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A new species of *Lenzitopsis* (Thelephorales, Basidiomycota) and its phylogenetic placement

Li-Wei Zhou [a,*], *Urmas Kõljalg* [b]

[a] *State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110164, PR China*
[b] *Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai St, Tartu 51005, Estonia*

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ABSTRACT

Lenzitopsis (Thelephorales, Basidiomycota), typified by *Lenzitopsis oxycedri*, was monotypic before we described the second species of this genus, *Lenzitopsis daii* in this study. *L. daii* resembles *L. oxycedri* in producing lenzitoid hymenophore as well as brown hyphae and echinulate spores, but it differs from the type species by its annual basidiocarps, amyloid spores and growth exclusively on *Juniperus chinensis* (Cupressaceae). In the phylogenetic perspective inferred with nuclear large subunit ribosomal DNA sequences, the two species were separated from each other and formed a strongly supported clade in the Thelephorales. The two *Lenzitopsis* species showed a more than 5% difference in internal transcribed spacer sequences. *Lenzitopsis* species are wood-decaying fungi, and this is the second genus of the order where mycorrhizal life style is unknown, besides of *Amaurodon*.

1. Introduction

Lenzitopsis Malençon & Bertault is a monotypic genus of the order Thelephorales, and *Lenzitopsis oxycedri* Malençon & Bertault is a rare species growing on living *Juniperus* (Cupressaceae) (Malençon and Bertault 1963; García-Manjón and Moreno 1981; Bernicchia 2000; Doğan et al. 2007). *Juniperus* occurs in arid and semi-arid regions of Northern Hemisphere and reaches Southern Hemisphere only in eastern Africa (Mao et al. 2010). *Lenzitopsis oxycedri*, so far, has been found only in Mediterranean region on *Juniperus foetidissima* (=*J. phoenicea*), *J. excelsa* (Turkey), *J. oxycedrus* (Morocco and Italy), and *J. thurifera* (Spain).

Ryvarden (1991) described a new genus *Lenzitella* Ryvarden to replace *Lenzitopsis* with the type species *Lenzitella malenconii* Ryvarden, a nom. nov. for *L. oxycedri*. However, Stalpers (1993) treated *Lenzitella* and *L. malenconii* as superfluous names and argued that "*Lenzitopsis* was published with one species only, which was newly described and thus the description of *L. oxycedri* meets the conditions for a *descriptio generico-specifico* (Art. 42.1) and thus both genus and species are validly published." *Lenzitopsis* and *L. oxycedri* as the current names have been accepted here as in the other study (Doğan et al. 2007).

Lenzitopsis was placed in Thelephoraceae because of brownish and echinulate spores (Malençon and Bertault 1963;

* *Corresponding author*. Tel./fax: +86 24 83970348.
E-mail address: liwei_zhou1982@163.com (L.-W. Zhou).
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Ryvarden and Gilbertson 1993; Stalpers 1993). Basidiocarps of *L. oxycedri* have been collected on the branches of living *Juniperus* trees, thus this fungus is regarded as a wood-decaying species. Other genera of the Thelephorales are well-known ectomycorrhizal taxa except for *Amaurodon* J. Schröt. species, which are probably non-mycorrhizal (Tedersoo et al. 2010). Resupinate basidiocarps of some *Amaurodon* species can also be found on living trees (U. Kõljalg unpublished). To understand the evolution of mycorrhizal association in the Thelephorales, it is critical to verify the phylogenetic position of *Lenzitopsis* in Thelephorales.

Based on molecular and morphological evidences we describe a new species of *Lenzitopsis* which grows on *Juniperus chinensis* in China. The phylogenetic placements of the two *Lenzitopsis* species were inferred with nuclear large subunit ribosomal DNA (LSU-rDNA) sequences.

