鲑 Sockeye salmon	Argentina
AY455777	*Saprolegnia parasitica*[4]	香鱼 Ayu fish	Japan
AM228840	*Saprolegnia parasitica*[5]	麦奇钩吻鲑 *Oncorhynchus mykiss*	Chile
AM228842	*Saprolegnia parasitica*[6]	麦奇钩吻鲑 *Oncorhynchus mykiss*	Chile
AM228843	*Saprolegnia parasitica*[7]	水 Water	Spain
AM228841	*Saprolegnia parasitica*[8]	麦奇钩吻鲑 *Oncorhynchus mykiss*	Chile
AM228839	*Saprolegnia parasitica*[9]	麦奇钩吻鲑 *Oncorhynchus mykiss*	Chile
AM228727	*Saprolegnia parasitica*[10]	水 Water	Spain
AM228724	*Saprolegnia hypogyna*[1]	水 Water	Spain
AM228814	*Saprolegnia hypogyna*[2]	褐鳟卵 *Salmo trutta* eggs	Spain
AM228846	*Saprolegnia hypogyna*[3]	水 Water	Spain
AM228815	*Saprolegnia hypogyna*[4]	褐鳟卵 *Salmo trutta* eggs	Spain
AY647188	*Saprolegnia hypogyna*[5]	*	UK

注: “*”表示来源或地区不明; 上标数字代表同名的不同分子种

Note: “*”represent that the source or region was unknown; Super-script numbers represent the different molecular sepecies with the same names

但在 7℃、18℃和 23℃水培 4 周均没有发现有性器官的形成。约 50%的藏卵器具有雄性器官, 雄器短而粗与藏卵器同枝,包括同体(Androgynous)(图版 I-6)和同枝(Monoclinous)(图版 I-7), 少数异枝(图版

I-5)。卵孢子分化中央型或亚中央型(图版 I-4、5、8), 每个藏卵器 3—25 个卵孢子不等(图版 I-4、8), 直径 18—30 μm。重复培养有性器官结构一致。

2.3 序列及系统发育分析

HSY 菌株 ITS 区域(包括 5.8S rDNA)有大约 744 bp, BLAST 结果显示其与 *S. litoralis*、卵形水霉 *S. oliviae*、棒形水霉 *S. longicaulis*、球形水霉 *S. bulbosa*、多子水霉 *S. ferax*、多形水霉 *S. mixta* 和异形水霉 *S. anomalies* 以及一株异株水霉(*S. diclina*)(AM228850)序列相似性均为 99%以上。图 1 显示了邻接法(NJ)和最大简约法(MP)所构建的系统发育树。从图可知, NJ 树和 MP 树在主要的拓扑结

