•研究报告•

中国西南高黎贡山绿僵菌物种多样性及其垂直分布特征

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摘要: 云南高黎贡山具有多样化的生态系统和生物资源。为探清该地区绿僵菌属(*Metarhizium*)真菌的物种多样性及其不同海拔的垂直分布特征, 沿海拔梯度(600–3,800 m)在7种典型植被类型(I: 干热河谷; II: 季风常绿阔叶林; III: 暖性针叶林; IV: 中山暖性常绿阔叶林; V: 山地苔藓矮林; VI: 寒温性灌丛或草甸; VII: 流石滩稀疏植被)中调查绿僵菌资源。从生境土壤中分离菌株, 通过多基因(*nrSSU*、*nrLSU*、*EF-1 α* 、*RPB1*和*RPB2*)系统发育分析进行物种鉴定。结果表明, 高黎贡山绿僵菌物种资源丰富, 获得的161株菌株分属于12个物种(*Metarhizium rileyi*, *M. viridulum*, *M. lepidiotae*, *M. brunneum*, *M. pingshaense*, *M. anisopliae*, *M. robertsii*, *M. guizhouense*, *M. indigoticum*, *M. pemphigi*, *M. campsosterni*和*Metacordyceps neogunnii*), 其中*M. indigoticum*为中国新记录种, *M. anisopliae* complex中的物种(8种)较集中; 同时还采集到了绿僵菌的近缘属*Nigelia*属物种*N. martiale*。高黎贡山绿僵菌广泛分布于除类型VII(海拔3,600–3,800 m)外的6种植被类型(海拔600–3,400 m)中。中低海拔植被类型(I–IV)中菌株数量较多(≥ 23 株)、物种多样性较高(4–9种), 而高海拔植被类型(V–VI)中菌株数量较少(2–8株)、物种较单一(1–2种)。中海拔的常绿阔叶林中绿僵菌资源最丰富, 其中季风常绿阔叶林(植被类型II)中的菌株数量(52株, 占总数的32.3%)和物种数(9种)最多; 中山湿性常绿阔叶林(植被类型IV)为其次(47株, 占总数的29.2%; 7种)。高黎贡山绿僵菌优势种现象明显, *M. brunneum*为最优势物种, 其菌株数占总数的46.6%, 在生境条件差异很大的6种植被类型(I–VI)中都存在, 说明该物种生态适应能力最强。

关键词: 高黎贡山; 绿僵菌; 系统发育分析; 物种多样性; 垂直分布

Species diversity and vertical distribution characteristics of *Metarhizium* in Gaoligong Mountains, southwestern China

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Abstract: Gaoligong Mountains in Yunnan Province, China have diverse ecological systems and biological resources. To understand the diversity and the vertical distribution of *Metarhizium* species at different elevations in this area, *Metarhizium* resources were investigated in seven typical vegetation types (i.e., I, dry-hot valley; II, monsoon evergreen broad-leaved forest; III, warm coniferous forest; IV, mod-montane humid evergreen broad-leaved forest; V, mountain moss dwarf forest; VI, cold shrubs of meadow; and VII, rocky beach sparsely vegetation) along an elevation gradient (600–3,800 m). Strains of *Metarhizium* were isolated from soils of the vegetations at different elevations and identified using multi-gene (*nrSSU*, *nrLSU*, *EF-1 α* , *RPB1*, and *RPB2*) phylogenetic analysis. Our result indicated that the species of *Metarhizium* in Gaoligong Mountains were abundant and the obtained 161 strains represented 12 *Metarhizium* species,

收稿日期: 2018-05-03; 接受日期: 2018-07-04

基金项目: 国家自然科学基金地区基金(31460153)、云南省科技厅地方本科高校基础研究联合专项资金面上项目(2017FH001-029)、云南省科技厅地方本科高校基础研究联合专项资金重点项目(2017FH001-126)和保山市第六批中青年学术和技术带头人项目(bszqnxshjsdtr201701)

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namely, *M. rileyi*, *M. viridulum*, *M. lepidiotae*, *M. brunneum*, *M. pingshaense*, *M. anisopliae*, *M. robertsii*, *M. guizhouense*, *M. indigoticum*, *M. pemphigi*, *M. campsosterni*, and *Metacordyceps neogunnii*, and most of them (8 species) belonged to *M. anisopliae* complex. Additionally, *Nigelia martiale*, a taxon close to *Metarhizium* was also found in this area. *M. indigoticum* is new to China. Fungi of *Metarhizium* in Gaoligong Mountains were widely distributed in six vegetation types at elevation between 600 m and 3,400 m, but not in type VII at the elevation of 3,600–3,800 m. The vegetations at the low and middle elevations (I–IV) harbored larger number of strains (≥ 23) and higher species diversity (4–9 species), whereas those at high elevations (V–VI) possessed fewer strains (2–8) and relatively limited species (1–2 species). *Metarhizium* resources were the most abundant in evergreen broad-leaved forests at the middle elevation. The monsoon evergreen broad-leaved forest (vegetation type II) possessed the largest number of *Metarhizium* strains (52 strains, 32.3% of the total) and species (9 species), while mid-montane humid evergreen broad-leaved forest (vegetation type IV) (47 strains, 29.2% of the total) was the next. The superior species phenomenon of *Metarhizium* was remarkable in Gaoligong Mountains, *M. brunneum* being the most dominant, at 46.6% of the total strain numbers. It widely exists in six vegetation types (I–VI) with very different habitat conditions, suggesting that this species has the highest ecologic adaptability.

