Mycological Progress (2019) 18:1231–1240
https://doi.org/10.1007/s11557-019-01519-w

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ORIGINAL ARTICLE

Are Trechisporales ectomycorrhizal or non-mycorrhizal root endophytes?

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Received: 18 March 2019 / Revised: 19 August 2019 / Accepted: 22 August 2019

Abstract

Trechispora (Hydnodontaceae) is considered as a soil-inhabiting fungus. However, some species in the genus are frequently forming basidiomes on soil, a typical feature of ectomycorrhizal fungi. Ectomycorrhizal basidiomes are found in neotropical and subtropical region, but taxonomical information and DNA sequences of root fungi and basidiomes from native Atlantic Rainforest are poorly reported. Basidiomes and soil samples including roots, humus layer, and mineral soil were collected in the Atlantic Rainforest, in Florianópolis (South of Brazil). Sequences of the ITS region were obtained from all sample types and subjected to phylogenetic reconstruction. Two sequences amplified from apparently ectomycorrhizal roots belonged to *Trechispora* and suggested a root-associated ecology, at least biotrophic and possibly ectomycorrhizal. The analysis of isotope abundance in the same Brazilian site and in French Guiana showed that *Trechispora thelephora* has high ^{15}N abundance and is often intermediate between ectomycorrhizal and saprotrophic species in ^{13}C abundance. This is congruent with a plant biotrophic ecology, perhaps ectomycorrhizal. Future investigations in subtropical regions are needed to determine whether such a mode of nutrition is widespread among *Trechispora*.

Keywords Atlantic rainforest · Biotrophic nutrition · Ectomycorrhizal fungi · Isotopic analysis · ITS · Phylogenetic analysis

Introduction

Trechispora P. Karst. (1980) belongs to Hydnodontaceae (Trechisporales) and includes about 46 species worldwide (Kirk et al. 2008). *Trechispora* basidiomes are resupinate, effuse-reflexed, or stipitate-pileate. They exhibit a wide range of hymenophore configurations (smooth, hydnoid and poroid representatives) and the hyphal system can be monomitic or dimitic. Micromorphological features include clamp connections, a fragile context, and absence of cystidia and ellipsoid, usually ornamented spores (Liberta 1973; Larsson 1994, 1996). *Trechispora* spp. are traditionally considered as soil-

Section Editor: Marc Stadler

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11557-019-01519-w) contains supplementary material, which is available to authorized users.

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inhabiting saprotrophic fungi (Hibbett et al. 2014). However, an unnamed fungus with affinities to Trechisporales was recently shown to form ericoid mycorrhizas (Vohnik et al. 2012). Moreover, Dunham et al. (2007) reported *Trechispora* mycelium associated with a root, indicating possible ectomycorrhizal (EcM) associations. In a study of EcM communities after transplanting *Asteropeia mcphersonii* seedlings from the wild to a nursery in Madagascar, Henry et al. (2017) found, after 8 months of growth, a tremendous increase in the abundance of Trechisporales on the roots, although the EcM status remained unclear in this study.

Ectomycorrhizal fungi are important in ecosystems due to their mutualistic association with many groups of plants (Heijden et al. 2015). In the EcM association, fungi form a hyphal mantle around root tips and penetrate between cortical cells to produce the so-called Hartig net (Smith and Read 2008). Fungi provide water and nutrients such as phosphorus or nitrogen while the plant provides sugars (Halling 2001; Smith and Read 2008; Heijden et al. 2015). EcM fungi also promote nutrients interchange between plants through their mycelium (Selosse et al. 2006), help the establishment of seed and seedling development, and protect plants from root pathogens (Tedersoo et al. 2010a). Most studies on EcM fungi are in the boreal and northern temperate regions where EcM fungi are associated with Pinaceae, Betulaceae, and Fagaceae. The distribution of EcM fungi in tropical regions remains poorly understood (Alexander and Selosse 2009; Roy et al. 2017; Corrales et al. 2018), and common EcM plant host genera in these habitats are *Guapira* Aubl., *Neea* Ruiz and Pav. and *Pisonia* L. (Nyctaginaceae); *Aldina* Endl., *Dicymbe* Spruce ex Benth., as well as other legumes (Fabaceae), *Pakaraimaea* Maguire and P.S. Ashton (Dipterocarpaceae); *Coccoloba* P. Browne (Polygonaceae) and *Gnetum* L. (Gnetaceae) (Moyersoen 2006; Suvi et al. 2010; Tedersoo et al. 2010b; Henkel et al. 2012).

