

篮状菌属二个中国新记录种

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孙剑秋, 张楷, 宋福行, 王龙. 篮状菌属二个中国新记录种[J]. 微生物学通报, 2022, 49(10): 4080-4089

Sun Jianqiu, Zhang Kai, Song Fuhang, Wang Long. Two species of *Talaromyces* new to China[J]. Microbiology China, 2022, 49(10): 4080-4089

摘要:【背景】调查我国滩涂土壤可培养真菌物种多样性, 丰富我国真菌物种资源。【目的】报道我国篮状菌属 2 个产子囊孢子的新记录种。【方法】采用形态学和基于 β -微管蛋白基因部分序列和 rDNA ITS1-5.8S-ITS2 序列系统学的多相分类学方法。【结果】分离鉴定出 3 种有性型篮状菌, 即阿根廷篮状菌 *Talaromyces argentinensis* (ZZ2-7-1h=CGMCC 3.16171)、巴塞篮状菌 *Talaromyces barcinensis* (ZZ2-1-1=CGMCC 3.16172) 和乌克兰篮状菌 *Talaromyces ucrainicus* (JS11-5=CGMCC 3.16173), 其中前 2 种为我国新记录种。阿根廷篮状菌在 25 °C 生长适度, 在 37 °C 生长局限, 形成絮状兼绳状菌落, 其菌丝体白色夹杂浅粉色, 产生稀少的橙黄色裸囊壳和壁具细密小刺的椭圆形子囊孢子。巴塞篮状菌在 25 °C 生长适度, 在 37 °C 不生长, 形成绒状菌落, 其菌丝体为白色兼黄色, 裸囊壳稀少, 较小, 呈皮黄色, 子囊孢子椭圆形, 壁具稀疏小刺。【结论】参考我国迄今已报道的篮状菌物种, 确定阿根廷篮状菌和巴塞篮状菌为我国篮状菌属的新记录种。

关键词: 霉菌; 青霉; 滩涂真菌; 子囊果

基金项目: 国家自然科学基金(31750001, 31870013); 科技部基础资源调查专项(2019FY100700); 中国科学院前沿科学重点研究项目(QYZDY-SSW-SMC029)

Supported by: National Natural Science Foundation of China (31750001, 31870013); National Project on Scientific Groundwork, Ministry of Science and Technology of China (2019FY100700); Key Research Program of Frontier Science of Chinese Academy of Sciences (QYZDY-SSW-SMC029)

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Received: 2022-01-30; Accepted: 2022-04-13; Published online: 2022-05-19

Two species of *Talaromyces* new to China

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Abstract: [Background] A survey of the species diversity of culturable fungi in tidal flats of China was carried out, aiming at enriching the fungal species resource in China. **[Objective]** To report two *Talaromyces* species producing ascospores new to China. **[Methods]** A polyphasic taxonomic method, which integrated morphological identification and the phylogeny based on sequences of β -tubulin gene (*BenA*) and rDNA ITS1-5.8S-ITS2, was employed. **[Results]** Three teleomorphic *Talaromyces* species were isolated and identified, namely, *T. argentinensis* (ZZ2-7-1h=CGMCC 3.16171), *T. barcinensis* (ZZ2-1-1=CGMCC 3.16172) and *T. ucrainicus* (JS11-5=CGMCC 3.16173), among which, the former two are new to China. *T. argentinensis* grows well at 25 °C and is restricted at 37 °C, presenting floccose and funiculose colonies with white mycelia mingled with a light pink tint and producing sparse orange-yellow gymnothecia with echinulate ellipsoidal ascospores. *T. barcinensis* grows well at 25 °C but does not grow at 37 °C, presenting velutinous colonies with white and yellow mycelia and bearing sparse, small gymnothecia in buff-yellow color with ellipsoidal, sparsely echinulate ascospores. **[Conclusion]** In review of the *Talaromyces* species reported from China hitherto, we confirmed that *T. argentinensis* and *T. barcinensis* are two new records of China.