Key words: Gaoligong Mountains; *Metarhizium*; phylogenetic analysis; species diversity; vertical distribution

在自然界中, 绿僵菌属(*Metarhizium*)真菌资源丰富、分布广泛。随着人们对绿色生态和绿色产品需求的增加, 绿僵菌在昆虫的自然生态控制和农林害虫生物防治中发挥着越来越重要的作用。在传统系统分类中, 人们主要依据菌落颜色、分生孢子、产孢结构等形态特征对绿僵菌进行分类。早期的研究认为, 绿僵菌属主要包括3个物种: 金龟子绿僵菌(*M. anisopliae*)、黄绿绿僵菌(*M. flavoviride*)和白色绿僵菌(*M. album*) (Tulloch, 1976; Driver et al, 2000)。之后, 根据形态特征陆续报道了一些新种和变种。随着现代分子生物学技术的进步, 绿僵菌属的分类鉴定取得了长足发展。近年来, 在绿僵菌物种的划分上, 多基因系统进化分析被认为较单基因分析更为准确(王萌等, 2014)。通过多基因分析, Bischoff等(2009)对金龟子绿僵菌复合种(*M. anisopliae* complex)进行了分类研究, 认为该复合种包含9个物种。Kepler等(2012)报道了绿僵菌有性型泛虫草属(*Metacordyceps*)包括15个物种和1个变种。至此, 绿僵菌的无性型主要归入*Metarhizium*, 有性型主要归入*Metacordyceps*。根据“一个物种一个名称”的原则, Kepler等(2014)通过多基因分析重新修订了绿僵菌的分类归属, 将*Metacordyceps*的所有物种、*Nomuraea*中绿色孢子的物种、*Chamaeleomyces*和*Paecilomyces*中的一些物种都归入*Metarhizium*中。随后, 又有若干新种陆续被报道(Sharma & Gautam, 2015; Chu et al, 2016; Nishi et al, 2017; Wen et al, 2017)。Luangsa-ard等(2017)报道了6个绿僵菌新种,

并将*Metarhizium martiale*归到新属*Nigelia* (*N. martiale*), 将*Paecilomyces reniformis*归到绿僵菌属(*Metarhizium reniforme*)。综合近年来的报道, 绿僵菌属已有近50个物种(附录1), 其中中国首次报道的绿僵菌物种很多, 例如无性型物种*Metarhizium pingshaense*、*M. guizhouense*和*M. cylindrosporum* (郭好礼等, 1986)和*M. synnematis*等(Chu et al, 2016), 以及有性型物种*Metacordyceps neogunnii* (Wen et al, 2017)、*Mc. guniujiangense* (Li et al, 2010)、*Mc. campsosterni* (Zhang et al, 2005)、*Mc. brittlebanksoides* (Liu et al, 2001)、*Mc. taii* (梁宗琦等, 1991)和*Mc. shibinensis* (Wen et al, 2015)等。

高黎贡山地处云南省西部中缅边境地区, 生态环境复杂多样, 是全球生物多样性研究热点地区之一(Huang et al, 2017)。该区具有典型的高山峡谷地貌, 海拔跨度650–5,000 m (Kavanaugh et al, 2014), 地形复杂, 立体气候明显, 特殊的自然环境孕育出多种不同的生态植被类型, 各植被类型由下至上形成了明显的垂直带谱(徐成东等, 2008)。高黎贡山复杂多样的植被和土壤条件下蕴藏着丰富的昆虫资源(Kavanaugh et al, 2014)和微生物资源(周慧, 2008)。关于高黎贡山动植物资源多样性和保护的研究很多(徐成东等, 2008; Li et al, 2011; Kavanaugh et al, 2014; Huang et al, 2017), 但关于其虫生真菌资源的研究却很少。调查高黎贡山不同植被生境中绿僵菌属真菌的物种多样性及其分布规律, 可为该区域绿僵菌资源的保护和利用提供科学依据。有鉴于

此,本研究从高黎贡山不同海拔地区选取典型的植被类型,从土样中分离获得绿僵菌菌株,进而提取菌株的DNA并进行测序,利用多基因系统发育树分析了高黎贡山绿僵菌的物种多样性及其分布特征。

1 材料与方法

1.1 土壤样品采集

在高黎贡山海拔600–3,800 m范围内选取7种典型的植被类型生境进行采样:(I)干热河谷,海拔600–1,100 m;(II)季风常绿阔叶林,海拔1,300–1,800 m;(III)暖性针叶林,海拔1,500–2,300 m;(IV)中山湿性常绿阔叶林,海拔1,900–2,600 m;(V)山地苔藓矮林,海拔2,800–3,200 m;(VI)寒温性灌丛或草甸,海拔3,200–3,500 m;(VII)流石滩稀疏植被,海拔3,600–3,800 m。

于2015–2017年间,每年7–9月进行取样。每种植被类型选取10个样地,每个样地采集3个土壤样品,共210个样品。样品采集地尽量选择不受或极少受人为活动干扰的区域,挑选阴湿的树下、石头旁、土堆旁肥沃的土壤。用小铲挖取土表10 cm以上的土壤,装入保鲜袋,用记号笔标号,记录采集地海拔、植被类型、采集地温湿度等信息。样品放置在4℃低温环境中,并在1周内处理。

1.2 绿僵菌菌株的分离

采用黄粉虫诱导分离绿僵菌菌株(图1M)。参考Keyser等(2015)的方法,每个土壤样品取约100 mL于无菌罐头瓶中,加入适量无菌水使其处于稍微潮湿状态。每瓶放入10只无菌培养的8龄黄粉虫幼虫。用无菌水洗掉死亡虫体表面土壤,在无菌湿滤纸片上保湿培养。挑取分生孢子转入蛋白胨马铃薯葡萄糖培养基(PPDA),25℃培养。纯化的菌株用试管斜面培养后于4℃保存。

1.3 绿僵菌菌株的鉴定

将保存的绿僵菌菌株转接到PPDA培养基上培养,进行形态学初步鉴定,然后通过5个基因序列(*nrSSU*、*nrLSU*、*EF-1 α* 、*RPB1*和*RPB2*)鉴定物种。

