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Arbuscular mycorrhizal structure and fungi associated with mosses

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Abstract We investigated the colonization and diversity of arbuscular mycorrhizal (AM) fungi associated with 24 moss species belonging to 16 families in China. AM fungal structures, i.e. spores, vesicles, hyphal coils (including intracellular hyphae), or intercellular nonseptate hyphae, were found in 21 moss species. AM fungal structures (vesicles, hyphal coils, and intercellular nonseptate hyphae) were present in tissues of 14 moss species, and spores and nonseptate hyphae on the surface of gametophytes occurred in 15 species. AM fungal structures were present in 11 of the 12 saxicolous moss species and in six of the ten terricolous moss species, but absent in two epixylous moss species. AM fungal structures were only observed in moss stem and leaf tissues, but not in rhizoids. A total of 15 AM fungal taxa were isolated based on trap culture with clover, using 13 moss species as inocula. Of these AM fungi, 11 belonged to *Glomus*, two to *Acaulospora*, one to *Gigaspora*, and one to *Paraglomus*. Our results suggest that AM fungal structures commonly occur in most mosses and that diverse AM fungi, particularly *Glomus* species, are associated with mosses.

Keywords Diversity · Moss · Mycorrhizal colonization · Trap culture

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Introduction

Bryophytes, as the first land plants, play an important role in the dynamics of understory vegetation, nutrient cycling, as well as soil structure, soil stability, and interception and retention of water (Lovato et al. 1995; Smith and Read 1997). Mycorrhizal fungus–bryophyte associations have been reported for a long time (Rayner 1927; Kelley 1950; Gerdemann 1968; Harley 1969). In particular, liverworts and hornworts can form symbioses with arbuscular mycorrhizal (AM) fungi (Turnau et al. 1999; Schüßler 2000; Russell and Bulman 2005).

Mosses, the largest living group of bryophytes, can play key ecological roles within plant communities, often as primary colonizers in early successional habitats. Moss carpets are known to be important seed beds for higher plants, and certain plant species have only been found to germinate in the presence of mosses (Bodenberg 1954). Mosses account for 75% of the annual phosphorus accumulation in aboveground parts of an Alaskan black spruce forest, although they comprise only 17% of the phosphorus pool in aboveground vegetation (Chapin et al. 1987). It was suggested that mycorrhizal hyphae might be an important avenue of phosphorus movement out of the moss carpet and a means by which the spruce competes with the overlying mosses for nutrients. Mosses also act as bioindicators of air and soil pollution (Ayrault et al. 2001; Poikolainen et al. 2004).

Although there have been a few studies of AM structures and fungi associated with mosses (Butler 1939; Dowding 1959; Rabatin 1980; Parke and Linderman 1980), research is still scanty and concerns a limited number of moss species. This is not compatible with the important ecological function of mosses in natural ecosystems. Thus, the

prevalence of mycorrhizal fungi in association with mosses needs to be further investigated systematically.

To better understand the AM structures and AM fungal diversity associated with mosses, 24 moss species were collected from different sites and habitats in China. The purpose was to study AM fungal structures in moss tissues and investigate which AM fungi are associated with mosses based on trap cultures.

Materials and methods

Sampling site and procedure

Moss samples (gametophytes) were collected from four sites, i.e., Wuling mountain of Hebei province, Wuzhi, Jianfeng, and Bawang mountains of Hainan province, the Botanical Garden of Beijing, and Dujiangyan of Sichuan province. The environmental conditions in the sampling sites are listed in Table 1. A total of 24 moss species belonging to 16 families were collected from August 2001 to September 2003. Of these, 11 were from Dujiangyan, five from Hainan province, five from Wuling mountain, and three from Beijing.

Whole gametophytes of mosses were collected from the different habitats, i.e., saxicolous, terricolous, and epixylous, rinsed with tap water to remove soil debris, air-dried, and stored in a refrigerator (4°C) for use in pot cultures. The moss individuals for staining were cleaned and preserved in 50% ethanol.

Observation of AM structure

The entire gametophyte was rinsed with tap water, cleared in 10% (*w*/*v*) potassium hydroxide (20 min, 92°C), acidified in lactic acid (3 min), and stained (20 min, 92°C) with 0.5% acid fuchsin (Berch and Kendrick 1982). A total of 50 fragments (about 1-cm long) of gametophytes were mounted on slides in lactic acid and examined with a compound microscope (Olympus BH-2) at ×100–400 to ascertain the presence of AM fungal structures, i.e., arbuscules, vesicles, hyphal coils, and intra- and intercellular nonseptate hyphae.

