

Ectomycorrhizas of *Coltricia* and *Coltriciella* (Hymenochaetales, Basidiomycota) on Caesalpinaceae, Dipterocarpaceae and Myrtaceae in Seychelles

Leho Tedersoo · Triin Suvi · Katy Beaver · Irja Saar

Received: 19 December 2006 / Revised: 19 February 2007 / Accepted: 4 April 2007 / Published online: 10 May 2007
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Abstract Ectomycorrhizal fungi, especially basidiomycetes, have repeatedly evolved from saprotrophic ancestors. Using rDNA internal transcribed spacer and large subunit sequences, we demonstrate that four species of *Coltricia* and *Coltriciella* form ectomycorrhiza with the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpinaceae) as well as the introduced *Eucalyptus robusta* (Myrtaceae) in Seychelles. *Coltricia* and *Coltriciella* species share a thin, orange-brown to dark brown mantle and extremely thick, clampless hyphae. Phylogenetic analyses placed *Coltriciella* monophyletic within *Coltricia*. This study provides further evidence that fruiting habit on dead wood does not indicate saprotrophic lifestyle.

Introduction

Ectomycorrhizal (EcM) fungal symbioses involve ca. 7,000 plant species and 7,000–10,000 species of basidiomycetes and ascomycetes (Taylor and Alexander 2005). In both phyla, EcM fungi have repeatedly arisen from saprotrophic or plant parasitic ancestors as indicated in recent phylogenetic analyses (LoBuglio et al. 1996; Hibbett et al. 2000). Such a switch to EcM lifestyle has evolved between 15 and 18 times within basidiomycetes (Binder et al. 2005).

The hymenochaetoid clade of homobasidiomycetes (sensu Binder and Hibbett 2002) comprises taxa with contrasting lifestyles and fruiting habits. Most members of the hymenochaetoid clade form tough, annual or perennial, conk-shaped to resupinate fruit-bodies on wood (Wagner and Fischer 2002a, b; Larsson et al. 2004, 2006; Binder et al. 2005). These wood-inhabiting taxa are predominantly saprobes or weak plant parasites, causing white rot (Hibbett and Donoghue 2001). Some basal members of the hymenochaetoid clade (e.g. *Rickenella* and *Cantharellopsis*) form biotrophic associations with mosses and produce tiny agaricoid fruit-bodies. Stipitate, more-or-less terrestrial fruit-bodies occur also in the *Coltricia*-*Coltriciella* lineage and in *Hymenochaete damicornis* (Link) Lév (Wagner and Fischer 2002a). However, fruit-bodies of these taxa, particularly of *Coltricia* and *Coltriciella*, are often associated with woody debris (Cunningham 1948; Grand and Vernia 2005), living roots (Corner 1991) or living tree trunks (Aime et al. 2003). Contrary to the observed woody habitat of fruit-bodies, Pachlewski and Chrusciak (1980) reported successful EcM synthesis experiments with *Coltricia perennis* (L.: Fr.) Murrill and *Pinus sylvestris* L., but provided no details of the association. Later, Danielson (1984) synthesised EcM and arbutoid mycorrhiza of *C. perennis* with *Pinus banksiana* Lamb. and *Arctostaphylos uva-ursi* (L.) Spreng, respectively. Danielson described the EcM morphology as highly conspicuous but, despite considerable sampling effort in the field, he failed to demonstrate the EcM habit of *C. perennis* in situ. In addition, fertile basidia formed in pure culture, which raised further doubts as to the true EcM lifestyle of *C. perennis* (Danielson 1984). Following mycelial connections in situ, Thoen and Ba (1989) identified brown, bristly EcM of *Coltricia cinnamomea* (Pers.) Murr. on *Uapaca guineensis* Müll. Arg. in Senegal. The authors also

L. Tedersoo (✉) · T. Suvi · I. Saar
Institute of Botany and Ecology, University of Tartu,
40 Lai Street,
51005 Tartu, Estonia
e-mail: leho.tedersoo@ut.ee

K. Beaver
Plant Conservation Action Group,
P.O. Box 392, Victoria, Mahé, Seychelles

noted the wide distribution of *C. cinnamomea* in many African vegetation types. In support of the potential EcM links of other members *Coltricia* and *Coltriciella*, Aime et al. (2003) observed their fruit bodies exclusively near EcM-forming *Dicymbe* sp. (Caesalpinaceae) in Guayanian neotropical forests that are generally dominated by arbuscular mycorrhizal plants. Contrary to wood rotting members of the hymenochaetoid clade and other taxa, *C. perennis* lacks most of the oxidative decay enzymes (Pachlewski and Chrusciak 1980; Hibbett and Donoghue 2001).