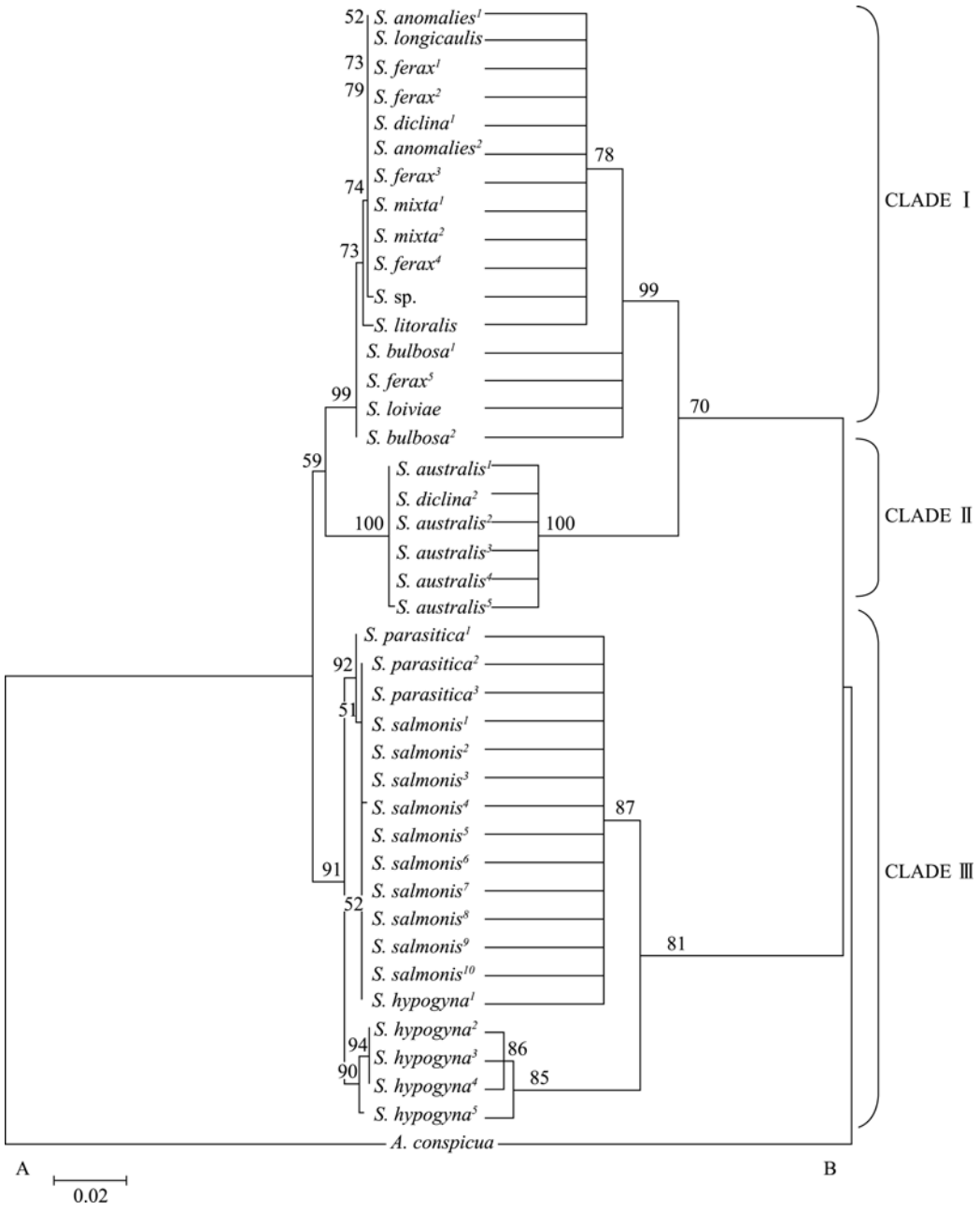

图 1 基于 ITS rDNA 的分子系统树显示了菌株 HSY(*S.* sp.)相对于其他同源水霉种的系统发育位置

Fig. 1 Phylogenetic positions of isolates HSY (*S.* sp.) in relation to other homologous sequenced *Saprolegnia* species based on ITS rDNA region

A. 示邻接法所建的 Neighbour-joining 树; B. 示最大似然法所建的 Maximum Parsimony 树; 枝系上的数值示 Bootstrap 值; 所有 Bootstrap 值均为 1000 次自举重复抽样百分比

A. It shows Neighbour-joining tree. B. It shows the Maximum Parsimony tree (MP); Bootstrap values are displayed on the branches; All bootstrap values are indicated at 1000 repetitions

