Chapter 16
Biogeography and Specificity of Ectomycorrhizal Fungi of *Coccoloba uvifera*

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16.1 Introduction

A range of biotic, abiotic and historical variables shape the structure and species richness of ectomycorrhizal (EcM) fungal communities. Host and fungal compatibility (i.e. host preference or specificity) that may vary widely across host taxa, has been increasingly shown to influence the structure and richness of EcM fungal assemblages at various taxonomic levels of plants (Ishida et al. 2007; Morris et al. 2008; Tedersoo et al. 2010b, 2013; Bahram et al. 2012; Põlme et al. 2013). To date, the influence of environment on EcM fungal richness and composition has received relatively limited attention and the biodiversity of several tropical plant groups has remained unknown. Unlike in most other organisms and fungi overall, EcM fungi exhibit greater diversity in temperate compared with tropical and arctic ecosystems (Tedresoo et al. 2012, 2014; Chap. 18). This has been ascribed to historical factors (the lack of earliest evolving Pinaceae hosts in lowland tropical habitats), rapid turnover of organic matter in tropical soils and low relative abundance of hosts.

The genus *Coccoloba* (Polygonaceae) comprises ca. 170 species of shrubs and trees with neotropical distribution (Howard 1960; Chap. 19). *Coccoloba* spp. are probably of South American origin, with greatest richness in northern Amazonia

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and the Atlantic rain forest (Howard 1961; Chap. 20). EcM associations were first described from sea grape (*Coccoloba uvifera* (L.) L.) in Cuba (Kreisel 1970) and thereafter in South American rain forest species (Moyersoen 1993; Bereau et al. 1997). The associated EcM fungi are poorly known, because host plants have remained unsettled in most South American mycological studies (Pegler 1983; Singer et al. 1983; Roy et al. 2016; but see Tedersoo et al. 2010b).

Sea grape is a tropical tree species with a native distribution from southern Florida to eastern Mexico to northeastern Brazil. Sea grape grows in immediate proximity of seashores, representing one of the first colonizers of sandy and rocky coastal areas. These habitats are characterized by high salinity, steady wind, seasonal drought and low soil nutrient availability. EcM symbiosis could potentially enhance plant tolerance of those harsh conditions, particularly of high salinity (Bandou et al. 2006). Séne et al. (2015) conducted a profound study sampling sporocarps and EcM root tips in sea grape communities and reported very limited EcM fungal diversity in Guadeloupe Island. The authors postulated four nonexclusive hypotheses to explain the low EcM fungal diversity: (1) isolation from mainland, (2) overall lower EcM fungal diversity at lower latitudes, (3) environmental filtering due to stressful conditions, (4) recent origin of EcM symbiosis within the Polygonaceae. In spite of profound local sampling effort in a small volcanic island, their study cannot be used to generalize about sea grape EcM communities over a wider geographic context.

In order to test the above hypotheses in a wider geographical and historical context, we sampled six additional sea grape communities around the Caribbean basin and compared these in the biogeographic perspective. We further addressed the potential specificity and origin of EcM fungi of *C. uvifera* by comparing the associated *Tomentella* species—the dominant group of mycobionts—to these from other hosts in North and South America.

16.2 Approaches

We performed root sampling in six study sites in the following locations: USA: Miami (June 2013; 26.0408°N; −80.1145°E), Mexico: Celestún (October 2015; 20.9336°N; −90.3748°E), Costa Rica: Cahuita (June 2013; 9.8590°N; −82.9458°E), Cuba: Cayo Santa Maria (December 2008; 22.6581°N; −79.0413°E), French Guiana: Montabo (November 2013; 4.9436°N; −52.2973°E) and Colombia: Los Naranjos (November 2014; 11.2973°N; −73.8946°E). From each site, roots from ten sea grape individuals were collected (except for the Cuba plot where 14 samples were collected). Randomly selected samples (15 × 15 cm to 10 cm depth) were situated at least 10 m apart.

Roots were cleaned from adhering soil in tap water and morphotyped under a stereomicroscope. EcM morphotypes were distinguished based on colour and roughness of mantle, presence of emanating hyphae and rhizomorphs. At least two EcM root tips from each morphotype per soil sample were stored in CTAB

buffer (1% cetyltrimethylammonium bromide, 100 mM Tris–HCL (pH 8.0), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid) for molecular analyses.

