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# 生态学报

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封面图说:自然奇观红海滩·辽宁省盘锦市——在辽河入海口生长着大片的潮间带植物碱蓬草,举目望去,如霞似火,蔚为壮观,人们习惯地称之为红海滩。粗壮的根系加快着海滩土壤的脱盐过程,掉下的茎叶腐质后肥化了土壤,它是大海的生态屏障。

彩图提供:段文科先生 中国鸟网 <http://www.birdnet.cn> E-mail:dwk9911@126.com

# 云南元江干热河谷五种优势植物的内生真菌多样性

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**摘要:** 从蔓草虫豆(*Atylosia scarabaeoides*)、余甘子(*Phyllanthus emblica*)和黄花稔(*Sida acuta*)等5种云南元江干热河谷植物的525个组织块中, 共分离得到内生真菌371株, 内生真菌的分离频率在0.61—0.92之间, 且所有植物叶内生真菌的分离频率都明显高于茎( $P<0.05$ )。经形态学鉴定, 内生真菌分属于拟茎点霉属(*Phomopsis* sp.)、离蠕孢属(*Bipolaris* sp.)和交链孢属(*Alternaria* sp.)等32个分类单元。拟茎点霉属为干热河谷植物优势内生真菌属, 从所有被调查植物的茎叶中都分离得到该属真菌, 且相对分离频率高达12.90%—50.54%。内生真菌群落组成的多样性和相似性分析结果表明, 云南元江干热河谷植物内生真菌多样性偏低、宿主专一性较小。

**关键词:** 元江干热河谷; 内生真菌; 多样性; 拟茎点霉属; 相似性

## Endophytic fungal diversity of five dominant plant species in the dry-hot valley of Yuanjiang, Yunnan Province, China

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**Abstract:** Fungi causing asymptomatic infections in living plant tissues have been called endophytic fungal. They comprise a diverse group of fungi and may protect their host plants against the insect pests and phytopathogens, increase hosts fitness in extreme environments. In order to document the ecological relationship between endophytic fungal and their host plants which grown in hot and dry stressed environments, the endophytic fungal diversity, the dominant fungi and their host specificity of 5 dominant plant species (*Atylosia scarabaeoides*, *Azadirachta indica*, *Phyllanthus emblica*, *Broussonetia papyrifera* and *Sida acuta*) which grown in the dry-hot valley of Yuanjiang Yunnan Province, southwest China were studied in present research works. A total of 371 endophytic fungal were isolated from 525 tissue segments of the 5 plant species. The isolation rate was 0.71. The highest isolation rate appeared in *S. acuta*, it was 0.92, while the lowest isolation rate appeared in *A. indica*, it was only 0.61. It was found that the isolation rate of leaves endophytic fungal was significantly higher than that of stems ( $P<0.05$ ) in the 5 plant species. Based on their morphology and the mechanism of spore production, the sporulating isolates were identified to genus level. Non-sporulating isolates were identified as mycelia sterile and sorted into different groups based on the colony surface texture, hyphal pigmentation and growth rates after two months sporulation. The isolated fungi were finally identified to 32 taxa including *Phomopsis*, *Colletotrichum* and *Alternaria* etc. The endophytic fungal richness of the 5 plant species was different. The highest endophytic fungal richness appeared in *A. scarabaeoides*, from which 20 endophytic fungal taxa were obtained, whereas the fungi from *A. indica* were just 11 taxa and showed the lowest richness. Each plant species harboured 1 to 2 dominant fungi. The dominant fungi of *A. scarabaeoides* were *Bipolaris* sp. and *Phomopsis* sp., the relative frequency was 38.81% and 14.93% respectively. *Phomopsis* sp. and an ascomycete species were the dominant fungi of *A. indica* and *P. emblica*, the relative frequency of *Phomopsis* sp. was 12.90% and 40.24%, the relative frequency of the ascomycete was 33.87% and 17.07% respectively. The dominant