构上是一致的, 两进化树结构均可分为 3 个大的聚类枝 CLADEⅠ、II、III。除了 MP 树中的 CLADEIII 支持率为 81%外, 其他枝系支持率均高达 91%—100%。CLADEIII 主要包括了水霉属著名的寄生种 *S. parasitica*、*S. salmonis* 和 *S. hypogyna*; 所有的 *S. australis* 菌株和一株 *S. diclina* (AM228811)聚集于 CLADEII; 菌株 HSY 和所有与其序列相似性达 99%的水霉种均归类于 CLADEⅠ。此外, 在 CLADEⅠ中, MP 树显示 HSY 菌株与 *S. litoralis*、*S. bulbosa*[1]、*S. oliviae*、*S. longicaulis*、*S. bulbosa*[2]、*S. ferax* 以及 *S. mixta* 枝系均互相平行, 而 NJ 树显示其与 *S. ferax* 和 *S. mixta* 系统发育位置更近, 虽然此支持率并不高(74%)。因此依据形态和分子分析结果, 将菌株 HSY 暂定为 *S. ferax*。

3　讨　论

3.1　无性繁殖

水霉属是水霉科重要属之一, 其分类系统最早由 de Bary & Woronin[21]和 de Bary [22]建立, 此后被 Coker & Mathews[15]进一步完善。新的种属鉴定及命名一般依据 Johnson[23]、Dick[24]、Seymour[5]、Johnson. *et al*. [25]以及不断出现的新种报道。水霉属与水霉科其他 5 个属在孢子囊或第一代游动孢子释放方式上有着截然不同的特点(图 2)。水霉属孢子囊粗大, 主要为棍棒形, 较常见的还有纺锤形, 第一代游动孢子释放便游开; 细囊霉属(*Leptolegnia*)虽然和水霉属释放孢子形式相似, 但其孢子囊很细, 只允许单排孢子成列车厢状排列; 丝囊霉(*Aphanomyes*)孢子

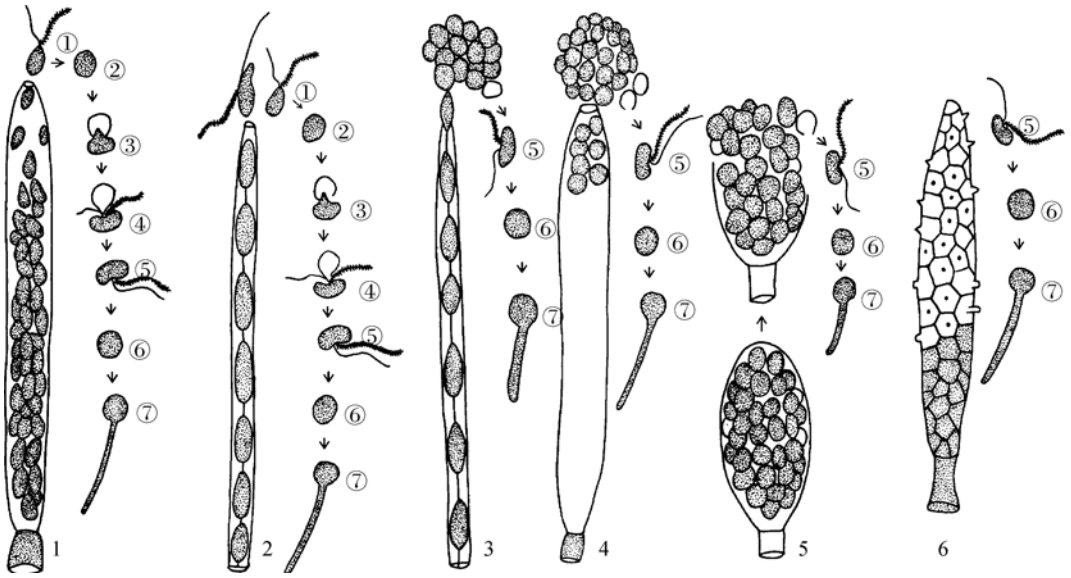

图 2　水霉属与水霉科其他 5 个相似属的无性生殖模式图

Fig. 2　Schematic drawing shows the asexual reproductions in *Saprolegnia* and other five similar genus of saprolegniaceae