1.4 基因组DNA提取、基因序列扩增及测序

用真菌基因组DNA提取试剂盒(北京百泰克生物技术有限公司)提取各菌株菌丝及分生孢子的基因组DNA。*nrSSU*、*nrLSU*、*EF-1 α* 、*RPB1*和*RPB2*的基因序列扩增引物,反应体系和反应程序参考Chen等(2013)。用1%琼脂糖胶检测DNA,对切胶纯

化后的样品直接进行测序;对部分测序拖带、杂合和非特异等测序结果,将样品进行克隆测序。引物合成和DNA测序由北京诺赛基因组研究中心有限公司完成。

1.5 测序数据处理及分析

利用Bioedit检测初始序列峰图的优劣,选取无双峰、无杂合等序列,利用Lasergene 7.1中EditSeq批量输出,克隆测序时去除载体,正反向测序时使用SeqMan进行序列拼接,而后使用Clustal X 2.1进行测序序列的多序列比对。

1.6 多基因系统发育分析

根据近年来对绿僵菌属研究的最新进展(Bischoff et al, 2009; Kepler et al, 2012, 2014; Luangsa-ard et al, 2017),检索并下载GenBank数据库中的绿僵菌5个基因序列(附录1)。在高黎贡山绿僵菌属每个物种中选取1–2个代表性菌株(共选取了18株菌),将其5个基因序列上传到GenBank获得序列号。因*Nigelia martiale* (原来为*Metarhizium martiale*)为Luangsa-ard等(2017)新修订的属,本研究将从高黎贡山采集到的*N. martiale*材料也一起进行了系统发育分析。

对每个基因序列数据矩阵进行比对,用“N”表示片段的缺失,对得到的5个基因数据矩阵序列进行拼接合并,以*Cordyceps militaris* (OSC 93623)、*Beauveria bassiana* (ARSEF 7518)、*Ophiocordyceps stylophora* (OSC 110999)和*O. acicularis* (OSC 110987)作为外类群,使用RAxML软件进行多基因系统发育分析。

2 研究结果

2.1 高黎贡山绿僵菌属物种多样性

从低海拔到高海拔的7种植被类型中,共采集到210份土壤样品。根据绿僵菌属物种的基本形态特征进行初步鉴定,然后结合菌株的5个基因的系统发育分析,确定了161株菌属于绿僵菌属物种(表1)。同时得到1个属于*Nigelia*属物种的材料(图1Q),但未分离得到纯菌株,作为绿僵菌的近缘属也一起进行了多基因分析。分离到的绿僵菌菌落颜色为白色(图1K、L)或黄白色(图1G),或产生淡绿色(图1J)、暗绿色(图1I)、翠绿色(图1H)、深绿色(图1D、F)、黑色(图1B、C、E)或棕黑色(图1A)的分生孢子层。

图1 高黎贡山绿僵菌不同物种的菌落及其他真菌材料。(A) *M. brunneum*的菌落; (B) *M. lepidiotae*的菌落; (C) *M. indigoticum*的菌落; (D) *M. campsosterni*的菌落; (E) *M. robertsii*的菌落; (F) *M. guizhouense*的菌落; (G) *M. anisopliae*的菌落; (H) *M. pingshaense*的菌落; (I) *M. viridulum*的菌落; (J) *M. pemphigi*的菌落; (K) *M. rileyi*的菌落; (L) *Mc. neogunnii*的菌落; (M) 绿僵菌的诱导分离; (N) *M. viridulum*的子座; (O) *M. rileyi*感染鳞翅目幼虫形成的孢子; (P) *M. pemphigi*感染叶甲形成的孢子; (Q) *N. martiale*的子实体。A-O和Q的比例尺 = 1 cm; P的比例尺= 0.5 cm。

Fig. 1 Colonies of *Metarhizium* species and other fungal materials in Gaoligong Mountains. (A) Colony of *M. brunneum*; (B) Colony of *M. lepidiotae*; (C) Colony of *M. indigoticum*; (D) Colony of *M. campsosterni*; (E) Colony of *M. robertsii*; (F) Colony of *M. guizhouense*; (G) Colony of *M. anisopliae*; (H) Colony of *M. pingshaense*; (I) Colony of *M. viridulum*; (J) Colony of *M. pemphigi*; (K) Colony of *M. rileyi*; (L) Colony of *Mc. neogunnii*; (M) The induction and isolation of *Metarhizium*; (N) Stroma of *M. viridulum*; (O) Spores of *M. rileyi* on the infected Lepidoptera larva; (P) Spores of *M. pemphigi* on the infected beetle larva; (Q) Fruit body of *N. martiale*. Bar of A-O and Q = 1 cm; Bar of P = 0.5 cm.

通过5个基因矩阵的拼接, 得到4,345个碱基对(bp)的序列, 其中包括*nrLSU* 852 bp, *nrSSU* 1,024 bp, *EF-1 α* 916 bp, *RPB1* 718 bp, *RPB2* 835 bp。对所有多基因序列(共102条)(附录1)采用RAxML软件构建系统发育树(图2), 共分成11个类群(Clades 1–11), 外类群物种序列明显形成一个独立的分支(Clade 11)。采自高黎贡山的编号BUM 713的子实体材料属于*Nigelia*属物种(*N. martiale*, 见图2 Clade 10), 该分支也与绿僵菌属序列明显区分开, 形成并列的近

缘分支。所有参与分析的绿僵菌序列分属于45个物种, 归为9个类群(Clades 1–9)。来自高黎贡山的18个代表菌株分属于12个绿僵菌属物种(*Metarhizium rileyi*、*M. viridulum*、*M. lepidiotae*、*M. brunneum*、*M. pingshaense*、*M. anisopliae*、*M. robertsii*、*M. guizhouense*、*M. indigoticum*、*M. pemphigi*、*M. campsosterni*和*Mc. neogunnii*), 被聚类到5个类群中(Clade 3、4、6、8和9)。其中物种数和菌株数最多的是Clade 4 (金龟子绿僵菌复合种, *M. anisopliae*

表1 高黎贡山绿僵菌属不同物种的菌株数量及生境条件。I: 干热河谷; II: 季风常绿阔叶林; III: 暖性针叶林; IV: 中山暖性常绿阔叶林; V: 山地苔藓矮林; VI: 寒温性灌丛或草甸; VII: 流石滩稀疏植被。

Table 1 Strain numbers and habitat conditions of *Metarhizium* species in Gaoligong Mountains. I, Dry-hot valley; II, Monsoon evergreen broad-leaved forest; III, Warm coniferous forest; IV, Mid-montane humid evergreen broad-leaved forest; V, Mountain moss dwarf forest; VI, Cold shrubs of meadow; VII, Rocky beach sparsely vegetation.