The first studies of EcM fungi and plants tracked the mycelium from the basidiomes to the roots, comparing hyphae found on the roots with the ones on the basidiomes, and used seed germination experiments to determine the presence or absence of EcM fungi (Rinaldi et al. 2008; Tedersoo et al. 2010a). Most EcM fungi studies now use metagenomics and next-generation sequencing of environmental samples (roots and soil), with the internal transcribed spacer (ITS rDNA) barcoding sequence (Rinaldi et al. 2008; Schoch et al. 2012) which is widely used in fungi molecular ecology studies (e.g., Haug et al. 2005; Wang and Qiu 2006; Suvi et al. 2010; Alvarez-Manjarrez et al. 2016). Published phylogenetic analysis based on ITS identified between 82 and 86 independent EcM lineages in Agaricomycetes, Endogonomycetes, and Pezizomycetes, some of which are rare or geographically restricted (Brundrett and Tedersoo 2018).

The 175 species of EcM fungi (Basidiomycota) currently recorded from Brazilian native forests (Roy et al. 2016) were essentially described from fruiting bodies deposited in herbaria. There are only few mentions of EcM root tip observations in natural habitats (although Singer and Araujo (1979) mentioned contact ectomycorrhizae), and none was confirmed by barcoding the fungus. Indeed, most published studies using environmental samples (roots or soil) were made on introduced pines and eucalyptus plantations (Yokomizo 1986; Giachini et al. 2000; Andrade et al. 2000; Giachini et al. 2004; Sulzbacher et al. 2016). In South America, the diversity of the EcM associated with native plant of the Atlantic Forest remains unknown. Recent collections in these forests have often included *Trechispora* basidiomes, especially the widely distributed *Trechispora thelephora* (Albee-Scott and Kropp 2010). This species is frequently found on sandy soils, where other EcM species also grow. This distribution suggests a different habit compared with other *Trechispora* species that can often grow as crusts on dead wood. The principal objective of this study was to test the EcM status of some subtropical *Trechispora* species. We used two strategies: (1) morphological and phylogenetic analyses of EcM root tips and *Trechispora* basidiomes and (2) isotopic analyses and comparison of *Trechispora* signature with other basidiomes collected at the very same site. The latter aspect takes advantage of the fact that EcM fungi have distinctive isotopic natural abundances for ^{15}N and ^{13}C compared with saprotrophic fungi (Mayor et al. 2009). EcM fungi preferentially transfer ^{14}N to the host plant and/or access soil N sources already poor in ^{14}N and are therefore enriched in ^{15}N compared with saprotrophs; they receive preferentially ^{12}C-enriched sugars from the plant and are therefore depleted in ^{13}C compared with saprotrophs (Zeller et al. 2007; Hobbie et al. 2012). For isotopic comparison, we extended our sampling to Amazonian forests of French Guiana where *Trechispora* basidiomes also occur (Schimann et al. 2019), often close to EcM hosts.

Materials and methods

Root tip sampling: study site and design

Study sites are located in the East of the Santa Catarina Island, municipality of Florianópolis, state of Santa Catarina. The samples were collected in two localities: Morro da Lagoa (defined as Atlantic Rainforest; S27° 35′ 20″ W48° 28′ 23′) and Dunas da Lagoa da Conceição (white sand dunes *restinga*, S27° 36′ 47″ W48° 27′ 10′). Collections were made once a month from September 2015 to June 2016. To search for root tips, we established five collection points in each locality at the base of *Guapira opposita* trees, known to be an EcM host. Five hundred grams of soil with roots were collected at each point. Each sample was a single soil core, sampled to 10 cm depth without the leaf litter layer. In total, we collected 10 soil samples per month. Samples were packed in plastic bags to

prevent desiccation. In the laboratory, roots were washed with tap water and root tips were observed using a dissecting microscope. EcM were tentatively recognized by the hyphal mantle and root modifications. EcM morphotypes were photographed and then dried in silica gel.

Basidiome sampling and morphological analysis

To identify EcM from root tips, surrounding basidiomes were also collected around soil samples at Morro da Lagoa da Conceição, (Florianópolis, southern Brazil). Collections were identified using standard morphological analysis (Largent et al. 1977; Mueller et al. 2004). The specimens were dried in a food dehydrator for 8 h at approximately 40 °C. Microscopic features were studied from dried material by mounting free-hand sections of the basidiomata in 5% KOH, Melzer's reagent, and Congo red. The specimens were deposited at the herbaria FLOR, at the Universidade Federal de Santa Catarina, Florianópolis, Brazil.

DNA extraction and sequencing

DNA was isolated from dried EcM and basidiomes. Root samples were disrupted using Precellys 24 (BioAmerica Inc). DNA was extracted with DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, but only 50 μl of AE buffer was added in step 10 to increase the final DNA concentration. DNA from basidiomes was extracted according to Doyle and Doyle (1987) modified by Góes-Neto et al. (2005). Nuclear ITS was amplified with primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). PCR final reaction volume was 30 μl and consisted of 15 μl of Master mix (Promega®), 1 μl of each primer (10 pMol) and 13 μl of DNA (1:10 dilution). DNAs were submitted to initial denaturation (5 min at 95 °C) followed by 35 amplification cycles (30 s at 95 °C, 30 s at 61 °C, 1 min at 72 °C) and a final extension (5 min at 72 °C). PCR products were visualized on 1% agarose gels and purified using polyethylene glycol protocol. Sequencing was performed in the Renné Rachou, Fundação Oswaldo Cruz (Fiocruz) investigation center (Belo Horizonte, Minas Gerais).