1. 水霉属, 大多产生棍棒状的孢子囊并形成两种形态的游动孢子, 第一代游动孢子出孢子囊口便游走; 2. 细囊霉属, 游动孢子形态及释放同水霉属, 但其孢子囊细窄, 游动孢子像火车厢样单排排列; 3. 丝囊霉属, 游动孢子囊形状及孢子排列同细囊霉, 但其第一代游动孢子出孢即形成静孢囊聚集在孢子囊口; 4. 绵霉属, 孢子囊形状同水霉属, 游动孢子释放同丝囊霉属; 5. 破囊霉属, 孢子囊为宽棍棒状, 第一代游动孢子在孢子囊里面即形成静孢囊, 初生的静孢囊通过孢子囊不规则破裂而被释放; 6. 网囊霉属, 孢子囊形状同水霉属, 但第一代游动孢子在孢子囊里面便形成静包囊, 且静包囊停留在孢子囊里面, 并通过孢子囊上的小孔释放次生的游动孢子(① 第一代游动孢子梨形, 具两根顶生的鞭毛②第一代静包囊③-④第一代静包囊释放第二代游动孢子⑤第二代游动孢子, 肾形, 具两根侧生的鞭毛⑥第二代静包囊⑦包囊的萌发)

1. *Saprolegnia*, characterized by clavate zoosporangia and diplanetic zoospores, the primary zoospores swim away when discharged from the zoosporangia; 2. *Leptolegnia*, the zoospores and their discharge are similar to *saprolegnia* but the zoosporangia narrow and the zoospores in it arranged one by one; 3. *Aphanomyes* has the *Leptolegnia*-like zoosporangia but primary zoospores encyst immediately on exiting the sporangial tip; 4. *Achlya*, zoosporangial shape resembles saprolegnia and zoospore escape pattern is similar with *Aphanomyes*; 5. *Thraustotheca*, zoosporangia disintegrate to release encysted primary zoospores which then germinate to release typical reniform secondary zoospores; 6. *Dictyuchus* hold clavata sporangia as in saprolegnia but the primary zoospores encyst within the zoosporangium and do not escape, instead forming a net of cells within the sporangium and germinating to release secondary zoospores singly through papillae in the sporangial walls (① Primary zoospores, pyriform, with two acrogenous flagella; ② Primary zoospores encysted; ③-④ The discharge of secondary zoospores; ⑤ The secondary zoospores, reniform, with two lateral flagella; ⑥ The secondary zoospores encysted; ⑦ Germinating zoospores)

囊虽和细囊霉属相似, 但其第一代游动孢子出囊便成静包囊停留在孢子囊出口处, 然后释放第二代游动孢子; 绵霉属(*Achlya*)在释放孢子方式和丝囊霉属相似, 但其孢子囊却和水霉属一样; 破囊霉属(*Thraustotheca*)孢子囊为宽棍棒状, 且第一代游动孢子在孢子囊里面即形成静孢囊, 初生的静孢囊通过孢子囊不规则破裂而被释放; 网囊霉属(*Dictyuchus*)孢子囊类似于水霉属, 第一代游动孢子在孢子囊里面便形成静包囊, 且静包囊停留在孢子囊里面, 通过孢子囊上的小孔释放次生的游动孢子并在孢子囊壁上留下多角形的网状结构。菌株 HSY 孢子囊粗大, 呈纺锤形、棍棒形且第一代游动孢子出口便游走, 此为典型的水霉属特征。