DNA was extracted from EcM root tips using Thermo Scientific Phire Plant Direct PCR Kit (Thermo Scentific, Waltham, MA, USA) according to the manufacturer's instructions. In the course of the study, PCR was performed by use of 5× HOT FIREPol Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia). In EcM root tips, fungal rDNA Internal Transcribed Spacer (ITS) region was amplified with a forward primer ITSOF-T (5′-acttggtcatttagaggaagt-3′) in combination with reverse primers LB-W (5′-cttttcatctttccctcacgg-3′) or TW13 (5′-ggtccgtgtttcaagacg-3′). In case of PCR failure, we combined ITSOF-T with universal primers ITS4 (5′-tcctccgcttattgatatgc-3′), or basidiomycete-specific primer ITS4B (5′-caggagacttgtacacggtccag-3′) and LR0B (5′-acccgctgaacttaagc-3′) in order to amplify a shorter fragment of fungal DNA. To improve sequence quality, some root tip extracts were re-amplified with taxon-specific primers (Tedersoo et al. 2008). PCR and sequencing were run following Põlme et al. (2013). Sequences were assembled, checked, trimmed and manually corrected in Sequencher 5.1 software (GeneCodes Corp., Ann Arbor, MI, USA).

Sequences were confirmed to belong to EcM fungal lineages (cf. Tedersoo et al. 2010a; Chap. 6) by use of BLASTn searches against the International Sequence Databases (INSD) or UNITE (Abarenkov et al. 2010a). Sequences were partitioned into operational taxonomic units (OTUs), defined as a group of sequences sharing at least 97% pairwise similarity. Sequences with sufficient length and quality were assigned to UNITE species hypothesis (SHs; Kõljalg et al. 2013) with 3% dissimilarity threshold. We also included the recently published data of Séne et al. (2015) from Guadeluope. This study covered four study plots and had a much higher per-site sampling effort.

Using the PlutoF web platform (Abarenkov et al. 2010b), we downloaded all *Tomentella* sequences originating from South, Central and Northeast America. Identical sequences from the same sampling plots and hosts were removed prior to phylogeny construction. All sequences were aligned using MAFFT software (Katoh and Standley 2013). The alignment was manually adjusted in AliView software (Larsson 2014) and Maximum likelihood analysis was performed in FastTree 2.1 (Price et al. 2010) using default settings, with *Odontia fibrosa* (UDB000284) as an outgroup (Tedersoo et al. 2015).

16.3 Fungal Diversity

Out of 147 EcM root tips subjected to molecular analyses, 131 (89%) yielded good quality sequences. These sequences (including material collected by Séne et al. 2015 from Guadeloupe) were clustered into 42 OTUs (Table 16.1). Altogether 33 of OTUs were accommodated to existing SHs at the 97% sequence similarity cutoff level. Of these SHs, 26 (78.8%) were exclusively associated with sea grape.

Table 16.1 Distribution of fungal species hypothesis and OTUs at 97% cutoff level, associating with *Coccoloba uvifera* individuals in seven sampling areas

EcM lineage	No. of sequences in SH	Species hypothesis or taxon code	Miami, USA	Cahuita, Costa Rica	Los Naranjos, Columbia	Cayo Santa, Maria Cuba	Montabo, French Guiana	Celestún, Mexico	Guadeloupe
/tomentella-thelephora	2	SH491687.07FU[a]	UDB023143						
/tomentella-thelephora	5	SH002563.07FU[a]	UDB023156			UDB004997			
/tomentella-thelephora	7	SH018148.07FU[a]	UDB023168			UDB010534			
/tomentella-thelephora	17	SH009884.07FU		UDB023176		UDB004975			FR682090
/tomentella-thelephora	2	SH494851.07FU[a]		UDB023210					
/tomentella-thelephora	7	SH009960.07FU	UDB023163		UDB023213				KF472143
/tomentella-thelephora	3	SH010081.07FU	UDB023147						
/tomentella-thelephora	4	SH490339.07FU[a]			UDB023211				
/tomentella-thelephora	6	SH489759.07FU[a]			UDB023216				
/tomentella-thelephora		*Tom.* Miami	UDB023145						
/tomentella-thelephora	1	SH494788.07FU[a]		UDB023189					
/tomentella-thelephora	4	SH493262.07FU			UDB023215				
/tomentella-thelephora	1	SH490338.07FU[a]			UDB023208				