In this study, we use sequence data from the rDNA internal transcribed spacer (ITS) and large subunit (LSU) regions of both EcM root tips and fruit bodies to demonstrate that four species of *Coltricia* and *Coltriciella* form EcM with both native and introduced trees in Seychelles. We provide morphological descriptions of EcMs to facilitate the morphology-based identification of these taxa in further studies.

Materials and methods

Sampling

Root samples were collected in the course of a more inclusive EcM community study in Seychelles between 28 February and 15 March 2006 (Tedersoo et al. 2007b). Coltricioid EcM morphotypes were retrieved from three host tree roots at five study sites. Only a single putative EcM host tree was present at each site. Briefly, Anse Major (Mahé Island; 4°37.3'S, 55°23.5'E; 40 m a.s.l.) is a steep coastal shrubland on exposed granite bedrock, dominated by the native *Intsia bijuga* (Colebr.) Kuntze (Caesalpinaceae; 20–50 years old; six scattered individuals), *Nephrosperma vanhoutteana* (Wendl. ex van Houtt) Balf. and thickets of the invasive *Chrysobalanus icaco* L. Vallée de Mai UNESCO World Heritage Site (Praslin Island; 4°20.0'S, 55°44.0'E; 180 m a.s.l.) comprises a virgin forest of endemic palms with a few clumps of *I. bijuga* (25–80 years old; 14 individuals) on eroded, yellow-to-red lateritic soils. L'Abondance (Mahé Island; 4°41.3'S, 55°29.1'E; 480 m a.s.l.) is an undisturbed forest dominated by the endemic *Vateriopsis seychellarum* Heim (Dipterocarpaceae; 20–200 years old; ca. 15 individuals), *Cinnamomum verum* L. and *Pandanus* spp. on shallow humus (2–7 cm) overlying coarse quartz sand. Casse Dent (Mahé Island; 4°39.3'S, 55°26.4'E; 410 m a.s.l.) is a plantation of *Camellia sinensis* (L.) Kuntze with a single planted *V. seychellarum* tree (40 years old) and multiple current-year seedlings on ploughed soils. Le Niol (Mahé Island; 4°37.5'S, 55°25.9'E; 210 m a.s.l.) comprises a *Eucalyptus robusta* Sm. (Myrtaceae; 29 years old; ca. 200 individuals) plantation, dominated by *C. verum* and a thick

herb layer of the invasive fern *Dicranopteris linearis* (Burm.) on strongly eroded red lateritic soils.

Root samples (15×15 cm to 5 cm depth) were taken from each site ($n=5-24$) between 28 February and 15 March 2006. Within 24 h, EcM roots were separated manually from adhering debris and mineral soil, and cut into fragments of ca. 3-cm. Twenty randomly selected root fragments from each sample were mounted into 10 ml 1% CTAB lysis buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 1% CTAB). Within 6 weeks, EcM root tips were further morphotyped and anatomotyped following Agerer (1991), and photographed using Axiovision 4.0 software (Zeiss, München, Germany). Plan views were recorded at 32–50 x magnification and mantle structure was recorded at 1,000 x magnification with Normarski differential interference contrast. Longitudinal and cross sections (10–20 µm thickness) of EcM root tips were cut using a cryo-microtome and checked for the presence of a Hartig net at 400–1,000 x magnification. The mantle images were edited and sharpened using Adobe Photoshop 5.5 software (Adobe Systems, San Jose, CA).

Molecular typing

Mycorrhizal root tips and pieces from vouchered *Coltricia* and *Coltriciella* fruit-bodies (Table 1) were subjected to DNA extraction using a High Pure PCR Template Preparation Kit for Isolation of Nucleic Acids from Mammalian Tissue (Roche, Indianapolis, IN), following the manufacturer's instructions. PCR was performed using primers ITS1F (5' cttggcatttagaggaagtaa 3') and TW13 (5' ggtccgtgttcaagacg 3'). In case of insufficient product, both the ITS and LSU regions were separately amplified using primer pairs ITS1F/ITS4 (5' tctcccgttattgatatgc 3') and ctb6 (5' gcatatcaataagcggagg 3')/LR5 (5' tctcggaggaaactcg 3'), respectively. Sequencing was performed using primers ITS4, ITS5 (5' ggaagtaaaagtcgtaacaagg 3'), ctb6 and LR5 or TW13. Contigs were assembled using Sequencher 4.0 (GeneCodes, Ann Arbor, MI). Mycorrhizal root tips with sequences of *Coltricia* and *Coltriciella* are presented in this paper. EcM root tip and fruit-body sequences of these two genera were submitted to EMBL (accession nos. AM412240–AM412260; Tables 1, 2).