Keywords: moulds; penicillia; mudflat fungi; ascocarps

相较于陆地环境, 滩涂环境可培养真菌物种多样性的研究较少, 而滩涂环境可能栖息着大量陆地环境罕见的物种或新物种, 栖息于该环境的某些物种可能产生新颖次级代谢产物^[1]。我国海岸线长约 32 000 km, 沿海滩涂面积约 2.17 万 km²^[2], 为了了解我国沿海滩涂的可培养真菌物种多样性、储备物种资源, 科技部启动了科技基础资源调查专项计划的“滩涂丝状真菌资源及多样性调查”项目。在该项目执行过程中, 我们从我国福建省漳州市和江苏省盐城市沿海滩涂分离得到了多种青霉(*Penicillium* Link)和篮状菌(*Talaromyces* C. R. Benjam.)。篮状菌属最初只包括产生裸囊壳(gymnothecium)和青霉状帚状枝无性繁殖结构的物种, 因此, 该属作为青霉的一个有性属被放在青霉的分类框架

中来研究^[3-5]。然而形态学、生态学及分子生物学特征显示, 篮状菌及其相关的青霉双轮对称组(sect. *Biverticillata-Symmetrica* Thom)^[4]或双轮亚属(Subgen. *Biverticillium* Diercks)^[5]的物种与青霉其他物种有相当大的差别^[6-11]。因此, 2012 年墨尔本法规认可了 *Talaromyces* 作为上述篮状菌及其相关青霉物种的合法属名^[12]。篮状菌属目前已发现约 175 种, 可分为 8 个组^[13-14]。其中篮状菌组(sect. *Talaromyces* Stolk and Samson)是该属物种最多的组, 全球已报道约 81 种^[13-17], 我国已报道该组 44 种^[14,17-29]; 糙刺孢篮状菌组(sect. *Trachyspermi* Yaguchi & Udagawa)全球已发现 29 种^[13-16,30-34], 我国已发现 10 种^[14,19,22-23,26,35]; 螺旋篮状菌组(sect. *Helici* Yilmaz, Frisvad & Samson)全球已报道 13 种^[13,22,36-37], 我国只报

道了3种^[19,22]。

篮状菌物种若存在有性阶段则通常产生壁具小刺的椭圆形子囊孢子,如黄色篮状菌[*T. flavus* (Klöcker) Stolk & Samson]、藤本篮状菌[*T. liani* (Kamyschko) N. Yilmaz et al.]和橙黄篮状菌(*T. aureolinus* L. Wang),但也有些物种产生壁具脊状纹饰的椭圆形子囊孢子,如柄篮状菌[*T. stipitatus* (Thom) C.R. Benj.]和鲜绿篮状菌[*T. viridis* (Stolk & G.F. Orr) Arx]^[4-5,15,17]。本文报道了从我国福建省漳州市和江苏省盐城市滩涂土壤分离到的3种产子囊孢子的篮状菌,即篮状菌组的阿根廷篮状菌(*T. argentinensis*)、螺旋篮状菌组的巴塞篮状菌(*T. barcinensis*)和糙刺孢篮状菌组的乌克兰篮状菌(*T. ucrainicus*),其中,阿根廷篮状菌和巴塞篮状菌为我国新记录种。

1 材料与方 法

1.1 样品来源和分离

土壤样品采自我国福建省漳州市九龙口(23°55'39.36"N, 117°25'33.6"E)和江苏省盐城市(33°37'28"N, 120°29'1"E)滩涂土壤。菌株分离采用倍比稀释涂布平皿法^[38-39],分离到多种青霉和篮状菌,其中有3株产子囊孢子的篮状菌经鉴定后保存于中国普通微生物菌种保藏中心(CGMCC, ZZ2-7-1h=CGMCC 3.16171, ZZ2-1-1=CGMCC 3.16172, JS11-5=CGMCC 3.16173)。

1.2 形态学研究方 法

总体生理学性状的观测使用查氏酵母精琼脂(Czapek yeast autolysate agar, CYA)和5%麦芽精琼脂(malt extract agar, MEA)培养基。培养基配方、培养条件、分生孢子、菌丝体、渗 出液和可溶性色素的描述分别参考 Raper 等^[4]、Pitt^[5]、Ridgway^[40]和 Samson 等^[41]。显微性状的研究挑取在 MEA 上 25 °C 培养 7 d 菌落的产孢结构,浸入在 85%乳酸水溶液中做普通光学显

