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ORIGINAL ARTICLE

Distribution of arbuscular mycorrhizal fungi in four semi-mangrove plant communities

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Abstract To better understand the diversity and species composition of arbuscular mycorrhizal fungi (AMF) in mangrove ecosystems, the AMF colonization and distribution in four semi-mangrove plant communities were investigated. Typical AMF hyphal, vesicle and arbuscular structures were commonly observed in all the root samples, indicating that AMF are important components on the landward fringe of mangrove habitats. AMF spores were extracted from the rhizospheric soils, and an SSU rDNA fragment from each spore morphtype was amplified and sequenced for species identification. AMF species composition and diversity in the roots of each semi-mangrove species were also analyzed based on an SSU-ITS-LSU fragment, which was amplified, cloned and sequenced from root samples. In total, 11 unique AMF sequences were obtained from spores and 172 from roots. Phylogenetic analyses indicated that the sequences from the soil and roots were grouped into 5 and 14 phylotypes, respectively. AMF from six genera including *Acaulospora*, *Claroideoglomus*, *Diversispora*, *Funneliformis*, *Paraglomus*, and *Rhizophagus* were identified, with a further six phylotypes from the Glomeraceae family that could not be identified to the genus level. The AMF genus composition in the investigated semi-mangrove communities was very similar to that in the intertidal zone of this mangrove ecosystem and other investigated mangrove ecosystems, implying possible fungal adaptation to mangrove conditions.

Keywords Arbuscular mycorrhizal fungi · Distribution · Diversity · Mangrove forest · Semi-mangrove plants · Wetland

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Introduction

Arbuscular mycorrhizal fungi (AMF) are some of the most abundant microorganisms in below-ground ecosystems (Parniske 2008). They form symbiotic relationships with most vascular plant species, supplying them with nutrients (particularly P and N) in exchange for photosynthates (Smith and Read 2008). It has been demonstrated that the presence and composition of AMF species have profound effects on the structure and function of plant communities (van der Heijden et al. 2008). It is therefore important to study their distribution in different environments.

The presence and importance of AMF in wetland ecosystems have been demonstrated in a number of recent studies (Wirsel 2004; Wang et al. 2010; Møller et al. 2013). It has been proposed that AMF are not only present but ubiquitous in these habitats (Ypsilantis and Sylvia 2007; Wang et al. 2010, 2011). The distribution of AMF species in some wetland types has been investigated (Wilde et al. 2009; Choudhury et al. 2010; Wang et al. 2010, 2011), but much remains to be learned about their diversity and species composition in wetland ecosystems.

Mangroves are important wetlands that are found around the world but whose continued existence is under threat. They occur in tropical and subtropical intertidal estuarine zones and

feature various salt-tolerant plants that fulfill a range of essential ecological functions and provide numerous valuable natural resources (Nagelkerken et al. 2008). Mangrove plants are usually categorized into two subgroups: true mangrove and semi-mangrove plants. True mangrove plants are often restricted to the typical intertidal mangrove habitats, while semi-mangrove plants grow on the landward fringes of mangrove habitats or terrestrial marginal zones that are subject only to spring or storm high tides (Wu et al. 2008). Several studies have reported the presence of AMF in the typical intertidal zones of mangrove habitats (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008; Wang et al. 2010). Some authors have also investigated the distribution of AMF species within these habitats (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008; Wang et al. 2010, 2011). However, the colonization and distribution of AMF in the terrestrial marginal zones of mangrove forests (i.e., the zones where semi-mangrove species are found) are less well studied. Therefore, to better understand the diversity and species composition of AMF in mangrove ecosystems, it is necessary to study their distribution in semi-mangrove plant communities.

The objectives of this study were to: (1) determine whether AMF were present in semi-mangrove communities; (2) identify the main abiotic factors that influence the formation of AM symbiosis in semi-mangrove communities; and (3) analyze the diversity and species composition of AMF in semi-mangrove communities.