Phylogenetic analyses

Additional fruit body sequences of *Coltricia*, *Coltriciella* and potential outgroup taxa (based on phylogenetic analyses in Larsson et al. 2006) were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov>). The sequences were aligned using MAFFT ver. 5.861 (Katoh et al. 2005). The initial alignment comprised 242 taxa, which was finally reduced to 42 taxa. The final alignment included 887 characters of which 248 were parsimony-informative.

Table 1 *Coltricia* and *Coltriciella* fruit bodies sequenced for this study

Species	Herbarium code ^a	Locality	EMBL sequence accession no.
<i>Coltricia cinnamomea</i> (Pers.) Murrill	TU103694	Australia, Tasmania, Warra LTER	AM412243
<i>C. cinnamomea</i>	MEL2059672	Australia, Victoria, Red Bluff Reserve	AM412244
<i>C. cinnamomea</i>	TU103352	China, Fujian, Fuzhou	AM412242
<i>Coltricia confluens</i> Keizer	TAA181460	Estonia, Ida-Viru Co., Sirgala	AM412241
<i>Coltricia aff. oblectans</i>	TU103621	Seychelles, Mahé, Le Niol	AM412246
<i>C. aff. oblectans</i>	TAA195036	Seychelles, Praslin, Vallée de Mai	AM412245
<i>Coltriciella dependens</i> (Berk. & MA Curtis) Murrill	MEL2096512	Australia, Capital Territory, Tidbinbilla Nature Reserve	AM412248
<i>C. dependens</i>	MEL2292349	Australia, New South Wales, Booderee National Park	AM412249
<i>C. dependens</i>	TU100506	Australia, Victoria, Healesville	AM412250
<i>C. dependens</i>	TU100518	Australia, Victoria, Healesville	AM412251
<i>C. dependens</i>	TU103378	China, Fujian, Fuzhou	AM412252
<i>C. dependens</i>	TU103611	Seychelles, Mahé, Casse Dent	AM412254
<i>C. dependens</i>	TAA195099	Seychelles, Mahé, L'Abondance	AM412253
<i>Coltriciella tasmanica</i> (Cleland & Rodway) DA Reid	TAA159956	Australia, Western Australia, Old Yanchep Rd.	AM412247

^a TU Tartu University Herbarium, MEL Melbourne Royal Botanical Gardens Herbarium, TAA Herbarium of Institute of Zoology and Botany

Parsimony and Bayesian analyses were performed using PAUP* ver. 4.0d81 (Swofford 2002) and Mr. Bayes ver. 3.1.1 (Ronquist and Huelsenbeck 2003), respectively. Parsimony analyses were run with tree-bisection-reconnection (TBR) branch swapping, gaps as missing data, maxtrees set to 10,000 and up to 100 saved trees per replicate. Bootstrap support was calculated based on 1,000 bootstrap replicates. For Bayesian analyses, model selection was performed using a program Mr. Modeltest ver. 2.2 (Nylander 2004). Bayesian analyses were performed with 2,000,000 generations and a burn-in value of 2,500.

Results

Phylogenetic placement

Amplification of both root tips and fruit bodies of *Coltricia* and *Coltriciella* was difficult using any of the primers — only 6 out of 13 (46%) extracted EcM root tips produced sequencable material. Sequencing of mycorrhizal root tip

DNA revealed four species of coltricioid fungi that possessed the ITS region between 886 and 932 bp, including 447–488 bp of ITS1. ITS sequences were highly divergent and poorly alignable among closely related species. All species of *Coltricia* and *Coltriciella* shared two hypervariable insertions in the LSU that were missing in all other members of the hymenochaetoid clade. With or without inclusion of these regions, *Coltricia* and *Coltriciella* formed a 100% supported lineage (Fig. 1). Phylogenetic analysis placed *Coltriciella* nested within the genus *Coltricia*, but with moderate bootstrap support (72%). Common taxa with large ranges (*C. cinnamomea* and *C. dependens*) were strongly polyphyletic or paraphyletic, suggesting the presence of several cryptic species. Sequence types L2367 and L2528 had ITS and LSU sequences identical with two Seychelles specimens of *Coltriciella dependens*. Phylogenetic analyses placed these and an additional sequence type, L2480, within *Coltriciella* spp. (Fig. 1). L2410 had ITS and LSU sequences identical with *Coltricia aff. oblectans* (AM412245). L2368 and L2450 had identical ITS sequences, but matched no fruit body

Table 2 List of ectomycorrhizal (EcM) root tips identified as *Coltricia* and *Coltriciella* species

