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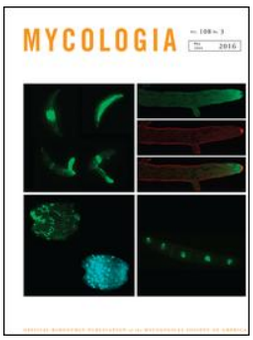

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Global diversity and phylogeny of *Onnia* (Hymenochaetaceae) species on gymnosperms

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ABSTRACT

Onnia includes white rotting polypores with annual basidiocarps, a duplex context, monomitic hyphal structure, hymenial setae, and hyaline, thin-walled, smooth basidiospores. Specimens of *Onnia*, originating mainly from East Asia, Europe, and North America, were studied using both morphology and phylogenetic analyses. Our concatenated data set was derived from 25 collections and included (i) 25 nuc rDNA internal transcribed spacer region sequences (ITS1-5.8S-ITS2 = ITS), 17 generated in this study; and (ii) 14 nuc rDNA 28S rDNA sequences, including the D1–D2 domains, 11 of them generated in this study. The resulting maximum likelihood and Bayesian phylogenies recovered all sampled collections of *Onnia* as a well-supported clade. In this clade, three previously accepted species, viz., *Onnia leporina, O. tomentosa*, and *O. triquetra*, received strong support, whereas three additional lineages with strong support represent the new species described in this paper, *O. subtriquetra, O. microspora*, and *O. tibetica*. Of the six *Onnia* species occurring on gymnosperms, *O. tomentosa* and *O. leporina* grow mainly on *Picea* and have circumboreal distribution in the Northern Hemisphere. In contrast, other species that mostly grow on *Pinus* are geographically restricted to limited regions, viz., *O. triquetra* in Europe, *O. subtriquetra* in North America, and *O. microspora* and *O. tibetica* in Asia.

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Hymenochaetales; polypore; taxonomy; wood-inhabiting fungi

INTRODUCTION

Onnia P. Karst. was described by Karsten (1889) with *Polyporus circinatus* Fr. (*O. tomentosa* [Fr.] P. Karst.) as its type. The genus is characterized by annual, sessile or stipitate fruiting bodies, a duplex context, monomitic hyphal system with generative hyphae bearing simple septa, the presence of mostly hooked hymenial setae, and hyaline, thin-walled, smooth, nonamyloid, nondextrinoid, and acyanophilous basidiospores (Niemelä 2005; Dai 2010). *Onnia* stands out as a small but distinct clade of Hymenochaetaceae according to recent molecular analyses (Wagner and Fischer 2002; Larsson et al. 2006; Dai 2010), although some mycologists treat it as a synonym of *Inonotus* P. Karst. (Gilbertson and Ryvarden 1986; Ryvarden 2005; Ryvarden and Melo 2014). Morphologically, *Onnia* resembles *Inonotopsis* Parmasto (part of *Inonotus* sensu lato) by having hyaline, thin-walled basidiospores, but the latter has resupinate basidiocarps that lack setae. Meanwhile, *Onnia* resembles *Coltricia* Gray in having stipitate basidiocarps, but the latter has thick-walled and yellowish basidiospores and lacks setae. Phylogenetically, *Onnia* is related to *Porodaedalea* (Wagner and Fischer 2002; Larsson et al. 2006; Dai 2010), but *Porodaedalea* has a perennial growth habit, pileate basidiocarps lacking a stipe, dimitic hyphal structure, and straight setae.

Most species of *Onnia* grow on gymnosperms (Ryvarden 2005; Dai 2010; Ryvarden and Melo 2014), with the most important pathogenic species on Pinaceae. A few species occur on angiosperms, e.g., *Onnia vallata* (Berk.) Y.C. Dai & Niemelä (Dai 2010), but no DNA data are available for *O. vallata*, which was placed in the genus according to its morphology. The present study focuses on species on gymnosperms.

Three species of *Onnia, O. leporina* (Fr.) H. Jahn (*I. leporinus* [Fr.] Gilb. & Ryvarden, *Pelloporus leporinus* [Fr.] Krieglst.), *O. tomentosa* (Fr.) P. Karst. (*I. tomentosus* [Fr.] Teng, *P. tomentosus* [Fr.] Quél.), and *O. triquetra* (Fr.) Imazeki (*I. triqueter* [Fr.] P. Karst., *P. triqueter* [Fr.] Quél.), grow on gymnosperms in the

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Northern Hemisphere (Gilbertson and Ryvarden 1986; Ryvarden 2005; Ryvarden and Melo 2014; Dai 2012), and *O. leporina* and *O. tomentosa* are pathogens on *Picea* and *Pinus* trees (Sinclair et al. 1987; Hunt and White 1998; Dai et al. 2007; Ryvarden and Melo 2014).

Previous phylogenetic analyses of *Onnia* in Hymenochaetaceae were based on limited sampling (Wagner and Fischer 2002; Dai 2010), and species diversity has mostly been assessed based on morphology. The aim of the present work is to investigate the phylogeny and taxonomy of *Onnia* species growing on gymnosperms of broad geographic origin. Three new species are described based on both morphological characters and molecular phylogenetic analysis.