Trap culture establishment

Each pot was filled with 300-ml (approximately 400 g) autoclaved (1 h, 15 psi) sandy loam and 5-g moss as AM fungal inoculum. Seeds of clover (*Trifolium repens* L.) were surface sterilized, germinated, and sown directly into the pot. A total of 13 moss species were used, and there were three replicates for each moss species in the trap culture. Plants were grown under greenhouse conditions and watered once every 2 days, with addition of 30% strength Hoagland's nutrient solution without phosphorus every 2 weeks. All plants were cultivated for 4 months; then, soil samples were air dried for spore isolation.

Spore isolation and identification of AM fungi

One hundred grams of each air-dried soil sample was used for spore isolation. AM fungal spores were isolated using the wet-sieving and decanting method of Gerdemann and Nicolson (1963), modified by Daniels and Skipper (1982). AM fungi were identified following the current taxonomic criteria (Schenck and Pérez 1990; Morton and Redecker 2001; Schüßler et al. 2001; Oehl and Sieverding 2004; Walker and Schüßler 2004), and using information from International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi on the internet (http://www.invam.caf.wdu.edu). At least 20 spores of each species were used for identification. Spores were first mounted in water, and morphological characteristics were measured. Melzer's reagent and cotton blue were used in the identification. The permanent slides were mounted in polyvinyl-lacto-glycerol, sealed with nail varnish, and stored in the Herbarium Mycologicum Academiae Sinicae in Beijing.

Results

AM fungal structure

A total of 24 moss species of 16 families were collected from the four sites in China. AM fungal structures, i.e., spores, vesicles, hyphal coils (including intracellular hy-

Table 1 A list of environmental conditions in the sampling sites in this study

	WL	HN	BG	DJY
Latitude /longitude (N/E)	40°29′–40°38′/117°17′–117°35′	18°14′–20°02′/109°31′–110°21′	39°55′/116°24′	30°44′/103°27′
Altitude (m)	1,800	316	45	1,900
Climate	Continental monsoon	Tropical	Temperate continental	Subtropical subalpine
Precipitation (mm/year)	720	1,464	624.7	1,800
Average temperature (°C)	7.6	23.8	12.8	8
Province	Hebei	Hainan	Beijing	Sichuan

Sampling sites: *WL* Wuling mountain; *DJY* Dujiangyan; *HN* Hainan province; *BG* Botanical Garden of Beijing, CAS

phae), or intercellular nonseptate hyphae, were found in 21 (88%) moss species (Fig. 1). AM fungal structures, i.e., vesicles, hyphal coils (including intracellular hyphae), or intercellular nonseptate hyphae, were present in 14 (58%) moss species, and spores and nonseptate hyphal strands along the surface of gametophytes occurred in 15 (63%) moss species. No AM fungal structures were found in three moss species (Table 2). AM fungal structures were present in 11 (92%) of the 12 saxicolous moss species, and six (60%) of the ten terricolous moss species, but absent from the two epixylous moss species. AM fungal structures, i.e., spores, vesicles, hyphal coils (including intracellular hyphae), or intercellular nonseptate hyphae, were only observed in moss stem and leaf tissues, but not in rhizoids. Arbuscules were not observed in this study (Table 2).

AM fungal species

To understand how many AM fungi associated with mosses, 13 moss species were selected and used as inocula in the trap cultures. After cultivation for 4 months, spores of 15 AM fungal taxa were isolated, of which 11 were identified to species, and four to genus level. Among the AM fungi isolated, 11 belonged to *Glomus*, two to *Acaulospora*, one to *Gigaspora*, and one to *Paraglomus*. *G. sinuosum* was present in nine (69%) moss species, followed by *G. ambisporum* in three species, and *G. fasciculatum*, *G. microaggregatum*, and *Glomus* sp.1 in two species. The other ten AM fungal species were only present in one moss species (Table 2).