Isolate	Locality	Host	EMBL sequence accession no.
L2367	Seychelles, Mahé, Le Niol	<i>Eucalyptus robusta</i>	AM412255
L2368	Seychelles, Mahé, Le Niol	<i>E. robusta</i>	AM412258
L2410	Seychelles, Praslin, Vallée de Mai	<i>Intsia bijuga</i>	AM412257
L2450	Seychelles, Mahé, Anse Major	<i>I. bijuga</i>	AM412259
L2480	Seychelles, Mahé, L'Abondance	<i>Vateriopsis seychellarum</i>	AM412260
L2528	Seychelles, Mahé, Casse Dent	<i>V. seychellarum</i>	AM412256

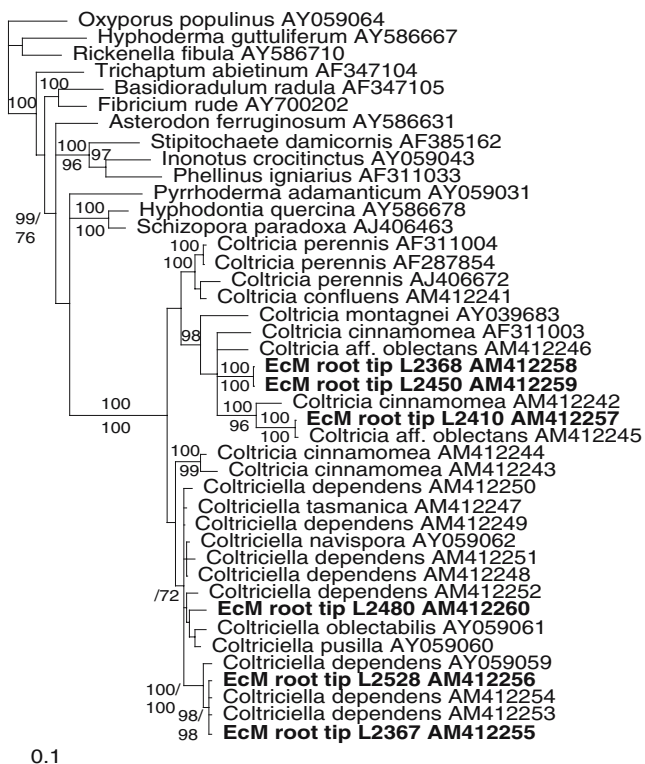


Fig. 1 Bayesian tree demonstrating the phylogenetic placement of six root tip isolates (**bold**) among *Coltricia* and *Coltriciella* species based on partial large subunit (LSU) sequences. Posterior probabilities ($\geq 95\%$) and bootstrap support ($\geq 70\%$) are shown above and below the branches, respectively

sequences. Based on LSU sequences, these isolates and L2410 clustered with several species of *Coltricia*, particularly *C. cinnamomea*, *C. aff. oblectans* and *C. montagnei*.

EcM mycorrhiza descriptions

Coltricioid EcM occurred as mycorrhizal root tips or in small clusters of up to six mycorrhizal root tips among other morphotypes. EcM of *Coltricia* and *Coltriciella* spp. were highly similar in gross morphology, sharing a thin (3–5 hyphal layers), orange-brown to dark brown mantle with abundant dark brown, thick-walled emanating hyphae and/or cystidia (Fig. 2a, f, k). None of the species possessed clamp connections. Hartig net was present (Fig. 2e), but sometimes poorly developed.

Coltriciella dependens (L2367 and L2528; Fig. 2a–e) Unramified ends straight or slightly bent, orange-brown to dark brown, 0.4–1.7 mm \times 0.18–0.23 mm (length \times diam.). Outer and middle mantle layers transitional between plectenchymatous and pseudoparenchymatous, with a unique labyrinthine pattern. Outer layer cells extremely thick-walled [(0.8) 1.2–1.7 (2.1) μm], 2–7 μm diam. Middle layer cells thick-walled (0.3–0.8 μm), 14–26 (34) \times 3.5–6 μm . Inner cells oblong, arranged in a rosette-like pattern, moderately

thick-walled [0.1–0.5 (0.7) μm], 15–35 (43) \times 3.6–6 (10) μm . Emanating hyphae moderately abundant, dark brown, stellately branching, thick-walled (0.4–0.7 μm), without warts, 2.5–4 (5) μm diam. Cystidia abundant, dark brown, with a basal cell, thick-walled at the base (0.4–0.9 μm), long (60–160 μm) and narrow (2–3 μm diam.).

