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The Phylogenetic Position of *Osteina obducta* (Polyporales, Basidiomycota) Based on Samples from Northern Hemisphere

Bao-Kai Cui [a], Josef Vlasák [b] and Yu-Cheng Dai*[a]

[a] Institute of Microbiology and Beijing Key Laboratory for Forest Pest Control, Beijing Forestry University, Beijing 100083, China.

[b] Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic.

*Author for correspondence; e-mail: yuchengd@yahoo.com

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ABSTRACT

Osteina obducta is a large bracket fungus growing mostly on gymnosperm wood in the northern hemisphere, especially on larch in China. It has been widely accepted as *Oligoporus obductus* in most publications. Six samples of *Osteina obducta* from Canada, China, Czech Republic, Russia and the USA were sequenced, and phylogenetic relationships among the species/genus and other brown rot species were inferred based on the ITS rDNA sequences. *Osteina obducta* is shown to represent a monophyletic lineage sister to *Oligoporus* in the Fomitopsidaceae. *Osteina obducta* should therefore be considered as the valid name for the species.

1. INTRODUCTION

Osteina was introduced by Donk [1] based on *Polyporus osseus* Kalchbr. The genus is characterized by sessile to distinctly stipitate basidiocarps, which are bone hard when dry, and have a monomitic hyphal system with clamped generative hyphae, hyaline and thin-walled basidiospores without any reactions in Melzer's and Cotton Blue reagents, and is a brown rot fungus. The genus is monotypic and based on *Osteina obducta* (Berk.) Donk; *Polyporus osseus* (described from Czech Republic) and *Polyporus obductus* Berk. (described from Canada) represent the same species and the latter name has priority. *Osteina obducta* however, has not been widely accepted, and the genus was treated as a synonym of *Oligoporus* Bref., and *Osteina obducta* was treated as *Oligoporus obductus* (Berk.) Gilb. & Ryvarden in most publications [2-5]. All previous studies on either *Osteina obducta* or *Oligoporus obductus* were based on the morphology, and no molecular data are available for the species. The phylogenetic relationships of *Osteina* and other brown rot polypore genera is unresolved. Taxonomy and phylogeny of polypores have been extensively studied in China, but most studies focused on the white-rot polypore groups [6-15], and only a few on the brown-rot polypores [16-18]. The aim of the present paper is to establish the phylogenetic position of the brown rot genus *Osteina* in the Fomitopsidaceae of Polyporales, and argue its correct name.

2. MATERIALS AND METHODS

Morphological studies. — The studied specimens are deposited at the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC), Institute of Applied Ecology, Chinese Academy of Sciences (IFP) and the private herbarium of J. Vlasák (JV) (http://mykoweb.prf.jcu.cz/polypores/index.html). Microscopic procedures follow [19]. Sections were studied at a magnification up to 1000 × using a Nikon E 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range and are given in parentheses. In the text the following abbreviations are used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, 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, n = number of spores measured from given number of specimens. Special color terms follow Petersen [20].

DNA extraction and sequencing. — A Phire Plant Direct PCR Kit (Finnzymes) procedure was used to extract total genomic DNA from the fruitbodies and for polymerase chain reaction (PCR). DNA sequencing was performed at Beijing Genomics Institute. All newly generated sequences were submitted to GenBank. Sequence data of nuclear ribosomal RNA regions were used to determine the phylogenetic positions of the new species. The PCR products were sequenced by the primers ITS4 and ITS5 for ITS.

Phylogenetic analysis. — In phylogenetic reconstruction, *Laetiporus sulphureus* (Bull.) Murrill (AY089742) was used as outgroup [21]. Sequences were aligned with additional sequences downloaded from GenBank using BioEdit [22] and ClustalX [23]. Alignment was manually adjusted to allow maximum alignment and to minimize gaps.

Phylogenetic analysis followed Li and Cui [10]. Maximum parsimony and Bayesian analysis were conducted for the ITS dataset. All characters were equally weighted and gaps were treated as missing data. For the maximum parsimony analysis, the tree construction procedure was performed in PAUP* version 4.0b10 [24]. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates [25]. Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated for each Maximum Parsimonious Tree (MPT) generated.