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fungi of *B. papyrifera* and *S. acuta* was *Phomopsis* sp., the relative frequency was 49.25% and 50.54% respectively. The results showed that *Phomopsis* was the dominant fungi of the dominant plant species in the dry-hot valley of Yuanjiang, Yunnan Province, China, its relative frequency got a range of 12.90% to 50.54%. The diversity index of endophytic fungal from *A. scarabaeoides*, *A. indica*, *P. emblica*, *B. papyrifera* and *S. acuta* were 0.77, 0.62, 0.65, 0.82 and 0.90 respectively, and it was little lower than that of some previous reports from tropical plants. The diversity index of endophytic fungal from leaves was 0.33 to 0.64, it was higher than that from stems (0.28—0.39). The similarity index of endophytic fungal from the 5 plant species ranged from 0.483 to 0.757, and the highest similarity index appeared in *A. scarabaeoides* and *S. acuta* (0.757), and the lowest similarity index appeared in *A. indica* and *P. emblica*, it was only 0.483. These results suggested that the host specificity of endophytic fungal from the dominant plant species in the dry-hot valley of Yuanjiang Yunnan Province, China was low.

**Key Words:** dry-hot valley; endophytic fungal; diversity; *Phomopsis* sp; similarity

植物内生真菌是指那些在其生活史的一定或全部阶段生活于健康植物组织和器官内部,而不使宿主植物表现出明显感染症状的真菌<sup>[1]</sup>。据估计,在自然界中真菌约有150万种,而其中一大部分以内生真菌的方式存在<sup>[2]</sup>。到目前为止,已有大量关于热带、亚热带和温带植物内生真菌多样性的研究报道<sup>[1-3]</sup>。近年,又有部分关于海洋红树林和盐碱地植物内生真菌多样性的相关报道<sup>[4-6]</sup>。然而,目前有关萨王纳植被植物内生真菌多样性的研究工作相对较少,仅见2003年一篇关于印度南部干旱有刺灌丛和干旱落叶林两种萨王纳植被类型中17科24种植物内生真菌多样性的研究报道<sup>[7]</sup>。

云南元江干热河谷是我国河谷型半萨王纳植被的典型代表<sup>[8]</sup>。由于开发时间早,人口密度大,植被破坏严重,已成为我国最为脆弱的生态环境之一。目前,有关干热河谷植被地上部分植物群落组成、发展、进化及演变过程,地下部分泡囊丛枝菌根真菌多样性等已有系统研究<sup>[9-11]</sup>,但有关其地上部分植物内生真菌多样性及其生态学功能的研究却未见任何报道。

本研究对云南元江干热河谷蔓草虫豆、余甘子和黄花稔等5种常见植物茎叶内生真菌进行分离、纯化和形态学鉴定,旨在探索云南干热河谷萨王纳植被植物内生真菌的群落组成、物种多样性及生态分布规律,为进一步研究元江干热河谷地区植物内生真菌的生态学功能及其在干热河谷植被恢复、资源开发和利用方面提供理论依据。

## 1 材料与方法

### 1.1 样地

云南省元江干热河谷位于云南省中南部,整体呈东南走向,大江西南侧与哀牢山系平行,高2500—3000m,成为西南季风和西风急流的风屏,使河谷处于雨影区的焚风效应地带,最终使得深陷的河谷形成以干热为典型特征的河谷型气候。采样点元江坝河谷,位于元江干热河谷的南部,海拔低(700—900m),焚风效应明显,属热带性干热河谷气候。年均温23.7℃,各月最高均温28.6℃,各月最低均温16.7℃,≥10℃的年积温为8710℃,年均降水量805mm,其中雨季占81%,年均蒸发量2750mm,是降水量3—4倍,年均相对湿度69%<sup>[8]</sup>。

### 1.2 植物样品

蔓草虫豆(*Atylosia scarabaeoides*)、印楝(*Azadirachta indica*)、余甘子(*Phyllanthus emblica*)、构树(*Broussonetia papyrifera*)和黄花稔(*Sida acuta*)5种植物样本于2008年9月采于云南省元江县元江坝干热河谷。

采样方法 在50m<sup>2</sup>样地里,每种植物随机选取健康植株10株,剪下3段2—3年生、带有叶片的茎段,取样后立即放入塑料袋中,贴上标签带回实验室,4℃冰箱保藏并在2d内处理完毕。