2. Material and methods

2.1. Morphological study

The studied specimens were deposited in the herbaria of Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Beijing Forestry University (BJFC) and Natural History Museum, Institute of Microbiology, University of Tartu (TU). The microscopic procedure follows Dai (2010). Sections were examined at magnification up to ×1000 using a Nikon Eclipse 80i microscope and phase contrast optics. The variation in the size of the spores was presented via excluding 5% of measurements from each end of the range, and the exclusions were given in parentheses. The abbreviations used in the text are as follows: IKI = Melzer's reagent, IKI− = neither amyloid nor dextrinoid, KOH = 5% potassium hydroxide, CB = Cotton blue, CB+ = cyanophilous, CB− = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, and *n* = number of spores measured from given number of specimens. Drawings were made with the aid of a drawing tube. Special colour terms follow Anonymous (1969) and Petersen (1996).

2.2. Molecular analyses

Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) was used to generate PCR products from herbarium specimens according to the manufacturer's instructions. To extract DNA, a small piece of specimen was incubated in 20 μl dilution buffer at room temperature for 3 min, and then 1.5 μl of the supernatant were used as a template for a 50 μl PCR reaction. LSU-rDNA region was amplified with primer pair LR0R and LR7 (Bunyard et al. 1994). Primers ITS5 and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) sequences. The PCR procedure included these steps: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 59 °C for 5 s for ITS region/48 °C for 5 s for LSU-rDNA region, and 72 °C for 5 s, and a final extension of 72 °C for 10 min. The products were purified, and then directly sequenced with the same primers in Beijing Genomics Institute, China. Sequence data for three collections of the new species of *Lenzitopsis*, Dai 10659, Yuan 2952 and Yuan 2959, were obtained.

Two European specimens of *L. oxycedri* (TU 115268 and TU 115337) were sequenced according to Kõljalg et al. (2009), and primer pairs ITSOF-T (ACT TGG TCA TTT AGA GGA AGT)/LB-z (AAA AAT GGC CCA CTA GAA ACT), ITS1F (CTT GG TCA TTT AGA GGA AGT AA)/LBw (CTT TTC ATC TTT CCC TCA CGG) and LR0R/LR7 were used. A high quality sequence was achieved only for the LSU-rDNA for specimen TU 115337, but high quality ITS and LSU-rDNA sequences were obtained for specimen TU 115268.

New sequences of *Lenzitopsis* along with specimen data were deposited into UNITE database under accession numbers UDB010601–UDB010608 (Abarenkov et al. 2010) and GenBank (accession numbers JN169793–JN169800).

2.3. Phylogenetic analysis

Besides the sequences from *Lenzitopsis* generated in this study, 59 other LSU-rDNA sequences available as ingroup and 2 outgroup sequences from *Dacrymyces* Nees (Dacrymycetales) were downloaded and included in the final dataset to construct phylogenetic trees. The dataset was aligned in ClustalX 2.0 (Larkin et al. 2007) with default parameters, and manually edited as necessary. Sequence alignment was deposited at TreeBase (http://www.treebase.org; accession number S11947). Maximum parsimony (MP) was analyzed using PAUP* 4.0b10 (Swofford 2001). All characters were of equal weight and gaps were treated as missing data. One thousand heuristic search replicates were performed. Maximum likelihood (ML) analyses were conducted using PhyML 3.0 (Guindon and Gascuel 2003) with 100 bootstrap replicates under the best-fit evolutionary model, which was selected by jModelTest (Guindon and Gascuel 2003; Posada 2008) according to corrected Akaike information criterion.

ITS sequence distances between two *Lenzitopsis daii* described below (Yuan 2959 and Yuan 2952) and one *L. oxycedri* (TU 115268) specimens were calculated with EMBOSS distmat developed by Tim Carver (http://emboss.bioinformatics.nl/cgi-bin/emboss/distmat).

3. Results

3.1. Taxonomy

Lenzitopsis daii L.W. Zhou & Kõljalg, sp. nov. Figs. 1 and 2

MycoBank no.: MB561768.

Basidiocarpium annuum, pileatum, sessile vel stipitatum omnino lateraliter, imbricatum. Facies hymenii hinnulea vel griseo-sepiacea; lamellula 1–2 per mm. Systema hypharum monomiticum, hyphae septatae fibulatae, hyphae contexti 3–5 μm in diam, hyphae lamellulae 2.8–3.5 μm in diam; sporae subglobosae, brunneolae, echinatae, crassitunicatae, 5–6.7 × 4.8–6 μm.