MATERIALS AND METHODS

Specimens examined were deposited in the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC), National Museum Prague of Czech Republic (PRM), the private herbarium of J. Vlasák (JV), and the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Macromorphological descriptions were based on field notes and herbarium specimens. Color terms followed Petersen (1996). Micromorphological data were obtained from dried specimens, as observed under a light microscope following the methods of Chen and Cui (2014). Sections were studied at a magnification of up to ×1000 using a Nikon E 80i microscope (Nikon, Tokyo, Japan) with phase-contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic characters, measurements, and drawings were made from slide preparations stained with Cotton Blue (CB) and Melzer's reagent (IKI). Spores were measured from sections cut from the tubes. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range and are given in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide; IKI– = both nonamyloid and nondextrinoid; CB– = acyanophilous; L = mean spore length (arithmetic average of all spores ± standard error); W = mean spore width (arithmetic average of all spores ± standard error); Q = variation in the L/W ratios between the specimens studied; n (a/b) = number of spores (a) measured from given number (b) of specimens.

A CTAB (cetyltrimethyl ammonium bromide) rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to extract total genomic DNA from dried specimens following the manufacturer's instructions with some modifications (Chen et al. 2015, 2016). For polymerase chain reactions (PCR), the DNA was amplified with the primers ITS4 and ITS5 for nuc rDNA internal transcribed spacer region (ITS1-5.8S-ITS2 = ITS) (White et al. 1990), and LR0R and LR7 for nuc rDNA 28S rDNA (= 28S) (Vilgalys and Hester 1990). The PCR profile for ITS was initial denaturation at 95 C for 3 min, followed by 35 cycles at 94 C for 40 s, 54 C for 45 s, and 72 C for 1 min, and a final extension at 72 C for 10 min. The PCR profile for 28S was initial denaturation at 94 C for 1 min, followed by 35 cycles at 94 C for 30 s, 50 C for 1 min, and 72 C for 1.5 min, and a final extension at 72 C for 10 min. PCR products were purified and sequenced at the Beijing Genomics Institute, China, with the same primers.

Seventeen ITS and 11 28S sequences were newly generated from specimens of *Onnia* submitted to GenBank (Supplementary Table 1). To construct the phylogeny of *Onnia, Porodaedalea pini* (Brot.) Murrill was selected as an outgroup, and *Phellinopsis andina* (Plank & Ryvarden) Rajchenb. & Pildain and *P. conchata* (Pers.) Y.C. Dai were selected as additional ingroups related to *Onnia* (Larsson et al. 2006; Zhou 2015; Zhou et al. 2016). The ITS and 28S regions were separately aligned using MAFFT 7 with G-INS-i option (Katoh and Standley 2013), and then the two resulting alignments were concatenated. The concatenated alignment was subjected to incongruence length difference (ILD) test (Farris et al. 1994) implemented in PAUP* 4.0b10 (Swofford 2002) with a heuristic search and 1000 bootstrap (BS) replicates. The ILD test generated a *P* value of 1.000, much greater than 0.01, which indicated that there was no incongruence between the ITS and 28S regions for phylogenetic analyses. Therefore, the concatenated alignment was used for subsequent phylogenetic analyses and was deposited in TreeBase (www.treebase.org; submission ID S19345).

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) algorithms following the best-fit evolutionary model estimated using jModelTest 2.1.4 (Guindon and Gascuel 2003; Darriba et al. 2012). The ML tree was constructed using raxmlGUI 1.2 (Stamatakis 2006; Silvestro and Michalak 2012) with an auto FC option in the bootstrap (BS) test (Pattengale et al. 2010). The BI analysis was conducted using MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003). Two independent runs were performed, each starting from random trees with four chains for 10 million generations. Trees were sampled every 1000 generations. The first quarter of sampled trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority consensus tree and calculate Bayesian posterior probabilities (BPPs).

RESULTS

The concatenated data set, with 25 ITS and 14 28S sequences derived from 25 specimens, resulted in an alignment of 1662 characters. The best-fit evolutionary model of this alignment for phylogenetic analyses was estimated as GTR+I+G. The BS test for ML analysis stopped after 250 replicates. After 10 million generations, the effective sample sizes of all parameters were greater than 6000 and the potential scale reduction factors approached 1.000, which indicated all chains converged in BI. The ML and BI algorithms generated congruent topologies in main lineages; thus, only the topology from ML algorithm was presented along with BS and BPP, respectively, greater than 50% and 0.8 at the nodes.

The current phylogeny placed all specimens of *Onnia* in a fully supported clade (Fig. 1). Within this clade, three previously accepted species, viz., *Onnia leporina, O. tomentosa*, and *O. triquetra*, received strong support. In addition, three additional lineages with strong support were identified. Combined with morphological characters, the three additional lineages are described as new species below. At the species level, *O. tomentosa* occupied a separate position from the other five species.

TAXONOMY

Onnia subtriquetra Vlasák & Y.C. Dai, sp. nov. Figs. 2, 3
MycoBank MB815593

Typification: USA. VIRGINIA: Woodbridge, Mason Neck State Park, on *Pinus*, 9 Oct 2004, *Josef Vlasák Jr.* (**holotype** PRM 944506). **Isotypes** JV0410/12-J, BJFC018941.