物种 Species	菌株数 strain number	菌株百分比 %	植被类型 Vegetation type	海拔跨度 Altitude span (m)	温度 Temperature (°C)	湿度 Humidity (%)
<i>Metarhizium brunneum</i>	75	46.6	I–VI	600–3,400	13–34	32–80
<i>M. pemphigi</i>	25	15.5	I, II, IV	800–2,119	20–32	39–70
<i>M. pingshaense</i>	11	6.8	I, IV, V	1,925–3,135	15–34	58–74
<i>M. guizhouense</i>	13	8.1	II, IV	1,540–2,280	18–30	57–74
<i>M. rileyi</i>	10	6.2	II, IV	1,780–2,495	17–27	49–54
<i>M. robertsii</i>	5	3.1	I, IV	723–1,981	22–32	52–63
<i>M. indigoticum</i>	4	2.5	II, III	1,512–1,981	22–30	58–59
<i>M. anisopliae</i>	4	2.5	II, III	1,370–1,900	25–30	60–76
<i>Metacordyceps neogunnii</i>	7	4.4	II	1,438	25	65
<i>M. campsosterni</i>	5	3.1	IV	2,022	22	58
<i>M. viridulum</i>	1	0.6	II	1,540	30	57
<i>M. lepidiotae</i>	1	0.6	III	1,765	25	58
<i>Nigelia martiale</i>	–	–	II	1,568	30	60
合计 Total	161	100	6	600–3,400	13–34	32–80

complex), 包括12个菌株、8个物种(*M. brunneum*、*M. pingshaense*、*M. guizhouense*、*M. robertsii*、*M. indigoticum*、*M. anisopliae*、*M. campsosterni*和*M. lepidiotae*)。其他分支的物种和菌株数都较少, Clade 3 (黄绿绿僵菌复合种, *M. flavoviride* complex)中包含2个菌株, 属于*M. pemphigi*; Clade 6中包含1个菌株, 属于*M. viridulum*; Clade 8中包含2个菌株, 属于*M. rileyi*; Clade 9中包含1个菌株, 属于*Mc. neogunnii*。

2.2 新记录种*Metarhizium indigoticum*形态描述

在PPDA培养基上, 25℃培养14 d, 菌落直径可达32–33 mm。单菌落为圆形(图3A, B), 初期为白色, 产孢时从中心向外长出黑色孢子层, 菌落边缘有一圈白色菌丝。在显微镜下观察, 菌丝分枝具隔、无色光滑, 菌丝宽3.57–1.62 (2.49 ± 0.52) μm 。分生孢子梗分枝或不分枝(图3D, E), 长24.43–8.82 (18.50 ± 7.05) μm , 基部粗2.70–2.21 (2.49 ± 0.21) μm 。分生孢子单细胞, 柱状或长椭圆形, 长11.31–7.79 (9.00 ± 0.81) μm , 粗3.96–2.23 (2.75 ± 0.44) μm (图3C)。分生孢子链的连接点在同一中心轴上, 单个孢子透明, 成堆时呈黑色。

2.3 高黎贡山不同绿僵菌属物种的分布特征

从高黎贡山土壤样品中分离鉴定到12个绿僵菌属物种(表1), 其中*M. brunneum* (图1A, M)的菌株

数量最多, 占总数的46.6%; 其分布区域的海拔跨度最大, 在600–3,400 m的6种植被类型(I–VI)中都有分布; 温度(13–34℃)和湿度(32–80%)的跨度也最大。其次是*M. pemphigi* (图1J), 占总数的15.5%, 海拔从800–2,119 m的3种植被类型(I、II和IV)中有分布。*M. pingshaense* (图1H)、*M. rileyi* (图1K)等10个物种的菌株数量都较少(< 7%), 分布范围也较窄。*M. pingshaense*在3种植被类型中出现; *M. guizhouense* (图1F)、*M. rileyi*、*M. robertsii* (图1E)、*M. indigoticum* (图1C)和*M. anisopliae* (图1G)这5种绿僵菌只分布于2种植被类型; 其他4种绿僵菌(*Mc. neogunnii* (图1L), *M. campsosterni* (图1D)、*M. viridulum* (图1I)、*M. lepidiotae* (图1B))都只在1种植被类型中发现, 其中*M. viridulum*和*M. lepidiotae*这2个物种数量最少, 分别只分离到1株菌。

2.4 不同植被类型中绿僵菌属物种的分布特征

高黎贡山绿僵菌分布广泛, 除海拔最高的植被类型VII (流石滩稀疏植被)中没有分离到绿僵菌菌株, 其他6种植被类型生境中都有绿僵菌分布, 但不同植被类型中绿僵菌的菌株数量和物种种类差异很大(图4)。总体来说, 中低海拔地区植被类型中分布的绿僵菌数量和物种种类较多, 高海拔地区植被类型中绿僵菌的数量和物种种类都较少。

图2 基于5个基因(*nrSSU*、*nrLSU*、*EF-1α*、*RPB1*和*RPB2*)核苷酸序列分析获得的绿僵菌属系统发育树。采自高黎贡山的材料被标为黑体字。

Fig. 2 Phylogenetic tree of *Metarhizium* based on nucleotide sequences of five loci (*nrSSU*, *nrLSU*, *EF-1α*, *RPB1* and *RPB2*) dataset. Those from Gaoligong Mountains are marked in bold.

