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Paxillus orientalis sp. nov. (*Paxillaceae, Boletales*) from south-western China based on morphological and molecular data and proposal of the new subgenus *Alnopaxillus*

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Abstract

The new species *Paxillus orientalis* is reported from Yunnan Province (south-western China). Based on morphological and molecular characters the novel taxon belongs to the sibling, holarctic alder-associated *P. rubicundulus* complex. Colour pictures of fresh basidiomes and line drawings are provided, accompanied by notes concerning its taxonomic and phylogenetic relationships within the genus. The new subgenus *Alnopaxillus* is established to accommodate *P. rubicundulus* and allied undescribed taxa characterized by basidiomes usually associated with *Alnus* and with a distinctly areolate–squamulose pileal surface.

Keywords

Basidiomycota Boletineae Chinese ectomycorrhizal fungi ITS sequences Molecular phylogeny Taxonomy

Introduction

Paxillus Fr. is a basidiomycete genus producing fleshy ectomycorrhizal sporophores characterized by medium to large size, drab colours ranging from ochraceous-yellow to chestnut brown or dark brown overall, a funnel-shaped pileus with typically involute margin at least in young specimens, deeply decurrent and easily detachable lamellae which are interveined towards the stipe, more or less intensely reddening/browning tissues on exposure or handling and rusty brown to reddish-brown spore print. Microscopically, taxa belonging to *Paxillus* are further characterized by broadly ellipsoid, smooth, cyanophilic spores, a trichodermal pileipellis consisting of filamentous hyphae, bilateral divergent hymenophoral trama, absence of lateral stipe stratum and presence of clamp

connections (Moser 1983; Singer 1986; Šutara 1992; Hahn and Agerer 1999a; Watling 2008; Hahn 2012; Knudsen and Taylor 2012).

Despite the clitocyboid habit of the basidiomes, it has long been demonstrated that *Paxillus*—based on morphological, chemical and structural features—is phylogenetically connected to boletes (Schmitt 1970; Watling 1970; Moser 1983; Kämmerer et al. 1985; Linzerkirchner and Besl 1993; Hahn and Agerer 1999b; Watling and Hills 2005; Agerer 2006; Knudsen and Taylor 2012). Recent molecular analyses support its placement within the order *Boletales* E.-J. Gilbert, suborder *Boletineae*, family *Paxillaceae* Lotsy (Binder 1999; Bresinsky et al. 1999; Jarosch 2001; Binder and Hibbett 2006; Drehmel et al. 2008; Vellinga et al. 2009; Nuhn et al. 2013).

Paxillus has been proven to be monophyletic only when the species occurring in the Northern Hemisphere (predominantly in temperate and boreal regions) are taken into consideration (Bresinsky et al. 1999; Jarosch and Bresinsky 1999; Watling 2002, 2008; Hedh et al. 2008, 2009; Vellinga et al. 2012). However, taxa reported from native habitats in the Southern Hemisphere group together in a separate lineage viz. *Austropaxillus* Bresinsky & Jarosch which is placed in the distantly related family *Serpulaceae* Jarosch & Bresinsky (Bresinsky et al. 1999).

In the light of present knowledge the genus *Paxillus* s.str. comprises about 15 validly published species-names worldwide (Kirk et al. 2008), a handful of taxa being detected over the past 25 years (Šutara 1991; Dessì and Contu 1999; Hahn and Agerer 1999a), even though, according to current literature, several phylogenetically recognized taxa are still waiting for formal description (Hedh et al. 2008; Vellinga et al. 2009; Gelardi et al. 2011). Basidiomes of *Paxillus* species are notoriously polymorphic and largely resemble each other (Hahn and Agerer 1999a), thus it is sometimes difficult to recognize a taxon in the field exclusively referring to macromorphological characters alone. Hence it is necessary both to examine microscopical features and to perform molecular analysis in order to confirm identification at species-level. Hedh et al. (2008), who recently addressed species concepts of the European representatives of *Paxillus* using a multi-gene analysis, pointed out that within the genus two distinctive clusters can be recognized (formerly reported by Jarosch and Bresinsky 1999), viz. *P. rubicundulus* P.D. Orton [= *P. filamentosus* (Scop.) Fr. ss. auct., *P. leptopus* Fr.] and the *P. involutus* (Batsch) Fr. complex, which comprises *P. involutus* I (= *P. involutus* s.str.), *P. involutus* II (not yet formally named), *P. obscurosporus* C. Hahn and *P. validus* C. Hahn. They also demonstrated that ITS sequences alone were able to differentiate the same phylogenetic species recovered from the multi-gene analysis (Hedh et al. 2008). Subsequently, based on ITS data referring to type material, Vellinga et al. (2012) proved that *P. validus* is a later synonym of *P. ammoniavirescens* Contu & Dessì and that *P. involutus* I and II also occur in North America.

As already pointed out by Vellinga and colleagues, so far there is scarce information about *Paxillus* species reported from Eastern Asia, with the exception of a single molecularly confirmed accession of *P. obscurosporus* from China (Vellinga et al. 2012). It is worth to point out, however, that Zang and Zeng (1978) described two new species from southwestern China which have not been reported again since the original descriptions. In addition, few accounts on unconfirmed records referring to European species are sporadically found in regional mycotas and local check-lists (Zang and Zeng 1978; Bi et al. 1994, 1997; Li and Song 2000; Mao 2000, 2009; Tian et al. 2000; Wu et al. 2011, etc.).