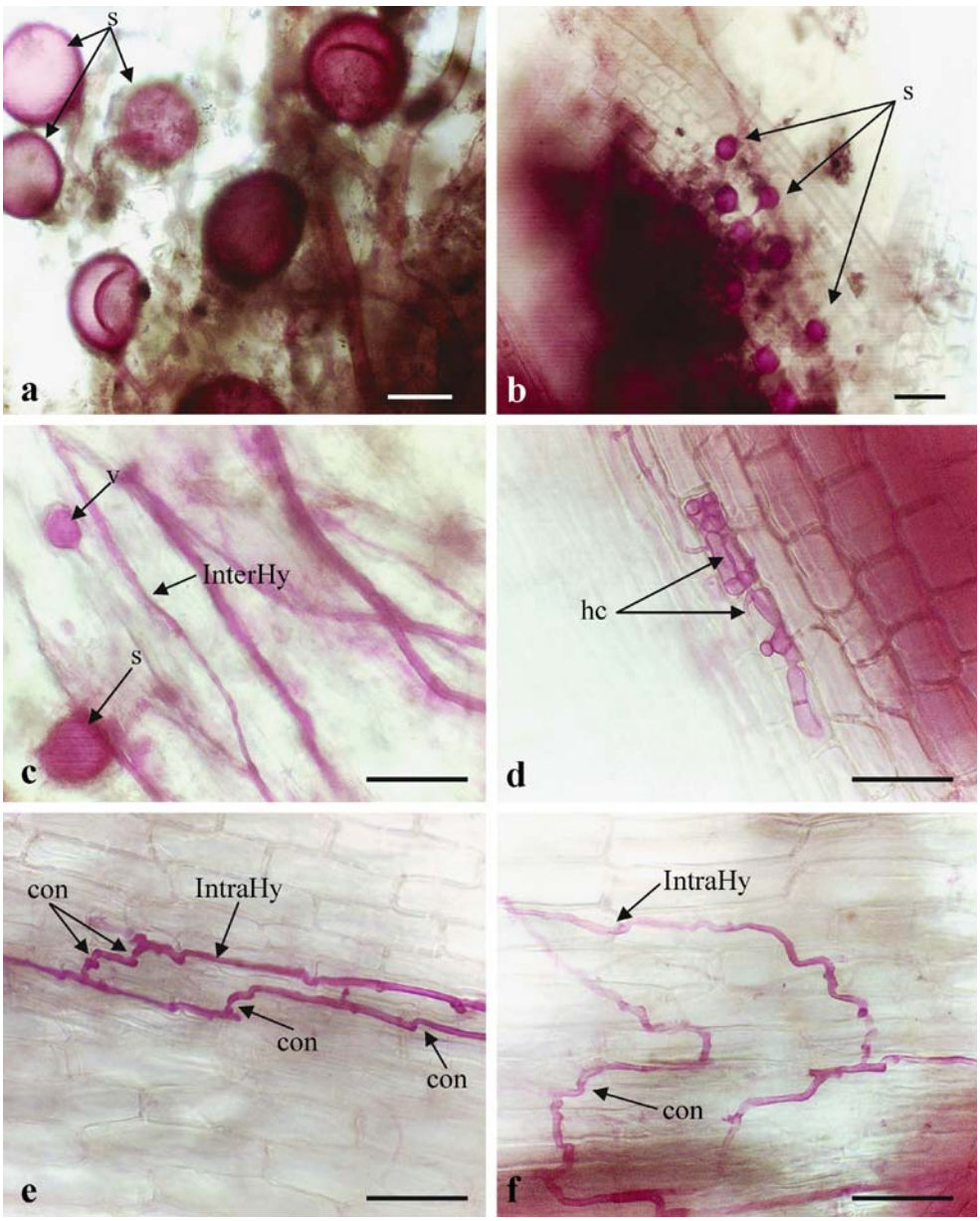

Fig. 1 Arbuscular mycorrhizal structures in mosses. **a** Spores (*s*) among stems of moss *Claopodium assurgens*. **b** Spores (*s*) among stems of moss *Ptychomitrium linearifolium*. **c** Intercellular hyphae (*InterHy*), vesicle (*v*) and spore (*s*) in the gametophyte stems of *Paraleucobryum enerve*. **d** Hyphal coils (*hc*) in the gametophyte stems of *P. enerve*. **e** and **f** Intracellular hyphae (*IntraHy*) and hyphal constriction (*con*) between moss cells in the gametophyte leaves of *P. enerve*. *Bars* 50 μm

Table 2 AM structures observed and AM fungi associated with mosses based on trap culture with clover