Phylogenetic analysis

Sequence were edited with Geneious, v6.8 (Kearse et al. 2012) and aligned with published sequences from GenBank and UNITE database with two Brazilian sequences from sclerotia structures recently related to Trechisporales (Sulzbacher et al. 2017) that were also included in the analysis (Table 1) using software MAFFT v7 (Katoh and Standley 2013). Manual adjustment was performed in MEGA 6.06 (Tamura et al. 2013). Substitution nucleotide model was estimated in jModelTest v2.1.6 (Darriba et al. 2012; Guindon and Gascuel 2003), available in CIPRES Science Gateway (Miller et al. 2010, http://www.phylo.org/), using Bayesian information criterion (BIC). Phylogenetic analysis using the Bayesian inference was performed using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003) available in CIPRES Science Gateway with 4 MCMC, 20 million generations, a burn-in of 25%, and using a three-partitioned model (ITS1, 5.8S, ITS2). The consensus tree was edited in FigTree v1.0.4. Posterior probabilities greater than 0.95 were considered significant. Maximum Likelihood analyses were performed under a GTRGAMMA model, using RAxML v 8.1.24 (Stamatakis 2006) available in CIPRES Science Gateway. To access the reliability of the nodes, 1000 multi-parametric bootstrapping replicates were computed. Bootstrap values greater than 70 were considered significant. Outgroup were three Hydnodontaceae species: *Porpomyces mucidus*, *Fibrodontia alba*, and *F. gossypina.* Alignment sequences are available in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23450).

Isotopic analysis: study sites and design

Natural abundance of ^{15}N and ^{13}C was established for Trechisporales species as well as for reference species from known guilds (namely EcM or saprotrophic) and growing at the same place. Samples consisted of tissues of basidiomes obtained from Morro da Lagoa da Conceição, Florianópolis, southern Brazil (site 3). Collections were made in November (spring in the Southern hemisphere), December, and February (summer). Specimens were also collected in two additional sites from French Guiana where basidiomes of *Trechispora* were frequently observed: site 1 (4° 05′ 29.9″ N 52° 40′ 38.0″ W) and site 2 (4° 04′ 47.9″ N 52° 41′ 05.8″ W), both located at Nouragues field station and visited in July 2011 and 2013 (summer). Sites in French Guiana were characterized by granite substratum, a very thin soil and a low canopy compared with surrounding forests (< 15 m). Our sampling encompassed basidiomes related to *Ramaria* spp., a clade in which basal species are likely saprotrophs, whereas the larger remaining part forms ectomycorrhiza (Hosaka et al. 2006; Rinaldi et al. 2008; Agerer et al. 2012). On both sites, ectomycorrhizal hosts were present such as *Coccoloba parimensis*, *Neea ovalifolia*, and *Guapira eggersiana* at site 1 and *G. salicifolia* at site 2. On each site, in a radius of 3 m around *Trechispora* basidiomes, all available basidiomes were collected and identified at least at the genus level. A minimum of five different species were collected per site (Online resource 1), and one to five basidiomes per species (mean, 3.68) were sampled and dried immediately after collection overnight in Gaz Herbarium oven. Three small portions of each basidiomes (cap or uppermost part whenever basidiomes were erected) were pooled to reach ca. 0.2 g. These

Table 1 *Trechispora* specimens and ITS sequence data used in the phylogenetic analysis