MrMODELTEST2.3 [26] was used to determine the best-evolution for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites [27]. Four Markov chains were run twice from random starting trees for two million generations sampling trees every 100 generations. The first 25% of trees was discarded as the burn-in.

A majority rule consensus tree of all remaining trees was calculated. Maximum parsimony (MP) bootstrap proportions higher than 75% and Bayesian posterior probabilities (BPP) more than 0.95 were considered as significant levels of support.

3. RESULTS

3.1 Taxonomy

Osteina obducta (Berk.) Donk, Schweiz. Z. Pilzk. 44: 86 (1966) Figure 1.

≡ *Grifola obducta* (Berk.) Aoshima & H. Furuk., Trans. Mycol. Soc. Japan 4: 91 (1963)

≡ *Oligoporus obductus* (Berk.) Gilb. & Ryvarden, Mycotaxon 22: 365 (1985)

≡ *Polyporus obductus* Berk., London J. Bot. 4: 304 (1845)

≡ *Polyporus osseus* Kalchbr., 1: 160 (1865)

≡ *Polyporus zelleri* Murrill, Western Polypores (5): 13 (1915)

≡ *Tyromyces obductus* (Berk.) Murrill, N. Amer. Fl. (New York) 9(1): 32 (1907)

Fruitbody — Basidiocarps annual, usually laterally stipitate, occasionally pileate with a narrow base, solitary or imbricate, watery to flesh and without special odour or taste when fresh, become bone hard when dry. Pilei more or less semicircular or fan-shaped, projecting up to 13 cm, 8 cm wide and 3 cm thick; margin acute, undulate, curved down when dry. Upper surface white when fresh, cream with age, margin usually paler than centre or base, smooth, azonate, become greyish brown and wrinkled after drying. Pore surface white to cream when fresh, becoming yellowish to yellowish brown up on drying; pores angular to irregular, 3-5 per mm; dissepiments thin, entire when juvenile, lacerate with age. Section: context white and flesh when fresh, azonate, become hard corky to rigid when dry, up to 2.7 cm thick. Tubes pale yellowsih and flesh when fresh, brittle and yellowish brown when dry, up to 3 mm long. Stipe cylindrical, white when fresh, pale greyish brown when dry, smooth, up to 7 cm long and 2 cm in diam.

Hyphal structure — Hyphal system monomitic; generative hyphae bearing clamp connections, IKI–, CB–; tissues unchanged in KOH.

Context — Generative hyphae hyaline, fairly thick-walled to distinctly thick-walled or subsolid, occasionally branched, flexuous, gelatinized, interwoven, 3-10 μm in diam. Hyphae in stipe similar to context.

Tubes — Generative hyphae hyaline, thin- to slightly thick-walled with a wide lumen, occasionally branched, straight to flexuous, gelatinized, interwoven, 2-5 μm in diam. Cystidia and cystidioles absent. Basidia distinctly clavate with four sterigmata and a basal clamp connection, 16-25 × 4-5.5 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores — Basidiospores cylindrical to cylindri-oblong, slightly tapering at apiculus, hyaline, thin-walled, smooth, IKI–, CB–, (3.9–)4–5.2(–5.5) × (1.9–)2–2.4(–2.6) μm, L = 4.66 μm, W = 2.19 μm, Q = 2.06–2.2 (n = 60/2).