Based on morphological and molecular (ITS) evidence, the present study aims at presenting a new species, *Paxillus orientalis*, recently found in two ecologically different mixed subtropical montane forests in Yunnan Province (south-western China). Such data demonstrate that *P. orientalis* is distinctly separated from any other known species, although exhibiting affinities with *P.*

rubicundulus s.l., a collective species widely distributed across the Northern Hemisphere and strictly associated with species of *Alnus* Mill.

Materials and methods

Collection sites and sampling

In September 2012, fresh material was collected by MG and EH at 2 different localities in Yunnan province, China. Specimens of the new species examined in this study are deposited in Herb. HKAS (holotype), in the first author's private herbarium (MG, isotype) and in Herb. ZT. Herbarium acronyms follow Thiers (2013) except "MG" that refers to Matteo Gelardi's personal herbarium. Author citations follow Index Fungorum, Authors of Fungal Names (www.indexfungorum.org/authorsoffungalnames.htm).

Morphological studies

Macromorphological description, macro-chemical reactions, habitat annotations and associated plant communities are based on detailed field notes of fresh basidiomes, colour terms in capital letters (e.g., Raw Umber, Plate III) are those of Ridgway (1912). Micromorphological features were observed on dried material; sections were either revived in water, 5 % potassium hydroxide (KOH) or in ammoniacal Congo Red. Anatomical features were measured by mounting preparations in ammoniacal Congo Red, colour and amount of pigmentation was described after examination in water and 5 % KOH. Measurements were made at 1000× magnification with a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Spores were measured from spore print and from the hymenophore of mature basidiomes. Values are given as (minimum) average ± standard deviation (maximum), Q= length/width ratio with minimum and maximum values in parentheses, Qm= average quotient (length/width ratio) ± standard deviation, while average spore volume was approximately estimated as a rotation ellipsoid ($V = 4/3*(length/2)*((width/2)*width)*\pi/2$ ± standard deviation). The notation [n/m/p] indicates that measurements were made on "n" randomly selected spores from "m" basidiomes of "p" collections. Metachromatic, cyanophilic and iodine reactions were also tested by staining the spores in Brilliant Cresyl blue, Cotton blue and Melzer's reagent, respectively. Line-drawings of microstructures were made from rehydrated material using a camera lucida.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 1 mg of two dried herbarium specimens (HKAS 63615 and ZT 13437), by using the Dneasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and Bruns 1993). Amplification reactions were performed in a T3000 thermal cycler (Biometra). The PCR amplification was performed according to Vizzini et al. (2013). The PCR products were purified with the Wizard SV Gel and PCR Clean-UP System (Promega) following manufacturer's instructions and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). Sequences were checked and assembled using Geneious v5.3 (Drummond et al. 2010) and submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Accession numbers are reported in Fig. 1.