Taxa	Voucher	Country	GenBank/Unite accession numbers	Source
Trechispora byssinella	BE 135	Sweden	UDB024785	Basidiome
T. cohaerens	TU 115568	Estonia	UDB016421	Basidiome
T. cohaerens	TU 110332	Estonia	UDB008249	Basidiome
T. cyatheae	FR0219442	Reunion	UDB024014	Basidiome
T. cyatheae	FR0219443	Reunion	UDB024016	Basidiome
T. echinocristallina	FR0219445	Reunion	UDB024018	Basidiome
T. echinocristallina	TU 110414	Papua New Guinea	UDB013050	Basidiome
T. hymenocystis	TL 11112	Denmark	UDB000778	Basidiome
T. hymenocystis	TU 117137	Estonia	UDB024146	Basidiome
T. invisitata	RGC 30–9	Sweden	UDB024810	Basidiome
T. laevis	TU 115551	Estonia	UDB016406	Basidiome
T. stevensonii	TU 115499	Estonia	UDB016467	Basidiome
T. thelephora	MVL 109	Brazil	KY769868	Basidiome
T. thelephora	1984a AMV	Colombia	KF937368	Basidiome
T. thelephora	1820 AMV	Colombia	KF937369	Basidiome
Trechispora sp.	TU 110068	Ecuador	UDB014099	Basidiome
Trechispora sp.	TU 110054	Ecuador	UDB014091	Basidiome
Trechispora sp.	TU 110037	Ecuador	UDB014080	Basidiome
Trechispora sp.	TU110042	Ecuador	UDB014082	Basidiome
Trechispora sp.	TU 108305	Gabon	UDB016782	Basidiome
Trechispora sp.	R15	Brazil	KY769820	Root
Trechispora sp.	R23	Brazil	KY769827	Root
Trechispora sp.	Sulz 345	Brazil	LT594980	Sclerotium
Trechispora sp.	Sulz 346	Brazil	LT594981	Sclerotium
Fungi	SD-120.2	USA	DQ365644	Root
Hydnodontaceae	R3	Brazil	KY769812	Root
Porpomyces mucidus	TU 109467	Estonia	UDB024361	Basidiome
Fibrodontia alba	TNM 24944	Taiwan	KC928274	Basidiome
Fibrodontia gossypina	GEL 5042	Reunion	DQ249274	Basidiome

subsamples were then ground in 1.5-ml Eppendorf tubes using two 2-mm-diameter glass balls in a Retsch MM301 vortex mixer (Retsch GmbH and Co.). ^{13}C and ^{15}N abundances were measured using an online continuous flow CN analyzer (NA 1500; Carlo Erba) coupled with an isotope ratio mass spectrometer (Delta S; Finnigan) at SSMIM (Muséum National d'Histoire Naturelle, Paris). Relative isotope abundances are denoted as δ values, which were calculated according to the following equation:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}}-1\right) \times 1000\ [‰]$$

where R_{sample} and R_{standard} are the ratios of heavy isotope to light isotope of the sample and the respective standard (Vienna Pee-Dee Belemnite or atmospheric N_2 for C and N, respectively). The standard deviations of the replicated standard samples were 0.022‰ for ^{13}C and 0.215‰ for ^{15}N. The isotopic signatures were compared within sites between species and guilds of fungi through an ANOVA followed by Tukey post hoc tests.

Results

One hundred soil samples from Santa Catarina island were collected and 48 root tips were isolated. Of these, 23 morphotypes were successfully sequenced. Seven were identified as Ascomycota and 16 as Basidiomycota (Online resource 2). From the Blast analysis, two morphotypes fell within *Trechispora* (R15 and R23, respectively, KY769820 and KY769825; sequences differing in 0.97%) and one morphotype within Hydnodontaceae (R3, KY769812) with no clear genus affiliation. All *Trechispora* morphotypes were found in spring only (September–October)

in both localities. Additionally, it was observed that two sclerotia structures related to *Trechispora* spp. (LT594980 and LT594981; sequences differing in 1.11%). We collected 11 basidiomes attributed to eight taxa identified as *Clavulinopsis* sp., *Lachnocladium* sp. 1, *Lachnocladium* sp. 2, *Lactifluus* aff. *venezuelanus*, *Leucoagaricus* sp., *Russula puiggarii*, *Suillus* sp., and *Trechispora thelephora*.

Trechispora thelephora morphological description

The specimens MLV109 and MVL113 (Fig. 1a, b) were collected in Morro da Lagoa and identified as *T. thelephora*. Basidiomes pileate-stipitate, upper surface light yellow brown, glabrous. Context thin pallid and not changing color when cut. Hymenophore hydnoid pinkish, teeth 1.0–0.5 mm in length, running part way down the stipe. Stipe glabrous, concolorous with the upper surface of the basidiome. Hyphal system monomitic, generative subhymenial hyphae with thin walls and clamped. Basidia clavate, with four sterigmata 14–26 × 5–7 μm. Basidiospores ellipsoid, echinulate, 4.0–5.0 × 3.4–4.5 μm, non-amyloid. Only for MVL109 was possible to obtain a readable ITS sequence.