Materials and methods

Study site and sample collection

The Qi'Ao mangrove forest was selected as the study site in order to facilitate comparisons of the AMF colonization intensity and species composition between true mangrove and semi-mangrove plant communities: the AMF colonization and species composition within the intertidal zones of this forest have been investigated previously (Wang et al. 2010, 2011). Four dominant semi-mangrove species, including *Heritiera littoralis* Dryand., *Pongamia pinnata* L., *Cerbera manghas* L. and *Hibiscus tiliaceus* L., are naturally distributed in the landward fringe of this habitat, where they are subjected only to spring or storm high tides. For each of the four investigated species, root samples and rhizosphere soil samples were collected from three individual plants. In all cases, the root samples collected were juvenile nutritive roots attached to the selected plants. The sampled replicates were separated by a distance of more than 10 m.

Soil analysis and assessment of AM colonization

Soil properties, including soil moisture, pH, electrical conductivity, organic matter content, total and available P contents, and available N content were measured using the method described by Page et al. (1982). The pH was measured in a 1:2.5 soil:water paste (w/v), using a digital pH meter (Basic PB-20, Sartorius, Goettingen, Germany). Electrical conductivity was measured in the centrifuged supernatant of a 1:5 soil:water (w/v) extract. Organic matter contents were determined by the Walkley-Black acid digestion method. Available N (extracted using 2 M KCl) was measured by titrating the distillates obtained after Kjeldahl sample preparation and analysis. Total P (digested with HNO_3) and available P (extracted with 0.05 M HCl–0.025 M H_2SO_4) was measured by molybdenum blue colorimetry.

AMF colonization intensity was quantified by the magnified intersection method (McGonigle et al. 1990): we scored 200 intersects on 40 root segments per root sample using a compound microscope (Zeiss, Axiostar Plus, Jena, Germany).

AMF spore extraction and primary morphological identification

AMF spores were extracted from the rhizosphere soil samples (20 g) using the wet-sieving and decanting method (An et al. 1990). Intact and healthy spores were counted, and classified initially into different morphological types based on their morphological characters according to Schenck and Peréz (1990) and INVAM. AMF spores for which no conclusive morphotype identity could be assigned based on morphology were assigned to different morphological types.

DNA extraction, PCR, and sequencing

One AMF spore of each morphological type was used for DNA extraction according to Schwarzott and Schüßler (2001). A nested PCR was then performed to amplify a fragment of the AMF SSU rDNA region, using the DNA extracts of the AMF spore as the template. The GeoA2-Geo11 primer pair (Schwarzott and Schüßler 2001) was used in the first round of PCR, which was performed with 20 μL reaction mixtures containing 2 μL template DNA, 2 μL 10× PCR buffer, 1.5 μM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer and 0.05 U μL^{-1} Takara LA TaqTM DNA Polymerase with proof-reading activity (Takara, Tokyo, Japan). The amplification program was as follows: 4 min of initial denaturation at 94 °C; 30 cycles of 30 s denaturation at 94 °C, 30 s annealing at 54 °C and 100 s elongation at 72 °C; and a final 10 min elongation period. The first PCR products were diluted 1:10 and used as templates for a second (nested) round of PCR conducted with 50 μL reaction mixtures and the AML1-AML2 primer pair (Lee et al. 2008). The same

conditions were applied as in the first-round PCR, except that the annealing temperature was set at 50 °C and elongation time was set at 50 s. Portions (2.0 μL) of the PCR products were analyzed by agarose gel electrophoresis (1.0 % w/v agarose, 100 V, 40 min), and ethidium bromide staining to check their integrity and yield. The second-round PCR products (AML1-AML2 fragment) with the expected length (approx. 800 bp) were purified using a High Pure Kit (Pearl, China) according to the manufacturer's protocol. They were then sequenced directly in both directions using the AML1 and AML2 primers. Sequencing reactions were performed using an ABI PRISM 3730XL automatic sequencer with a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturers' instructions.