微镜载片观测^[41]。

1.3 分子系统学研究方 法

基因组 DNA 的提取、 β -微管蛋白基因(β -tubulin gene, *BenA*)和 rDNA ITS1-5.8S-ITS2 的 PCR 引物及扩增方法分别参考 Wang 等^[42]、Glass 等^[43]、White 等^[44], PCR 产物由诺赛生物技术有限公司进行双向直通测序。原始序列用软件 BioEdit 7.0.9^[45]校对编辑并提交到 GenBank (ZZ2-7-1h=CGMCC 3.16171: *BenA*=OK104788, ITS=OK087304, *Rpb2*=OK104792; ZZ2-1-1=CGMCC 3.16172: *BenA*=OK104789, ITS=OK087305; JS11-5=CGMCC 3.16173: *BenA*=OK104791, ITS=OK087307, *Rpb2*=OK104794)。

由于 GenBank 无 *T. barcinensis* 模式菌株 CBS 649.95 的钙调蛋白基因(calmodulin gene, *CaM*)和 RNA 多聚酶第二大亚基基因(DNA-dependent RNA polymerase II second largest subunit gene, *Rpb2*)序列,本文作者经多次尝试也未扩增出我国菌株 CGMCC 3.16171 的这 2 个基因序列。因此,分子系统学分析只能依据 *BenA* 和 ITS 序列,将其连接成 *BenA*-ITS 组合序列进行统一分析。选择篮状菌组 18 个种、螺旋篮状菌组 5 个种和糙刺孢篮状菌 19 个种的模式菌株和本研究中的 3 个菌株,以岛蓝状菌组 [sect. *Islandici* (Pitt) Yilmaz, Frisvad & Samson] 的鸽色篮状菌 (*T. columbinus* S.W. Peterson & Jurjević) 作为外群采用最大似然法(maximum likelihood, ML)分析,并进行 1 000 次自展(bootstrap)计算各演化支的支持率^[46-47]。

2 结果与分析

PCR 扩增的 *BenA* 序列约 400 bp, ITS 序列约 560 bp。连接后的 *BenA*-ITS 组合序列矩阵去掉空格共约 687 个位点(site), ML 最适替代模型为 K2+GI。基于该组合序列矩阵的分子系统

学分析显示, 菌株 ZZ2-7-1h=CGMCC 3.16171 与阿根廷篮状菌的模式菌株 NRRL 28750 聚在一起, bootstrap 值为 100%; 菌株 ZZ2-1-1=CGMCC 3.16172 与巴塞篮状菌的模式菌株 CBS 649.95 聚在一个分支, bootstrap 值为 88%; 菌株 JS11-5=CGMCC 3.16173 与乌克兰篮状菌的模式菌株 CBS 162.67 聚在一起, bootstrap 值为 100%。菌株 CGMCC 3.16173 的 *BenA* 序列在糙刺孢篮状菌组的分析中与包括模式菌株在内的 4 株乌克兰篮状菌同处一个演化支, bootstrap 支持率为 100%。通过分子系统学和形态学相结合的分析, 支持这 3 个菌株的鉴定, 参考我国已报道的篮状菌组、糙刺孢篮状菌组和螺旋蓝状菌组物种, 确定阿根廷篮状菌和巴塞篮状菌为我国新记录种(图 1-3)^[14,17-29]。

2.1 阿根廷篮状菌(图 2)

Talaromyces argentinensis (Jurjevic & S.W. Peterson, Fungal Biology 123: 745-762, 2019^[48].)