图3 中国新记录种*Metarhizium indigoticum* (菌株BUM 1512.8)的形态特征。(A)菌落正面; (B)菌落背面; (C)分生孢子链; (D, E)产孢结构。C-E的比例尺 = 5 μm 。

Fig. 3 Morphology of the new record species in China, *Metarhizium indigoticum* (strain BUM 1512.8). (A) Obverse of the colony on PPDA; (B) Reverse of the colony on PPDA; (C) Conidia chains; (D, E) Conidiogenous structures. Bar of C-E = 5 μm .

图4 高黎贡山不同植被类型中绿僵菌的物种及菌株数量。L: 低海拔; M: 中海拔; H: 高海拔。

Fig. 4 Species and strain numbers of *Metarhizium* in different vegetation types of Gaoligong Mountains. L, Low elevation; M, Middle elevation; H, High elevation.

在中海拔地区, 植被类型II (季风常绿阔叶林) 中的菌株数量最多(52株, 占总数的32.3%, *N. martiale*的1份材料未分离到菌株), 物种也最多, 包括8个绿僵菌属物种(*M. brunneum*、*M. pemphigi*、*M. guizhouense*、*M. rileyi*、*M. anisopliae*、*M. indigoticum*、*M. viridulum*和*Mc. neogunnii*)和1个*Nigelia*属物种(*N. martiale*)。植被类型IV (中山湿性常绿阔叶林)中绿僵菌的分布也较广泛, 分离到47株菌(占总数的29.2%), 属于7个物种(*M. brunneum*、*M. pemphigi*、*M. rileyi*、*M. pingshaense*、*Mc. campsos-terni*、*M. guizhouense*和*M. robertsii*)。植被类型III (暖性针叶林)中分布的绿僵菌相对较少, 分离到23个菌株(占总数的14.3%), 属于4个物种(*M. brunneum*、*M. anisopliae*、*M. indigoticum*和*M. lepidiotae*)。在低海拔高温环境(植被类型I, 干热河谷)中分离到29株菌(占总数的18.0%), 属于4种绿僵菌(*M. brunneum*、*M. pemphigi*、*M. robertsii*和*M. pingshaense*)。在高海拔低温环境中, 植被类型V (山地苔藓矮林)中只分离到8株菌(占总数的5.0%), 属于2种绿僵菌(*M. brunneum*和*M. pingshaense*)。植被类型VI (寒温性灌丛或草甸)中分布的绿僵菌物种和数量最少, 只分离到2株菌(占总数的1.2%), 属于1个物种(*M. brunneum*)。

3 讨论

本研究结果表明, 高黎贡山森林生态系统中蕴涵着丰富的绿僵菌资源, 其物种多样性较高、分布较广。除最高海拔的植被类型VII (流石滩稀疏植被)外, 在不同海拔梯度的6种植被类型中都发现有绿僵菌的分布。通过多基因发育分析共鉴定到12个绿僵菌属物种和1种*Nigelia*属物种。其中, 绿僵菌物种共归入5个类群中(Clades 3、4、6、8、9), 其中多数物种(8个种)集中在*M. anisopliae* species complex中, 其他4个类群中分别只有1个物种。*M. indigoticum* (Kepler et al, 2014)为中国新记录种, 其余物种均为国内已报道过的种(黄勃等, 2004; Zhang et al, 2005; 梁宗琦, 2007; 章西, 2015^①; Luangsa-ard et al, 2017; Wen et al, 2017)。

高黎贡山绿僵菌丰富的物种多样性在很大程度上

上归因于其植被类型的多样性。陈名君(2008)研究认为, 在森林生态系统中, 虫生真菌的物种和数量因海拔高度、地理位置、植被等因素的不同而具有极大的差异。高黎贡山不同海拔植被类型中的绿僵菌属真菌的物种与分布差异也很大, 表现出明显的垂直地带性分布特点。总体来说, 中低海拔地区的植被类型中出现的绿僵菌物种种类和数量较多, 而高海拔地区植被类型的绿僵菌物种较单一、数量较少。中海拔地区的植被类型II (季风常绿阔叶林)中分布的绿僵菌物种最丰富, 囊括了大多数种类, 分离到的菌株数量也最多; 其次为植被类型IV (中山湿性常绿阔叶林), 包括了7个物种。常绿阔叶林是高黎贡山分布最广的植被类型, 包括季风常绿阔叶林(属于亚热带湿润气候)和中山湿性常绿阔叶林(属于暖性湿润型气候)(何蓉等, 2004; 苏文苹等, 2014), 海拔分布从1,100–2,800 m, 其植被覆盖率和土壤肥力较高, 植物和昆虫的物种多样性均丰富。何蓉等(2004)研究认为, 中山湿性常绿阔叶林中土壤的养分和通透性高于季风常绿阔叶林。本研究中, 季风常绿阔叶林(植被类型II)的绿僵菌物种和菌株数量都较多, 推测可能是因为其海拔相对较低, 气温(夏季温度26℃–30℃)相对较高, 更有利于绿僵菌生长与繁殖。但高黎贡山生态系统成分复杂, 取样面积和取样时间也可能造成一定影响。植被类型I (干热河谷)和植被类型III (暖性针叶林)中的菌株数量和物种种类相对较少, 可能原因是低海拔的干热河谷受焚风气候影响, 干旱少雨, 持续的高温(31℃–34℃)和较低的湿度(32–74%)对绿僵菌的生长和繁殖较为不利; 而暖性针叶林虽处在中海拔地区, 气候接近常绿阔叶林, 但土壤相对贫瘠, 植被物种组成较简单, 主要为马尾松(*Pinus massoniana*)、云南松(*P. yunnanensis*)、水杉(*Metasequoia glyptostroboides*)等, 可能对绿僵菌造成了一定不利影响。在高海拔地区, 植被类型VI (寒温性灌丛或草甸)温度低(12℃–14℃), 绿僵菌难以生长, 分布较少, 仅分离到1种(*M. brunneum*); 植被类型VII (流石滩稀疏植被)中温度极低(10℃–13℃), 植被覆盖率低, 微生物资源也较贫乏, 本次研究未分离到绿僵菌菌株。

高黎贡山优势种现象非常明显。金龟子绿僵菌复合种(Clade 4)中的*M. brunneum*为最优势物种(占总菌株数的46.6%), 而且在每种植被类型中均为最优势的物种。黄绿绿僵菌复合种(Clade 6)中的*M.*

① 章西 (2015) 中国绿僵菌分子分类的研究. 硕士学位论文, 安徽农业大学, 合肥.