AMF species composition and diversity in the roots was analyzed by pooling all root samples from a single semi-mangrove species and extracting their DNA by the CTAB method. Because all attempts at amplifying the target AML1-AML2 region from the root DNA were unsuccessful, an alternative AMF fragment covering part of the SSU, the whole ITS and part of the LSU rDNA region (SSU-ITS-LSU) was used to analyze the distribution and diversity of AMF species in the roots. This SSU-ITS-LSU region has previously proven to be suitable in field-based studies of AMF communities (Wang et al. 2011), and was recommended as a DNA barcoding region for AMF (Stockinger et al. 2010). The target fragments from each of the four pooled root samples were amplified using a nested PCR procedure, after which the second-round PCR products (ca. 1.5 kb) were purified and used to construct clone libraries (Wang et al. 2011). Approximately 40 positive clones were selected randomly from each semi-mangrove root sample and used to construct a SSU-ITSLSU library. All clones from each library were then sequenced directly in both directions using the M13F and M13R primers.

Sequence analyses and construction of phylogenetic trees

The forward and reverse sequences from each clone were first assembled into a consensus sequence and compared to those available in the GenBank database using the BLAST tool to determine whether they were derived from Glomeramycota. Obtained AMF sequences from spores (AML1-AML2) and roots (SSU-ITS-LSU) were aligned separately and edited using LASERGENE SEQMAN (DNA Star, http://www.dnastar.com). Multiple alignments were performed with ClustalX 1.83 (Thompson et al. 1997), and primer sequences were excluded before further analyses.

The obtained AMF sequences were grouped into OTUs with sequence similarities of ≥97 % using the Mothur program (Schloss et al. 2009). Phylogenetic analyses were performed using the MEGA 4 program (Tamura et al. 2007). For the sequences obtained from AMF spores, a neighbor-joining (NJ) tree were constructed using all obtained AML1-AML2 sequences from this study and the representative AMF sequences from GenBank. The reliability of clades in the NJ analysis was assessed using nonparametric bootstrapping in MEGA (Kimura's two-parameter model; 1,000 replicates). Similar phylogenetic analyses were applied to the SSU-ITS-LSU sequences obtained from the roots, except that only one representative sequence (derived using the Mothur program) from each OTU was used for construction of the NJ tree. The AMF "phylotypes" identified in this work were defined primarily according to the topology of the phylogenetic trees. For the SSU-ITS-LSU fragment, the average pairwise distances (calculated based on the K2P model using MEGA 4) between different OTUs were considered in cases where it was difficult to decide whether two phylogenetically adjacent sequences or monoclades should be placed in the same phylotype or not. Two sequences or monoclades were separated into different phylotypes if their pairwise distances were greater than 0.055, as discussed by Wang et al. (2011). The sequence alignments in construction of both phylogenetic trees are presented in the Supplementary Materials (Alignments S1 and S2). All of the sequences obtained from this study were deposited in GenBank under accession numbers KF304581–KF304591 and KJ484643–KJ484814.

Statistical analyses

AMF phylotype richness (N) was calculated as the number of phylotypes recorded in each soil sample or pooled root sample. To justify the assumption of normality and homogeneity of variances before ANOVA, the AMF percentage colonization data were transformed by arcsine $x^{1/2}$ and spore densities were transformed by ln (x+1). A parametric one-way ANOVA, followed by a least significance difference (LSD) test, was used to determine differences in the soil properties among the different semi-mangrove communities, and the AMF colonization intensity and phylotype richness among the investigated semi-mangrove species. Correlation analysis was also used to evaluate the relationships between soil variables and AMF colonization rates or spore density. All statistical analyses were conducted using SPSS 16.0 (SPSS, Chicago, IL).