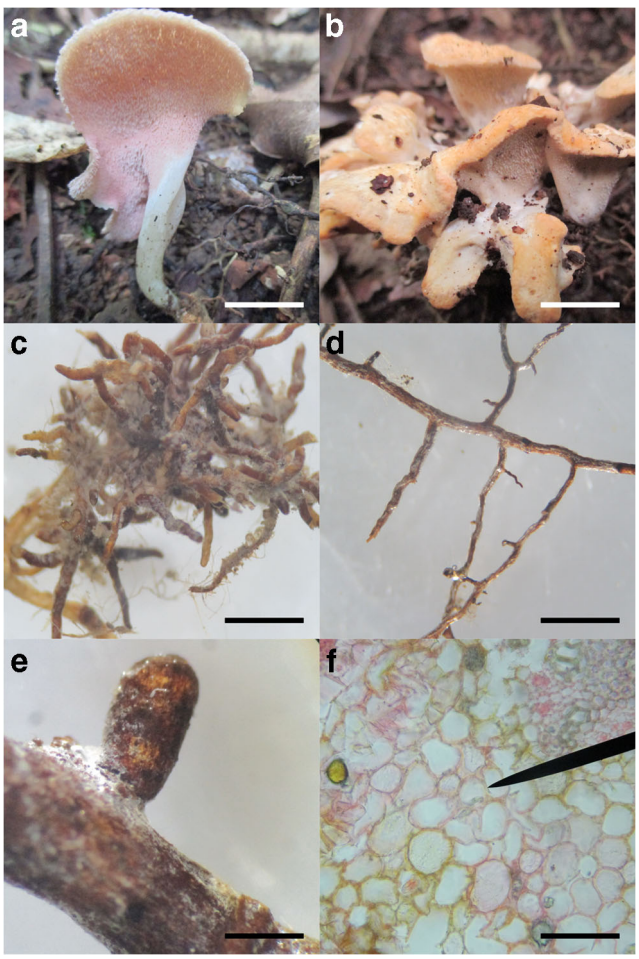

Fig. 1 Trechisporales basidiomes and root colonization. **a** MVL 109 *Trechispora thelephora* basidiome. **b** MVL113 *T. thelephora* basidiome. **c** EcM root tip R3. **d** EcM root tip R15. **e** EcM root tip R23. **f** R23 cross section with root endophytic hyphae (arrow). Scale bars: 2 cm (**a**, **b**), 2 mm (**c**–**e**), and 20 μm (**f**)

Morphology of root colonization

Morphotype R3 has intricate brown roots and a gray mycelium layer on the root surface (Fig. 1c). Morphotype R15 has long thin light brown roots and a white mycelium thin layer on the root surface, forming a discontinuous mantle (Fig. 1d). Morphotype R23 has a mantle similar to morphotype R15; however, morphotype R23 has dark brown short thick roots (Fig. 1e). In morphotype R23 cross section, we observed very few septate intracellular hyphae on the root cortex (Fig. 1f), but not a Hartig net which would confirm an EcM association. Intraradical colonization was very weak, with very few hyphae. Yet root tissues were fleshy and alive, which support a biotrophic colonization.

Molecular analysis and phylogenetic positioning in Trechisporales

Bayesian inference (Fig. 2) and maximum likelihood topologies (Online resource 3) showed that morphotypes R15 and R23 are sisters to each other and cluster in an unresolved order with *T. hymenocystis* and *T. thelephora* (for this whole cluster, pp. = 1/bs = 99%). The *T. thelephora* cluster included the basidiome specimen MVL109 and two Colombian specimens of *T. thelephora* (pp = 0.99/bs = 71%). The two sclerotium sequences were close to each other and within other *Trechispora* species. Morphotype R3 was part of Hydnodontaceae, but relations with other species and inclusion in the genus *Trechispora* were uncertain.

Isotopic analysis

Trends of values in isotopic abundance were very different depending on the sampling site. At site 1 in "trail to the inselberg" (French Guiana), *T. thelephora* ^{13}C abundance was not distinct from that of other EcM species, but higher than that of saprotrophs (significant difference only for a *Ramaria* species which maybe saprotrophic); its ^{15}N abundance was within the range for EcM fungi, similar to the saprotrophic *Lachnocladium* and significantly higher than the saprotrophic *Ramaria* (Fig. 3a). Thus, at site 1 where EcM species were as expected enriched in ^{15}N but depleted in ^{13}C compared with saprotrophs, *T. thelephora* behaved rather as an EcM species, or intermediately between the two guilds. At site 2 in "trail to the turbine" (French Guiana), *T. thelephora* did not differ in ^{13}C abundance from the saprotrophic *Stipitochaete* and *Hymenochaete*, as well as from EcM *Coltricia* and *Cantharellus* (Fig. 3b); its ^{15}N abundance did not differ from