/tomentella-thelephora		*Tom.* Costa Rica 1		UDB023171					
/tomentella-thelephora		*Tom.* Costa Rica 2		UDB023174					
/tomentella-thelephora	32	SH009872.07FU[a]	UDB023141			UDB010547			KF472135
/tomentella-thelephora		*Tom.* Cuba				UDB010532			
/tomentella-thelephora	4	SH027506.07FU[a]				UDB010544			
/tomentella-thelephora		*Tom.* Guadeloupe 1							KF472141
/tomentella-thelephora		*Tom.* Guadeloupe 2							KF472148
/tomentella-thelephora	1	SH007321.07FU[a]							KF472158
/boletus		*Boletus* Miami 1	UDB023149						
/boletus		*Boletus* Miami 2	UDB023158						
/boletus	3	SH490763.07FU[a]			UDB023206				
/boletus	1	SH460556.07FU[a]				UDB004996			
/inocybe	18	SH032645.07FU		UDB023179					
/inocybe	1	SH491686.07FU[a]		UDB023188					
/inocybe	1	SH029289.07FU[a]				UDB004995			
/inocybe	2	SH029290.07FU[a]						UDB031216	FR682085
/clavulina	2	SH489802.07FU[a]	UDB023151						
/clavulina	1	SH490342.07FU[a]	UDB023161						
/clavulina	2	SH490812.07FU[a]			UDB023224				
/paxillus-gyrodon	1	SH023513.07FU[a]							KF472137

(continued)

Table 16.1 (continued)

EcM lineage	No. of sequences in SH	Species hypothesis or taxon code	Miami, USA	Cahuita, Costa Rica	Los Naranjos, Columbia	Cayo Santa, Maria Cuba	Montabo, French Guiana	Celestún, Mexico	Guadeloupe
/paxillus-gyrodon	1	SH023512.07FU[a]							KF472152
/pisolithus-scleroderma	40	SH003700.07FU[a]	UDB023152	UDB023172		UDB004993	UDB023202	UDB031196	FR682092
/pisolithus-scleroderma	10	SH003702.07FU[a]	UDB023157	UDB023185	UDB023207	UDB004971			
/sebacina	11	SH016792.07FU				UDB004981			
/sebacina	1	SH494787.07FU[a]			UDB023214				
/ cantharellus	1	SH030040.07FU[a]							FR682089
/ cenococcum	318	SH027498.07FU			UDB023209				KF472151
/russula-lactarius	1	SH004059.07FU[a]							FR682087
/serendipita		*Ser*. Guadeloupe							KF472155
		Total OTU richness	13	9	11	10	1	2	14

[a]Species hypothesis that are exclusively associated with *Coccoloba uvifera*

Only five fungal OTUs found from newly sampled sites overlapped with the Guadeloupe study, whereas eight OTUs remained exclusive to Guadeloupe.

The /tomentella-thelephora was by far most taxon-rich phylogenetic lineage of EcM fungi comprising 21 OTUs. Other sea grape-associating lineages were represented with the following number of OTUs: /boletus (four), /inocybe (four), /clavulina (three), /pisolithus-scleroderma (two), /sebacina (two), /paxillus-gyrodon (two), /russula-lactarius (one), /cantharellus (one), /serendipita (one; questionable mycorrhizal status) and /cenococcum (one). In spite of taxonomical richness of the /tomentella-thelephora lineage, *Scleroderma bermudense* (SH003700.07FU) and *Scleroderma* sp. (SH003702.07FU) were the most common individual taxa that were present in six and four sites out of seven, respectively.

Florida constituted the most OTU-rich site harbouring 13 EcM fungal taxa, followed by Cuba (11 OTUs), Colombia (10) and Costa Rica (9). Interestingly, Mexico and French Guiana sites harboured only two and one EcM fungal OTU, respectively (Fig. 16.1). In comparison, altogether 14 EcM OTUs were identified from root tips from four plots in Guadeloupe, with 4–9 EcM fungal OTUs per plot (Séne et al. 2015), but this can be ascribed to more extensive sampling effort on a local scale. None of the sea grape-associated OTUs overlapped with EcM fungal