Results

AMF colonization and spore density

AMF structures were observed in the roots of all investigated semi-mangrove species. Hyphal, vesicle and arbuscular structures were commonly found, and their colonization rates were above 10 % for all root samples (Table 1). The AMF hyphal

Table 1 Arbuscular mycorrhizal fungi (AMF) colonization intensity, spore density (SD) and phylotype richness in the roots (N_{root}) and rhizosphere soil (N_{soil}) of the four studied semi-mangrove plant communities. *TC%*, *HC%*, *VC%* and *AC%* represent the total percentage of AM colonization and the percentage of hyphal colonization, vesicle colonization and arbuscular colonization, respectively; *SD* density of AMF spores extracted from 20 g air-dried soil. Values with different lower case letters in the same column are significantly different across all host species at the $P<0.05$ level (mean±SE, $n=3$)

Species	TC%	HC%	VC%	AC%	SD	N_{soil}	N_{root}
Heritiera littoralis	63.7±2.7 a	54.5±5.5 a	38.2±4.1 a	14.7±2.6 b	27.3±1.2 a	2.33±0.33 a	5
Pongamia pinnata	46.5±6.7 a	43.0±5.8 a	20. 7±2.3 b	16.3±2.2 b	17.3±5.5 b	1.67±0.33 a	4
Cerbera manghas	57.2±5.9 a	53.0±4.4 ab	17.2±4.6 b	29.8±3.9 a	11.3±6.9 bc	2.0±0.58 a	8
Hibiscus tiliaceus	33.3±5.1 b	27.9±3.4 b	13.8±4.0 b	20.4±7.3 ab	3.0±0.6 c	1.33±0.33 a	5

(54.5 %), vesicle (38.2 %) and total (63.7 %) colonization intensities for *Heritiera littoralis* were the highest of the four investigated species, while those for *Hibiscus tiliaceus* were the lowest. The degree of AMF vesicle colonization in *Heritiera littoralis* (38.2 %) was significantly greater than that in the other species ($P<0.05$), and the total AMF colonization in *Hibiscus tiliaceus* (33.3 %) was significantly lower than that of the other species ($P<0.05$). AMF spores were also observed in all the soil samples (Table 1). Their density in the rhizosphere of *Heritiera littoralis* was significantly higher than in that of the other species ($P<0.05$). The AMF spore density in the rhizosphere of *Hibiscus tiliaceus* was significantly lower than that of *Heritiera littoralis and P. pinnata* ($P<0.05$).

Properties of rhizosphere soil

The properties of the rhizosphere soils collected from the different semi-mangrove plant communities are shown in Table 2. In general, the soil properties in the rhizospheres of *Heritiera littoralis*, *P. pinnata* and *C. manghas* were similar. However, the electrical conductivity and contents of organic matter and available N in the *Hibiscus tiliaceus* community were significantly lower than those for the other studied species ($P<0.05$). Correlation analysis revealed a significant positive correlation between total AMF colonization rates and the soil organic matter content ($R^2=0.603$, $P=0.038$, $n=12$) and the AMF spore density ($R^2=0.600$, $P=0.039$, $n=12$).

AMF species composition and distribution

The collected AMF spores were assigned to 1 of 16 types based on their morphological characters. The target sequence fragment (ca. 800 bp) was amplified successfully from all of these morphological types. Some of the amplified sequences were identical to one-another, resulting in the identification of 11 unique AMF sequences. These sequences were grouped into 5 operational taxonomic units (OTUs) based on sequence similarities of 97–100 % using the Mothur program. Phylogenetic analyses (Fig. 1) showed that these OTUs (phylotypes) should belong to four genera, including *Funneliformis* (two phylotypes), *Claroideoglomus* (one phylotype), *Diversispora* (one phylotype) and *Acaulospora* (one phylotype). AMF spores from *Funneliformis* and *Acaulospora* were dominant and were both found in three of the four semi-mangrove communities. The numbers of AMF spores and phylotypes obtained from each semi-mangrove community are presented in Table 3. Two or three AMF spore phylotypes were found for each community, and there were no significant differences between the communities with respect to phylotype richness ($P>0.05$).