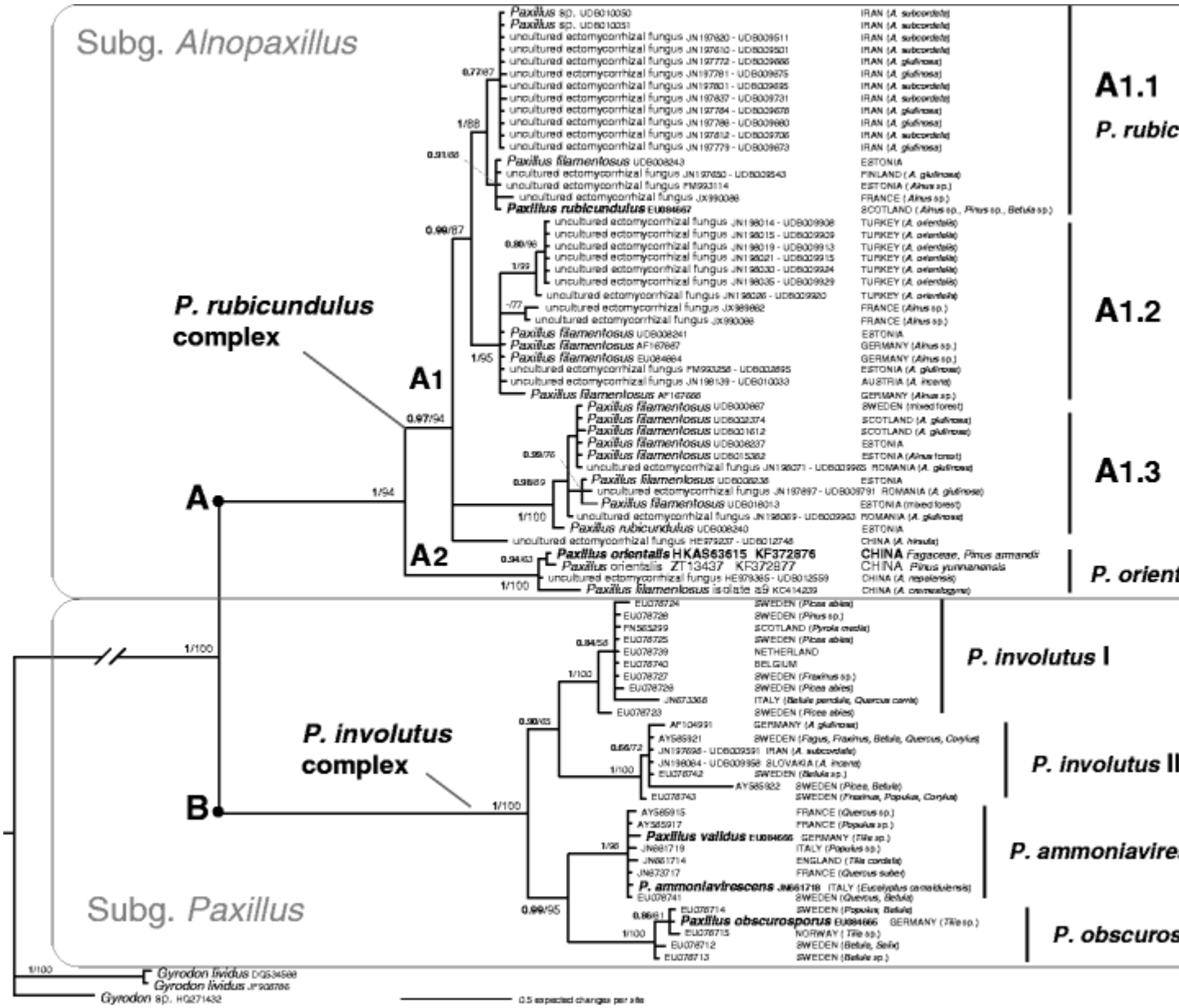

Fig. 1

Bayesian phylogram obtained from the general nrITS sequence alignment of *Paxillus* species. *Gyrodon* species were used as outgroup taxa. Support values in either the Bayesian (Posterior probabilities values [BPP]) or Maximum likelihood (ML bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.70 (in *bold*) and MLB values over 50 % are given above clade branches. Sequences from type collections are in *bold*

Sequence alignment, dataset assembly and phylogenetic analysis

The ITS sequences obtained in this study were compared to those available in the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/) databases by using the Blastn algorithm. Based on the Blastn results, sequences from voucher specimens and from environmental samples were selected according to the outcomes of recent phylogenetic studies on *Paxillus* (Jarosch and Bresinsky 1999; Hedh et al. 2008; Gelardi et al. 2011; Vellinga et al. 2012).

Alignment was generated using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignment was imported into MEGA 5.10

(Tamura et al. 2011) for manual adjustment. *Gyrodon lividus* (DQ534569 and JF908786), and *Gyrodon* sp. (HQ271432) were used as outgroup taxa because *Gyrodon* is sister to *Paxillus* (Binder and Hibbett 2006; Moreau et al. 2013; Nuhn et al. 2013). Best-fit models were estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada 2008), to provide a substitution model for the alignment. Phylogenetic analyses were performed using the Bayesian inference (BI) and maximum likelihood (ML) approaches. The BI was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with 4 incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run over 10 million generations, under GTR + Γ evolutionary model. Trees were sampled every 1,000 generations resulting in an overall sampling of 10,001 trees; the first 2,500 trees were discarded as "burn-in" (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP).

ML estimation was performed through RAxML v.7.3.2 (Stamatakis 2006) with 100 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-× 12345" as a random seed to invoke the novel rapid bootstrapping algorithm.

BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP values over 0.70 and MLB over 50 % are reported in the resulting tree (Fig. 1). Branch lengths were estimated as mean values over the sampled trees.

Pairwise % identity values (P%IV) of ITS sequences were calculated using MEGA 5.10 (Tamura et al. 2011).

Results

Molecular analysis

Both analysis methods applied yielded the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Fig. 1). The ITS data matrix included a total of 81 sequences (2 newly generated, 40 from GenBank, 12 from UNITE and 27 present in both GenBank and UNITE databases). The alignment comprised 795 characters and contains 358 variable sites.

Two well-supported major clades, A (BPP = 1/MLB = 94 %) and B (BPP = 1/MLB = 100 %) were distinguished within *Paxillus*. Clade A (P%IV = 94.2), consisting of 2 sister clades A1 (BPP = 0.97/MLB = 94 %) and A2 (BPP = 1/MLB = 100 %), was described as a new subgenus (see below). Clade A1 (including 3 subclades A1.1-A.1.3 and the Chinese uncultured ectomycorrhizal fungus HE979237 - UDB012748 from an ectomycorrhizal root tip of *Alnus hirsuta* Turczaninow ex Rupr.), comprises sequences from voucher specimens and from environmental samples associated with *Alnus* species: it is herewith named the *P. rubicundulus* complex and shows a P%IV of 95.0. Subclade A1.1, supported by 1 of BPP and 88 % of MLB, encompasses the sequence (EU084667) of *P. rubicundulus* type collection (Scotland), the sequence from an Estonian *P. filamentosus* basidiome and 15 sequences from environmental samples (12 from Iran, 1 from Estonia, 1 from Finland and 1 from France). These sequences share a P%IV of 99.6. Subclade A1.2 (BPP = 1/MLB = 95 %; P%IV = 98.8) comprises 15 sequences, 4 from basidiomes (2 from Estonia and 2 from Germany) and 11 from environmental samples (7 from Turkey, 2 from France, 1 from Estonia and 1 from Austria). Subclade A1.3 (BPP = 1/MLB = 100 %; P%IV = 99.4) consists of 11 sequences, 8 from basidiomes (Scotland, Sweden and Estonia) and 3 from environmental samples (Romania).