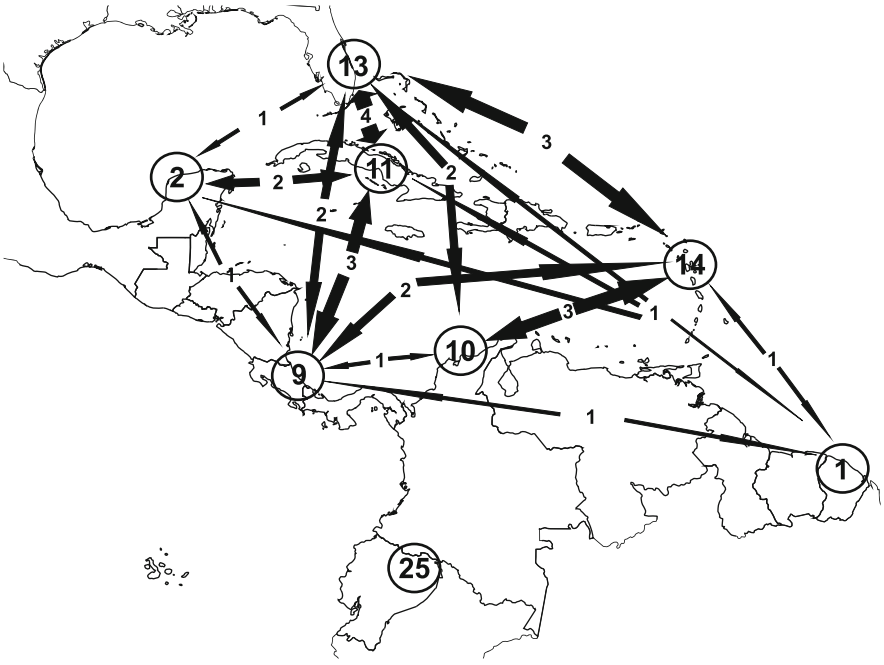

Fig. 16.1 Map of study sites indicating number of fungal OTUs in each sampling area. Shared OTUs between areas are shown with *arrows*. Note that inland *Coccoloba* species from Ecuador (Tedersoo et al. 2010b) do not share any fungal OTUs with *Coccoloba uvifera* communities from the coastal areas

taxa associated with the five sampled *Coccoloba* spp. in Ecuador (Tedersoo et al. 2010b).

The overall OTU richness detected over a broader geographic scale increased nearly three-fold compared to the single study conducted in Guadeloupe (Séne et al. 2015). In addition to formerly reported EcM lineages, we found /sebacina, /clavulina and /boletus lineages from our sampling sites, which were not found in Guadeloupe. Interestingly, several EcM fungal lineages such as /paxillus-gyrodon, /serendipita, /russula-lactarius and /cantharellus were not found outside Guadeloupe. The two Guadeloupean species of *Melanogaster* belonging to the /paxillus-gyrodon lineage are remarkable, because this genus and the entire EcM lineage is not known to associate with hosts of tropical origin. Species of *Cantharellus* fruited in abundance in another *C. uvifera* stand ca. 3 km distant from the Colombian site, but ITS sequencing of these fruit-bodies failed, indicating either primer bias or extensive length of the ITS region (Tedersoo et al. 2016). It is likely that additional sampling effort would have revealed these taxa from the mainland as well and that the true EcM fungal species richness associated with sea grape is considerably higher than the currently reported 42 fungal OTUs (Fig. 16.2). However, the coarse structure of EcM fungal communities was relatively similar to that previously reported in Guadeloupe i.e. /tomentella-therephora being most taxon rich lineage and *Scleroderma bermudense* being the most abundant fungal taxon. Sites in French Guiana and Mexico were extremely species-poor, comprising only one and two fungal OTUs respectively. In French Guiana, *S. bermudense* colonized sea grape root systems in all samples. The French Guiana site was characterized by intense anthropogenic disturbance in addition to a small host tree population. However, the Florida

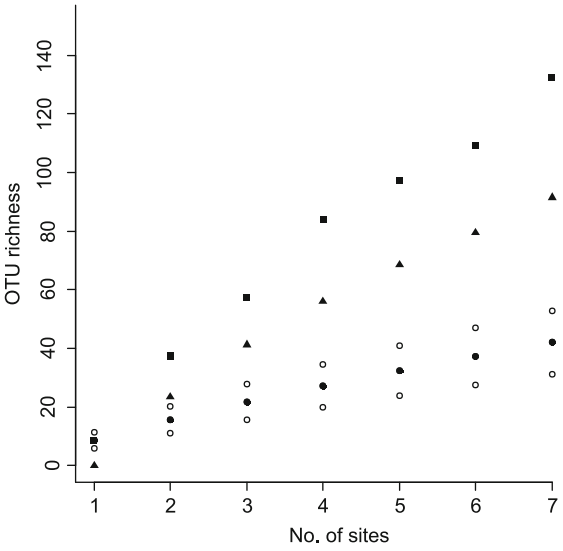

Fig. 16.2 Rarefied OTU accumulation curve of *Coccoloba uvifera* associated EcM fungi found in Caribbean basin. *Closed circles* and *open circles* represent the rarefied curve and its 95% confidence intervals, respectively. *Triangles* and *squares* represent the values of Chao2 and Jacknife2 minimum richness estimators, respectively. The values were calculated based on 999 permutations using EstimateS 9 (Colwell 2013)

site with the highest OTU richness was also characterized by substantial anthropogenic impact but with considerably larger host population.