Table 2 Soil properties of rhizosphere soils from different semi-mangrove plant communities. *EC* Electrical conductivity, *OM* organic matter, *AN* available nitrogen, *AP* available phosphorus, *TP* total phosphorus; values with different lower case letters in the same line are significantly different at the $P<0.05$ level (mean± SE, $n=3$)

Soil property	Semi-mangrove plant communities			
	Heritiera littoralis	*P. pinnata*	*C. manghas*	*Hibiscus tiliaceus*
Moisture (%)	23.7±1.8 a	26.2±1.8 a	26.0±2.0 a	22.5±1.2 a
pH	7.59±0.37 ab	6.64±0.13 b	6.87±0.28 b	7.89±0.18 a
EC (ds m^{-1})	1.74±0.03 a	1.48±0.16 a	1.41±0.46 a	0.54±0.01 b
OM (g kg^{-1})	24.9±3.0 a	30.5±1.6 a	29.2±3.0 a	9.5±1.8 b
AN (mg kg^{-1})	63.1±1.9 b	82.0±3.8 a	75.8±1.7 a	35.8±1.6 c
AP (mg kg^{-1})	31.2±1.6 ab	26.2±2.1 b	42.2±7.5 a	34.2±2.9 ab
TP (mg kg^{-1})	0.38±0.03 ab	0.34±0.11 ab	0.42±0.02 a	0.31±0.07 b