Type: CHINA. Sichuan Province, Dujiangyan County, Qingchengshan, approx. 30°54′N, 103°35′E, on living tree of *J. chinensis*, 1.XI.2006, Yuan 2959 (holotype in IFP, isotype in TU). INSDC accession numbers: JN169795 (LSU-rDNA); JN169799 (ITS). UNITE accession numbers: UDB010605 (LSU-rDNA); UDB010606 (ITS).

Fig. 1 – Basidiocarps of *Lenzitopsis daii* on *Juniperus chinensis* (Holotype, photo by Yu-Cheng Dai).

Etymology: *daii* (Lat.): in honor of a Chinese mycologist Yu-Cheng Dai.

Fruitbody: Basidiocarps annual, pileate, sessile or with a rudimentary lateral stipe, mostly imbricate, corky or leathery and without odor or taste when fresh, fragile when dry. Pilei semicircular to fan-shaped, projecting up to 2 cm long, 3 cm wide, and 1 cm thick at base. Pileal surface dark, velutinate, azonate; margin narrow, pinkish buff, undulating, curved down when dry. Hymenophore fawn to grayish brown, irregular, lenzitoid, lamella 1–2 per mm; dissepiments fairly thick, entire to lacerate. Context pinkish buff, corky, up to 2 mm thick, lamella concolorous with context, corky, up to 8 mm deep.

Hyphal structure: Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–, tissue darkening but otherwise unchanged in KOH. Context: Generative hyphae hyaline to pale yellowish, slightly thick-walled, frequently branched, regularly arranged, 3–5 μm diam, some irregular crystals present on hyphae. Lamella: Generative hyphae hyaline to pale yellowish, thin- to slightly thick-walled, occasionally branched, subparallel along the lamella, 2.8–3.5 μm diam. Cystidia and cystidioles absent. Basidia narrowly clavate, with four sterigmata and a basal clamp connection, 28–45 × 4–6.5 μm; basidioles in shape similar to basidia, but distinctly shorter than basidia, 21–30 × 3.5–5.4 μm. Spores: Basidiospores subglobose, pale brownish, thick-walled,