在 CYA 上 25 °C 培养 7 d, 菌落直径 30-33 mm, 薄, 表面具少量辐射状和同心环状皱纹, 边缘整齐; 绒状; 无分生孢子; 在中部可见少量未成熟裸囊壳, 皮黄色(buff yellow, R. Pl. IV); 菌丝体白色, 在中部呈海贝粉色(seashell pink, R. Pl. X IV); 无渗出液和可溶性色素; 菌落背面呈浅皮黄色至浅赭皮黄色(light buff to light ochraceous-buff, R. Pl. X V)。

在 MEA 上 25 °C 培养 7 d, 菌落直径 45-48 mm, 稍厚, 表面平坦, 边缘流苏状; 短絮状兼绳状; 无分生孢子; 中央可见少量帝国黄色(empire yellow)裸囊壳(R. Pl. IV); 菌丝体在边缘为白色, 其余为绣球花粉色(hydrangea pink, R. Pl. X X VII); 无渗出液和可溶性色素; 菌落背面肉桂红色(cinnamon rufous, R. Pl. X IV)。

在 CYA 上 37 °C 培养 7 d, 菌落直径 5-8 mm, 全部为白色夹杂粉色菌丝体。

在 CYA 上 5 °C 培养 7 d, 不生长。

裸囊壳球形, 250-400 μm, 帝国黄色(empire yellow, R. Pl. IV), 21 d 后成熟; 子囊球形至近球形, 10-12 μm, 内含 8 个子囊孢子; 子囊孢子椭圆形, (4-6)×(3.5-5) μm, 壁具小刺。

主要特征: 生长适度, 在 MEA 上形成短絮状兼绳状菌落, 裸囊壳呈帝国黄色, 菌丝体白色夹杂粉色; 子囊孢子椭圆形, 壁具小刺。

分布和基物: 福建省漳州市九龙口滩涂土壤(ZZ2-7-1h=CGMCC 3.16171)。

2.2 巴塞篮状菌(图 3)

Talaromyces barcinensis (Yaguchi & Udagawa, Transactions of Mycological Society of Japan 34: 15-19, 1993^[36].)

在 CYA 上 25 °C 培养 7 d, 菌落直径 33-34 mm, 薄, 表面平坦, 边缘整齐; 绒状; 无分生孢子; 在中部可见少量未成熟裸囊壳, 皮黄色(buff yellow, R. Pl. IV); 菌丝体在边缘为白色, 其余为皮黄色至玉米黄色(buff yellow to maize yellow, R. Pl. IV); 无渗出液和可溶性色素; 菌落背面中部呈橙红色(orange rufous, R. Pl. II), 外围浅至早金莲黄色(capucine yellow, R. Pl. III)。

在 MEA 上 25 °C 培养 7 d, 菌落直径 37-40 mm, 较薄, 表面平坦, 边缘流苏状; 质地绒状兼短絮状; 无分生孢子; 菌丝体在边缘呈白色, 其余呈萘黄色(naphthalene yellow, R. Pl. X IV); 无渗出液和可溶性色素; 菌落背面呈浅黄色。

在 CYA 上 37 °C 培养 7 d, 不生长。

在 CYA 上 5 °C 培养 7 d, 不生长。

裸囊壳球形, 120-240 μm, 皮黄色, 21 d 后成熟; 子囊球形至椭圆形, 5-8 μm, 内含 8 个子囊孢子; 子囊孢子椭圆形, (2.5-3.5)×(2-2.5) μm, 壁近光滑至稍粗糙。