Coltricia aff. oblectans (L2410; Fig. 2f–j) Unramified ends straight or slightly bent, orange-brown to dark brown, 0.4–3.6 \times 0.25–0.4 mm. Outer and inner mantle layers transitional between pseudoparenchymatous and plectenchymatous. Outer layer cells oblong, thick-walled (0.2–0.7 μm), arranged in a stretched mosaic-like pattern, 5–7 μm diam. Inner layer cells thin-walled (< 0.2 μm), 3.5–6 μm diam. Emanating hyphae abundant, dark brown, straight, moderately thick-walled (0.3–0.5 μm), without warts, 5–11 μm diam. Cystidia abundant, dark brown, covering the whole outer mantle layer, always unicellular, stout, cylindrical, moderately thick-walled (0.3–0.4 μm), 30–120 \times 7–12 μm .

Coltriciella sp. (L2480; Fig. 2k–o) Unramified ends straight or slightly bent, orange-brown to dark brown, 0.3–11 \times 0.18–0.22 mm. Outer and inner mantle layers transitional between pseudoparenchymatous and plectenchymatous. Outer layer cells oblong, irregular, forming a net-like pattern, thick-walled (0.3–0.6 μm), 3–6 μm diam. Inner layer cells more elongated, forming a mosaic-like pattern, sometimes arranged in a rosette, moderately thick-walled [0.1–0.3 (0.8) μm], 2.5–6 μm diam. Emanating hyphae abundant, dark brown, thick-walled (0.3–0.6 μm), without warts, 4–6 μm diam. Cystidia scarce, dark brown, with a basal cell (4–5 μm diam.), multicellular, slender, moderately thick-walled (0.3–0.4 μm), 60–100 \times 2.5–3.5 μm .

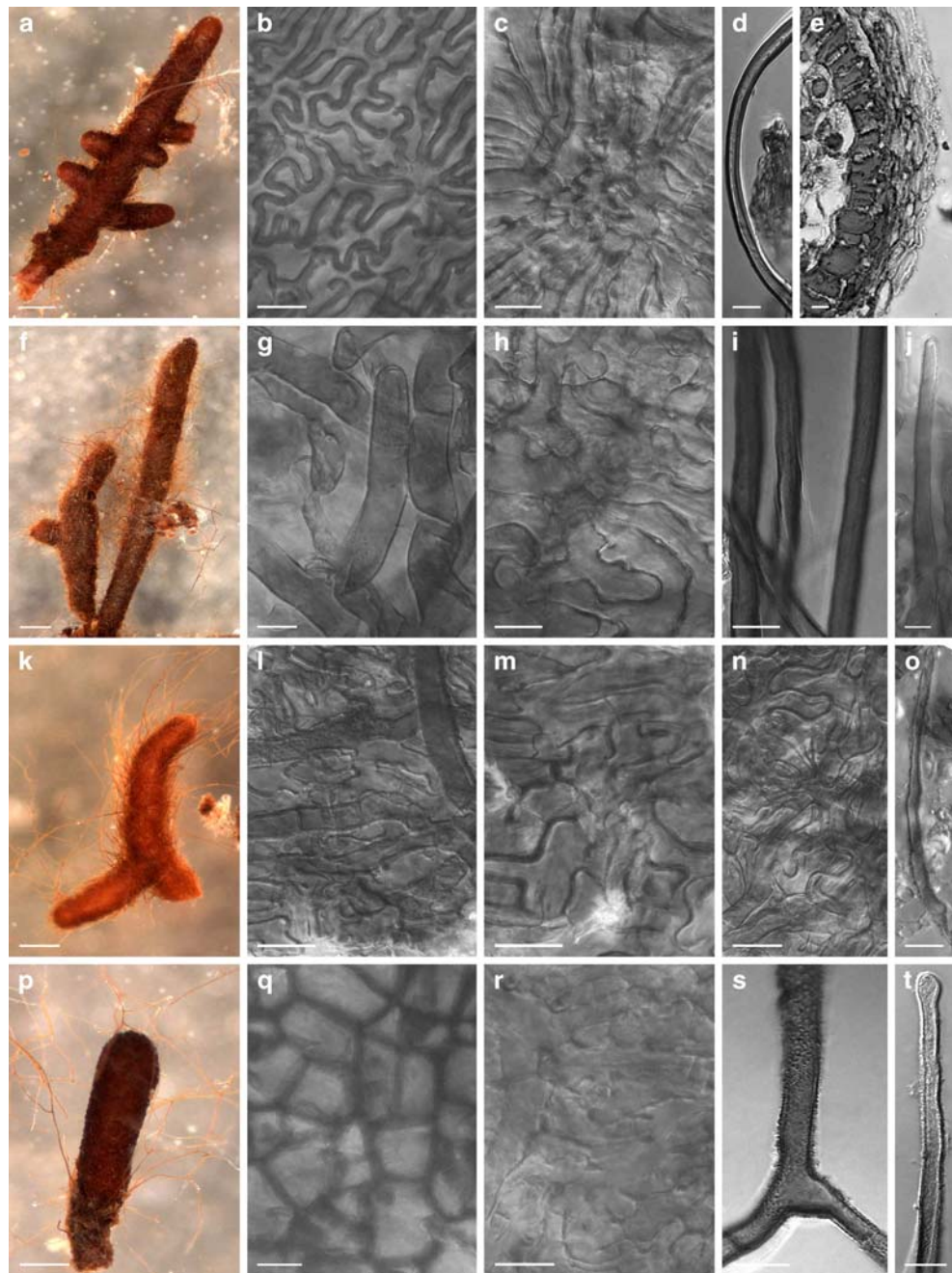
Coltricia sp. (L2368 and L2450; Fig. 2p–t) Unramified ends straight, club-like, red-brown to dark brown, 0.3–1.1 \times 0.19–0.27 mm. Outer and middle mantle layers distinctly pseudoparenchymatous. Cells rectangular to oval, extremely thick-walled [0.5–1 (1.3) μm], 15–25 (31) \times 8–19 μm . Inner mantle layer intermediate between plectenchymatous and pseudoparenchymatous. Inner layer cells relatively thin-walled (< 0.2 μm), 3.5–6 μm diam. Emanating hyphae moderately abundant, dark brown, covered with abundant warts, stellately branching, thick-walled (0.4–0.7 μm), 5–10 μm diam. Cystidia absent.