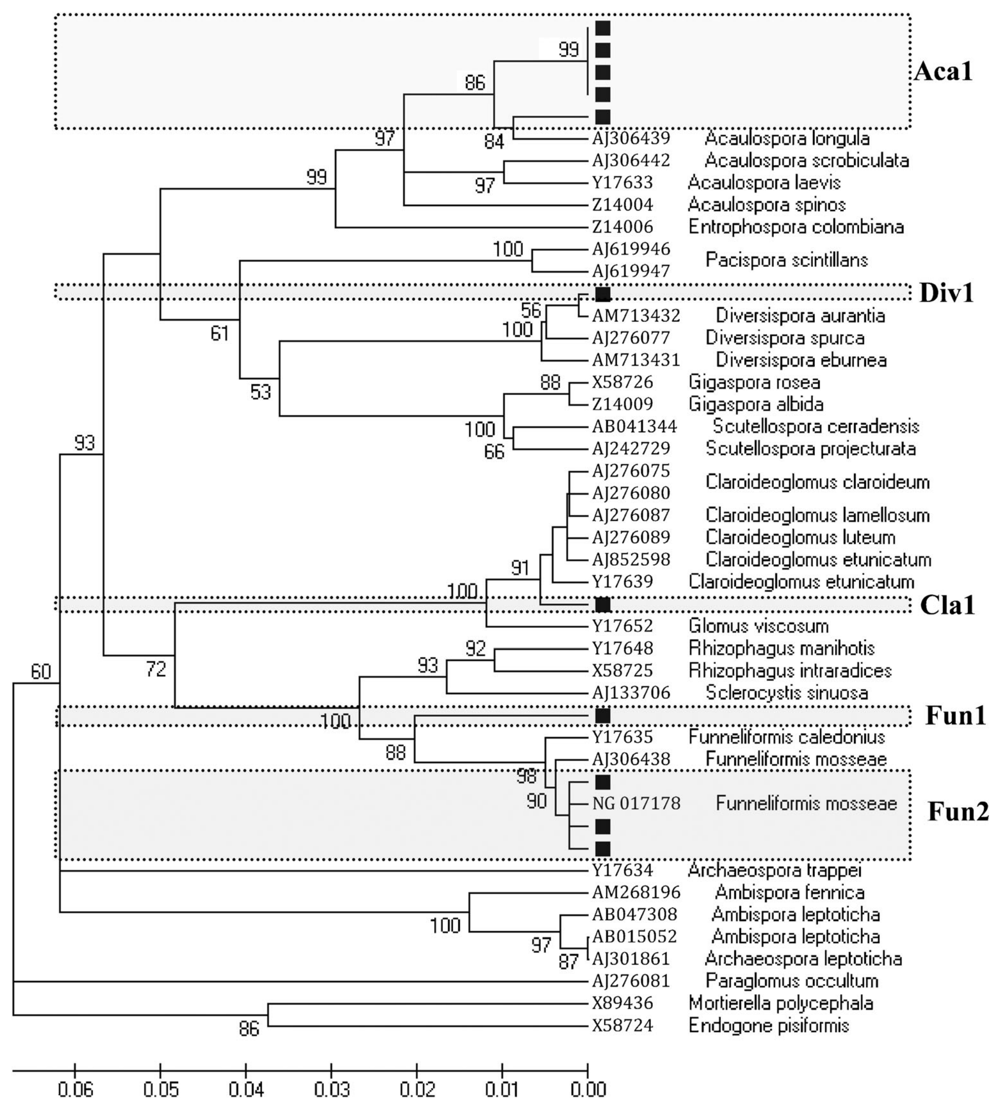

Fig. 1 Phylogenetic relationships between all of the obtained arbuscular mycorrhizal fungi (AMF) sequences (■) and the representative sequences from GenBank. The AMF DNA fragments (partial SSU rDNA sequences of ca. 800 bp, amplified using the AML1–AML2 primers) were obtained from AMF spores collected from rhizosphere soil samples from the four studied semi-mangrove species. The values above the branches are bootstrap values (1,000 replicates); only support greater than 50 % is shown

The Fun2 (79 spores), Div1 (4 spores) and Aca1 (70 spores) AMF spore phylotypes were associated strongly with *Funelliformis mosseae*, *Diversispora aurantium* and *Acaulospora longula*, respectively. Cla1 (7 spores) exhibited high sequence similarities with *Claroideoglomus lamellosum* (99.4 %, AJ276087), *C. claroideum* (99.4 %, AJ276080) and *C. etunicatum* (99.3 %, AJ852598). Fun1 (17 spores) was also related to an uncultured *Funneliformis* sequence (DQ396781) that could not be identified at the species level.

The target SSU-ITS-LSU fragment was amplified successfully from each of the four pooled root samples. In total, 173 positive clones were sequenced from four libraries, and a total of 172 AMF sequences were obtained (one sequence not belonging to the Glomeromycota was excluded). The 172 sequences were grouped into 26 OTUs based on 97–100 % sequence similarities using the Mothur program, and finally sorted into 14 phylotypes based on phylogenetic analyses (Fig. 2). These included one phylotype from *Paraglomus* (Par1, 4 sequences), seven from *Rhizophagus* (Rhi1-Rhi7, 17 sequences), and six Glomeraceae phylotypes (Glome1-Glome6, 151 sequences) that could not be identified at the genus level (Fig. 2). The abundance matrix of AMF sequences (clones) observed for each phylotype within the roots of the four semi-mangrove species are shown in Fig. 2. Four to eight phylotypes were detected in the root samples for each semi-mangrove species.

Discussion

To date, studies on AMF colonization in estuarine mangrove habitats have focused primarily on the intertidal zones (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008; Wang et al. 2010). It has been demonstrated that AMF are very common in these habitats, and that their colonization rates are higher in drier areas than in wetter, more anaerobic

Table 3 Relative abundance matrix of AMF spores obtained in each phylotype within rhizospheric soil samples of each semi-mangrove species

Phylotype	Semi-mangrove communities				
	Heritiera littoralis	*P. pinnata*	*C. manghas*	*Hibiscus tiliaceus*	Total
Fun1	0	0	17	0	17
Fun2	37	35	7	0	79
Cla1	0	0	0	7	7
Div1	2	0	0	2	4
Aca1	43	17	10	0	70
Total	82	52	34	9	177

areas (Miller 2000; Wang et al. 2010). It was therefore unsurprising that all of the semi-mangrove species examined in this work, which were distributed in the landward areas of the mangroves, were colonized heavily by AMF. Previous studies indicated that AMF vesicle and arbuscule structures were rare in roots collected from the low intertidal zones, but hyphal structures were common (Sengupta and Chaudhuri 2002; Wang et al. 2010). In this work, typical hyphal, vesicle and arbuscular structures were all commonly associated with the roots of the studied species, indicating that AMF are important components in the landward fringes of mangrove habitats. The ubiquity of vesicular and arbuscular structures in this work also supports our previous suggestion that the scarcity of such structures in the intertidal regions of mangroves is due to their comparatively high sensitivity to moist and saline environments (Wang et al. 2010).