3.2 有性器官培育

菌株 HSY 有性器官表现为藏卵器表面光滑; 卵细胞中央型或少数亚中央型, 卵细胞个数多数超过 3 个; 几乎一半成熟的藏卵器旁均可发现雄性器官; 雄器主要表现为同体或同枝, 少数异枝; 藏卵器柄的长度通常大于或等于藏卵器直径; 藏卵器柄无螺旋盘曲现象; 藏卵器柄和雄器枝很少形状不规则。依据 Johnson *et al*. [25]形态鉴定检索表发现, 除了雄枝多寡有差别外, 菌株 HSY 有性器官特征与 *S. litoralis* 和 *S. ferax* 的非常相似。另外, 通过进一步实验我们发现, 很多种有性器官形成的温度通常是一个温度段, 如 15℃正常产生雄器和藏卵器, 其在 14℃或 16℃也可产生有性器官, 只不过这两个温度藏卵器较少或雄器较少或者两者均较少。因此我们推断: *S. litoralis* 和 *S. ferax* 在不同温度下, 均能产生数量不等的有性器官。在藏卵器柄大于或等于藏卵器直径的情况下, 侧生和顶生实际上是一个相对的概念, 基本上均可看成是顶生, 除非藏卵器的柄很短甚至基本不存在, 此时侧生才能有效区别于顶生。因此, 仅从形态上是难以区分菌株 HSY 和已知种 *S. litoralis* 和 *S. ferax* 的。

3.3 序列及系统发育分析

ITS 序列比较结果显示 HSY 菌株与已知形态种: *S. litoralis*、*S. bulbosa*[1]、*S. oliviae*、*S. Longicaulis*、*S. bulbosa*[2]、*S. ferax*、*S. mixta* 和 *S. anomalies* 以及一株 *S. Diclina* (AM228850)序列相似性均在 99%以上。在系统发育分析中, 所有的种类分为 3 个群。HSY 菌株及与之相似的形态种均聚类于 CLADE Ⅰ (NJ 99%、MP 99%)。MP 树中, 所有位于 CLADE Ⅰ中的菌株为姊妹种互相平行; NJ 树中, 虽然 CLADEI 内呈现出许多小的分枝, 但支持率都不高(52%—74%), 且 *S. ferax* 的不同基因种 (相似性 99%—100%)贯穿于不同的小枝系。综合形态学和 ITS 区分子信息, 我们将菌株 HSY 鉴定为 *S. ferax*, 理由有三: 一是 *S. ferax* 分布广泛, 其与 *S. litoralis* 区别仅在雄枝多寡和藏卵器侧生与顶生两方面, 但是雄枝丰度本来就多变, 且在藏卵器柄大于或等于藏卵器直径时, 侧生基本可以看生顶生, 也就是说 *S. litoralis* 与 *S. ferax* 在形态上不能区分为两个种; 二是在具有 HSY 相似有性器官的种中, *S. ferax* 是最早被命名的水霉种; 三是 HSY 与 *S. ferax* 在 ITS 区相似性在 99%以上, 此与同名的 *S. ferax* 之间相似性一致。CLADE Ⅱ中包含常见的腐生水霉 *S. australis*, 各菌株序列相似性均在 99%以上; CLADEⅢ中包含常见的寄生种 *S. salmonis*、*S. parasitica* 序列相似性也均在 99%以上, Diéguez-Uribeondo, *et al*.[7]建议将其统一命名为 *S. parasitica*。CLADE Ⅰ中各形态种序列相似性也均在 99%以上, 而且实验证明 *S. ferax* 的有性器 官特别是藏卵器在不同的环境中形态并不是固定 的[26], 因此 CLADE Ⅰ中各种亦有可能是同一种或是同一种的不同亚群, 当然此结论需结合形态学及大量的分子数据进一步论证。

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SAPROLEGNIA IDENTIFICATION BASED ON THEIR MORPHOLOGICAL CHARACTERISTICS AND ITS rDNA REGION