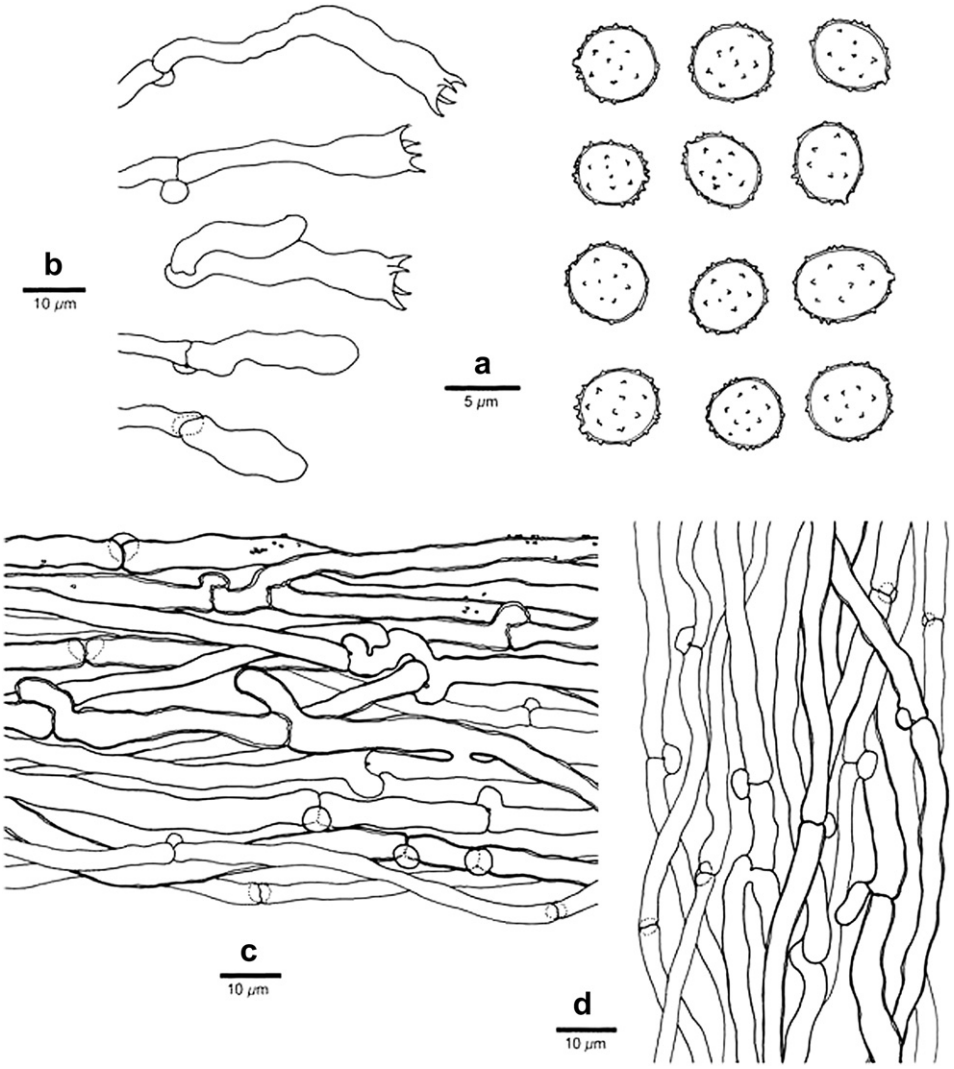

Fig. 2 – Microscopic structures of *Lenzitopsis daii* (Holotype). a: Basidiospores. b: Basidia and basidioles. c: Hyphae from context. d: Hyphae from lamella.

echinulate, amyloid, slightly CB+ when juvenile, 5–6.7(–7) × 4.8–6 μm, L = 5.99 μm, W = 5.10 μm, Q = 1.14–1.2 (n = 120/4).

Type of rot: White rot.

Additional specimens examined: China, Beijing, Summer Palace, on living tree of *J. chinensis*, 25 VII 2005, Dai 6610 (IFP); China, Beijing Botanical Garden, on living tree of *J. chinensis*, 27 IX 2008, Dai 10659 (BJFC); China, Sichuan Province, Dujiangyan County, Qingchengshan, on living tree of *J. chinensis*, 1 XI 2006, Yuan 2952 (IFP), Yuan 2956 (IFP).

3.2. *Molecular phylogeny*

Five new LSU-rDNA sequences from *Lenzitopsis* species (GenBank accession numbers JN169793–JN169797), and 61