16.4 Environmental Filtering and Host Specificity

Séne et al. (2015) proposed that the impoverished EcM fungal richness detected in Guadeloupe could partly result due to a founder effect and isolation from mainland. Although our sampling intensity is too low for comprehensive statistical comparison, the mainland and Guadeloupe sea grape communities harboured comparable EcM fungal richness, largely refuting this hypothesis. In spite of phylogenetic and geographical proximity, the absence of shared OTUs with inland *Coccoloba* species from neotropical forest, which harboured higher EcM diversity (Tedersoo et al. 2010b), supports the hypothesis of environmental filtering, also proposed by Séne et al. (2015) as an alternative. This explanation coincides well with the fact that vast majority of the SHs detected were exclusively associated with the sea grape. Similarly, *Pisonia grandis* (Nyctaginaceae) inhabiting small and often guano-rich Indian Ocean and Pacific islands harbours species poor and highly specific EcM assemblage (Suvi et al. 2010; Hayward and Horton 2012). Therefore, putative host specificity in such stressful habitats is most likely bounded with environmental filtering (but see Hayward and Horton 2012). The strong intrageneric ecological specificity in EcM fungi associated with *Coccoloba* contrast to the largely genus-level specific EcM fungi of *Alnus* (Põlme et al. 2013). Interestingly, environmental filtering is likely to have an important role in driving specificity of EcM interactions in both cases (Huggins et al. 2014). Nevertheless, we are unable to confidently disentangle the cause and consequence between the ecological host specificity and environmental filtering, because genetic and physiological compatibility between host and symbiont is likely to evolve mutually in extreme habitats over extended periods of time. Séne et al. (2015) pointed out that EcM origin in *Coccoloba* is relatively recent and this also holds true for *Pisonia* (Chap. 19), possibly explaining low EcM diversity in both groups. The fact that numerous EcM host taxa, with much broader range of mycobionts, have diverged in a comparable time frame (Chap. 19), makes this hypothesis disputable. Taken together, the hypothesis of strong environmental filtering seems the most plausible explanation for the low fungal diversity in *C. uvifera*.

16.5 Biogeography of Thelephoraceae

Using the PlutoF workbench, we were able to recover 832 sequences belonging to the */tomentella-thelephora* lineage originating from eastern North America, northern South America and Central America. After removal of redundant sequences originating from the same host and study site, 525 sequences were subjected to a

phylogenetic analysis (Fig. 16.3). In the large Thelephoraceae phylogram, sea grape-associated sequences clustered together more commonly with sequences originating from Central America and North America rather than with those from South America. This conflicts with the putative South American origin of the genus (Raven and Axelrod 1974; Chap. 20) and previously established patterns in Russulaceae (De Crop et al. 2017) and Sclerodermataceae (Wilson et al. 2012) putatively associating with *Coccoloba* spp. In terms of host identity, Thelephoraceae sequences associated with *C. uvifera* most often clustered together with sequences from *Pinus* spp. and to a lesser extent with those from *Quercus* spp. This suggests potential host shifts from phylogenetically distant host taxa. Previous studies focusing on the mycobionts of introduced plants support that host shifts for EcM fungi may occur between distantly related taxa such as Fagales and Pinales in a very short time frame (Bahram et al. 2013). Currently, the distribution of *Coccoloba* spp. overlaps with that of *Pinus* spp. and Fagales from Central Mexico to Nicaragua and in Cuba (Chap. 20). Furthermore, the fossil record indicates much greater overlap among the geographic range of Pinales, Fagales and *Coccoloba* during the Oligocene and Eocene (Gray 1960; Graham and Jarzen 1969), when *Coccoloba* spp. were distributed up to Central USA in the north, whereas Fagales spp. were more widely distributed in the Caribbean islands. In particular, *C. uvifera* shares its habitat with *Pinus* spp. in coastal sand dunes, although these species do not co-exist in present-day communities, except perhaps in the Bahamas. Nevertheless, the results of phylogenetic analysis should be interpreted with caution, because South America remains relatively under sampled compared to Central America and especially North America, which may slightly overestimate the links between Pinaceae and *C. uvifera* and particularly the genus *Coccoloba* in general.

We also tested, whether the habitats historically influenced by Pinaceae and Fagales (Florida, Costa Rica, Mexico, Cuba) harbour more OTUs of Thelephoraceae shared with northern hosts than the sites far from natural Pinaceae and Fagales habitats (Guadeloupe, Colombia, French Guiana). Contrary to our expectations, there were no differences between the sites with shared and unshared habitats ($n = 34$; $\chi^2 = 0.123$; $P = 0.726$). The wide distribution of EcM fungi of potentially northern temperate origin indicates their effective dispersal and good adaptation to tropical climate.