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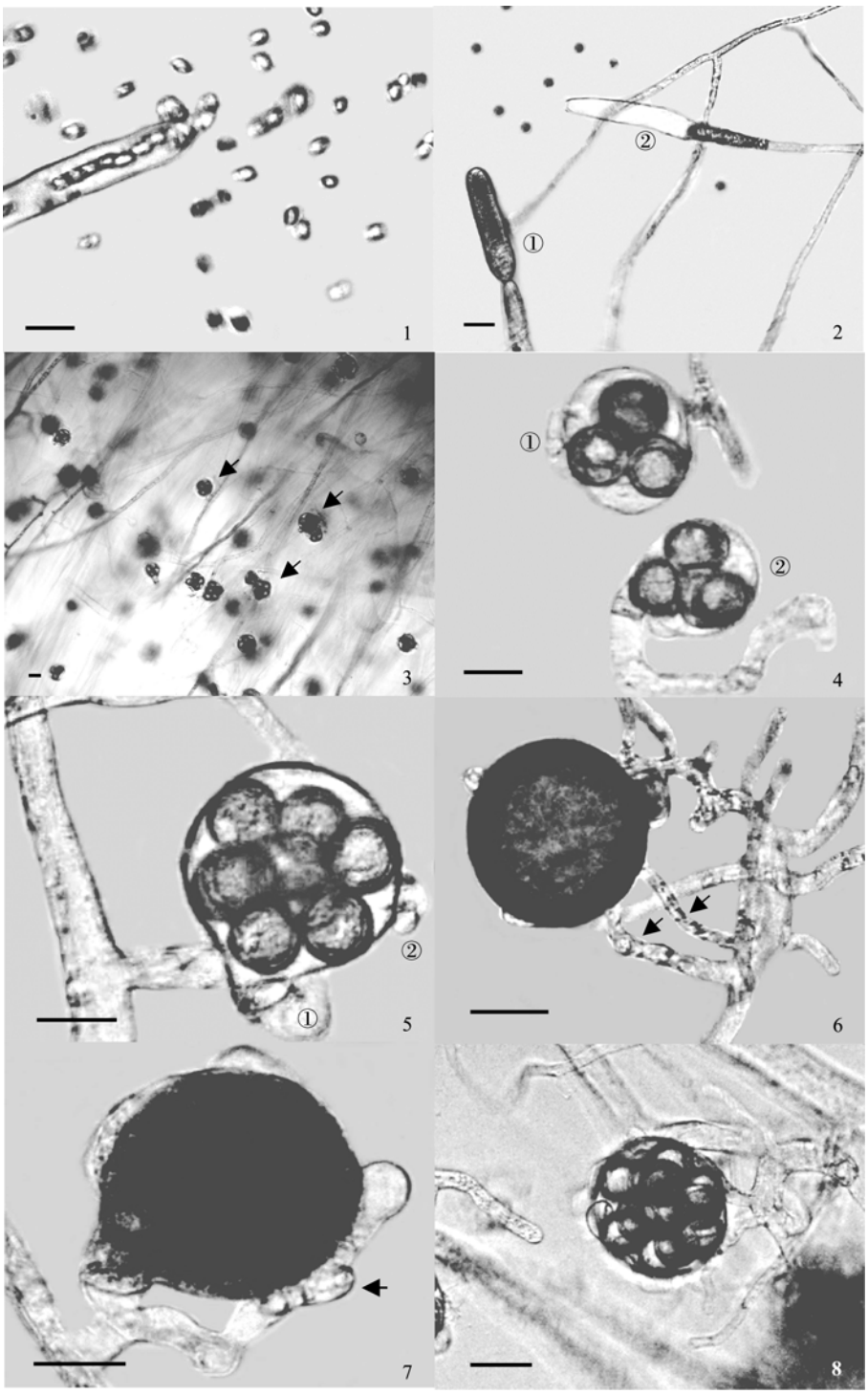
Abstract: *Saprolegnia* is one of the main genus of water molds responsible for fungal infections of freshwater fish and their eggs. These infections are usually termed "saprolegniasis", which can cause severe losses of freshwater fish in both nature and commercial fish farms. However, identification of the causative agent is often unmanageable, especially to those isolated from fish lesions. Traditionally, the generic definition was mainly based on the asexual characters, especially the mode of zoospores discharge, and species differentiation was mainly on the features of sexual reproductive organs including oogonia, antheridia, antheridial origin and oospore. However, many of these characters may be variable in one species or similar in different species and some strains either lose the ability to produce sexual reproduction or cannot be induced to form them under laboratory conditions. So, it is difficult to make definitive identification only using traditional morphological criteria in species level.