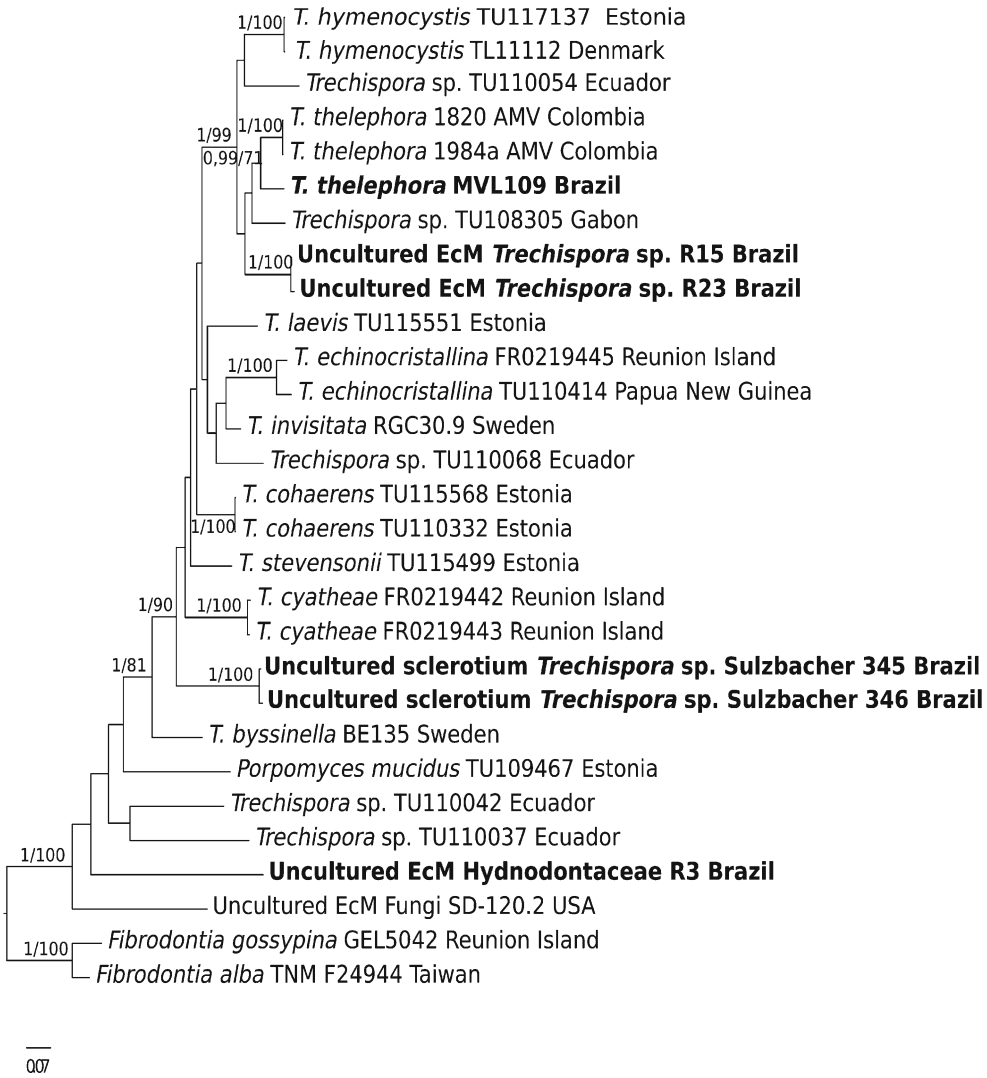

Fig. 2 Bayesian inference tree showing phylogenetic relationships between Brazilian *Trechispora* and other species in the Hydnodontaceae family

saprotrophic species (but *Hymenochaete* and *Lentinus*) and the EcM *Coltricia*, but was significantly lower than the EcM *Cantharellus* and a likely EcM *Ramaria* species (Fig. 3b). Thus, at site 2 where EcM and saprotrophic species were not markedly separated, no clear trend can be proposed for *T. thelephora*. Finally, at site 3, *T. thelephora* ^{13}C abundance was not distinct from saprotrophic *Leucoagaricus* and EcM *Lactifluus* sp.1 and significantly more enriched than saprotrophic *Lachnocladium* and EcM *Lactifluus* sp.2 (Fig. 3c); its ^{15}N abundance was higher than all other fungi from the same site. Thus, at site 3 in Morro da Lagoa (Brazil), where EcM species were as expected enriched in ^{15}N but depleted in ^{13}C compared with saprotrophs, *T. thelephora* behaved as an EcM species or intermediary between the two guilds. *Trechispora thelephora* showed inconsistent positions between sites compared with *Lachnocladium* (at sites 1 and 3), grouping with EcM or placing between EcM and saprotrophic fungi, with high ^{15}N values (see the Online resource 1 for all isotopic values).

Discussion

Our phylogenetic analysis and morphological observations confirmed that at least two root tips were colonized by fungi of the genus *Trechispora* (R15 and R23). Dunham et al. (2007) found an EcM root morphotype identified as *T.* cf. *stellulata* in Oregon, an EcM status supported by other molecular ecological data (Rosenthal et al. 2017). This first observation already suggested that some *Trechispora* species (at least *T. stellulata*) could be EcM, but Rinaldi et al. (2008), in a review of mycorrhizal lineages, still consider the evidence with a question mark; similar doubts were expressed in a review by Tedersoo et al. (2010a). In this context, we report new phylogenetic, morphological, and isotopic data about *Trechispora*.