16.6 Conclusions

Our regional-scale study provides strong evidence that sea grape harbors distinct and relatively species-poor EcM fungal communities throughout its range. These associated fungi are highly distinct from the mycobionts of other *Coccoloba* spp., suggesting that strong environmental filtering due to salinity and perhaps high pH may cause the high observed ecological specificity. Close affinities of *C. uvifera*-associated *Tomentella* spp. with those from the phylogenetically distant Pinaceae

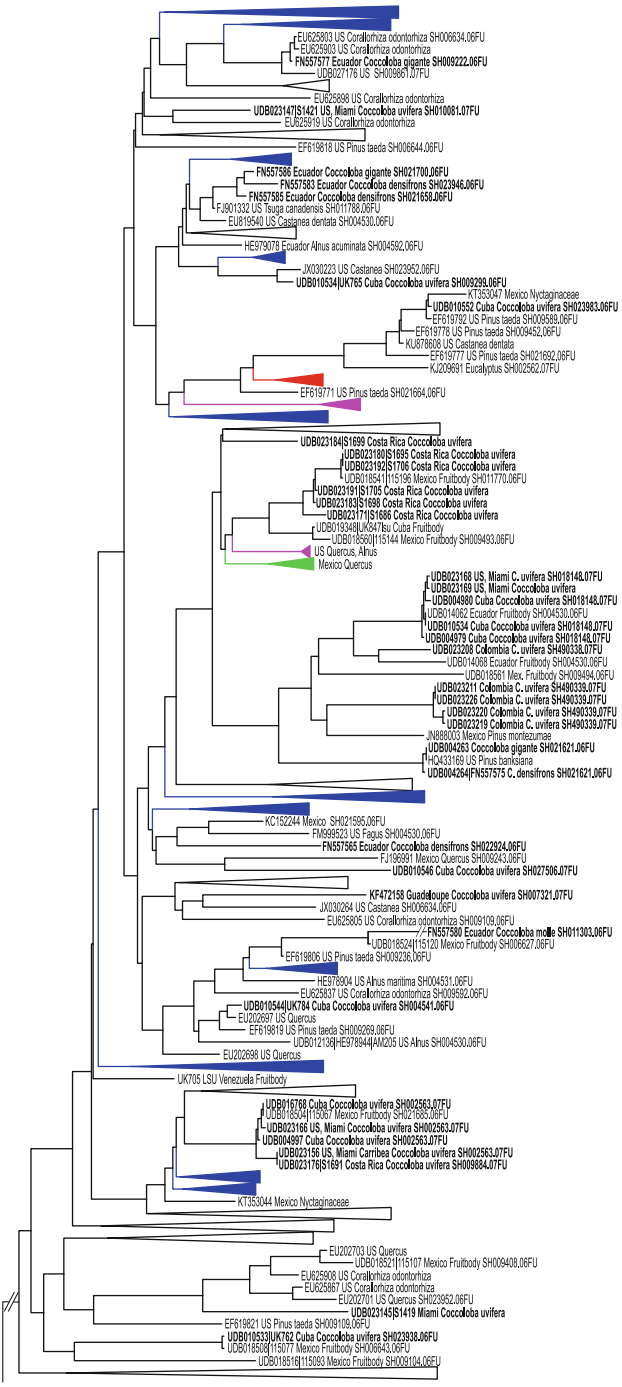

Fig. 16.3 Maximum likelihood phylogram of Thelephoraceae sequences from South, Central and Northeast America. Clades that do not contain *Coccoloba* associated Thelephoraceae sequences have been collapsed. Clades highlighted in *purple*, *red* and *green* represent Northeast, Central and South American groups, respectively. *Blue*, *yellow* and *orange* represent the mixture of North and