In this study, we identified one *saprolegnia* isolate HSY obtained from yellow catfish (*Pelteobagrus fulvidraco*) based on the morphological and molecular characteristics. Results showed Isolate HSY grew luxuriantly on hemp seeds in water at 18—23℃ and were measured 1.5—4.0cm in diameter. The hyphae were stout, sparingly branched and measured between 20—58μm in diameter. Zoosporangia had formed abundantly and were often fusiform, clavate, straight or bent, lateral or terminal and renewed internally, measured 110—490 × 25—62μm. Primary zoospore discharge was typically saprolegnoid, pyriform; secondary zoospores emerged from the encysted, reniform; encysted zoospores were globose and measured 9—12μm in diameter. Oogonia were formed abundantly within three weeks at 15℃, not 18℃ and 23℃, mainly terminal, rarely lateral, and measured 75—108μm in diameter. Oogonial walls were sooth, and stalks were stout and bent. The attachment of antheridial cells was moderate (mainly androgynous and monoclinous, rarely diclinous). The oosproes were centric or subcentric, 3—25 per oogonium, and meansured 18—30μm. These morphological were very similar to *S. litoralis* and *S. ferax*.

Comparing 744 base pairs of internal transcribed spacer (ITS) region and the 5.8S rDNA, we found a group of currently identified *saprolegnia* species including *S. litoralis* and *S. ferax* shared an almost identical ITS sequence (above 99% similarity) with that of isolate HSY. Then, thirty-nine available sequences for representative *Saprolegnia* spp. formed three phylogenetically separate clades. Isolate HSY fell into clade I which also comprised a group of isolates showing high similarities among ITS sequences but different in their morphological features. Moreover, both NJ and MP tree showed that almost all these species in clade I were parallel to each other. These suggested the isolates in clade I might be the same or closely related phylogenntic species and showed how unsatisfactory oogonium morphology appeared to be as a predictor of genetic relatedness.

Consequently, in our opinions, HSY should be identified as *S. ferax* because *S. litoralis* and *S. ferax* could not be differentiated only by morphology; secondly, *S. ferax* was the first named one which has the similar sexual organs with isolate HSY; finally, the similarity of the ITS regions of *S. ferax* and HSY was above 99%. Meanwhile, the present results evidently suggested that *Saprlegnia* classification only based on sexual organs were likely to lead to artificial species. Many species once identified as different might be just one thing in fact.

Key words: *Oomycetes*; *Saprolegnia*; Taxonomy; ITS rDNA; Phylogenetics



图版 I　Plate I

1. 第一代游动孢子的释放; 2. 成熟的孢子囊(①)和刚刚释放完孢子的空孢囊(②); 3. 15℃水培养一周所形成的大量的有性器官(箭头示藏卵器); 4. 卵孢子分化良好的球形(①)和梨形藏卵器(②); 5. 球形的藏卵器①示弯曲的藏卵器主枝②示异枝雄器; 6. 球形藏卵器附有同枝雄器(箭头示雄器); 7. 球形藏卵器附有同体雄器(箭头示雄器); 8. 卵孢子分化中央型. 标尺均为 50 μm

1. The discharge of primary zoospores; 2. The matured zoosporangia (①) and emptied zoosporangia (②); 3. Large numbers of sexual organs were produced after incubating one week in water at 15℃ (arrowhead show the oogonia); 4. The spores of the global oogonium(①)and pyriform oogonuium (②) were well differentiated; 5. The global oogonium with curved stalk (①) and diclinous antheridial hyphae (②); 6. The global oogonium attached by monoclinous antheridial hyphae; 7. The global oogonium attached by androgynous antheridial hyphae; 8. The centre differentiated spores. Scale bar=50 μm