Etymology: *Subtriquetra* (Latin), referring to the similarity to *Onnia triquetra*.

Basidiocarps annual, pileate to laterally substipitate, solitary, hard corky upon drying. Pilei dimidiate to fan-shaped, projecting up to 5 cm, 8 cm wide and 20 mm thick at base. Pileal surface cinnamon to yellowish brown when dry, homogeneous, scrupose when juvenile, distinctly velutinate with age, concentric zones lacking; margin sharp, curved down when dry. Pore surface grayish yellow to fawn when dry; pores angular, 2–3 per mm; dissepiments thin, slightly lacerate. Context: Duplex, upper layer cinnamon-buff, spongy, up to 12 mm thick, lower layer honey-yellow, hard corky, up to 3 mm thick, a demarcation zone between the two layers indistinct. Tubes: Pinkish buff, paler than context and pore surface, hard corky to brittle, up to 5 mm long. Stipe very short to almost lacking; pores decurrent on stipe.

Hyphal system monomitic, generative hyphae simple septate, IKI–, CB–; tissues darkening but otherwise unchanged in KOH. Context: Hyphae in upper layer pale yellowish to golden yellow, thin-walled, rarely branched with frequent simple septa, regularly arranged, 4–7 µm diam; hyphae in the lower layer yellowish to golden brown, thin- to slightly thick-walled, occasionally branched with frequent simple septa, regularly arranged, more or less agglutinated, 4–6 µm diam; hyphae in stipe similar to those in context. Tubes: Tramal hyphae hyaline to yellowish, thin- to slightly thick-walled, occasionally branched and frequently septate, parallel along the tubes, slightly agglutinated, 2.5–3.5 µm diam.

Hymenium: Setae hooked, sharp-pointed, dark brown, thick-walled, very deep-rooting, 70–180 × 15–25 µm; cystidia and cystidioles absent; basidia clavate, with four sterigmata and a simple septum at the base, 14–19 × 4–6

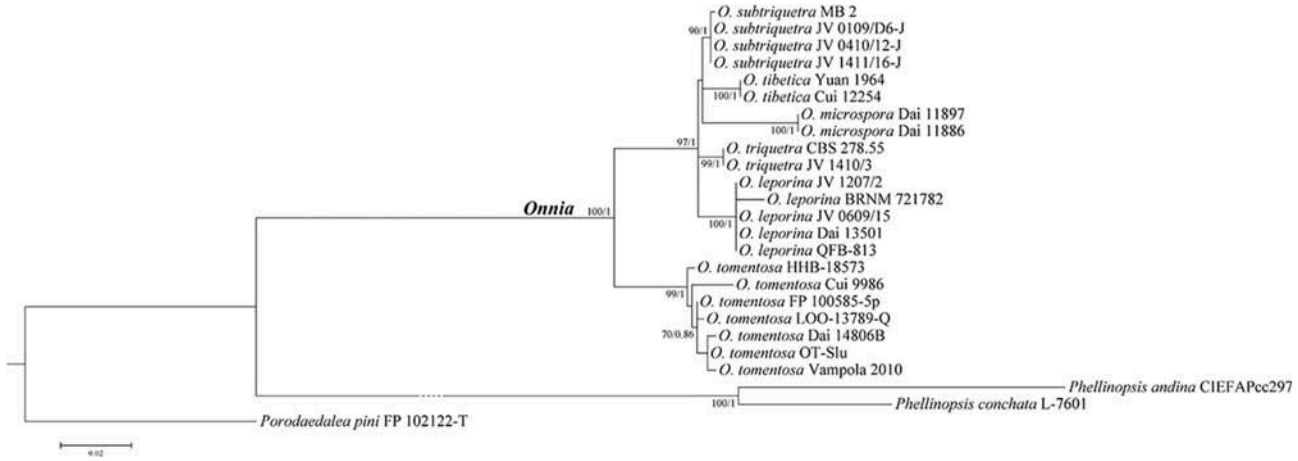

Figure 1. Phylogeny of *Onnia* inferred from the concatenated data set of ITS and 28S regions. The topology is from maximum likelihood algorithm, and the bootstrap values from maximum likelihood algorithm and Bayesian posterior probabilities from Bayesian inference algorithm, respectively, greater than 50% and 0.8 are labeled at the nodes.