Clade A2 encompasses our 2 *P. orientalis* sequences and 2 other sequences from Chinese samples, HE979365 - UDB012559 ("uncultured ectomycorrhizal fungus") from an ectomycorrhizal root tip of *Alnus nepalensis* D. Don (Yunnan) and KC414239 ("*Paxillus filamentosus* isolate a9") from a basidiome collected under *A. cremastogyne* Burkill (Sichuan, He Xin-Sheng, pers. comm.); the 4 sequences show a P%IV of 98.6.

In clade B (P%IV = 95.5), herewith named the *P. involutus* complex according to Vellinga et al. (2012), the 4 phylogenetic species recognized by Hedh et al. (2008), Gelardi et al. (2011) and Vellinga et al. (2012) are also recovered in our nrITS sequence analyses (Fig. 1), with high support for *P. involutus* I (BPP = 1/MLB = 100 %), *P. involutus* II (BPP = 1/MLB = 100 %), *P. ammoniavirescens* (BPP = 1/MLB = 98 %) and *P. obscurosporus* (BPP = 1/MLB = 100 %).

Taxonomy

Paxillus* subgenus *Alnopaxillus Vizzini & Gelardi, **subg. nov**.

Mycobank MB 805069

Etymology: the name of the epithet is derived from *Alnus* (alder) and *Paxillus* and refers to the ectomycorrhizal association of members of the subgenus with *Alnus* species.

The subgenus is characterized by species with a (sub)squamulose pileus surface, yellowish to deep yellow context, spore length less than 8 µm in average and the occurrence usually in association with *Alnus* species (subg. *Alnus*). The new subgenus has so far only been reported from Europe and Asia.

Typus: *Paxillus rubicundulus* P.D. Orton, Notes R. bot. Gdn Edinb. 29: 110 (1969).

Paxillus orientalis Gelardi, Vizzini, E. Horak & G. Wu, **sp. nov.** (Figs. 2 and 3)

Fig. 2

Paxillus orientalis. Basidiomata. **a** Holotype, HKAS 63615, photo by M. Gelardi; **b** ZT 13437, photo by E. Horak. *Scale bars* 5 cm