### 1.3 内生真菌的分离纯化

从每种植物样品中随机选取15段带有叶片的小茎段在自来水下冲洗干净,按下列程序进行表面消毒:体

积分数 75% 乙醇漂洗 2 min、无菌水冲洗 3 次;25% 的 NaClO 溶液漂洗 2 min、无菌水冲洗 3 次,置于无菌滤纸上吸干水分。将表面消毒后的样品剪成 0.5 cm×0.5 cm 的片段,从每种植物样品中随机选取 54 个茎片和 54 个叶片贴到 PDA 平板上,置于 27℃ 恒温培养箱中黑暗培养 3—30 d,隔天观察,发现组织块周围有菌落长出,则将菌转接入斜面,经纯化后得到内生真菌菌株<sup>[2]</sup>。

#### 1.4 内生真菌的鉴定

依据菌落形态、产孢方式、孢子形态特征和产孢结构进行鉴定<sup>[12]</sup>。不产孢的菌株则接种到促孢培养基上进行促孢培养,并定期检查其产孢情况,产孢后按上述方法进行鉴定。经促孢培养后仍不产孢的菌株则根据菌落表面特征、菌丝颜色、菌丝生长速率等分为无孢类群的不同组。

#### 1.5 统计分析方法

内生真菌的分离频率可以衡量植物组织中内生真菌的丰富程度和每个组织块受多重侵染的频率,而相对分离频率可以衡量植物组织中某种内生真菌的优势度,分别按以下方法计算<sup>[13]</sup>:

$$\text{分离频率} = \frac{\text{样本组织块中得到的菌株数}}{\text{全部供试样本组织块数}}$$

$$\text{相对分离频率} (\%) = \frac{\text{样本中分到的某种内生真菌的菌株数}}{\text{分离到的总菌株数}}$$

5 种植物叶内生真菌的分离频率与茎内生真菌的分离频率之间的比较采用 SPSS 软件,进行 t 检验,当  $P < 0.05$  时认为差异显著。

多样性指数( $H'$ )可以反映每种植物内生真菌的物种多样性程度,按 Shannon-Weiner 指数公式计算<sup>[14]</sup>:

$$H' = - \sum P_i \times \ln P_i$$

式中, $P_i$  是指某种内生真菌的菌株数占全部内生真菌菌株数的百分数。

相似性系数( $CS$ )可以比较两种植物之间内生真菌种类组成的相似程度,按 Sorenson 系数公式计算<sup>[14]</sup>:

$$CS = 2j / (a+b)$$

式中, $j$  是两种植物共同具有的内生真菌种类数, $a$  是一种植物内生真菌的种类数, $b$  是另一种植物内生真菌的种类数。

## 2 结果

### 2.1 内生真菌分离结果

从蔓草虫豆、印楝和余甘子、构树和黄花稔 5 种元江干热河谷植物的 525 个组织块中共分离得到内生真菌 371 株,分离频率分别为 0.62、0.61、0.77、0.62 和 0.92,平均分离频率为 0.71。其中黄花稔内生真菌的分离频率最高,为 0.92,而其它 4 种植物的较为接近,在 0.61—0.77 之间(表 1)。经 SPSS 软件统计分析,得出  $P=0.043$ ,因此从组织水平来看,5 种植物叶片内生真菌的分离频率都明显高于茎内生真菌的分离频率。

表 1 5 种干热河谷植物内生真菌的分离频率

Table 1 Isolation rate of endophytic fungal from 5 plant species of dry-hot valley

宿主植物 Host plant	科 Family	组织块数 Tissue number			菌株数 Strain number			分离频率 Isolation rate		
		叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total
蔓草虫豆 <i>A. scarabaeoides</i>	蝶形花科 Papilionaceae	54	54	108	39	28	67	0.72	0.52	0.62
印楝 <i>A. indica</i>	楝科 Meliaceae	52	49	101	32	30	62	0.62	0.61	0.61
构树 <i>B. papyrifera</i>	桑科 Moraceae	54	54	108	43	24	67	0.80	0.44	0.62
余甘子 <i>P. emblica</i>	大戟 Euphorbiaceae	54	53	107	47	35	82	0.87	0.66	0.77
黄花稔 <i>S. acuta</i>	锦葵科 Malvaceae	54	47	101	58	35	93	1.07	0.74	0.92