Moss	Intracellular nonseptate hyphae (coils)	Intercellular nonseptate hyphae	Vesicle	Nonseptate hyphae on tissue surface	Spores on tissue surface	AM species	Site	Habitat
Leucobryaceae								
Leucobryum glaucum (Hedw.) Aongstr.	(+)	+	–	–	–	*Glomus* sp.1	DJY	Terricolous
Leucobryum javense (Brid.) Mitt.	–	(+)	++	+	(+)	/	DJY	Saxicolous
Pottiaceae								
Trichostomum crispulum Bruch	–	+	–	–	–	*Glomus aggregatum* Schenck & Sm. *G. australe* (Berk.) Berch *G. sinuosum* (Gerd. & Bakshi) Almeida & Schenck	WL	Saxicolous
Timmiella anomala (B.S.G.) Limpr.	–	–	–	++	–	*G. fasciculatum* (Thaxt.) Gerd. & Trappe *G. sinuosum*	WL	Saxicolous
Andreaeaceae								
Andreaea mamillosula Chen	–	–	–	+	++	/	BG	Terricolous
Funariaceae								
Funaria hygrometrica Hedw.	–	–	–	–	–	/	BG	Terricolous
Hypnaceae								
Gollania ruginosa (Mitt.) Broth.	–	(+)	+	++	–	/	DJY	Saxicolous
Taxiphyllum taxiramenum (Mitt.) Fleisch.	–	–	–	–	–	*Acaulospora* sp. *Glomus* sp.2	DJY	Terricolous
Rhizogoniaceae								
Rhizogonium dozyanum Lac.	–	(+)	+	–	–	/	DJY	Terricolous
Sematophyllaceae								
Brotherella falcatula Broth.	+	++	–	–	–	/	DJY	Saxicolous
Neckeraceae								
Homalia trichomanoides (Hedw.) B.S.G.	–	++	++	–	–	*G. ambisporum* Sm. & Schenck *G. fasciculatum* *G. sinuosum* *G. microaggregatum* Koske, Gemma & Olexia *Glomus* sp.1	HN	Terricolous
Homalia sp.	–	–	–	++	–	*G. etunicatum* Becker & Gerd. *G. sinuosum*	WL	Epixylous
Brachytheciaceae								
Brachythecium plumosum (Hedw.) B.S.G.	–	–	–	++	–	*G. ambisporum* *G. sinuosum*	WL	Epixylous
Bryhnia novae-angliae (Sull. et Lesq.) Grout	–	–	–	++	(+)	*G. ambisporum* *G. sinuosum*	WL	Saxicolous

Table 2 (continued)

Moss	Intracellular nonseptate hyphae (coils)	Intercellular nonseptate hyphae	Vesicle	Nonseptate hyphae on tissue surface	Spores on tissue surface	AM species	Site	Habitat
						Paraglomus occultum (Walker) Morton & Redecker		
Cirriphyllum piliferum (Hedw.) Grout	(+)	++	–	++	–	*G. sinuosum*	HN	Saxicolous
Dicranaceae								
Paraleucobryum enerve (Thed.) Loesk.	++	+	–	++	+	*G. sinuosum*	HN	Terricolous
Trichocoleaceae								
Trichocolea tomentella (Ehrh.) Dumortier	+	++	++	++	(+)	/	DJY	Terricolous
Trichocoleopsis sacculata (Mitt.) Sh. Okam.	+	++	+	+	+	/	DJY	Saxicolous
Hypnodendraceae								
Hypnodendron reinwardtii (Reinw. et Hornsch.) Lindb.	–	–	–	–	–	*G. liquidambaris* (Wu & Chen) Almeida & Schenck *G. microaggregatum*	HN	Saxicolous
Ptychomitriaceae								
Ptychomitrium linearifolium Reim.	–	+	(+)	++	++	*Gigaspora* sp.	DJY	Saxicolous
Thuidiaceae								
Claopodium assurgens (Sull. et Lesq.) Card.	–	–	–	++	(+)	*Glomus lamellosum* Dalpé, Koske & Tews *G. sinuosum* *Acaulospora laevis* Gerd. & Trappe	HN	Saxicolous
Thuidium cymbifolium (Doz. et Molk.) Doz. et Molk.	+	+	+	–	–	/	DJY	Saxicolous
Bartramiaceae								
Bartramia halleriana Hedw.	–	+	–	++	–	/	DJY	Terricolous
Grimmiaceae								
Rhacomitrium canescens (Timm.) Brid.	–	–	–	+	++	/	BG	Terricolous

Relative 'intensity' of development of AM structures: ++ always present in significant numbers; + always present; *(+)* rare; – not detected; / no trap culture

Sites: *WL* Wuling mountain of Hebei province; *DJY* Dujiangyan of Sichuan province; *HN* Hainan province; *BG* Botanical Garden of Beijing, CAS