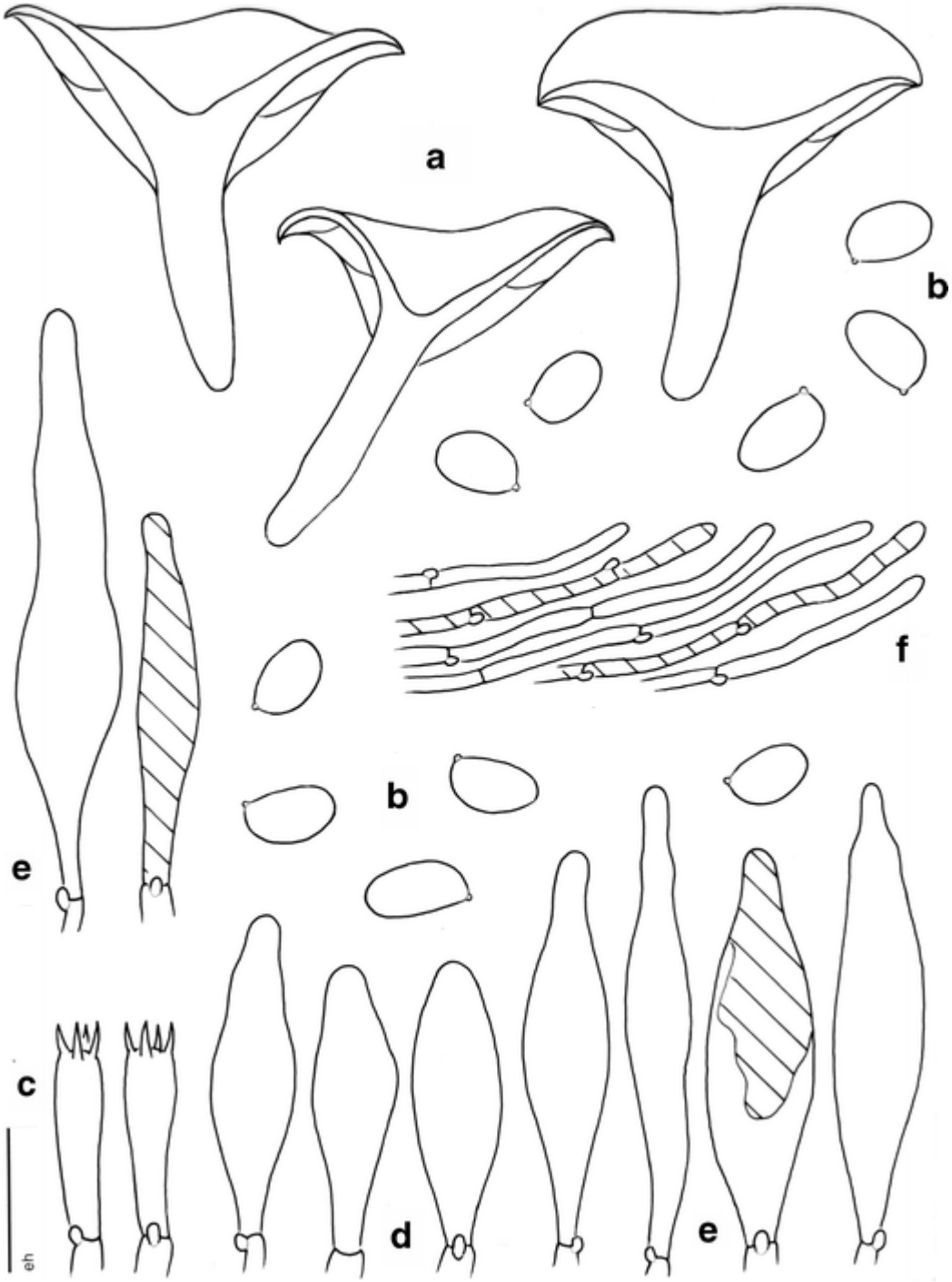

Fig. 3

Paxillus orientalis. Habit and microscopic characters (from HKAS 63615 and ZT 13437). **a** Basidiomes; **b** spores; **c** basidia; **d** cheilocystidia; **e** pleurocystidia; **f** pileipellis. Line drawings by E. Horak. *Scale bar* **a** = 2 cm; **b = 10** μm; **c–e** =20 μm; **f** = 40 μm

Mycobank MB 805070

Etymology the specific epithet “orientalis” (eastern) is derived from Latin and refers to the occurrence of the new species in the Far East.

Original diagnosis:

Paxillus orientalis differs from *P. rubicundulus* by the not or only occasionally faintly ribbed/striate pileus margin, the alutaceous-pinkish to beige-pinkish shades in the exposed tissues delimiting pileal scales, the smooth stipe exhibiting pinkish hues, the paler coloured hymenophore and context, the slightly smaller spores, the occurrence not only under *Alnus* but also in association with other fagaceous and conifer trees in subtropical montane forests on acidic soil and the different ITS sequence.

Holotype (here designated): China, Yunnan province, Binchuan County, Jizu Mount, N 25°57'39", E 100°23'24", 2280 m alt., 20 September 2012, leg. M. Gelardi and Q. Zhao, HKAS 63615; MG 488a (isotype).

Detailed description

Macrocharacters – Basidiomes medium-small sized. Pileus 4.0–5.5 (–9.5) cm broad, firstly plane but slightly depressed at centre, soon expanding and then deeply funnel-shaped or infundibuliform, regularly to hardly unevenly shaped, with a small, obtuse central umbo in young specimens, moderately fleshy, firm but softer with age; margin strongly inrolled and remaining involute at least up to maturity then expanding and finally completely flattened, occasionally faintly costate-dentate at the rim, entire at first but progressively lobed and rippled with growth, not or only indistinctly exceeding beyond the lamellae; surface matt, dry, loosely covered with innate adpressed and more or less radially arranged squamules or subrecurved fibrils sometimes tending to decrease in number outwards, finely velutinous at margin; scales dark brown to soot brown or brownish-olive (Raw Umber, Plate III; Prout's Brown, Plate XV; Saccardo's Olive, Plate XVI) on a pale beige-pinkish to ochraceous-alutaceous (Capucine Buff, Plate III; Pale Salmon Color, Plate XIV; Pale Ochraceous buff, Plate XV; Pale Pinkish Cinnamon, Plate XXIX) ground colour, gradually fading and becoming cream-beige (Ivory Yellow, Plate XXX) in old specimens; slowly changing to rusty brown (Vinaceous-Russett, Plate XXVIII) on handling or when injured; subcuticular layer cream-beige (Ivory Yellow, Plate XXX). Hymenophore lamellate, lamellae distinctly arcuate-decurrent and easily detachable from pileus, crowded, straight, rather short and as long as the pileus context thickness (up to 0.5 cm high), intermixed with several lamellulae, concolorous edges entire, moderately furcate or interveined near attachment on stipe so as to suggest a subporoid appearance; at first beige to cream-yellowish (Marguerite Yellow, Colonial Buff, Plate XXX) in age fading to ochraceous-russet or rusty-brown (Ochraceous Tawny, Plate XV; Vinaceous-Russett, Plate XXVIII) due to mature spores, staining dark rusty-brown (Cameo Brown, Plate XXVIII) on bruising or exposure. Stipe up to 4.3 × 1.4 cm, longer than or as long as the pileus diameter in young basidiomes but always shorter than pileus width at maturity, central to slightly off-centre, solid, firm, dry, straight or curved, cylindrical, less frequently tapering or enlarging towards the base, not rooting and tending to incorporate soil particles; surface smooth, glabrous, veils absent; initially cream-yellowish (Marguerite Yellow, Plate XXX) in the upper third, beige-pinkish (Pale Salmon Color, Plate XIV) elsewhere, increasingly darker with age, finally dark rusty-brown to fuscous (Cameo Brown, Rood's Brown, Plate XXVIII) on handling; rusty-orange (Orange-Cinnamon, Plate XXIX) in places eroded by slugs; basal mycelium beige-olivaceous (Reed Yellow, Plate XXX). Rhizomorphs not recovered on dried material. Context firm, later soft textured in the pileus, more fibrous in the stipe, cream-beige to cream-yellowish (Ivory Yellow, Colonial Buff, Plate XXX) throughout, quickly turning pale pink to reddish (Salmon Color, Carrot red, Plate XV) on exposure, red-brown (Russett, Plate XV) in rind of the lower half of stipe, finally fading to drab ochraceous-pinkish (Pale Ochraceous Buff, Plate XV); dry specimens sooty brown to brownish-pinkish (Raw Umber, Amber Brown, Plate III). Smell strong and pleasant, sourish, fruity. Taste mild to bitterish. Spore print rusty-brown. Macrochemical reactions: 10 % KOH: dark purple-vinaceous on all parts of the basidiome.