### 2.2 内生真菌的组成

经鉴定,从 5 种植物分离得到的 371 株内生真菌分属于拟茎点霉属(*Phomopsis* sp.)、离蠕孢属(*Bipolaris*

sp.)和交链孢属(*Alternaria* sp.)等32个分类单元(表2)。其中90株为无孢类群,分属于3个不同的无孢类群组,其它281株中,有241株为半知菌,40株为子囊菌。

5种植物中定植的内生真菌种类和数量各不相同,其中蔓草虫豆中内生真菌种类最多,有20种,而印楝最少,仅11种。蔓草虫豆的优势内生真菌是离蠕孢属和拟茎点霉属,相对分离频率分别为38.81%和14.93%。印楝和余甘子的优势菌为子囊菌和拟茎点霉属,相对分离频率都超过了12.90%,有的甚至达到40.24%(表2)。构树和黄花稔的优势菌是拟茎点霉属,相对分离频率分别达到49.25%和50.54%。从以上结果可以看出,拟茎点霉属是元江干热河谷木本植物中分布较广的内生真菌类群,也是各植物茎叶中的优势类群。

蔓草虫豆、印楝、构树、余甘子和黄花稔5种植物内生真菌多样性指数分别为0.77、0.62、0.65、0.82和0.90。此外,5种植物叶片内生真菌的多样性指数(0.33—0.64)都明显高于相应植物茎中内生真菌的多样性指数(0.28—0.39)(表2)。

表2 干热河谷植物内生真菌的相对分离频率和多样性( $H'$ )

Table 2 Relative frequency and diversity ( $H'$ ) of endophytic fungal from 5 plant species of dry-hot valley