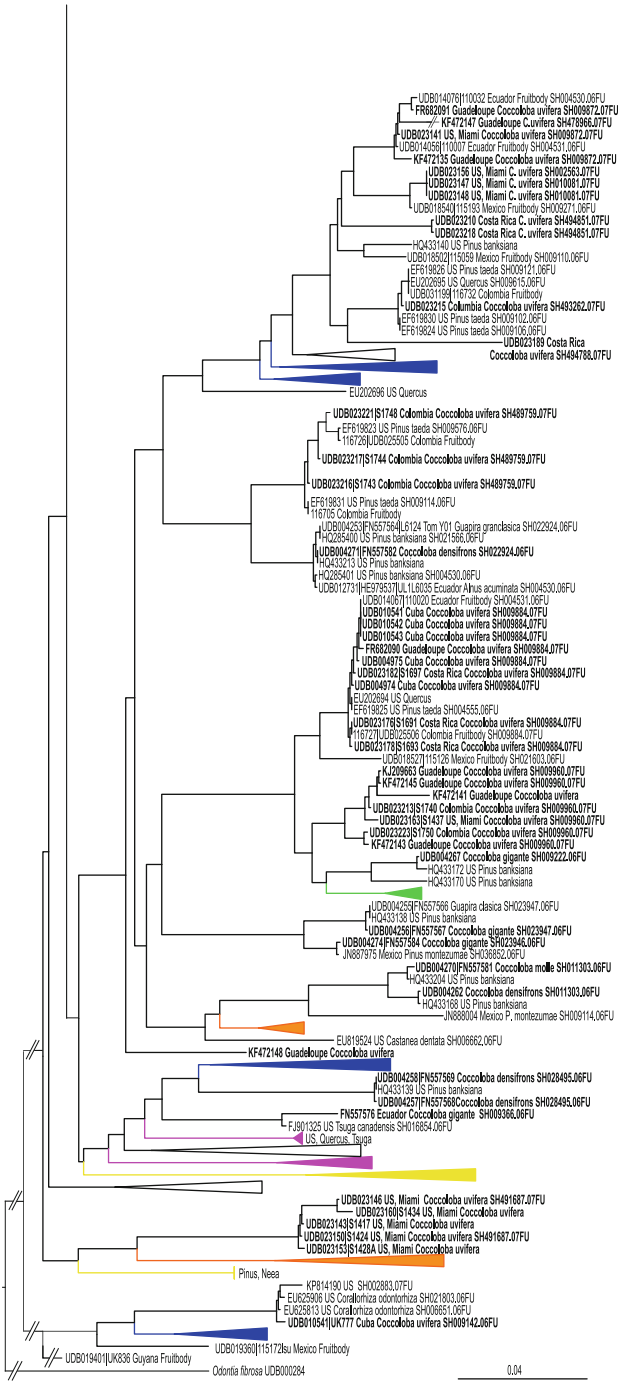

Fig. 16.3 (continued) Central American groups, North and South American groups, and Central and South American groups, respectively. *Transparent clades* represent a mixture of all three regions. *Bold* names indicate Thelephoraceae sequences associating with various *Coccoloba* species. The sequence name includes accession number, country, associating host species and species hypothesis (if available)

and Fagaceae hosts supports multiple host shifts and/or broadening of host range due to more commonly shared habitats in the past. Future phylogeographic studies including more samples from South America are needed to enlighten the evolutionary history of EcM symbiosis in Central and South America.

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References

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K, Peintner U, Kõljalg U (2010a) The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytol 186:281–285

Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M, Kurina O, Ostonen I, Jõgeva J, Halapuu S, Põldmaa K, Toots M, Truu J, Larsson K-H, Kõjlag U (2010b) PlutoF—a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. Evol Bioinforma 6:189–196

Bahram M, Põlme S, Kõljalg U, Zarre S, Tedersoo L (2012) Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. New Phytol 193:465–473

Bahram M, Kõljalg U, Kohout P, Mirshahvaladi S, Tedersoo L (2013) Ectomycorrhizal fungi of exotic pine plantations in relation to native host trees in Iran: evidence of host range expansion by local symbionts to distantly related host taxa. Mycorrhiza 23:11–19

Bandou E, Lebailly F, Muller F, Dulormne M, Toribio A, Chabrol J, Courtecuisse R, Plenchette C, Prin Y, Duponnois R, Thiao M (2006) The ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape (*Coccoloba uvifera* L.) seedlings. Mycorrhiza 16:559–565

Bereau M, Gazel M, Garbaye J (1997) Les symbioses mycorhiziennes des arbres de la foret tropicale humide de Guyane francaise. Can J Bot 75:711–716

Colwell RK (2013) EstimateS: statistical estimation of species richness and shared species from samples. Version 9. User's guide and application published at: http://purl.oclc.org/estimates

De Crop E, Nuytinck J, Van de Putte K, Wisitrassameewong K, Hackel J, Stubbe D, Hyde KD, Roy M, Halling RE, Moreau PA, Eberhardt U (2017) A multi-gene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. Persoonia 38:58–80

Graham A, Jarzen DM (1969) Studies in neotropical paleobotany. I. The Oligocene communities of Puerto Rico. Ann Mo Bot Gard 56:308–357

Gray J (1960) Temperate pollen genera in the Eocene (Claiborne) Flora, Alabama. Science 132:808–810