Microcharacters – Spores [92/4/2] (5.0–) 6.3 ± 0.71 (–8.4) × (3.5–) 4.2 ± 0.36 (–5.1) µm, Q = (1.28–) 1.30–1.77 (–1.90), Qm = 1.50 ± 0.10, V = 61 ± 17 µm^3, asymmetric, broadly elliptical to ovoid or subovoid, more rarely subcylindrical (especially in spores born on 2-spored basidia) in side view, ovoid to elliptical-ovoid in face view, smooth, with a short apiculus and without suprahilar depression, apex rounded, moderately thick-walled (0.5–0.7 µm), straw yellow coloured in water and 5 % KOH, with one large oil drop when mature, inamyloid, cyanophilic and showing a faint metachromatic reaction, germ pore absent. Basidia (20–) 24–37 × 5–7 (–10) µm (*n* = 15), cylindrical to vaguely cylindrical-clavate, moderately thick-walled (0.6–0.8 µm), predominantly 4-spored but also 3- or 2-spored, sterigmata 3–7 µm long, subhyaline to pale yellowish in water and 5 % KOH, containing straw-yellow oil guttules, basal septa clamped; basidioles subcylindrical to subclavate, about the same size as basidia, usually clamped. Cheilocystidia (35–) 40–73 (–81) × 7–16 µm (*n* = 20), frequent, polymorphic, fusiform to ventricose-fusiform, less frequently broadly lageniform or cylindrical-fusiform, sometimes with tapered and long neck, with obtuse apex, smooth, moderately thick-walled (0.6–1.0 µm), containing a straw yellow to brownish-yellow refringent plasmatic pigment in water and 5 % KOH, bright yellow to brownish-yellow in Melzer, without epiparietal encrustations. Pleurocystidia (34–) 43–72 (–80) × 8–19 (–22) µm (*n* = 20), frequent, shape, size and colour as cheilocystidia but sometimes a little broader and gradually tapering towards a subacute or subcapitate obtuse apex. Pileal surface (pileipellis) a non-gelatinized trichoderm consisting of erect and strongly interwoven, long, filamentous and sinuous, frequently branched hyphae with prominent clamps at septa, with age tending to become a cutis; terminal elements (24–) 28–102 (–130) × (2.5–) 4–7 (–10) µm, long, cylindrical or slender subclavate, apex rounded, moderately thick-walled (up to 1 µm), hyaline to more often honey yellow to brownish-yellow in water and 5 % KOH, smooth or more rarely with a poorly detectable zebra-pattern epiparietal encrustation; subpellis composed of cylindrical hyphae 5–12 µm broad, smooth, thin-walled, plasmatic and encrusting pigment absent. Stipital surface (stipitipellis) a texture of slender, parallel to loosely intermingled and longitudinally running, smooth, cylindrical adpressed hyphae, 3–17 µm broad, yellowish to honey yellow in water and 5 % KOH. Caulocystidia (28–) 30–50 (–53) × 2–10 µm (*n* = 20), abundant at apex of stipe, subcylindrical-irregular to subfusoid with elongated neck or slender subclavate, moderately thick-walled (up to 0.8 µm), hyaline or with yellow-brown plasmatic pigment in water and 5 % KOH; fertile caulobasidia extremely rare (only 1- and 3-spored observed!); oleiferous hyphae not observed. Lateral stipe stratum under caulohymenium absent. Stipital trama made up by longitudinal and densely arranged, subparallel to moderately interwoven, filamentous, smooth or more rarely encrusted by a granular brownish pigment, inamyloid hyphae, (2.5–) 4–21 µm across. Hymenophoral trama bilateral-divergent, with somewhat divergent and loosely arranged, partially gelatinized hyphae [lateral strata hyphae in transversal section not touching and (2–) 4–6 (–8) µm distant from each other, (2–) 4–13 (–15) µm broad], hyaline in water and 5 % KOH; lateral strata (15–) 30–40 (–55) µm thick, mediostratum 30–40 µm thick, consisting of a tightly adpressed, not gelatinous bundle of hyphae, 4–13 (–15) µm broad; in Congo red the mediostratum is darker than the lateral strata. Costiferous hyphae relatively frequent in the hymenophoral trama, slender, cylindrical, empty, clamped at septa, moderately thick-walled (up to 1.2 µm) and up to 20 µm broad, with transversal "weak ribs" originating from the inner surface of the hyphal wall and easily discernible in Congo red (see Clémençon 1983). Clamp connections present and abundant in all tissues. Hyphal system monomitic.

Habitat – Gregarious or in troops, solitary or subcaespitose, growing on soil among litter in subtropical montane mixed forests dominated by *Castanopsis delavayi* Franch., *Cyclobalanopsis glaucoides* Schottsky, *Lithocarpus* sp., *Keteleeria evelyniana* Mast., *Pinus yunnanensis* Franch. and *P. armandii* Franch. on acidic red soil (pH 5.9-6,5) (Li et al. 2013), also reported in association with *Alnus nepalensis* and *A. cremastogyne* (see Discussion below), autumn.

Known distribution – So far known only from southwestern China (Yunnan and Sichuan provinces), distribution limits unknown.