*pemphigi*为第二优势物种(占总菌株数的15.5%),在3种植被类型(I、II和IV)中均有分布。其他10个绿僵菌物种的菌株数量都较少(分别占总数的0.6–6.8%),10个种的菌株数只占总数的37.9%。*M. brunneum*普遍存在于从低海拔高温低湿环境的干热河谷到高海拔低温、强紫外线辐射的寒温性灌丛或草甸等6种环境条件差异很大的植被类型中,说明该物种的生态适应能力较强,有望从中筛选出耐逆能力强的优良生防菌株。该优势种在植被类型I、II、III中的菌株数都超过了16株($\geq 10\%$),说明低海拔植被环境中较高的温湿度气候条件有利于其生存。其他物种都只出现在某几个植被类型中。*M. pemphigi*和*M. pingshaense*只在3种植被类型分离到,*M. guizhouense*、*M. rileyi*、*M. robertsii*、*M. indigoticum*和*M. anisopliae*只在2种植被类型中分离到,其他5个物种都只存在于1种植被类型。可见,除*M. brunneum*外,高黎贡山多数绿僵菌物种都具有明显的植被类型偏好性,分布范围相对较窄。

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附录 Supplementary Material

附录1 近年来报道的绿僵菌属物种及用于系统发育分析的多基因序列信息

Appendix 1 *Metarhizium* species in recent reports and voucher information for the multigene phylogenetic analysis

<http://www.biodiversity-science.net/fileup/PDF/2018131-1.pdf>

附录1 近年来报道的绿僵菌属物种及用于系统发育分析的多基因序列信息。采自高黎贡山的材料被标为黑体字。

Appendix 1 *Metarhizium* species in recent reports and voucher information for the multigene phylogenetic analysis. Those from Gaoligong Mountains are marked in bold.

编号 No.	物种 Species	菌株号 Strain code	基因库登录号 GenBank accession number					参考文献 References
			nrSSU	nrLSU	EF-1 α	RPB1	RPB2	
1	<i>Metarhizium acridum</i>	ARSEF 324	–	–	EU248844	EU248896	EU248924	Bischoff et al, 2009
	<i>M. acridum</i>	ARSEF 7486	–	–	EU248845	EU248897	EU248925	Bischoff et al, 2009
2	<i>M. album</i>	ARSEF 2082	DQ518775	DQ522560	DQ522352	DQ522398	DQ522452	Kepler et al, 2014
	<i>M. album</i>	ARSEF 2179	–	–	KJ398807	KJ398618	–	Kepler et al, 2014
3	<i>M. anisopliae</i>	ARSEF 7450	–	–	EU248852	EU248904	EU248932	Bischoff et al, 2009
	<i>M. anisopliae</i>	BUM 1900	MH143837	MH143820	MH143854	MH143869	MH143884	This study
	<i>M. anisopliae</i>	ARSEF 7487	–	–	DQ463996	DQ468355	DQ468370	Bischoff et al, 2009
4	<i>M. atrovirens</i>	TNM F10184	JF415950	JF415966	–	JN049884	–	Kepler et al, 2012
5	<i>M. bibionidarum</i>	NBRC 112661	–	–	LC126076	LC125908	LC125924	Nishi et al, 2017
	<i>M. bibionidarum</i>	CBS648.67	–	–	LC126075	LC125907	LC125923	Nishi et al, 2017
6	<i>M. blattodeae</i>	MY00896	HQ165657	HQ165719	HQ165678	HQ165739	HQ165638	Luangsa-ard et al, 2017
	<i>M. blattodeae</i>	NHJ11597	HQ165662	HQ165724	HQ165683	HQ165743	HQ165643	Luangsa-ard et al, 2017
7	<i>M. brasiliense</i>	ARSEF 2948	–	–	KJ398809	KJ398620	–	Kepler et al, 2014
8	<i>M. brittlebankisoides</i>	G97025	–	–	–	–	–	Liu et al, 2001
9	<i>M. brunneum</i>	ARSEF 2107	–	–	EU248855	EU248907	EU248935	Bischoff et al, 2009
	<i>M. brunneum</i>	ARSEF 4179	–	–	EU248854	EU248906	EU248934	Kepler et al, 2014
	<i>M. brunneum</i>	BUM 44	MH143834	MH143817	MH143851	MH143866	MH143881	This study
10	<i>M. campsosterni</i>	HMIGD 20885	–	–	–	–	–	Zhang et al, 2005
	<i>M. campsosterni</i>	BUM 828	MH143843	MH143826	–	–	–	This study
	<i>M. campsosterni</i>	BUM 10	MH143832	MH143815	MH143849	MH143864	MH143879	This study
11	<i>M. carneum</i>	CBS 239.32	EF468843	EF468988	EF468789	EF468894	EF468938	Sung et al, 2007
12	<i>M. chaityaphumense</i>	BCC19020	HQ165654	HQ165716	HQ165675	HQ165737	HQ165635	Luangsa-ard et al, 2017
	<i>M. chaityaphumense</i>	BCC19021	HQ165655	HQ165717	HQ165676	HQ165738	HQ165636	Luangsa-ard et al, 2017
13	<i>M. chlamydosporia</i>	CBS 504.66	AF339593	AF339544	EF469069	EF469098	EF469120	Sung et al, 2007
	<i>M. chlamydosporia</i>	CBS 101244	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424	Spatafora et al, 2007
14	<i>M. cylindrosporum</i>	ARSEF 6926	–	–	KJ398814	KJ398625	–	Kepler et al, 2014
15	<i>M. dhauladharensis</i>	SK-2014	–	–	–	–	–	Sharma et al, 2015
16	<i>M. flavoviride</i>	ARSEF 2133	–	–	DQ463999	DQ468358	DQ468373	Bischoff et al, 2009
	<i>M. flavoviride</i>	ARSEF 2025	–	AF138269	KJ398804	KJ398614	DQ468374	Bischoff et al, 2006
17	<i>M. frigidum</i>	ARSEF 7445	–	–	KJ398818	KJ398628	–	Bischoff et al, 2006
	<i>M. frigidum</i>	ARSEF 4124	–	–	DQ464002	DQ468361	DQ468376	Bischoff et al, 2009
18	<i>M. globosum</i>	ARSEF 2596	–	–	EU248846	EU248898	EU248926	Bischoff et al, 2009
19	<i>M. granulomatis</i>	UAMH 11176	HM635078	–	KJ398782	KJ398593	–	Kepler et al, 2014
	<i>M. granulomatis</i>	UAMH 11028	HM195304	HM635076	KJ398781	–	–	Kepler et al, 2014
20	<i>M. guizhouense</i>	CBS 258.90	–	–	EU248862	EU248914	EU248942	Bischoff et al, 2009
	<i>M. guizhouense</i>	ARSEF 6238	–	–	EU248857	EU248909	EU248937	Bischoff et al, 2009
	<i>M. guizhouense</i>	BUM 119	MH143835	MH143818	MH143852	MH143867	MH143882	This study
	<i>M. guizhouense</i>	BUM 334	MH143838	MH143821	MH143855	MH143870	MH143885	This study
21	<i>M. guniujiangense</i>	GNJ02052704	–	–	–	–	–	Li et al, 2010