Discussion

AM fungal structures, i.e., vesicles, hyphal coils (including intracellular hyphae), or intercellular nonseptate hyphae, were observed in the tissues of most mosses. In particular, intracellular hyphae penetrated the leaves of the moss gametophytes. Fungal hyphae were first observed in capsules of the moss *Buxbaumia* by Peklo (1903, cited by Rayner 1927). Hyphae and vesicles of a nonseptate endotrophic fungus were reported in partially decomposed fragments of *Sphagnum* in Alberta, Canada from a peat bog (Butler 1939), and the fungus was considered to be closely allied to AM fungi commonly observed in roots of higher plants. Dowding (1959) described the occurrence of an AM fungus *Endogone fasciculata* (=*G. fasciculatum*) in Alberta swamps, which was found in mud, in and around plant roots and "between the leaves of mosses". Rabatin (1980) reported the association of *G. tenuis* and unidentified "coarse" AM fungi with field-collected specimens of the *Pogonatum*; however, penetration into the moss tissue was not observed. Hyphae, vesicles, and spores of *G. epigaeum* was found in

Funaria hygrometrica only when grown with a "companion" plant, asparagus (Parke and Linderman 1980). Similarly, the tissues of *F. hygrometrica*, which were collected from natural environment away from higher plants, were not infected by AM fungi in the present study.

AM fungal structures were observed in moss stem and leaf tissues, but not in rhizoids in the present and previous studies (Dowding 1959; Rabatin 1980; Parke and Linderman 1980). The unusual sites of AM colonization of the mosses are not surprising when one considers that the moss stems and leaves are composed of rather unspecialized parenchymatous cells morphologically similar to the undifferentiated cortical cells of higher plant roots. The hollow rhizoids that are generally devoid of fungal structures are primarily anchoring organs (Parke and Linderman 1980).

Several AM fungal species, i.e., 15 taxa belonging to four genera, were obtained in the trap cultures using 13 mosses as inocula, regardless of whether AM fungal structures were observed or not in the moss tissues. Similarly, when mats of *Lembophyllum* collected from tree branches were added to the planting medium of *Coprosma robusta*, the seedlings became colonized by *Rhizophagustenuis* (=*G. tenuis*), although no mycorrhizal structures had been observed in the moss (Johnson 1977).

Glomus most commonly formed association with mosses, and *G. sinuosum* was formed in most trap cultures. *Glomus* species were also the most common AM fungi of mosses in previous studies. The fruiting structures of *G. fasciculatum* were produced between the leaves of mosses (Dowding 1959). Fine hyphae and spores of *G. tenuis* were found among the leaves and stems of *Pogonatum* sp. (Rabatin 1980), and *G. epigaeum* produced sporocarps in the moss layer in a pot culture (Daniels and Trappe 1979). Hyphae, vesicles, and spores of *G. epigaeum*, *G. fasciculatum*, and *G. mosseae* were produced in *F. hygrometrica*, also in pot culture (Parke and Linderman 1980). In addition, other three AM fungal genera, i.e., *Acaulospora*, *Gigaspora*, and *Paraglomus*, were also isolated in the trap culture in the present study, although they were rare. This result indicates that many genera of AM fungi can be associated with mosses.

There was no relationship between AM fungal structures or AM fungal species of mosses and sampling sites with different environment conditions. This result indicated that AM fungi and AM fungal structures associated with mosses occurred widely and were not affected by environment conditions.

Although several researches showed that AM fungal structures occurred within moss tissues, little convincing evidence certified that they were mycorrhizal (Butler 1939; Dowding 1959; Rabatin 1980). However, there was not a mutualistic symbiosis between *F. hygrometrica* and AM fungi in pot culture, but *F. hygrometrica* could be infected by *G. epigaeum* only when grown with a "companion" plant, asparagus (Parke and Linderman 1980). In our study, although we collected moss samples away from other plants as possible, we were not sure that other plant roots did not spread into mosses collected and whether AM fungi associated with mosses were from other higher plants, because AM fungal hyphae can grow some distance and apparently grow into the moss layer as a desirable place to sporulate. Therefore, we cannot certify that the mosses formed a mutualistic symbiosis with AM fungi in the present study. Further study employed that more AM fungi and moss species need to be carried out to test whether AM fungi can colonize moss plants and form mutualistic symbiosis, as the paper of Parke and Linderman (1980), where inoculations of AM fungi were made in the presence or absence of higher nurse plants.

In conclusion, AM fungal structures, e.g., vesicles, hyphal coils (including intracellular hyphae), or intercellular nonseptate hyphae, were present in most mosses examined. Fifteen AM fungal taxa belonging to four genera were isolated based on the trap culture with clover. Mosses colonized by several AM fungi, and *Glomus* was the dominant genus.

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