We observed three root morphotypes colonized by Trechisporales falling at two different phylogenetic positions, including one within the genus *Trechispora*. We could not identify the collected specimens to the species level because

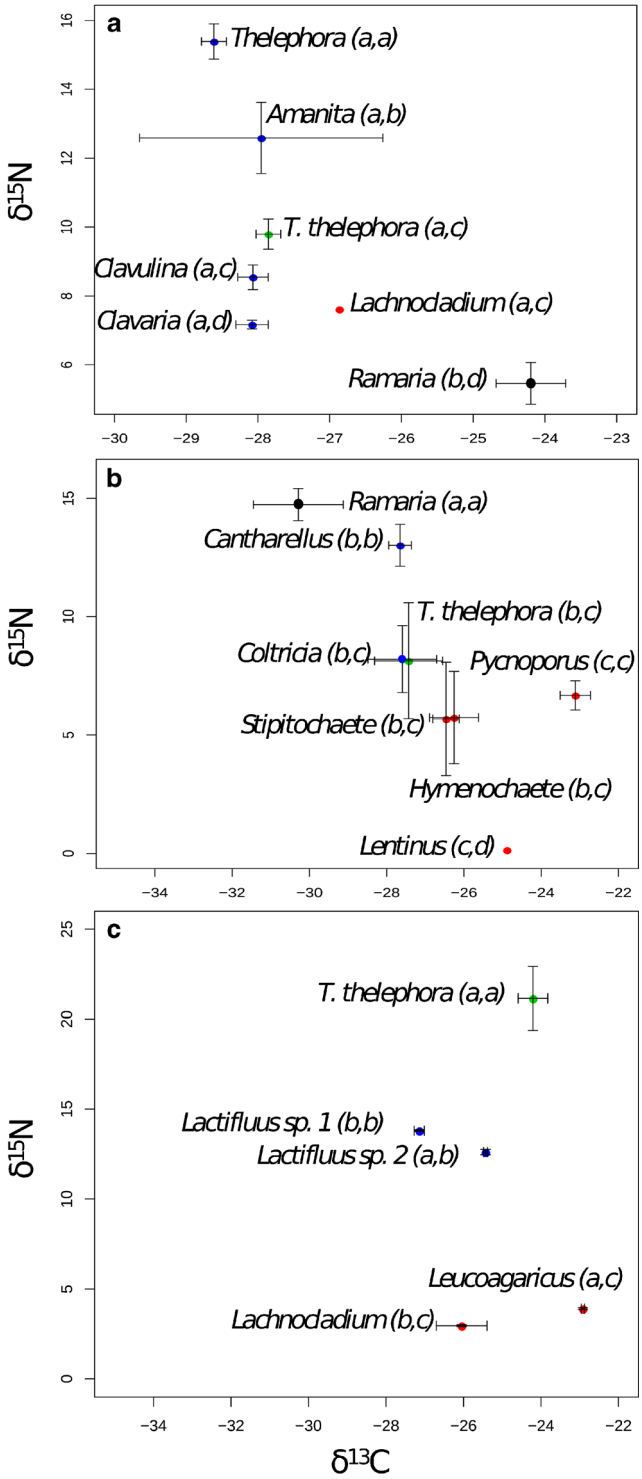

Fig. 3 Natural isotopic (^{13}C and ^{15}N) abundance of Trechisporales (green), together with reference species ectomycorrhizal (blue), wood saprotrophs or parasites (red), and *Ramaria* species that can be ectomycorrhizal or saprotrophs depending on the taxa (black; $n = 5$ repetitions per species). The threes investigated sites are the following: **a** Nouragues field station, "trail to the inselberg"; **b** Nouragues field station, "trail to the turbine"; **c** Morro da Lagoa (BR). Different letters in parentheses denote different significant means for, successively, ^{13}C and ^{15}N, according to Tukey's HSD test at alpha risk of 0.05; bars represent 95% confidence intervals of the mean

existing molecular databases have few reference sequences for neotropical fungi (many sequences used in this study are from European material), and also likely because this family is understudied everywhere. Also in the neotropics, more exploration is pending. Future research must be aimed at identifying *Trechispora*-associated plants in the Brazilian Atlantic Forest and in whole America (Rosenthal et al. 2017). In such a group of fungi where some species are corticioid, with the notable exception of *T. thelephora*, one should be prepared that some species form poorly (or not) visible crust basidiomes. The identification of their plant host range is also a work in progress: according to what is known on neotropical EcM plants, members of Nyctaginaceae have high probabilities to form associations with EcM fungi in Santa Catarina Island (Haug et al. 2005; Tedersoo et al. 2010b; Alvarez-Manjarrez et al. 2018). *Guapira opposita* is the only species of Nyctaginaceae family known in the Lagoa da Conceição dunes (Falkenberg 1999); therefore, this is possibly the symbiotic plant species associated with *Trechispora* at this site.