附录1 (续) Appendix 1 (continued)

编号 No.	物种 Species	菌株号 Strain code	基因库登录号 GenBank accession number					参考文献 References
			nrSSU	nrLSU	EF-1 α	RPB1	RPB2	
22	<i>M. indigoticum</i>	TNS-F 18553	JF415968	JF415952	JF416010	JN049886	JF415992	Kepler et al, 2012
	<i>M. indigoticum</i>	TNS-F 18554	JF415952	JF415969	JF416011	JN049887	JF415993	Kepler et al, 2012
	<i>M. indigoticum</i>	BUM 1512.8	KY264192	KY264190	KY264186	KY264194	KY264196	This study
	<i>M. indigoticum</i>	BUM 2600	KY264193	KY264191	KY264187	KY264195	KY264197	This study
23	<i>M. kalasinense</i>	BCC53581	KC011174	KC011182	KC011188	—	—	Luangsa-ard et al, 2017
	<i>M. kalasinense</i>	BCC53582	KC011175	KC011183	KC011189	—	—	Luangsa-ard et al, 2017
24	<i>M. khaoyaiense</i>	BCC 12687	JF415971	—	KJ398796	JN049889	—	Kepler et al, 2012
	<i>M. khaoyaiense</i>	BCC 14290	JF415970	—	KJ398797	JN049888	—	Kepler et al, 2012
25	<i>M. koreanum</i>	ARSEF 2039	—	—	KJ398806	KJ398616	—	Kepler et al, 2014
	<i>M. koreanum</i>	ARSEF 2038	—	—	KJ398805	KJ398615	—	Kepler et al, 2014
26	<i>M. kusanagiensis</i>	TNS-F 18494	JF415972	JF415954	JF416014	JN049890	—	Kepler et al, 2012
27	<i>M. liangshanense</i>	EFCC 1523	EF468961	EF468814	EF468755	—	EF468918	Sung et al, 2007
28	<i>M. lepidiotae</i>	ARSEF 7412	—	—	EU248864	EU248916	EU248944	Bischoff et al, 2009
	<i>M. lepidiotae</i>	BUM 66.1	MH143831	MH143814	MH143848	MH143863	MH143878	This study
29	<i>M. majus</i>	ARSEF 1946	—	—	EU248867	EU248919	EU248947	Kepler et al, 2014
	<i>M. majus</i>	ARSEF 1914	—	—	KJ398801	KJ398610	—	Bischoff et al, 2009
30	<i>M. marquandii</i>	CBS 182.27	EF468845	EF468990	EF468793	EF468899	EF468942	Sung et al, 2007
31	<i>M. minus</i>	ARSEF 1764	AF280635	AF280632	DQ464006	KJ398609	DQ468380	Kepler et al, 2014
	<i>M. minus</i>	ARSEF 2037	AF339531	AF339580	DQ464007	DQ468366	DQ468381	Sung et al, 2007
32	<i>M. novozealandicum</i>	ARSEF 4661	—	—	KJ398811	KJ398622	—	Kepler et al, 2014
	<i>M. novozealandicum</i>	ARSEF 4674	—	—	KJ398812	KJ398623	—	Kepler et al, 2014
33	<i>M. owariense</i>	NBRC 33258	HQ165730	HQ165669	HQ165689	HQ1665747	—	Kepler et al, 2012
34	<i>M. pemphigi</i>	ARSEF 7491	—	—	KJ398819	KJ398629	DQ468379	Kepler et al, 2014
	<i>M. pemphigi</i>	ARSEF 6569	—	—	KJ398813	KJ398624	DQ468378	Kepler et al, 2014
	<i>M. pemphigi</i>	BUM 1	MH143829	MH143812	MH143846	MH143862	MH143877	This study
	<i>M. pemphigi</i>	BUM 39.4	KY087813	KY087817	KY087821	KY087825	KY087827	This study
35	<i>M. pingshaense</i>	CBS 257.90	—	—	EU248850	EU248902	EU248930	Bischoff et al, 2009
	<i>M. pingshaense</i>	ARSEF 4342	—	—	EU248851	EU248903	EU248931	Kepler et al, 2014
	<i>M. pingshaense</i>	BUM 68.1	MH143833	MH143816	MH143850	MH143865	MH143880	This study
	<i>M. pingshaense</i>	BUM 70.9	MH143840	MH143823	MH143857	MH143872	MH143887	This study
36	<i>M. prachinense</i>	BCC47950	KC011172	KC011180	KC011186	KC011184	—	Luangsa-ard et al, 2017
	<i>M. prachinense</i>	BCC47979	KC011173	KC011181	KC011187	KC011185	—	Luangsa-ard et al, 2017
37	<i>M. pseudoatrovirens</i>	TNSF 16380	JF415977	—	—	JN049893	JF415997	Kepler et al, 2012
38	<i>M. purpureogenum</i>	ARSEF 12571	—	—	LC126078	LC125913	LC125920	Nishi et al, 2017
	<i>M. purpureogenum</i>	ARSEF 12570	—	—	LC126079	LC125911	LC125922	Nishi et al, 2017
39	<i>M. rileyi</i>	ARSEF 1972	—	—	KJ398803	KJ398613	—	Kepler et al, 2014
	<i>M. rileyi</i>	CBS 806.71	—	AY526491	EF468787	EF468893	EF468937	Sung et al, 2007
	<i>M. rileyi</i>	BUM 901	MH143844	MH143827	MH143860	MH143875	MH143890	This study
	<i>M. rileyi</i>	BUM 9.24	MH143839	MH143822	MH143856	MH143871	MH143886	This study
40	<i>M. reniforme</i>	ARSEF 429	HQ165671	HQ165733	HQ16569	—	HQ165650	Luangsa-ard et al, 2017
	<i>M. reniforme</i>	ARSEF 577	HQ165672	HQ165734	HQ165691	—	HQ165651	Luangsa-ard et al, 2017
41	<i>M. robertsii</i>	ARSEF 727	—	—	DQ463994	DQ468353	DQ468368	Bischoff et al, 2009
	<i>M. robertsii</i>	BUM 723	MH143836	MH143819	MH143853	MH143868	MH143883	This study