Discussion

Phylogeny and ecology

We provided morphological and molecular evidence for in situ EcM habit of *Coltricia* and *Coltriciella* spp. within the

Fig. 2 Morphological and anatomical characters of *Coltricia* and *Coltriciella* ectomycorrhiza (EcM). **a–e** *Coltriciella dependens* (L2367 and L2528) on *Vateriopsis seychellarum*; **a** plan view; **b** outer mantle layer; **c** inner mantle layer; **d** emanating hyphae; **e** Hartig net (cross section); **f–j** *Coltricia* aff. *oblectans* (L2410) on *Intsia bijuga*; **f** plan view; **g** outermost mantle layer with abundant cystidia; **h** inner mantle layer; **i** emanating hyphae; **j** cystidia; **k–o** *Coltriciella* sp. (L2480) on *V. seychellarum*; **k** plan view; **l** outer mantle layer; **m** middle mantle layer; **n** inner mantle layer; **o** cystidia; **p–t** *Coltricia* sp. (L2368 and L2450) on *I. bijuga*; **p** plan view; **q** outer mantle layer; **r** inner mantle layer; **s, t** emanating hyphae. Bars **a, f, k, p** 1 mm; **b–e; g–j; l–o; q–t** 10 μ m



predominantly saprotrophic hymenochaetoid clade. Thus, our results complement earlier reports on EcM formation between *C. perennis* and *Pinus* spp. in axenic synthesis experiments (Pachlewski and Chrusciak 1980; Danielson 1984). In addition, *C. perennis* possessed ratios of stable ^{13}C and ^{15}N isotope concentrations similar to that of most EcM basidiomycetes and clearly different from saprobes (Tedersoo et al. 2007a). These results suggest that *Coltricia* and *Coltriciella* spp. may function as typical EcM fungi.

Based on nuclear LSU sequence data and Bayesian analyses, *Hyphodontia-Schizopora* lineage is a sister taxon to *Coltricia-Coltriciella* lineage (Larsson et al. 2006). Most

members of the *Hyphodontia-Schizopora* lineage form resupinate fruit bodies on wood. Indeed, the majority of basidiomycetous EcM lineages have resupinate fruiting saprotrophic ancestors (Larsson et al. 2004), suggesting that habitat in close contact to soil and roots in dead wood provides preconditions for the development of EcM lifestyle in general.

The genera *Coltricia* and *Coltriciella* are distributed world-wide, especially in tropical ecosystems (Ryvarden and Johansen 1980; Corner 1991). The panglobal distribution suggests that *Coltricia-Coltriciella* is an ancient taxon and members of this lineage likely form EcM associations

in all continents. So far, their presence in below-ground EcM communities may have been simply overlooked, for two reasons. First, coltricioid taxa form relatively small and thin EcMs that are low in abundance on a fine scale in Seychelles and Tasmania (Tedersoo 2007; Tedersoo et al. 2007b). Second, high mutation rates in the ITS and LSU regions and long ITS sequences may contribute to low success in DNA amplification and sequencing, especially from stored specimens (this study).