Are some Trechisporales EcM partners, as claimed by Rosenthal et al. (2017), and does this apply to the observed species? Phylogenetic studies have revealed that EcM associations occur in > 80 lineages of Dikarya (Tedersoo and Smith 2013), and small, poorly abundant groups of EcM fungi likely remain to be discovered. Although the number of root tips collected was low for each morphotype in this study, they provide very similar morphology, with a poorly developed mantle compared with typical EcM mantles from boreal and north temperate regions (Agerer 1997; Smith and Read 2008) and no developed Hartig net. Yet, few hyphae penetrated in the roots among living tissues and were observed in the intercellular spaces. The roots of *Asteropeia mcphersonii* seedlings colonized by Trechisporales in Madagascar (Henry et al. 2017) revealed very similar morphologies, with few intraradical hyphae and loose mantle (Ducousso et al. unpubl. data). On the one hand, it is possible that EcM interactions in the tropics may be devoid of usual EcM morphology, as suggested by the absence of Hartig net in some Caesalpiniaceae (Smith and Read 2008) or even some Nyctaginaceae (Smith and Read 2008; Hayward and Hynson 2014); for example, Kariman et al. (2014) reported a beneficial symbiosis with mineral provision to the plant between the fungus *Austroboletus occidentalis* and *Eucalyptus marginata*, thanks to hyphae localized in rhizospheric soil, and no internal root colonization. On the other hand, we may face a different, non-EcM colonization: Tedersoo et al. (2010a) mention that, although they do not consider the taxon as EcM, root tips are recovered by mycelial mats formed by Trechisporales. The exact status of several tropical root associations is questionable: for example, Corrales et al. (2018) report the case of *Ticodendron incognitum* (Ticodendraceae) that may form symbioses with EcM fungi without a typical EcM morphology. We add here an evidence for root colonization by

Trechispora. We consider that some Trechisporales, such as the ones we observed here, cause a symptom close to the "SRH" category *sensu* Brundrett and Tedersoo (2019), i.e., superficial root hyphae with Hartig net absent.

Isotopic data for *T. thelephora* showed high ^{15}N abundance (as expected for EcM fungi, or above) and grouped for ^{13}C abundance with most EcM fungi at sites 1 and perhaps 2 (but without clear trend at sites 3). This is congruent with an EcM niche, but an endophytic one is also possible. Although the latter guild is poorly documented, endophytic fungi tend to be similarly intermediate between saprotrophic and EcM fungi. By endophytes, we mean organisms that for all or part of their life cycle grow within living plant tissues, causing an unapparent infection, and especially do not form mycorrhizae, nor cause any obvious disease symptoms (Wilson 1995). *Hygrocybe* (waxcaps), which is root endophytes, has isotopic patterns resembling EcM fungi more than saprotrophs, with consistently high ^{15}N abundance that also indicates a different mode of nitrogen nutrition (Tello et al. 2014; Halbwachs et al. 2018). The same isotopic features were claimed for orchid mycorrhizal fungi from the Seredipitaceae, Ceratobasidiaceae, and Tulasnellales (Selosse and Martos 2014, and references therein).

Whatever the exact status of the interaction between Trechisporales and roots, it may fall into interactions that are difficult to affiliate to existing categories: an endophytic strategy in some species of a group (this study) and an EcM status in others (Rosenthal et al. 2017) is not unexpected if one considers the evolution of niches in fungi, which gives sense to the diversity of ecological strategies of closely related species (Selosse et al. 2018). Especially, the occurrence of endophytic and EcM species in a given family is often observed (e.g., Selosse et al. 2009) and is considered to support the "waiting room hypothesis" (i.e., the idea that root endophytism as an evolutionary niche predisposing to sometimes become mycorrhizal; Selosse et al. 2009; Heijden et al. 2015).

Further studies should focus on identifying the plants that are involved in this symbiosis with *Trechispora*, as well as trying to better understand the morphology of the root tip colonization, e.g., using fluorescent in situ hybridization. Our study suggests at least a biotrophic nutrition mode for some Trechisporales taxa. Also, it contributes to increasing the knowledge about fungi and interactions in the Brazilian Atlantic forest, a biodiversity hot spot strongly threatened by habitat loss and fragmentation.

Acknowledgments The first author thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master's scholarship. We thank Lina Ribeiro Venturieri and Cony Decock for help on collecting field trips, Professor Mayara Caddah for help in *Guapira opposita* field identification, and one anonymous reviewer for insightful comments on an earlier version of this paper. Isotopic analyses were made at SSMIM (MNHN Paris) with technical assistance of Denis Fiorillo (UMR 7209 CNRS).

Funding information The sampling in French Guiana was funded by Nouragues field station projects and by Labex grants CEBA (ANR 10-LABX-0025) and TULIP (ANR 10-LABX-0041).

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