主要特征: 生长适度, 形成绒状菌落, 菌丝体白色夹杂萘黄色; 裸囊壳较小, 呈皮黄色, 21 d 后成熟; 子囊孢子椭圆形, 较小, 壁近光滑至稍粗糙。

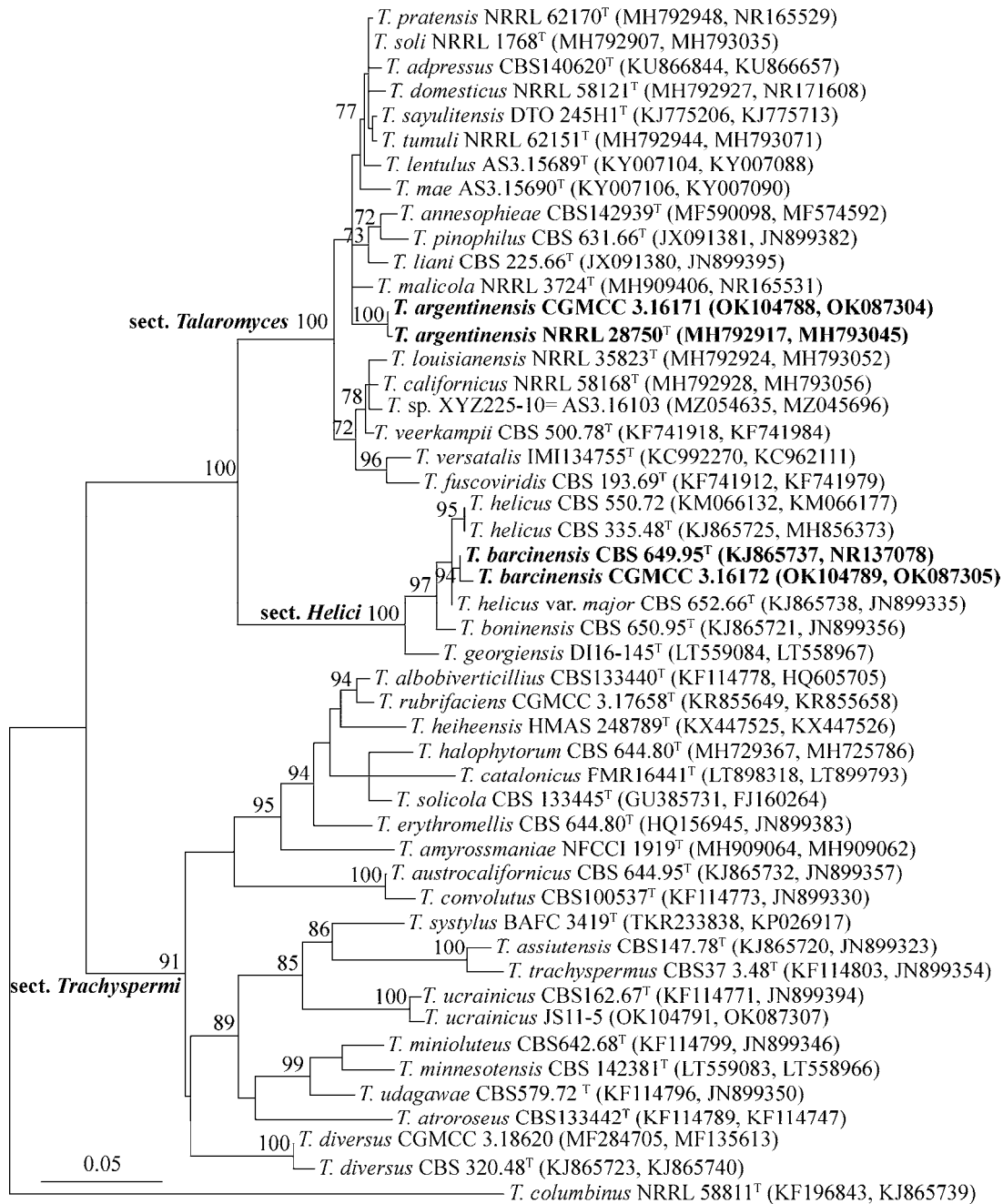


图 1 基于 *BenA*-ITS 序列构建的 ML 系统发育树 Bootstrap 值 $\geq 70\%$ 的分支标注在分支处；模式菌株用“^T”标记；新记录种用粗体标记；菌株 *BenA* 和 ITS 序列的 GenBank 登录号放在括号内；物种所属的 3 个组标注在分支处并用粗体显示；*T. columbinus* 作为外群。标尺 0.05 为每核苷酸位点替代率

Figure 1 The ML tree based on the *BenA*-ITS sequences. Bootstrap percentages $\geq 70\%$ are indicated at the nodes; ex-type strains are indicated with ^T; new record species are indicated in boldface; the GenBank accession numbers of *BenA* and ITS sequences of each strains are in parentheses; the three sections the species belonging to are indicated at each nodes in boldface; *T. columbinus* is as the outgroup. Bar=0.05 substitutions per nucleotide position.