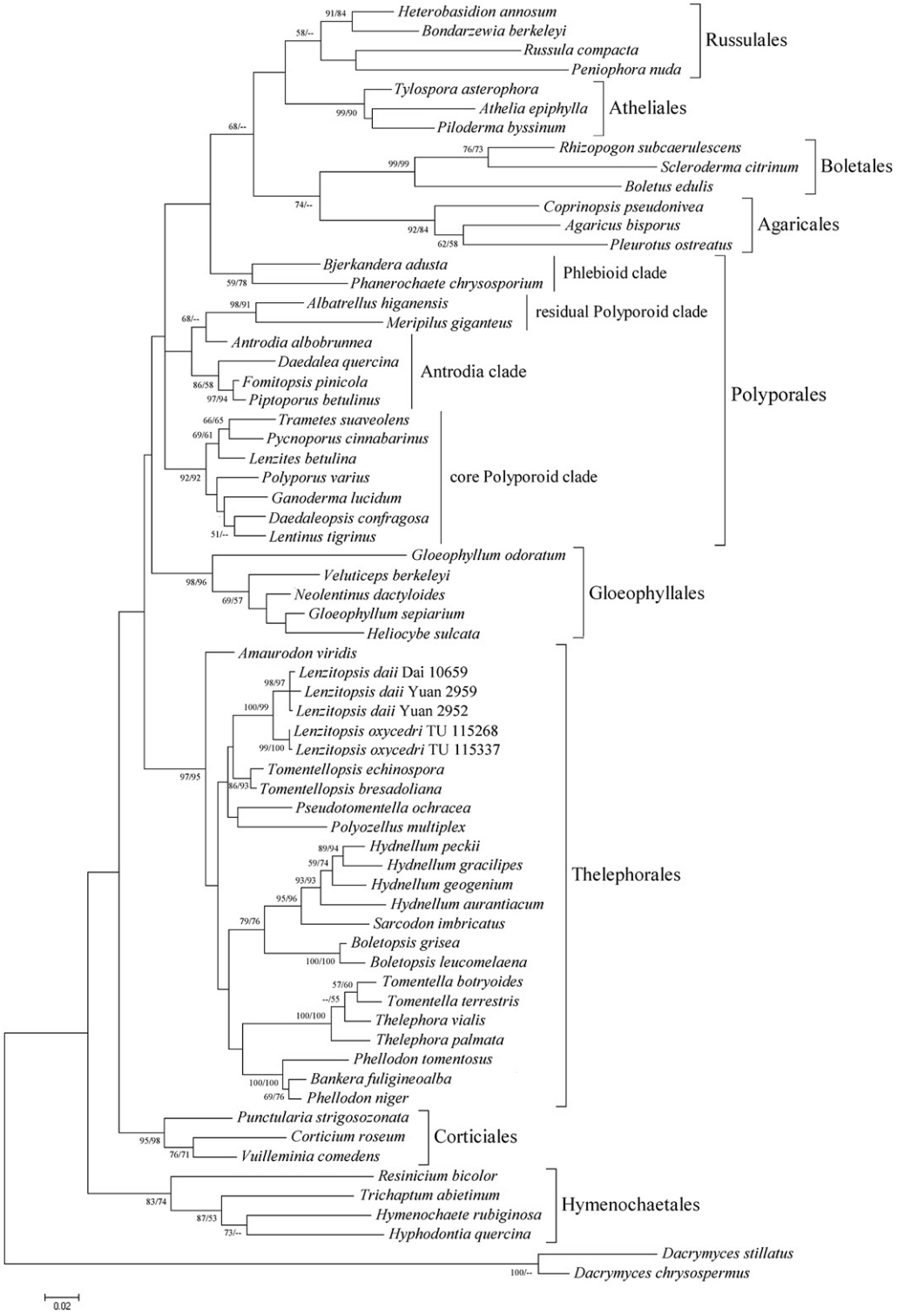

Fig. 3 – Phylogenetic position of *Lenzitopsis daii* and *L. oxycedri* inferred with nuclear LSU-rDNA regions. Topology from ML tree and bootstrap values (not less than 50%) from both ML (the former) and MP (the latter) trees.

LSU-rDNA sequences from other species were included for phylogenetic analyses (Fig. 3). The aligned dataset of LSU-rDNA contained 900 characters, of which 467 were constant, 98 parsimony-uninformative and 335 parsimony-informative. MP analysis generated 12 equally best trees of 2223 steps (CI = 0.315 and RI = 0.514; Supplementary Fig. 1). jModelTest suggested TrN + I + G as the best-fit evolutionary model. The detailed parameters were as follows: unequal base frequencies of $A = 0.2517$, $C = 0.1844$, $G = 0.2872$ and $T = 0.2767$, substitution rates of AC = AT = CG = GT = 1.0000, AG = 4.0866 and CT = 9.1279, a proportion of invariable sites of 0.3230, and a gamma distribution parameter of 0.5810.

The ML tree with bootstrap values from both ML and MP tree is shown in Fig. 3. The monophyly of Thelephorales was well supported with 97% and 95% bootstrap values in ML and MP trees, respectively. All five sequences of *Lenzitopsis* species nested in this order. Moreover, three sequences of *L. daii* and two sequences of *L. oxycedri*, respectively, formed two well supported clades, which clustered together with 100% and 99% bootstrap values in ML and MP trees, respectively.

Based on distance matrix shown in Table 1, the ITS sequences of *L. daii* and *L. oxycedri* specimens (GenBank accession numbers JN169798–JN169800) were more than 5% different. Two specimens of *L. daii* contained almost identical ITS sequences (0.31% difference) but differed from that of *L. oxycedri* by 5.35 and 5.36%, respectively.