Despite additional evidence for EcM habit, the autecology of *Coltricia* and *Coltriciella* spp. remains poorly understood. Unlike saprotrophic and most EcM fungi, *C. perennis* lacks laccase and tyrosinase activities in spot tests with pure cultures, suggesting that at least the studied isolate is not adapted to decomposition (Pachlewski and Chrusciak 1980). This lends further evidence to the view that fruiting habit on decomposed wood does not necessarily indicate saprotrophic lifestyle. Similarly, wood-inhabiting *Lactarius* sect. *Panuoidei* were originally considered saprotrophic or root parasitic (Singer 1984). However, these taxa were later proven to be EcM by molecular analyses of associated EcM root tips (Henkel et al. 2000). Also, the ubiquitous ‘inconspicuous, wood-inhabiting’ resupinate theleporoid, athelioid and sebacinoid fungi were long considered saprotrophic. But why do some EcM fungi fruit preferentially on dead wood or the butts of living trees? We hypothesise that tree trunks provide a more exposed place for releasing spores (Henkel et al. 2006) or a safe site against flooding. Contrary to *Lactarius* spp. and similar to resupinate fungi, fruit bodies of most *Coltricia* spp. are long-lived and may use dead wood as a relatively stable substrate for physically supporting fruit bodies. In addition, and perhaps more importantly, dead wood provides a substantial moisture reserve during short dry periods (Harvey et al. 1978, 1981). Thus, the mycelium of coltricioid taxa could potentially provide water from dead wood to fruit bodies shortly after the rainy season. Unlike the elevated abundance of resupinate theleporoid, athelioid and sebacinoid taxa in dead wood in boreal forests (Tedersoo et al. 2003), we found no evidence for substrate preference of coltricioid or any other taxa in Seychelles (Tedersoo et al. 2007b).

Ectomycorrhiza morphology and anatomy

The morphological and anatomical characters of *Coltricia* and *Coltriciella* EcM presented here resemble brief descriptions of *C. perennis* and *C. cinnamomea* EcM (Danielson 1984; Thoen and Ba 1989). Thus, *Coltricia* and *Coltriciella* spp. studied so far share orange-brown to dark brown EcM mantle with moderately to extremely thick cell walls. Clampless emanating hyphae and/or cystidia (‘bristles’) are usually abundant, forming a dense brown web around the

mycorrhizal root tips. Both outer and inner mantle layers are transitional between plectenchyma and pseudoparenchyma, except in *Coltricia* sp. (L2368 and L2450), which has distinctly pseudoparenchymatous outer layer cells. The EcM of coltricioid taxa most strongly resemble members of the *Genea-Humaria*, *Wilcoxina-Trichophaea hybrida* and *Geopora* lineages of Pezizales with respect to plan morphology and mantle structure. *Wilcoxina* and *Geopora* spp. form mantles intermediate between pseudoparenchymatous and plectenchymatous, but emanating hyphae are scarce and only 2–4 layers of mantle cells are present (Tedersoo et al. 2006). *Coltricia* sp. (L2368 and L2450) was nearly identical to the EcM of *Humaria hemisphaerica* and *Genea* spp, differing only by the lack of anastomoses in the outer mantle cells and somewhat thinner cell walls (Brand 1991; Tedersoo et al. 2006). The EcM of coltricioid taxa also resemble certain *Tomentella* species that differ by a thicker mantle with more differentiated layers (Agerer 2006; L.T. personal observation). In addition, most *Tomentella* spp. possess clamp connections in emanating hyphae and moderately thick cell walls in the outer mantle layer.

In conclusion, accumulating evidence suggests that several species of *Coltricia* and *Coltriciella* form anatomically conspicuous EcM with several host plants. Further experimental studies are needed to establish the ecophysiological traits of individual species and their position in the mutualism-parasitism continuum (Egger and Hibbett 2004).

Acknowledgements We thank D. Dogley, J. Mougall, Y. Juliette and staff of the Forestry Section (Ministry of Environment and National Resources, Seychelles) for help, information and support in Seychelles; U. Kõljalg for sequencing *C. tasmanica*; H. Tamm and B. Kullman for assistance in sectioning; E. Parmasto for identifying the specimens from Seychelles and China; two referees and E. Parmasto for constructive comments on earlier versions of the manuscript. This study was funded by the Estonian Science Foundation grant no. 6606.