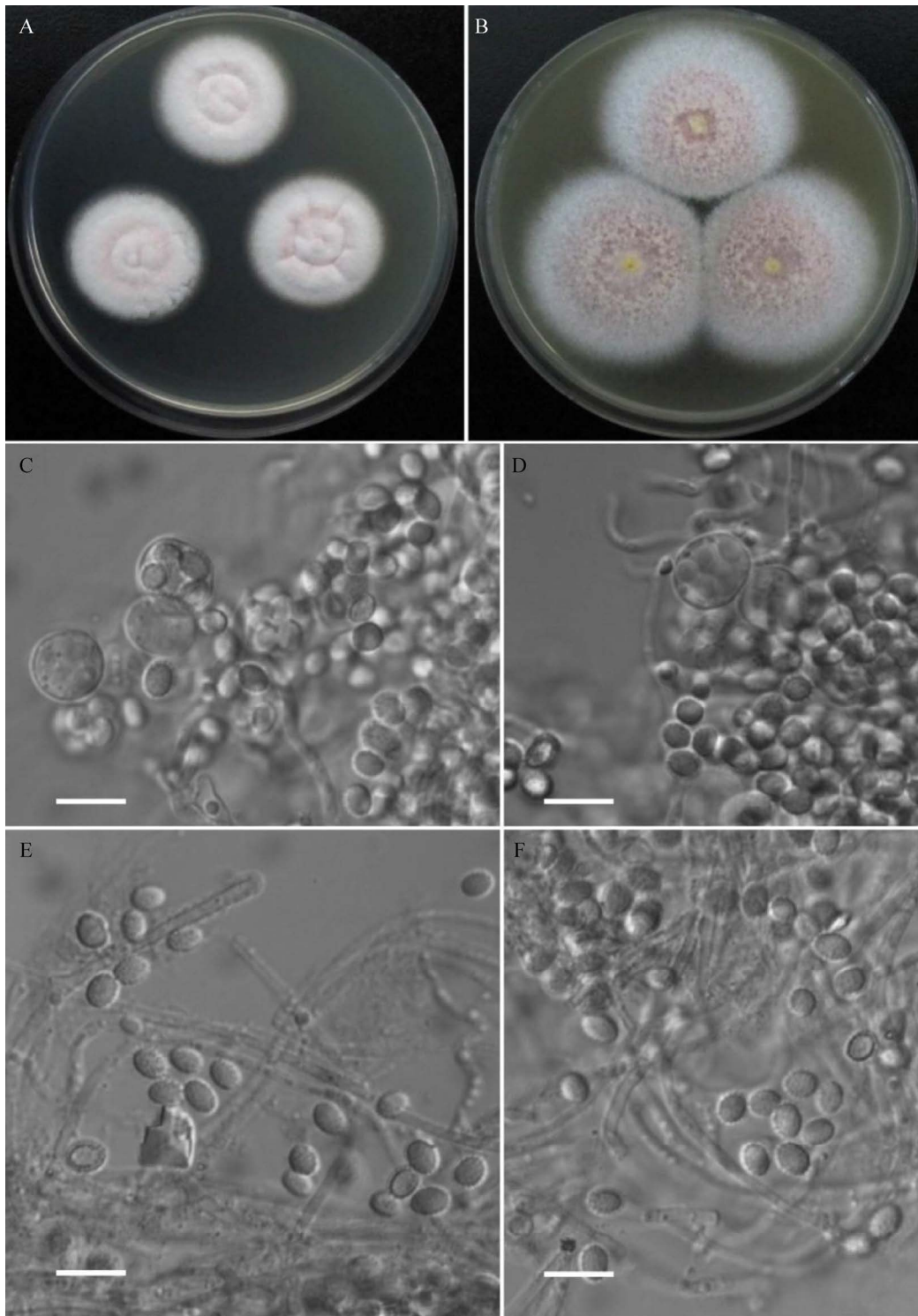


图 2 阿根廷篮状菌(*Talaromyces argentinensis*) CGMCC 3.16171 的形态特征 A、B: 在 CYA 和 MEA 上 25 °C 培养 7 d 的菌落; C: 成熟子囊; D: 未成熟子囊; E、F: 子囊孢子。标尺: 10 μm

Figure 2 Morphological characters of *Talaromyces argentinensis* CGMCC 3.16171. A, B: Colonies on CYA and MEA at 25 °C after 7 d; C: Mature asci; D: Immature asci; E, F: Ascospores. Scale bar: 10 μm.