分类单元 Taxa	内生真菌的相对分离频率 Relative Frequency (RF≥1%)/%															
	蔓草虫豆 <i>A. scarabaeoides</i>			印楝 <i>A. indica</i>			构树 <i>B. papyrifera</i>			余甘子 <i>P. emblica</i>			黄花稔 <i>S. acuta</i>			
	叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total	叶 Leaf	茎 Stem	合计 Total	
<i>Aspergillus</i> sp.	5.97	1.49	7.46	—	—	—	—	—	—	3.66	—	3.66	—	—	—	
Ascomycetes	—	—	—	29.03	4.84	33.87	1.49	—	1.49	17.07	—	17.07	4.30	—	4.30	
<i>Alternaria</i> sp.	1.49	—	1.49	6.45	11.29	17.74	—	—	—	2.44	—	2.44	—	—	—	
<i>Bipolaris</i> sp. 1	8.96	4.48	13.43	—	—	—	1.49	1.49	1.49	1.22	—	1.22	1.08	1.08	1.08	
<i>Bipolaris</i> sp. 2	11.94	7.46	19.40	—	—	—	—	—	—	1.22	1.22	1.22	1.08	1.08	2.15	
<i>Bipolaris</i> sp. 3	2.99	2.99	5.97	—	—	—	1.49	—	1.49	—	1.22	1.22	1.08	—	1.08	
<i>Botrytis</i> sp.	—	—	—	—	—	—	—	2.99	2.99	—	—	—	—	—	—	
<i>Catinula</i> sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Cephalosporium</i> sp.	—	1.49	1.49	1.61	1.61	3.23	2.99	—	2.99	—	—	—	1.08	1.08	2.15	
<i>Cloeosporium</i> sp.	—	—	—	—	1.61	1.61	—	—	—	—	—	—	1.08	3.23	4.30	
<i>Colletotrichum</i> sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	1.08	1.08	
<i>Clasterosporium</i> sp.	—	—	—	—	—	—	—	1.49	1.49	—	—	2.44	—	—	—	
<i>Fusarium</i> sp.	—	1.49	1.49	—	—	—	—	—	—	—	—	—	3.23	1.08	4.30	
<i>Gilmania</i> sp.	—	1.49	1.49	—	—	—	—	—	—	—	—	—	1.08	—	1.08	
<i>Hendersonia</i> sp.	—	—	—	—	—	—	1.49	—	1.49	—	—	—	—	—	—	
<i>Ovulariopsis</i> sp.	—	—	—	—	—	—	—	—	—	—	1.22	1.22	—	—	—	
<i>Phomopsis</i> sp. 1	11.94	1.49	13.43	4.84	—	4.84	26.87	5.97	32.84	12.20	18.29	30.49	19.35	19.35	38.71	
<i>Phomopsis</i> sp. 2	—	—	—	—	6.45	6.45	—	—	—	—	3.66	3.66	—	1.08	1.08	
<i>Phomopsis</i> sp. 3	—	1.49	1.49	—	1.61	1.61	14.93	1.49	16.42	2.44	3.66	6.10	9.68	1.08	10.75	
<i>Peyronellaea</i> sp.	—	1.49	1.49	—	1.61	1.61	—	1.49	1.49	—	—	—	—	—	—	
<i>Penicillium</i> sp.	1.49	—	1.49	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Rhinocladiella</i> sp.	—	1.49	1.49	3.23	3.23	6.45	—	—	—	—	—	—	2.15	—	2.15	
<i>Rhizopus</i> sp.	4.48	—	4.48	—	—	—	—	—	—	—	—	—	3.23	—	3.23	
<i>Septonema</i> sp.	1.49	—	1.49	—	—	—	—	—	—	1.22	—	1.22	1.08	—	1.08	
<i>Sirodesmium</i> sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Stemphylium</i> sp.	—	—	—	—	—	—	—	—	—	—	—	1.22	—	—	—	
<i>Trichocladium</i> sp.	—	1.49	1.49	—	—	—	—	—	—	—	—	—	0	—	—	
<i>Trichoderma</i> sp.	—	—	—	—	—	—	—	—	—	1.22	—	1.22	—	—	—	
<i>Trichothecium</i> sp.	1.49	1.49	2.99	—	—	—	—	—	—	1.22	—	1.22	—	—	—	
Mycelia sterile 1	1.49	4.48	5.97	6.45	12.90	19.35	13.43	1.49	14.93	7.32	9.76	17.07	7.53	5.38	12.90	
Mycelia sterile 2	4.48	5.97	7.46	—	3.23	3.23	1.49	17.91	19.40	1.22	3.66	4.88	3.23	2.15	5.38	
Mycelia sterile 3	—	1.49	1.49	—	—	—	—	1.49	1.49	2.44	—	2.44	3.23	—	3.23	
多样性 Diversity ( $H'$ )	0.47	0.39	0.77	0.33	0.36	0.62	0.43	0.28	0.65	0.53	0.37	0.82	0.64	0.38	0.90	

### 2.3 植物内生真菌的相似性系数

从表3可以看出,5种干热河谷植物内生真菌的相似性系数在0.483—0.757之间,其中蔓草虫豆与黄花稔的相似性系数最高,为0.757,而印楝与余甘子的相似性系数最低,为0.483。

表3 5种干热河谷植物内生真菌的相似性系数

Table 3 CS of endophytic fungal from 5 plant species of dry-hot valley

	蔓草虫豆 <i>A. scarabaeoides</i>	印楝 <i>A. indica</i>	构树 <i>B. papyrifera</i>	余甘子 <i>P. emblica</i>	黄花稔 <i>S. acuta</i>
蔓草虫豆 <i>A. scarabaeoides</i>					
印楝 <i>A. indica</i>	0.533				
构树 <i>B. papyrifera</i>	0.563	0.583			
余甘子 <i>P. emblica</i>	0.649	0.483	0.581		
黄花稔 <i>S. acuta</i>	0.757	0.621	0.581	0.611	

### 3 讨论

从云南元江干热河谷5种常见植物中共分离到内生真菌371株,内生真菌的分离频率在0.61—0.92之间,与已报道的一些热带地区植物内生真菌的分离频率相似。如仙人掌内生真菌的分离频率为0.806<sup>[15]</sup>,可可树的为0.8<sup>[16]</sup>,印度南部干旱有刺灌丛和干旱落叶林的为0.93和0.86<sup>[17]</sup>,这说明元江干热河谷植物中也定植有相当丰富的内生真菌。此外,研究结果显示元江干热河谷植物叶内生真菌的分离频率均明显高于茎( $P<0.05$ ),这可能与干热河谷植物叶的比表面积大,蒸发量大,热量散发快,客观上造成了一个局部有利于真菌生长繁殖的场所有关。