Hayward JA, Horton TR (2012) Edaphic factors do not govern the ectomycorrhizal specificity of *Pisonia grandis* (Nyctaginaceae). Mycorrhiza 22:647–652

Howard RA (1960) Studies in the genus *Coccoloba* IX, a critique of the South American species. J Arn Arbor 41:357–390

Howard RA (1961) Studies in the genus *Coccoloba*, X. New species and a summary distribution in South America. J Arn Arbor 42:87–95

Huggins JA, Talbot J, Gardes M, Kennedy PG (2014) Unlocking environmental keys to host specificity: differential tolerance of acidity and nitrate by *Alnus*-associated ectomycorrhizal fungi. Fungal Ecol 12:52–61

Ishida TA, Nara K, Hogetsu T (2007) Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. New Phytol 174:430–440

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson L, Lindahl BD, Lücking R, Martin MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KB, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson K-H (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277

Kreisel H (1970) El papel de los hongos en la veetacion forestal de Cuba. Bol Soc Mex Mic 4:39–43

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30:3276–3278

Morris MH, Smith ME, Rizzo DM, Rejmánek M, Bledsoe CS (2008) Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. New Phytol 178:167–176

Moyersoen B (1993) Ectomicorrizas i micorrizas vesiculo-arbusculares en Caatinga Amazonica del Sur de Venezuela. Sci Guaianae 3:1–82

Pegler DN (1983) Agaric Flora of the lesser Antilles. Her Majesty's stationary Office, London (Kew Bull. Additional Ser. IX)

Põlme S, Bahram M, Yamanaka T, Nara K, Dai YC, Grebenc T, Kraigher H, Toivonen M, Wang PH, Matsuda Y, Naadel T (2013) Biogeography of ectomycorrhizal fungi associated with alders (*Alnus* spp.) in relation to biotic and abiotic variables at the global scale. New Phytol 198:1239–1249

Price MN, Dehal PS, Arkin AP (2010) FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490

Raven PH, Axelrod DI (1974) Angiosperm biogeography and past continental movements. Ann Mo Bot Gard 61:539–673

Roy M, Schimann H, Braga-Neto R, Da Silva RA, Duque J, Frame D, Wartchow F, Neves MA (2016) Diversity and distribution of ectomycorrhizal fungi from Amazonian lowland white-sand forests in Brazil and French Guiana. Biotropica 48:90–100

Séne S, Avril R, Chaintreuil C, Geoffroy A, Ndiaye C, Diédhiou AG, Sadio O, Courtecuisse R, Sylla SN, Selosse MA, Bâ A (2015) Ectomycorrhizal fungal communities of *Coccoloba uvifera* (L.) L. mature trees and seedlings in the neotropical coastal forests of Guadeloupe (Lesser Antilles). Mycorrhiza 25:547–559

Singer R, Araujo I, Ivory MH (1983) The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. Nova Hedw 77:1–352

Suvi T, Tedersoo L, Abarenkov K, Beaver K, Gerlach J, Koljalg U (2010) Mycorrhizal symbionts of *Pisonia grandis* and *P. sechellarum* in Seychelles: identification of mycorrhizal fungi and description of new *Tomentella* species. Mycologia 102:522–533

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U (2008) Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490

Tedersoo L, May TW, Smith ME (2010a) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M (2010b) Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. ISME J 4:465–471

Tedersoo L, Bahram M, Toots M, Diedhiou AG, Henkel TW, Kjøller R, Morris MH, Nara K, Nouhra E, Peay GK, Polme S, Ryberg M, Smith ME, Kõljalg U (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Mol Ecol 21:4160–4170

Tedersoo L, Mett M, Ishida TA, Bahram M (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytol 199:822–831

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Villarreal-Ruiz L, Vasco-Palacios A, Quang Thu P, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Ratkowsky D, Pritsch K, Riit T, Põldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo L, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, De Kesel A, Dang T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K (2014) Global diversity and geography of soil fungi. Science 346:1078

Tedersoo L, Harend H, Buegger F, Pritsch K, Saar I, Kõljalg U (2015) Stable isotope analysis, field observations and synthesis experiments suggest that *Odontia* is a non-mycorrhizal sister genus of *Tomentella* and *Thelephora*. Fungal Ecol 11:80–90

Tedersoo L, Liiv I, Kivistik PA, Anslan S, Kõljalg U, Bahram M (2016) Genomics and metagenomics technologies to recover ribosomal DNA and single-copy genes from old fruitbody and ectomycorrhiza specimens. MycoKeys 13:1–20

Wilson AW, Binder M, Hibbett DS (2012) Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletales, Basidiomycota). New Phytol 194:1079–1095