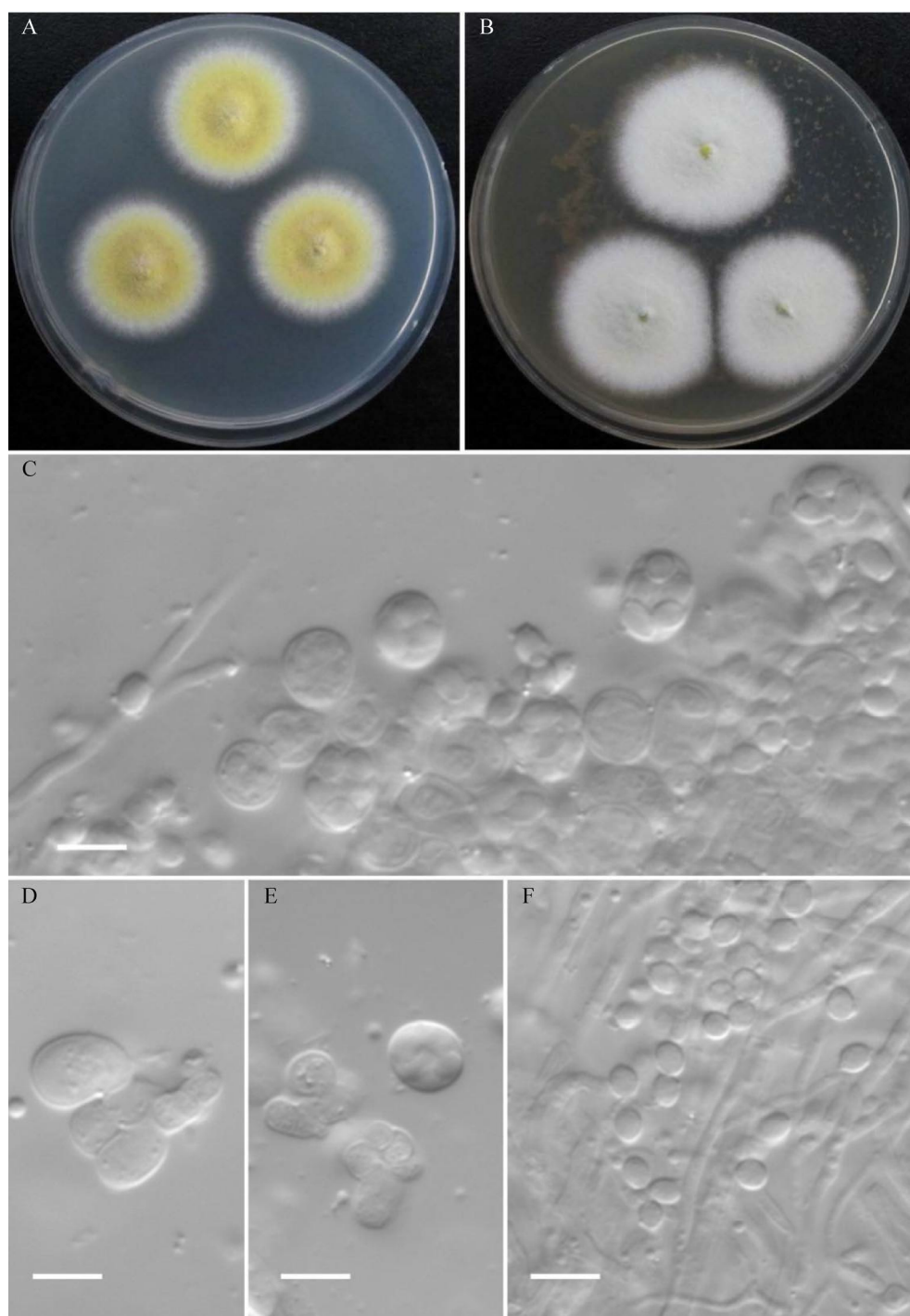


图3 巴塞篮状菌(*Talaromyces barcinensis*) CGMCC 3.16172 的形态特征 A、B: 在 CYA 和 MEA 上 25 °C 培养 7 d 的菌落; C: 成熟子囊; D: 原基和正在发育的子囊, E: 原基和未成熟子囊; F: 子囊孢子。标尺: 10 μm