As the most widespread and ecologically important plant symbionts, AMF colonization may be affected by a variety of biotic and abiotic factors (e.g., Liu et al. 2012a, b; 2013). Møller et al. (2013) found that in a eutrophic wetland, a high soil organic matter content induced night-time anoxia in both the soil and plant tissues, thus reducing root growth and AMF colonization. The results obtained in this work and in previous studies on the intertidal zones of the same habitat (Wang et al. 2010) suggest that the soil organic matter content correlates positively with the intensity of AMF colonization ($P<0.05$).

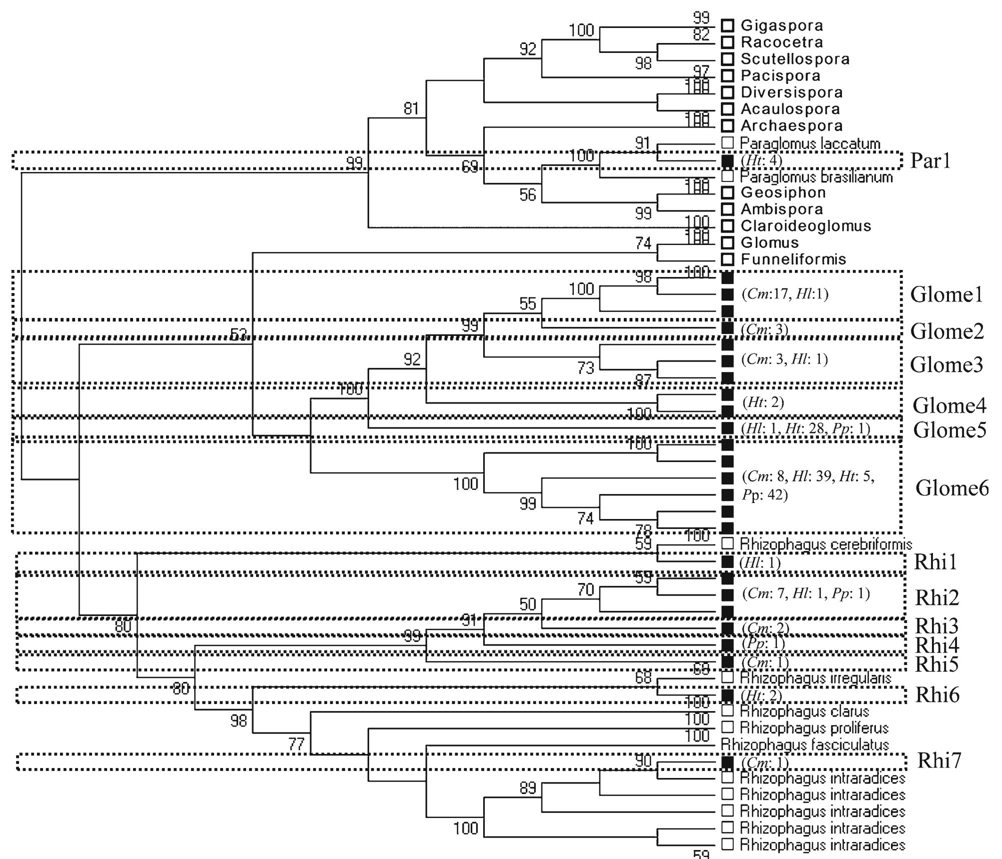

Fig. 2 Phylogenetic relationships between the AMF sequences obtained from roots (■) and the representative sequences from GenBank (□). The AMF DNA fragments (partial SSU, ITS region and partial LSU rDNA sequences of ca. 1.5 kb) were obtained from the roots of four semi-mangrove species. The number of sequences obtained from each of the four semi-mangrove species are shown behind the ■ marks (*Cm Cerbera manghas*, *Ht Hibiscus tiliaceus*, *Hl Heritiera littoralis*, *Pp Pongamia pinnata*). The values above the branches are bootstrap values (1,000 replicates); only support greater than 50 % is shown