附录1 (续) Appendix 1 (continued)

编号 No.	物种 Species	菌株号 Strain code	基因库中登录号 GenBank Accession Number					参考文献References
			nrSSU	nrLSU	EF-1 α	RPB1	RPB2	
42	<i>M. samlanense</i>	BCC17091	HQ165665	HQ165727	HQ165686	—	HQ165646	Luangsa-ard et al, 2017
	<i>M. samlanense</i>	BCC17093	HQ165666	HQ165728	HQ165687	HQ165746	HQ165647	Luangsa-ard et al, 2017
43	<i>Metacordyceps synnematis</i>	GZUHXCHL12	—	—	—	—	—	Chu et al, 2016
44	<i>M. takense</i>	BCC30934	HQ165658	HQ165720	HQ165679	HQ165740	HQ165639	Luangsa-ard et al, 2017
	<i>M. takense</i>	BCC30939	HQ165659	HQ165721	—	HQ165741	HQ165640	Luangsa-ard et al, 2017
45	<i>M. viride</i>	ARSEF 2456	—	—	KJ398808	KJ398619	—	Kepler et al, 2014
	<i>M. viride</i>	CBS 659.71	HQ165735	HQ165673	HQ165692	—	HQ165652	Luangsa-ard et al, 2017
46	<i>M. viridulum</i>	ARSEF 6927	—	—	KJ398815	KJ398626	—	Kepler et al, 2014
	<i>M. viridulum</i>	BUM 721	MH143842	MH143825	MH143859	MH143874	MH143889	This study
47	<i>M. yongmunense</i>	EFCC 2135	—	EF468979	EF468834	EF468769	EF468877	Kepler et al, 2012
	<i>M. yongmunense</i>	EFCC 2131	EF468833	EF468977	EF468770	EF468876	—	Kepler et al, 2012
48	<i>Mc. shibinensis</i>	GZUHSB 13050311	KR153588	—	KR153589	KR153590	—	Wen et al, 2015
49	<i>Mc. neogunnii</i>	GZUH SB13050305	KU729724	—	KU729729	KU729734	—	Wen et al, 2017
	<i>Mc. neogunnii</i>	GZUH HS14061253	KU729725	—	KU729730	—	—	Wen et al, 2017
	<i>Mc. neogunnii</i>	BUM 415	MH143845	MH143828	MH143861	MH143876	MH143891	This study
50	<i>Nigelia aurantiaca</i>	BCC19475	GU979935	GU979944	GU979953	GU979962	GU979968	Luangsa-ard et al, 2017
	<i>N. aurantiaca</i>	BCC20179	GU979936	GU979945	GU979954	GU979963	GU979969	Luangsa-ard et al, 2017
51	<i>N. martiale</i>	EFCC 6863	—	JF415974	JF416015	—	—	Kepler et al, 2012
	<i>N. martiale</i>	BUM 713	MH143841	MH143824	MH143858	MH143873	MH143888	This study
52	<i>Cordyceps militaris</i>	OSC 93623	AY184977	AY184966	DQ522332	DQ522377	—	Sung et al, 2007
53	<i>Beauveria bassiana</i>	ARSEF 7518	HQ880975	HQ880975	HQ880975	HQ880834	HQ880834	Chen et al, 2013
54	<i>Ophiocordyceps stylophora</i>	OSC 110999	EF468982	EF468837	EF468777	EF468882	EF468931	Luangsa-ard et al, 2017
55	<i>O. acicularis</i>	OSC 110987	EF468950	EF468805	EF468744	EF468852	—	Luangsa-ard et al, 2017