Examined material of *P. orientalis* – CHINA, Yunnan province, Binchuan County, Jizu Mount, N 25°57’39”, E 100°23’24”, 2280 m alt., on ground among litter under *Castanopsis delavayi*, *Cyclobalanopsis glaucoides*, *Keteleeria evelyniana*, *Pinus armandii* and *Lithocarpus* sp., 20 September 2012, M. Gelardi and Q. Zhao, HKAS 63615 (holotype) and MG 488a (isotype); Yunnan province, Xiaguan County, Shaba town, Dapoqing, N 25°34’33”, E 100°08’12”, 2050 m alt., on soil under *Pinus yunnanensis*, 18 September 2012, E. & A. Horak (ZT 13437).

Additional examined material – P. rubicundulus. SCOTLAND, Rannoch, 22 October 1966, P.D. Orton 2905 (Herb. K, holotype, annot. EH). ITALY, Trentino, Pergine Valsugana town, Pudro Lake, N 46°04’00”, E 11°14’00”, 510 m alt., on soil among litter under *Alnus glutinosa* L. with the presence of *Populus nigra* L. and *P. tremula* L., 26 August 2010, M. Floriani & M. Gelardi (MG 337a).

Discussion

Paxillus phylogeny and infrageneric classification

According to our ITS analysis (Fig. 1) there are 2 main and strongly supported species lineages in *Paxillus*, the subg. *Alnopaxillus* consisting of the *P. rubicundulus* complex (clade A1) and of *P. orientalis* (clade A2), and the subg. *Paxillus* (clade B) comprising the species referred to the *P. involutus* complex. Subg. *Alnopaxillus*, recorded in Asia and Europe, is characterized by basidiomes with a squamulose pileus surface, yellowish to deep yellow context, usually in association with *Alnus* species (*Betulaceae*) and by spores in average shorter than 8 µm (see Hahn 2000). In particular, all samples of the *P. rubicundulus* complex are strictly associated with species of *Alnus* subgenus *Alnus* [= subg. *Gymnothyrsus* (Spach) Regel], whilst *P. orientalis* was found under both fagaceous host trees or *Pinus* spp. (our collections) but also with *Alnus* spp. of subg. *Alnus*, sect. *Cremastogyne* (Winkl.) Murai (see Chen and Li 2004), as the sequence KC 414239 filed under “*Paxillus filamentosus* isolate a9” (*Alnus cremastogyne*, Jiange County, Guangyuan city, Sichuan Province, China; He Xin-Sheng, pers. comm.) and another sequence (HE 979365–UDB 012559) from an environmental sample (“uncultured ectomycorrhizal fungus”, ectomycorrhizal root tip of *Alnus nepalensis*, Yunnan Province, China), cluster with our collections and are supported as contaxic in the molecular analysis (Fig. 1).

By contrast, species of the *P. involutus* complex (characterized by a smooth pileus surface, whitish to light yellow context and spores longer than 8 µm in average, mainly distributed in Europe and North America) usually show no host specificity and are associated with a wide range of host trees belonging to the *Betulaceae*, *Corylaceae*, *Fagaceae*, *Pinaceae*, *Salicaceae*, *Tiliaceae*, etc. (Hedh et al. 2008; Gelardi et al. 2011; Vellinga et al. 2012). Only *P. involutus* II seems to display a preference for *Betula* spp. (see also Vellinga et al. 2012) even though, according to our analysis, 3 collections are also associated with *Alnus* [AF104991, Germany, *A. glutinosa*; JN197698 - UDB009591, Iran, *A. subcordata* C.A. Mey.; JN198064 - UDB009958, Slovakia, *A. incana* (L.) Moench].

According to our data the *P. rubicundulus* complex shows a high molecular divergence (P%IV = 95.0), probably suggesting that a cryptic speciation process has taken place (which means lack of really clear-cut morphological divergence in spite of genetic isolation), as already reported for the *P. involutus* complex on the basis of intercompatibility tests (Fries 1985), morphology

(Hahn and Agerer, 1999a) and molecular data (Jarosch and Bresinsky 1999; Jarosch 2001; Hedh et al. 2008; Gelardi et al. 2011; Vellinga et al. 2009, 2012).

At least three taxa are hidden in the *P. rubicundulus* complex (Fig. 1): subclade A1.1, which contains sequences from basidiomes as well as from environmental samples and includes the sequence of the *P. rubicundulus* type collection (EU 084667, Orton 2905 in Herb. E), is here named *P. rubicundulus* s.str.; subclades A.1.2 and A.1.3 represent new, yet undescribed phyletic lines; finally, the uncultured Chinese ectomycorrhizal fungus HE 979237 – UDB 012748, isolated from *Alnus hirsuta*, occupies an uncertain position. Future phylogenetic and morphological data including the analysis of fresh material belonging to these subclades might provide additional evidence for recognizing these phyletic lines as independent taxa likely representing new species.

For the time being the suggested speciation events in the A1 subclades do not seem strictly correlated to the geographical origin of samples or to the different associated *Alnus* species (Fig. 1). For example, collections from Estonia or samples in association with *A. glutinosa* are scattered over all subclades.

Paxillus orientalis and allied taxa

In the phylogenetic analysis (Fig. 1) *Paxillus orientalis* occupies an independent position and is sister to the *P. rubicundulus* complex.