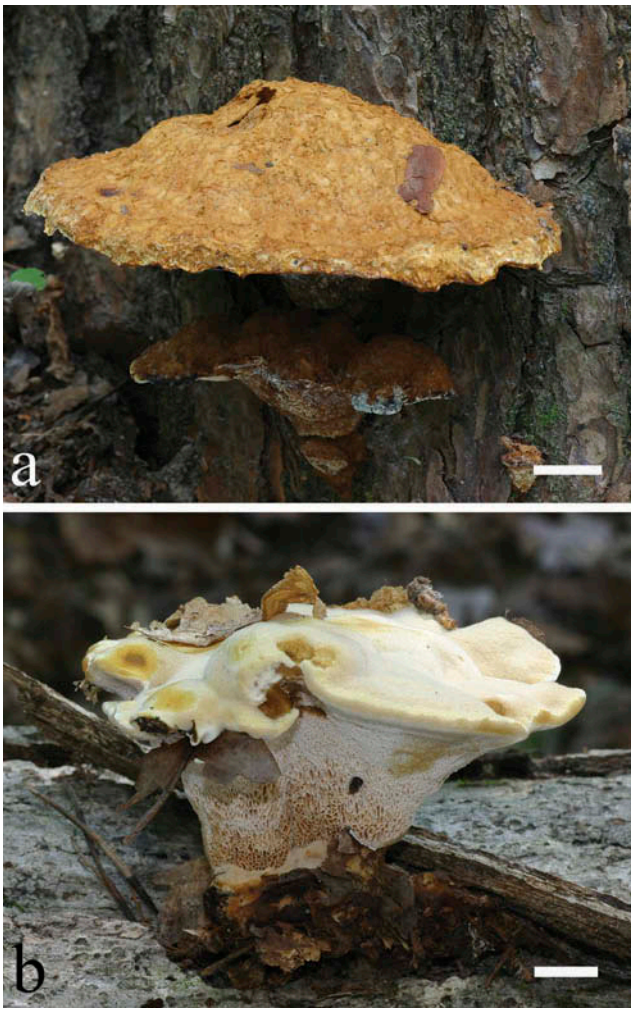

Figure 2. Basidiocarps of *Onnia subtriquetra*. a. Holotype. b. JV1411/16-J. Bars = 1 cm.

μm; basidioles dominant, in shape similar to basidia, but distinctly smaller. Basidiospores oblong-ellipsoidal, hyaline, thin-walled, smooth, some with a big guttule, IKI–, CB–, (5–)5.5–6.5 × 3–4 μm, L = 6.14 ± 0.03 μm, W = 3.7 ± 0.05 μm, Q = 1.6–1.7 (n = 60/2).

Other specimens examined: USA. MARYLAND: Pokomoke City, Pokomoke State Forest, on *Pinus*, Sep 2001, *Josef Vlasák Jr.* (PRM 944507, JV0109/D6-J); MICHIGAN: base of living *Pinus*, 1999, *Banik MB-number 2* (CFMR); NEW JERSEY: Bass River State Forest, on *Pinus*, Nov 2014, *Josef Vlasák Jr.* (PRM 944508, JV1411/16-J).

Notes: Onnia subtriquetra is similar to *O. triquetra*, but the hymenial setae in the latter project 40–50 μm beyond the hymenium and cannot be observed with a 5× lens, and its basidia and basidioles are almost in the same size, whereas the hymenial setae in *O. subtriquetra* project 50–80 μm beyond the hymenium and can be observed by 5× lens, and its basidia are distinctly longer than basidioles.

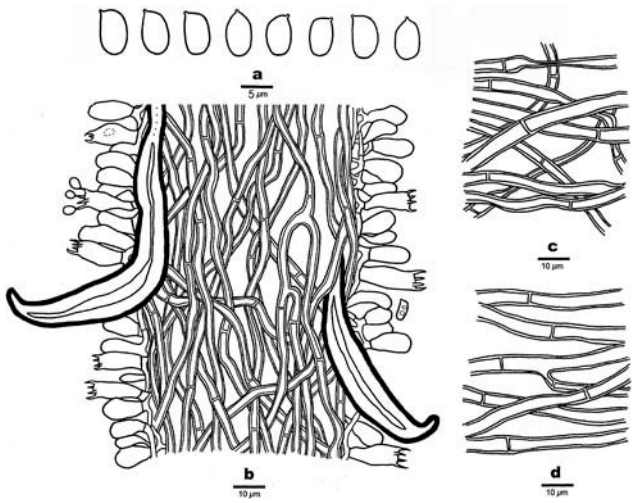

Figure 3. Microscopic structures of *Onnia subtriquetra*, drawn from holotype. a. Basidiospores. b. Section of trama. c. Hyphae from upper tomentum. d. Hyphae from context.

Moreover, the two species are not closely related in the phylogenetic tree (Fig. 1).

Onnia microspora Y.C. Dai & L.W. Zhou, sp. nov. Figs. 4,5
MycoBank MB815594

Typification: CHINA. ANHUI: Huangshan, Huangshan Nature Reserve, on root of living *Pinus massoniana*, 21 Oct 2010, *Y.-C. Dai 11897* (**holotype** BJFC008999).

Etymology: *Microspora* (Latin), referring to the small basidiospores.

Basidiocarps annual, pileate to laterally substipitate or stipitate, solitary, without odor or taste and corky when fresh, becoming hard corky upon drying. Pilei dimidiate to fan-shaped, projecting up to 4 cm, 6 cm wide and 9 mm thick at center. Pileal surface golden brown to buff-yellow when fresh, becoming yellowish brown to cinnamon-buff, homogeneous and distinctly velutinate, concentric zones lacking; margin sharp to

Figure 4. Basidiocarps of *Onnia microspora*, Dai 11886. Bar = 1 cm.