经鉴定,371株内生真菌分属于32个分类单元(表2)。其中拟茎点霉属(*Phomopsis* sp.)、交链孢属(*Alternaria* sp.)、刺盘孢属(*Colletotrichum* sp.)等是热带地区植物常见的内生真菌属<sup>[18-20]</sup>,而派伦霉属(*Peyronellaea* sp.)、短梗蠕孢霉属(*Trichocladium* sp.)和顶生线隔孢霉属(*Septonema* sp.)等则较少见。从表2可以看出,拟茎点霉是元江干热河谷植物中分布最广的内生真菌,从所有被调查植物的茎叶中都分离得到,且通过形态特征初步确定有3个种,显示了一定的物种多样性。此外,拟茎点霉属还是元江干热河谷植物的优势内生真菌属,在5种植物中其相对分离频率都超过了12.90%,构树和黄花稔中甚至高达49.25%和50.54%。之前,拟茎点霉属(*Phomopsis* sp.)也曾被报道为热带植物常见的优势属<sup>[17,20-21]</sup>和萨王纳植被植物的优势内生真菌属<sup>[7,22]</sup>,这说明拟茎点霉属真菌较其它种属的真菌而言,可能对干热生态环境有着更强的耐受力和适应力,更容易在宿主植物中生长、传播和蔓延。在长期的生态适应过程中,拟茎点霉与其宿主植物之间建立了一种互惠共生的关系,植物为内生真菌提供了栖息地和碳水化合物,而内生真菌可能通过产生一些化合物来促进宿主植物叶片增厚、根系生长、调节气孔开闭和增强植物抗氧化保护系统等措施来增强宿主植物的抗旱能力。当然,拟茎点霉对其宿主植物抗旱性增益效果及其增益机制将在下一步研究工作中进行探究。拟盘多毛孢(*Pestalotiopsis* sp.)是热带植物常见的一类内生真菌<sup>[23-25]</sup>,它曾被报道为金沙江干热河谷(云南禄劝段)小桐子(*Jatropha curcas*)的优势内生真菌属,相对分离频率达到27.5%<sup>[22]</sup>。然而,在本研究中却没有分离得到,是季节差异、还是地域差异导致了元江干热河谷植物与其它干热河谷植物或其它热带植物内生真菌区系的不一致,有待进一步研究。

与Suryanarayanan等<sup>[7]</sup>对印度南部萨王纳植被植物内生真菌多样性偏低的研究结果一致,云南元江干热河谷植物内生真菌多样性也偏低,多样性指数在0.62—0.90之间,明显低于已报道的其它热带地区植物。如Paulus等<sup>[26]</sup>报道澳大利亚热带雨林4种常见植物内生真菌的多样性指数在2.96—3.76之间,Suryanarayanan等<sup>[27]</sup>报道中美洲热带落叶林植物红鸡蛋花在不同月份内生真菌多样性指数在1.67—2.6之间。当然,由于本研究采用传统组织块培养法分离内生真菌,在培养基中生长缓慢或不长的菌株就可能被忽视,无孢类群菌株也没有通过分子生物学方法进一步鉴定到属,内生真菌多样性可能会比实际偏低。在今后的研究中,可直

接利用DNA序列分析法分析样品,并利用DNA测序鉴定无孢类群菌株,以便更确切的反映干热河谷植物内生真菌的多样性<sup>[28-30]</sup>。

从内生真菌相似性分析结果来看,除了余甘子与印楝内生真菌的相似性系数小于0.5外,其它植物之间内生真菌的相似性都较高,表明干热河谷植物内生真菌的宿主专一性较小,这与Suryanarayanan等<sup>[7]</sup>对印度南部萨王纳植被植物内生真菌宿主专一性的报道一致。

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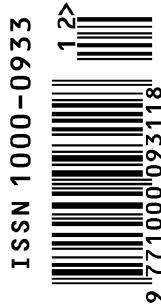
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