Within the genus *Paxillus* the new taxon is recognizable on account of the relevant morphological features as follow: (a) basidiomes medium-small sized; (b) pileal surface covered with rough, soot brown to brownish-olive fine squamules on a pale pinkish-beige ground colour; (c) pileus not or only occasionally ribbed-sulcate at margin; (d) stipe smooth, pinkish-beige coloured but cream to yellowish at apex, darkening in age; (e) context cream-yellowish turning pinkish to reddish on bruising or exposure; (f) rusty-brown spore print, without reddish tinge when fresh; (g) spores small in size, (5.0–) 6.3 ± 0.71 (–8.4) × (3.5–) 4.2 ± 0.36 (–5.1) µm, Qm = 1.50 ± 0.10; h) pileipellis a trichoderm consisting of filamentous hyphae, undifferentiated terminal cells 5–10 µm broad; (i) stipitipellis mostly producing irregularly shaped sterile cells (caulocystidia) and scattered caulobasidia. Concerning the ectomycorrhizal relationships, the two contaxic sequences retrieved from GenBank (HE979365 - UDB012559 and KC414239) were isolated from root tips of *Alnus nepalensis* and from specimens associated with *A. cremastogyne*, respectively. It is remarkable, however, that our collections of *P. orientalis* were found in mixed broadleaf-conifer forests dominated by fagaceous trees (HKAS63615) or with *Pinus yunnanensis* (ZT 13437). In both localities no alder was observed in the next neighborhood; accordingly *P. orientalis* might likely be, similarly to *Paxillus* species of subg. *Paxillus* but unlike the other taxa of subg. *Alnopaxillus*, a multi-host generalist able to associate with *Alnus* spp. as well as with a range of different host trees.

Paxillus rubicundulus s.str. has revealed to be the closest relative to *P. orientalis* but differs from the new species by the strikingly ribbed-costate pileus margin over the entire life cycle, the brown-yellowish to olive-brown pileus always lacking pinkish or alutaceous tinges amongst the scales, the faintly pruinose apex of stipe lacking pinkish hues, deeper yellow coloured gills and context and the slightly larger spores [according to Hahn (2000) who studied the holotypic material 6.99 × 4.50 µm on average, Qm = 1.55, V = 76 µm^3, n = 60; pers. obs. on MG337a, (5.3–) 6.9 ± 0.76 (–8.7) × (3.5–) 4.5 ± 0.36 (–5.4) µm, Qm = 1.50 ± 0.12, V = 76 ± 19 µm^3 (36/2/1)]. In addition, *P. rubicundulus* is a highly specific species, viz. an obligate host fungus strictly associated with *Alnus* spp. (Kotlába and Pouzar 1960; Watling 1967; Orton 1969; Moser 1983; Szczepka 1987; Outen 1988; Breitenbach and Kränzlin 1991; Šutara 1992; Courtecuisse and Duhem 1994; Dessì and Contu 1999; Hahn and

Agerer 1999a; Hahn 2000, 2012; Noordeloos 2000; Watling and Hills 2005; Eyssartier and Roux 2011; Knudsen and Taylor 2012; pers. obs.).

P. yunnanensis M. Zang, previously described by Zang and Zeng (1978) from southwestern China, once was supposed to be a member of *Omphalotus* Fayod, but re-examination of the type material (Yang and Feng 2013) indicates that it probably belongs to *Tricholomopsis* Singer. However *P. rhytidophyllus*, also published by M. Zang, could be a true representative of *Paxillus* s.str. It differs from *P. orientalis* in having a reddish-brown to dingy brown pileus surface, a predominantly subporoid hymenophore, significantly larger basidiospores [(8.5–) 9.0–11.5 × (6–) 6.5–8 µm, Q = 1.46 ± 0.09] and smaller cheilocystidia (Zang and Zeng 1978; Yang and Feng 2013).

The new Chinese entity *P. orientalis* is characterized by features not observed in the following taxa reported from Europe and North America such as *P. involutus* s.l., *P. vernalis* Watling (a taxon probably conspecific with *P. obscurosporus* according to Vellinga et al. 2012), *P. ammoniavirescens P. albidulus* Šutara (probably an albinotic form of a species in the *P. involutus* complex, see Gelardi et al. 2011, Vellinga et al. 2012) and *P. obscurosporus*. As compared to the enumerated species the most distinguishing characters are the smaller dimensions, the squamulose-scaly pileus, the occurrence in subtropical forests in association with Asian host trees and most of all the significantly shorter spores (Watling 1969; Šutara 1991; Dessì and Contu 1999; Hahn and Agerer 1999a; Gelardi et al. 2011; Hahn 2012; Knudsen and Taylor 2012). Moreover, the remarkable reddish-vinaceous hues of the fresh spore print, as reported in literature for *P. vernalis* and *P. obscurosporus* (Watling 1967, 1969; Hahn and Agerer 1999a; Noordeloos 2000; Bresinsky 2006; Kibby 2008) are not observed in *P. orientalis*.

Finally, none of the *Paxillus* species described by Corner (1971), Horak (1980) and Heinemann and Rammeloo (1985, 1986) from tropical habitats in SE-Asia or elsewhere is closely related to *P. orientalis* as it has been demonstrated that they belong to other paxilloid genera such as *Austropaxillus*, *Tapinella* E.-J. Gilbert or *Gyrodon* Opat. (Pegler and Young 1981; Šutara 1992; Hahn 1996; Bresinsky et al. 1999; Hahn and Agerer 1999b; Binder and Hibbett 2006).

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