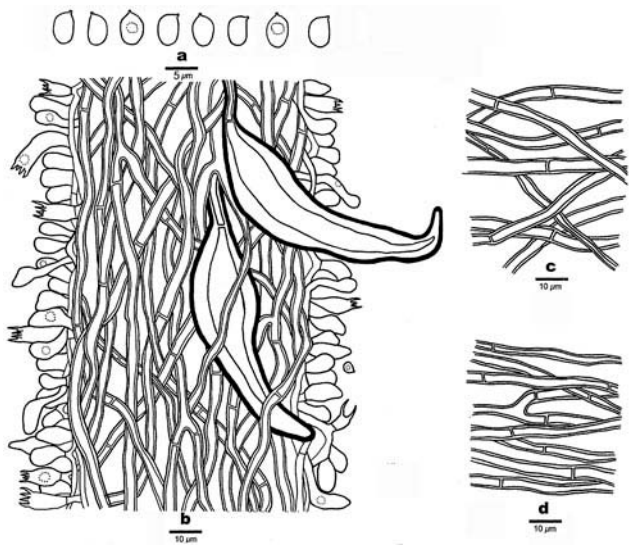

Figure 5. Microscopic structures of *Onnia microspora*, drawn from holotype. a. Basidiospores. b. Section of trama. c. Hyphae from upper tomentum. d. Hyphae from context.

blunt, curved down when dry. Pore surface ashy white to grayish brown when fresh, becoming pinkish buff to buff-yellow when dry; pores angular, 3–5 per mm; dissepiments thin, entire to slightly lacerate. Context: Duplex, upper layer cinnamon, spongy, up to 3 mm thick, lower layer buff, hard corky, up to 3 mm thick, a demarcation zone between the two layers indistinct. Tubes: Clay-buff to fawn, slightly darker than context and pore surface, hard corky, up to 3 mm long. Stipe clay-buff, woody hard when dry, velutinate, up to 1 cm long, 5 mm diam; pores decurrent on stipe.

Hyphal system monomitic, generative hyphae simple septate, IKI–, CB–; tissues darkening but otherwise unchanged in KOH. Context: Hyphae in upper layer pale yellowish to golden brown, thin- to slightly thick-walled, frequently branched with frequent simple septa, loosely interwoven, 4–5 µm diam; hyphae in the lower layer yellowish to golden brown, thin- to slightly thick-walled, rarely branched, with frequent simple septa, regularly arranged, more or less agglutinated, 3–5 µm diam; hyphae in stipe similar to those in context. Tubes: Tramal hyphae hyaline to yellowish, thin- to slightly thick-walled, frequently branched and septate, parallel along the tubes, agglutinated, 2.5–6 µm diam.

Hymenium: Setae hooked, sharp-pointed, dark brown, thick-walled, deep-rooting, 40–80 × 11–23 µm; cystidia and cystidioles absent; basidia clavate, with four sterigmata and a simple septum at the base, 9–17 × 4.8–6 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoidal, hyaline, thin-walled, smooth, IKI–, CB–, 4–5.5 × 3–4 µm, L = 4.9 ± 0.05 µm, W = 3.5 ± 0.05 µm, Q = 1.35–1.45 (n = 60/2).

Other specimens examined: CHINA. ANHUI: Huangshan, Huangshan Nature Reserve, on root of living *Pinus*, 21 Oct 2010, *Y.-C. Dai 11886* (BJFC008988). ZHEJIANG: Lin'an, Tianmushan Nature Reserve, on *Pinus*, 9 Oct 2005, *B.-K. Cui 2540* (BJFC001322) and *B.-K. Cui 2563* (BJFC001328); 11 Oct 2005, *B.-K. Cui 2665* (BJFC001321), *B.-K. Cui 2670* (BJFC001323), *B.-K. Cui 2679* (BJFC001324), *B.-K. Cui 2690* (BJFC001327), and *B.-K. Cui 2695* (BJFC001325); 16 Oct 2004, *Y.-C. Dai 6424* (IFP003473); 12 Oct 2005, *B.-K. Cui 2737* (IFP014965), *B.-K. Cui 2741* (IFP014966), and *B.-K. Cui 2746* (BJFC001326).

Notes: In the phylogenetic tree, two samples of *Onnia microspora* form a distinct lineage with strong support (100% ML, 1.00 BPPs; Fig. 1) and in a clade with *O. subtriquetra* and *O. tibetica*. Morphologically, *O. microspora* has a similar macromorphology to *O. triquetra* and both species grow on *Pinus*, explaining why specimens were first identified as *O. triquetra* by Dai (2010, 2012). However, after further study of the Chinese specimens, *O. microspora* differs from *O. triquetra* in both molecular sequences and morphology. *Onnia microspora* has smaller pores (4–5/mm vs. 2–4/mm) and shorter basidiospores (4.1–5.4 × 3–4 µm vs. 5.5–7 × 3–4 µm) than *O. triquetra* (Ryvarden and Melo 2014).

Onnia tibetica Y.C. Dai & S.H. He, sp. nov. Fig. 6
MycoBank MB815595

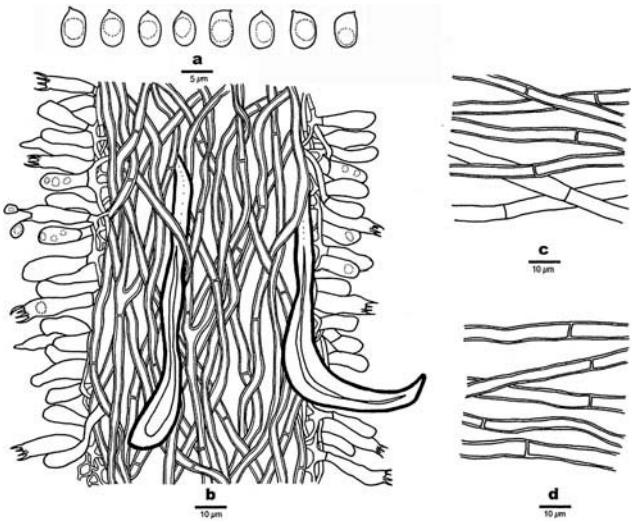

Figure 6. Microscopic structures of *Onnia tibetica*, drawn from holotype. a. Basidiospores. b. Section of trama. c. Hyphae from upper tomentum. d. Hyphae from context.