Figure 3 Morphological characters of *Talaromyces barcinensis* CGMCC 3.16172. A, B: Colonies on CYA and MEA at 25 °C after 7 d; C: Mature asci; D: Initials and the developing asci; E: Initials and immature asci; F: Ascospores. Scale bar: 10 μm .

分布和基物: 福建省漳州市九龙口滩涂土壤(ZZ2-1-1=CGMCC 3.16172)。

3 讨论与结论

阿根廷篮状菌此前只发现于阿根廷和加纳, 而且 GenBank 只记录了 Peterson 等^[48]论文中包括模式菌株的 2 株菌的序列。我国菌株 CGMCC 3.16171 与这 2 株菌在生长速度和菌落形态上几乎无差别, 它们在 MEA 上均形成絮状兼绳状菌落, 产生少量黄色裸囊壳; 其子囊和子囊孢子的形态和大小无差别, 但国外的这 2 株菌在 25 °C 培养 7 d 时产生少量分生孢子, 而我国菌株则未产生分生孢子。CGMCC 3.16171 与模式菌株 NRRL 28750 在 *BenA* 序列上无差别, 与另外一个菌株 NRRL 28758 有 2 个核苷酸的差别(OK104788 vs. MH792917 和 MH792918), 在 ITS 序列上与模式菌株 NRRL 28750 只有 1 个核苷酸的差别, 而与 NRRL 28758 有 5 个核苷酸的差别(OK087304 vs. MH793045 和 MH793046), 在 *Rpb2* 序列上与模式菌株 NRRL 28750 只有 1 个核苷酸的差别, 与 NRRL 28758 有 6 个核苷酸的差别(OK087304 vs. MH793108 和 MH793109)。因此, 我国菌株 CGMCC 3.16171 与模式菌株亲缘关系更近。

Yilmaz 等^[15]把 *T. barcinensis* 作为 *T. helicus* (Raper & Fennell) C.R. Benj. 的异名, 但 *T. barcinensis* 与 *T. helicus* 在子囊发育过程中的原基(initials)有着本质不同, 即 *T. barcinensis* 的原基由不规则膨大的细胞构成, 而 *T. helicus* 的原基则是由产囊体(ascogonium)和螺旋缠绕其上的雄器(antheridium)构成。因此, Yilmaz 等^[15]将 *T. barcinensis* 作为 *T. helicus* 的异名是不正确的。巴塞篮状菌此前只发现于西班牙、日本和尼泊尔。我国菌株 CGMCC 3.16172 的菌落形态、子囊和子囊孢子大小及形状均与模式菌株 CBS

649.95 的无明显差别, 但模式菌株在 25 °C 培养 7 d 时产生稀疏分生孢子, 而 CGMCC 3.16172 则未产生分生孢子。另外模式菌株的子囊孢子壁稀疏粗糙, 但 CGMCC 3.16172 的分生孢子近于光滑或只是稍粗糙^[36]。CGMCC 3.16172 与模式菌株的 *BenA* 序列无差别(OK104789 vs. KJ865737), ITS 序列有 3 个碱基的差别(OK087305 vs. MH862547)。GenBank 中未记录其他巴塞篮状菌菌株的 *BenA* 序列, 但有 3 个菌株的 ITS 序列(MT510027、KJ942582、KC800596), 由于 ITS 无法区分巴塞篮状菌及其近缘种螺旋篮状菌(*T. helices* C.R. Benj.), 因此这 3 个菌株不能确定是巴塞篮状菌。Yilmaz 等^[15]的研究表明, *BenA* 序列基本可以区分目前发现的所有篮状菌物种, 通过上述 *BenA* 序列的比较, 确定菌株 CGMCC 3.16171 和 CGMCC 3.16172 鉴定无误。

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