4. Discussion

Lenzitopsis daii specimens all have dark pileal surface, irregular and lenzitoid hymenophore, brown hyphae and echinulate spores, and these features overlap with the concept of the genus *Lenzitopsis* (Ryvarden and Gilbertson 1993). However, annual basidiocarps and amyloid spores of *L. daii* make it different from *L. oxycedri* that produces perennial basidiocarps and IKI− spores. In addition, *L. daii* produces slightly smaller spores (5–6.7 × 4.8–6 μm) than *L. oxycedri* (6–7 × 5–7 μm). Moreover, *L. daii* was collected exclusively on living *J. chinensis*, while *L. oxycedri* is restricted to other *Juniperus* trees. Besides the differences in morphology and ecology, molecular analyses of LSU-rDNA sequences split the two species into two well supported clades. This topology strongly differentiated *L. daii* and *L. oxycedri* from each other. The ITS sequences of the two species are also different by deviating from each other more than 5% (Table 1). It is common practice in molecular ecology studies that the 3% difference of ITS sequences is set as a species threshold value (Tedersoo et al. 2003; Izzo et al. 2005). Smith et al. (2007) studied intraspecific variation of ITS in 68 basidiomycete and ascomycete species. The results showed that in all 27 species of their study, the intraspecific variation was generally low (0.16–2.85%, mean = 0.74%). In the present study authors were able to sequence only one specimen of *L. oxycedri*, therefore, could not find data on its intraspecific variation of ITS sequences. However, it was assumed that more than 5% ITS sequence difference combined with LSU-rDNA sequence and morphological/ecological data is sufficient for the safe delimitation of the two species.

Table 1 – Distance matrix of the alignment of ITS sequences from two *Lenzitopsis daii* and one *L. oxycedri* specimens.

		1.	2.	3.
1.	*L. oxycedri* (TU 115268; JN169800)	0.00	5.35	5.36
2.	*L. daii* (Yuan 2959; JN169799)		0.00	0.31
3.	*L. daii* (Yuan 2952; JN169798)			0.00

Macroscopically, both *L. daii* and *L. oxycedri* resemble *Gloeophyllum sepiarium* (Wulfen) P. Karst. and *Daedaleopsis tricolor* (Bull.) Bondartsev & Singer in having lamellate hymenophoral surface; however, the basidiocarps of the two latter species are corky to hard corky. Furthermore, microscopically they have dimitic hyphal structure and smooth basidiospores. But *Lenzitopsis* basidiocarps might be buried in the herbaria under these taxon names.

Although *J. chinensis* is commonly planted in China, its wood seems not attractive to wood-inhabiting fungi, and only eight polypores were recorded on this tree species even if more than 1200 wood-decaying fungi have been found in China (Dai 2011, 2012). *L. daii* was found exclusively on *J. chinensis* in two localities in China; however, its pathogenicity was uncertain (Dai et al. 2007). In Sichuan, the type locality with a warm and semi-arid climate, the specimens of *L. daii* were collected in natural mixed forests, while the Beijing specimens were found on planted *J. chinensis*.

Based on phylogenetic analyses of LSU-rDNA, *Lenzitopsis* is classified in Thelephorales. The monophyly of Thelephorales is well supported, but the lower-level relationship is unresolved which is common when only LSU-rDNA data are utilized (Larsson et al. 2004). Several phylogenetic studies included a few taxa of Thelephorales (Hibbett and Binder 2002; Larsson et al. 2004; Binder et al. 2005; Matheny et al. 2007), but there is no in-depth molecular study of this order. One reason for that is availability of very few living cultures as most members of Thelephorales are difficult to cultivate. Another probable reason is that it is difficult to obtain high quality DNA from more than five-year-old thelephoroid basidiocarps. Without a robust phylogeny of the whole Thelephorales, for now it is unclear whether *Lenzitopsis* belongs to Thelephoraceae or Bankeraceae, which are the only two legitimate and currently used families in Thelephorales (Kirk et al. 2008).

Declaration

All experiments in this manuscript comply with the current laws of China and Estonia.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.myc.2012.06.002.

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