Typification: CHINA. XIZANG (TIBET): Bomi County, Tongmai, on root of living *Pinus*, 22 Sep 2014, *B.-K. Cui 12254* (**holotype** BJFC017168).

Etymology: Tibetica (Latin), referring to the occurrence in Tibet, China.

Basidiocarps annual, laterally substipitate, solitary, without odor or taste and corky when fresh, becoming woody hard to bone hard upon drying. Pilei more or less semicircular to fan-shaped, projecting up to 7 cm, 10 cm wide and 10 mm thick at center. Pileal surface clay-buff to fawn when dry, distinctly velutinate, concentric zones lacking; margin sharp, curved down when dry. Pore surface fuscous to dark brown when dry, shinning; sterile margin very narrow to almost lacking; pores angular, 2–4 per mm; dissepiments thin, slightly lacerate. Context: Duplex, upper layer cinnamon, spongy, up to 3 mm thick, lower layer fawn, hard corky, up to 2 mm thick, a demarcation zone between the two layers indistinct. Tubes: Buff-yellow, slightly paler than context and pore surface, hard corky to brittle, up to 5 mm long. Stipe clay-buff to fawn when dry, distinctly velutinate, up to 4 cm long, 10 mm diam, duplex, outer layer cinnamon, spongy, up to 2 mm thick, inner part fawn, hard corky, a demarcation zone between the two parts indistinct; pores decurrent on stipe.

Hyphal system monomitic, generative hyphae simple septate, IKI–, CB–; tissues darkening but otherwise unchanged in KOH. Context: Hyphae in upper layer pale yellowish to golden brown, thin- to slightly thick-walled, rarely branched with frequent simple septa, regularly arranged, 5–8 μm diam; hyphae in the lower layer yellowish to golden brown, thin- to slightly thick-walled, rarely branched, with frequent simple septa, regularly arranged, agglutinated, some hyphae bearing an oily-like substance, 4–7 μm diam; hyphae in stipe similar to those in context. Tubes: Tramal hyphae hyaline to yellowish, thin- to slightly thick-walled, frequently branched and septate, parallel along the tubes, agglutinated, 2.5–6 μm diam.

Hymenium: Setae hooked, sharp-pointed, dark brown, thick-walled, deep-rooting, 70–150 × 10–18 μm; cystidia absent; cystidioles present, mostly fusoid, hyaline, thin-walled, 18–28 × 3–5 μm; basidia clavate, with four sterigmata and a simple septum at the base, 15–22 × 4–6 μm; basidioles dominant, in shape similar to basidia, but slightly smaller. Basidiospores ellipsoidal, hyaline, thin-walled, smooth, with a big guttule, IKI–, CB–, 5–6 × 3–4 μm, L = 5.31 ± 0.04 μm, W = 3.47 ± 0.05 μm, Q = 1.4–1.6 (n = 60/2).

Other specimens examined: CHINA. SICHUAN: Daocheng County, Julong, on ground of *Pinus* forest, 8 Aug 1984, *H.-S. Yuan 1964* (BJFC001319).

Notes: Onnia subtriquetra is closely related to *O. tibetica* in the phylogenetic tree (Fig. 1), and both species grow mostly on *Pinus*. However, *Onnia tibetica* is distinguished from *O. subtriquetra* in having cystidioles and shorter basidiospores (5–6 μm in *O. tibetica* vs. 5.5–6.5 μm in *O. subtriquetra*). *Onnia tibetica* is similar to *O. tomentosa* in field and macromorphology, but the latter has straight hymenial setae, lacks cystidioles, and grows mostly on *Picea* (Ryvarden and Melo 2014). In addition, *O. tibetica* is distant from *O. tomentosa* in the phylogenetic analyses (Fig. 1).