References

- Agerer R (1991) Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Techniques for the Study of Mycorrhiza. Academic, London, pp 25–73
- Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5:67–107
- Aime MC, Henkel TW, Ryvarden L (2003) Studies in neotropical polypores 15: new and interesting species from Guyana. Mycologia 95:614–619
- Binder M, Hibbett DS (2002) Higher level phylogenetic relationships of Homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. Mol Phyl Evol 22:76–90
- Binder M, Hibbett DS, Larsson K-H, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Syst Biodiv 3:113–157
- Brand F (1991) *Genea hispidula*. In: Agerer R (ed) Colour Atlas of Ectomycorrhizae, plate 57. Einhorn, Schwäbisch Gmünd

- Corner EJH (1991) *Ad Polyporaceae* VII. The xanthochroic polypores. *Nova Hedwigia* 101:1–175
- Cunningham GH (1948) New Zealand Polyporaceae. 6. The genus *Coltricia*. *Plant Dis Div Bull* 77:1–13
- Danielson RM (1984) Ectomycorrhizal associations in jack pine stands in northeastern Alberta. *Can J Bot* 62:932–939
- Egger KN, Hibbett DS (2004) The evolutionary implications of exploitation in mycorrhizas. *Can J Bot* 82:1110–1121
- Grand LF, Vernia CS (2005) Biogeography and hosts of poroid decay fungi in North Carolina: species of *Coltricia*, *Coltriciella* and *Inonotus*. *Mycotaxon* 91:35–38
- Harvey AE, Jurgensen MF, Larsen MJ (1978) Seasonal distribution of ectomycorrhizae in a mature Douglas fir/larch forest soil in Western Montana. *For Sci* 24:203–208
- Harvey AE, Jurgensen MF, Larsen MJ (1981) Organic reserves: importance to ectomycorrhizae in forest soils of western Montana. *For Sci* 27:442–445
- Henkel TW, Aime MC, Miller SL (2000) Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal status. *Mycologia* 92:1119–1132
- Henkel TW, Aime MC, Mehl HK, Miller SL (2006). *Cantharellus pleurotoides*, a new and unusual basidiomycete from Guyana. *Mycol Res* 110:1409–1412
- Hibbett DS, Donoghue MJ (2001) Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. *Syst Biol* 50:215–242
- Hibbett DS, Gilbert L-B, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* 407:506–508
- Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33:511–518
- Larsson K-H, Larsson E, Kõljalg U (2004) High phylogenetic diversity among corticioid homobasidiomycetes. *Mycol Res* 108:983–1002
- Larsson K-H, Parmasto E, Fischer M, Langer E, Nakasone KK, Redhead SA (2006) Hymenochaetales: a molecular phylogeny of the hymenochaetoid clade. *Mycologia* 98:926–936
- LoBuglio KF, Berbee ML, Taylor JW (1996) Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among the ascomycetes. *Mol Phyl Evol* 6:287–294
- Nylander JAA (2004) MrModeltest 2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden
- Pachlewski R, Chrusciak E (1980) Aktywnosc enzymatyczna mikorozowych II (in Polish). *Acta Mycol* 16:97–103
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Ryvarden L, Johansen I (1980) A preliminary polypore flora of East Africa. *Fungiflora*, Oslo
- Singer R (1984) Tropical Russulaceae II. *Lactarius* section *Panuoidei*. *Nova Hedwig* 40:435–447
- Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer, Sunderland
- Taylor AFS, Alexander IJ (2005) The ectomycorrhizal symbiosis: life in the real world. *Mycologist* 19:102–112
- Tedersoo L (2007) Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. PhD Thesis, University of Tartu, Tartu, Estonia
- Tedersoo L, Kõljalg U, Hallenberg N, Larsson K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytol* 159:153–165
- Tedersoo L, Hansen K, Perry BA, Kjølner R (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. *New Phytol* 170:581–596
- Tedersoo L, Pellet P, Kõljalg U, Selosse M-A (2007a) Parallel evolutionary paths to mycoheterotrophy in understory Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. *Oecologia*. 151:206–207
- Tedersoo L, Suvi T, Beaver K, Kõljalg U (2007b) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Instia hijuga* (Caesalpinaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). *New Phytol* (in press). DOI 10.1111/j.1469-8137.2007.02104.x
- Thoen D, Ba AM (1989) Ectomycorrhizas and putative ectomycorrhizal fungi of *Azelia africana* and *Uapaca guineensis* in southern Senegal. *New Phytol* 113:549–559
- Wagner T, Fischer M (2002a) Classification and phylogenetic relationships of *Hymenochaete* and allied genera of the Hymenochaetales, inferred from rDNA sequence data and nuclear behaviour of vegetative mycelium. *Mycol Progress* 1:93–104
- Wagner T, Fischer M (2002b) Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* 94:998–1016