This implies that adding appropriate quantities of organic matter to the soils of oligotrophic wetlands such as the Qi'Ao mangrove forest would strengthen the symbiotic relationships between AMF and wetland plants. Soil organic matter may have positive effects on this symbiosis because it enables the proliferation of AMF hyphae and provides a source of nitrogen (Hodge and Fitter 2010), which is taken up by the AMF and transferred to the host plant in exchange for sugars (Leigh et al. 2009). Because AMF can greatly enhance the growth of mangrove plants by increasing their absorption of nutrient elements (Wang et al. 2010), the positive effects of organic matter enrichment on AMF colonization may be useful in the protection and re-establishment of mangrove habitats.

AMF species composition and diversity is sensitive to a range of factors (Wang et al. 2010, 2011; Liu et al. 2012a, b), all of which should be accounted for in field studies on AMF communities. It should be noted that the analyses of AMF spore density, species composition and diversity in soils and roots were all based on samples collected on a single sampling occasion. AMF spores were obtained from all of the rhizosphere soil samples, but their densities were relatively low. This is consistent with the results of previous sampling campaigns in the intertidal zones of this mangrove habitat (unpublished data from Yutao Wang) and other mangroves (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008; D'souza and Rodrigues 2013). AMF spore density may be affected by many factors, including edaphic factors, seasonal variation, host species, etc. (e.g. Gai et al. 2012; Sivakumar 2013). The relatively low AMF spore density in mangrove forests could be due to tidal currents (although these are not common in semi-mangrove communities) and the saline environment in these habitats; further studies will be required to clarify this point.

Based on the molecular identification of the obtained AMF spores, each individual soil sample contained between one and three AMF phylotypes, and a total of five phylotypes were found in the rhizosphere soil. The species (phylotype) diversity of AMF spores in the soil was similar to that observed in the Sundarban mangrove swamp in the Ganges river estuary in India (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008). However, it was greater than the diversity observed in the intertidal zone of the habitat considered in this work, in which only three AMF species could be directly identified in 45 rhizosphere soil samples collected from nine mangrove species (Wang et al. 2010). Conversely, the level of diversity detected in the roots (14 phylotypes based on four root samples from four host species) was similar to that seen in the intertidal zones of this habitat (Wang et al. 2011) and was clearly greater than the AMF spore diversity in the rhizosphere soil. It is important to note that measures of AMF diversity obtained using different methods must be compared with caution. According to the results of phylogenetic analyses and our experiences, both of the "phylotype" criteria applied in the analysis of the soil and the root samples in this work should correspond roughly to AMF "species". The greater diversity of AMF in the roots compared to the soil can be explained by the presence of AMF species that do not produce spores, since most of the sequences obtained from the root samples derived from un-described or uncultured AMF. Similar results have also been reported in terrestrial ecosystems (Liu et al. 2012b) and the intertidal zone of the habitat considered in this work (Wang et al. 2010, 2011).

Based on sequence similarity and phylogenetic analyses, the AMF spores isolated in this work belonged to four genera (*Funneliformis*, *Claroideoglomus*, *Diversispora* and *Acaulospora*), three of which were previously associated with the former genus "*Glomus*". The AMF sequences obtained from the roots also mostly belonged to what was previously known as "*Glomus*" with the exception of four sequences from *Paraglomus*. The genus composition was very similar to that in the intertidal zone of the same area (Wang et al. 2010, 2011) and that in the Sundarban (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008) and Goan mangroves (D'souza and Rodrigues 2013). This suggests that these fungi may have adapted to mangrove conditions. It has been suggested that the AMF in the intertidal zone areas of the estuarine mangrove forests may come from adjacent terrestrial habitats (Sengupta and Chaudhuri 2002; Wang et al. 2010). The high similarity in genus composition between the intertidal zones and the terrestrial marginal zones in the Qi'Ao mangrove forest, and the observation of sequences from *F. mosseae* (Fun2) and *Rhizophagus intraradices* (Rhi7), both of which were also found in the intertidal zone of this habitat (Wang et al. 2010, 2011), both support this hypothesis.

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