Specimens examined of other species: ***Onnia leporina***. CHINA. HEILONGJIANG: Yichun, Wuying, Fenlin Nature Reserve, on *Picea*, 10 Aug 2000, *R. Penttilä 13448* (BJFC013495); JILIN: Antu County, Changbaishan Nature Reserve, on *Picea*, 9 Sep 2013, *Y.-C. Dai 13501* (BJFC014963). CZECH REPUBLIC. Hluboká nad Vltavou, Libochovka, on *Picea*, Sep 2006, *Josef Vlasák* (PRM 944511, JV0609/15), 22 July 2012, *Josef Vlasák* (PRM 944510, JV1207/2). FINLAND. Sompion Lappi, Sodankylä, on *Picea*, 4 Aug 1998, *Y.-C. Dai 2765* (BJFC001316). ***Onnia tomentosa.*** CHINA. INNER MONGOLIA: Genhe, Great Xingan Nature Reserve, on *Larix*, 27 Aug 2009, *Y.-C. Dai 11022* (IFP008502); JILIN: Antu County, Changbaishan Nature Reserve, on *Larix*, 13 Sep 2014, *Y.-C. Dai 14806b* (BJFC017920), 25 Aug 2005, *Y.-C. Dai 6960* (BJFC001318); on *Picea*, 8 Aug 2011, *B.-K. Cui 9986* (BJFC010879); 4 Aug 2008, *Y.-C. Dai 10244* (IFP008401). CZECH REPUBLIC. Sucha District, Jihlava, on the ground in conifer wood, 1 Oct 2010, *Marek Brom* (PRM 944514, JV1010/1.10, MJ75/10). FINLAND. Etelä-Häme, Lammi Biological Station, ground of *Picea* forest, 9 Sep 1997, *Y.-C. Dai 2591* (BJFC001320). USA. ALASKA: On *Picea*, 2000, *HHB-18673* (CFMR); NEW HAMPSHIRE: The Bowl, White Mt., on ground, Sep 2008, *Josef Vlasák* (PRM 944512, JV0809/77); WASHINGTON: Queets River, Queets, on ground, Sep 2003, *Josef Vlasák* (PRM 944513, JV0309/10-J). ***Onnia triquetra***. CZECH REPUBLIC. Strunkovice n.Vol., on *Pinus*, Aug 2002, *Josef Vlasák* (JV0208/14), Bezdrev cabins, Hluboká, Oct 1995, on *Pinus, Josef Vlasák* (PRM 944509, JV9510/1A).

DISCUSSION

In this study, most previously accepted species of *Onnia* were subjected to morphological examination and phylogenetic analyses (Niemelä 2005; Dai 2010). Three new *Onnia* species, *O. subtriquetra, O. microspora*, and *O. tibetica*, are described based on morphological differences and molecular phylogenetic analyses.

All six species of *Onnia* included in the current phylogenetic analyses formed a fully supported clade (Figs. 1). Previous studies recovered *Onnia leporina, O. tomentosa*, and *O. triquetra* in a single clade but without statistical support (Larsson et al. 2006; Zhou 2015; Zhou et al. 2016). Those phylogenies were inferred from 5.8S region combined with the D1–D2 domains of 28S region (Larsson et al. 2006) or even the single D1–D2 domains of 28S region (Zhou 2015; Zhou et al. 2016). Those regions are very conserved and normally are more suitable than ITS1 and ITS2 regions for inferring phylogeny on a larger taxonomic scale, such as Hymenochaetales in Larsson et al. (2006) and Hymenochaetaceae in Zhou (2015) and Zhou et al. (2016). Our data set focused only on *Onnia* rather than the family or order and included the more variable ITS1 and ITS2 regions along with the 28S region for phylogenetic analyses. This extra data enabled the genus *Onnia* to be recovered as a clade with full support for the first time.

At the species level, the six species of *Onnia* formed two strongly supported clades: one composed of *O. tomentosa*, and the other with the remaining five species (Figs. 1). *Onnia leporina* and *O. tomentosa* have a broad distribution in temperate and boreal forests of Asia, Europe, and North America, and some sequence variability existed in specimens of these two species from different geographic regions, which was indicated by some branching within the species clade. Ecologically, *O. leporina* and *O. tomentosa* grow mainly on species of *Picea* and have circumboreal distribution in North America, Europe, and North Asia, whereas *Onnia triquetra, O. subtriquetra*, and *O. microspora* and *O. tibetica* occur mostly on species of *Pinus* and seem to have limited distribution. The former two species are geographically restricted to Europe and North America, respectively, and the latter two species restricted to Asia. No distinct variability among collections of these four species was observed in the current phylogenetic analyses.

The classification of *Onnia flavida* (Berk.) Y.C. Dai was proposed by Dai (2010) based on its morphology. Recent phylogenetic analyses showed it to be clearly separated from *Onnia* and other genera of *Inonotus* s. l., and the new genus *Cylindrosporus* was proposed for it (Zhou 2015). *Polyporus cumingii* Berk. and *Polystictus incisus* Lloyd were originally described from the Philippines, but their hosts were not mentioned (Ryavrden 1976, 1992). These two species were recombined later as *Onnia cumingii* (Berk.) Imazeki and *Onnia incisa* (Lloyd) Imazeki without explanation (Imazeki 1943, 1952). However, the type of *Polyporus cumingii* represents a specimen of *Phylloporia spathulata* (Hook.) Ryvarden (Ryvarden 1976) and *Polyporus cumingii* is a synonym of *Microporellus obovatus* (Jungh.) Ryvarden (Ryvarden 1992). Thus, they can be excluded from *Onnia. Polyporus orientalis* Lloyd was described from Japan and recombined as *Onnia orientalis* (Lloyd) Imazeki, again without explanation (Imazeki 1943), but according to Ryvarden (1990), it is a synonym of *Onnia vallata*. Presently, there are no sequence data for *O. vallata* and its classification is accepted provisionally until such data confirm its position.

KEY TO SPECIES OF *ONNIA* ON GYMNOSPERMS

1. Growing on *Picea* 2
1′. Growing on *Pinus*.. 3
2. Basidiocarps pileate to laterally stipitate; setae hooked ..... *O. leporina*
2′. Basidiocarps centrally or laterally stipitate; setae straight ..... *O. tomentosa*
3. Cystidioles present.. ..... *O. tibetica*
3′. Cystidioles absent. 4
4. Pores 3–5/mm; basidiospores 4–5.5 µm long; occurring in East Asia.. .... *O. microspora*
4′. Pores 2–4/mm; basidiospores 5–7 µm long; occurring in Europe or North America 5
5. Hymenial setae projecting 50–80 µm beyond hymenium, observable with a 5× lens; basidia longer than basidioles; occurring in East North America ..... *O. subtriquetra*
5′. Hymenial setae projecting 40–50 µm beyond hymenium, not observable with a 5× lens; the same size of basidia and basidioles; occurring in Europe ..... *O. triquetra*

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LITERATURE CITED

Chen JJ, Cui BK. 2014. *Phlebiporia bubalina* gen. et. sp. nov. (Meruliaceae, Polyporales) from southwest China with a preliminary phylogeny based on rDNA sequences. Mycological Progress 13:563–573.

Chen JJ, Cui BK, Dai YC. 2016. Global diversity and molecular systematics of *Wrightoporia* s. l. (Russulales, Basidiomycota). Persoonia 37:21–36.

Chen JJ, Cui BK, Zhou LW, Korhonen K, Dai YC. 2015. Phylogeny, divergence time estimation, and biogeography of the genus *Heterobasidion* (Basidiomycota, Russulales). Fungal Diversity 71:185–200.

Dai YC. 2010. *Hymenochaetaceae* (Basidiomycota) in China. Fungal Diversity 45:131–343.

Dai YC. 2012. Polypore diversity in China with an annotated checklist of Chinese polypores. Mycoscience 53:49–80.

Dai YC, Cui BK, Yuan HS, Li BD. 2007. Pathogenic wood-decaying fungi in China. Forest Pathology 37:105–120.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Gilbertson RL, Ryvarden L. 1986. North American polypores. Oslo, Norway: FungiFlora. 433 pp.

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

Hunt RS, White T. 1998. First report of *Inonotus tomentosus*, the cause of tomentosus root disease, from the Yukon Territory. Plant Disease 82:264.

Imazeki R. 1943. Genera of Polyporaceae of Nippon. Bulletin of the Tokyo Science Museum 6:1–111.

Imazeki R. 1952. A contribution to the fungous flora of Dutch New Guinea. Bulletin of the Government Forestry Experimental Station, Meguro 57:87–128.

Karsten PA. 1889. Kritisk öfversigt af Finlands Basidsvampar (Basidiomycetes; Gastero- & Hymenomycetes). Bidrag till Kännedom af Finlands Natur och Folk 48:1–470.

Katoh K, Standley DM. 2013. MAFFT: multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30:772–780.

Larsson KH, Parmasto E, Fischer M, Langer E, Nakasone K, Redhead S. 2006. Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. Mycologia 98:926–936.

Niemelä T. 2005. Polypores, lignicolous fungi. Norrlinia 13:1–320.

Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2010. How many bootstrap replicates are necessary? Journal of Computational Biology 17:337–354.

Petersen JH. 1996. Farvekort. The Danish Mycological Society´s colour-chart. Greve, Danmark: Foreningen til Svampekundskabens Fremme. 6 pp.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Ryvarden L. 1976. Type studies in the Polyporaceae 7. Species described by J.M. Berkeley from 1836 to 1843. Kew Bulletin 31:81–103.

Ryvarden L. 1990. Type studies in the Polyporaceae 22. Species described by C.G. Lloyd in *Polyporus*. Mycotaxon 38:83–102.

Ryvarden L. 1992. Type studies in the Polyporaceae 23. Species described by C.G. Lloyd in *Lenzites, Polystictus, Poria* and *Trametes*. Mycotaxon 44:127–136.

Ryvarden L. 2005. The genus *Inonotus*. Synopsis Fungorum 21:1–149.

Ryvarden L, Melo I. 2014. Poroid fungi of Europe. Synopsis Fungorum 31:1–455.

Silvestro D, Michalak I. 2012. raxmlGUI: a graphical frontend for RAxML. Organisms Diversity and Evolution 12:335–337.

Sinclair WA, Lyon HH, Johnson WT. 1987. Diseases of trees and shrubs. Ithaca, NY: Cornell University Press. 575 pp.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sunderland, MA: Sinauer Associates.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus species*. Journal of Bacteriology 172:4238–4246.

Wagner T, Fischer M. 2002. 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.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols, a guide to methods and applications. San Diego, CA: Academic Press. pp. 315–322.

Zhou LW. 2015. *Cylindrosporus flavidus* gen. et comb. nov. (Hymenochaetales, Basidiomycota) segregated from *Onnia*. Phytotaxa 219:276–282.

Zhou LW, Vlasák J, Dai YC .2016. Taxonomy and phylogeny of *Phellinidium* (Hymenochaetales, Basidiomycota): a redefinition and the segregation of *Coniferiporia* gen. nov. for forest pathogens. Fungal Biology 120:988–1001.