Material examined: CHINA — Heilongjiang Province, Tahe County, Huzhong Nature Reserve, root of *Larix*, 18 Aug 2003, YC Dai 4756 (IFP 003369) & 4796 (IFP 003370). Yichun County, Fenglin Nature Reserve, *Betula*, 1 Aug 2011, BK Cui 9832 (BJFC 010725); *Picea*, 2 Aug 2011, BK Cui 9865 (BJFC 010758); *Pinus*, 1 Aug 2011, BK Cui 9825 (BJFC 010718); Inner Mongolia Autonomous Region, Arshan County, Arshan Nature Reserve, rotten *Larix* wood, 31 July 2005, BK Cui 2017 (IFP 003341); Genhe County, Great Hinggan Nature Reserve, rotten *Larix*, 27 Aug 2009, YC Dai 11024 (IFP 008504). Jilin Province, Antu County, Changbaishan Nature Reserve, root of *Larix*, 10 Aug 1997, YC Dai 2360 (IFP 003340); 13 Sept 2007, YC Dai 9519 (IFP 003348); 1 Aug 2008, YC Dai 10076 (IFP 008243); *Pinus*, 8 Aug 2011, BK Cui 9957 (BJFC 010850).

Xinjiang Autonomous Region, Buerjin County, Kanasi Nature Reserve, rotten *Larix*, 12 Aug 2004, YL Wei 1444 (IFP 003382). CZECH REPUBLIC — Moravia, Chroustov, *Pinus*, 22 July 1996 Laznicka (H). Obora, Hluboká, Velký Kameník, *Larix*, Aug 2002, JV 0208/8 (JV). RUSSIA — Khabarovsk Reg.: Solnechny Dist., Suluk-Makit, *Larix*, 20 Aug 2011 Spirin 4238 (H). USA — Pennsylvania, Ricketts Glen Sate Park, Wilkes-Barre, *Tsuga*, July 2003, JV 0307/6-J (JV). Washington, Olympic Peninsula, Soleduck River, gymnosperm wood, 3 July 1957 Lowe 7954 (H).

Figure 1. A basidiocarp of *Osteina obducta* (Dai 13178).

3.2 Phylogeny

The ITS dataset included sequences from 69 fungal samples representing 39 taxa. Five ITS sequences were newly yielded in this study (GenBank accession numbers KF147855–KF147859). The dataset had an aligned length of 641 base pairs, among which 193 characters are constant, 22 are variable and parsimony-uninformative, and 138 are parsimony-informative. Maximum Parsimony analysis yielded five equally parsimonious trees (TL = 605, CI = 0.4165, RI = 0.7965, RC = 0.3318, HI = 0.5835), a strict consensus tree of these trees is shown in Figure 2. The 50% majority consensus tree generated by the Bayesian analysis showed a similar topology to the strict consensus MP tree with an average standard deviation of split frequencies = 0.006587, and only the topology from MP analysis is presented, while both bootstrap values and BPPs are shown at the nodes (Figure 2).

The six collections of *Osteina obducta* from Canada, China, Czech Republic, Russia and USA in northern hemisphere were resolved as a well-supported lineage in the ITS-based phylogenetic analysis with a strong support (MP = 99%, BPP = 1.00), and represent a monophyletic clade sister to *Oligoporus* in the Fomitopsidaceae. So *Osteina obducta* should be considered as the valid name rather than *Oligoporus obductus*.

4. Discussion

The spores in the type of *Polyporus osseus* (from Czech Republic) are oblong, 4.5–6 × 1.7–2 μm [28], and they are cylindric-oblong, 4–6 × 2–2.5 μm in *Polyporus zelleri* from North America [29], while spores in China are cylindrical to cylindri-oblong, slightly shorter (4–5.2 × 2–2.4 μm), but their dimensions all overlap.

Polyporus zelleri Murrill was described from Washington in North America, and it was confirmed as a synonym of *Osteina obducta* [2,30]. *Osteina obducta* was addressed in *Leucoporus* Quél., *Scutiger* Paulet, *Leptoporus* Qu□l., *Grifola* Gray and *Tyromyces* P. Karst. [1], but none of these could satisfactorily accommodate the species as mentioned by Donk [1], because *Leucoporus* is a synonym of *Polyporus* P. Micheli and *Scutiger* is an invalid name [3]. *Leptoporus* is similar to *Osteina* by a monomitic hyphal structure and causing a brown rot, but it is distinguished by its simple septate generative hyphae. *Grifola* and *Tyromyces* cause a white rot, so they are distinctly different from *Osteina*.

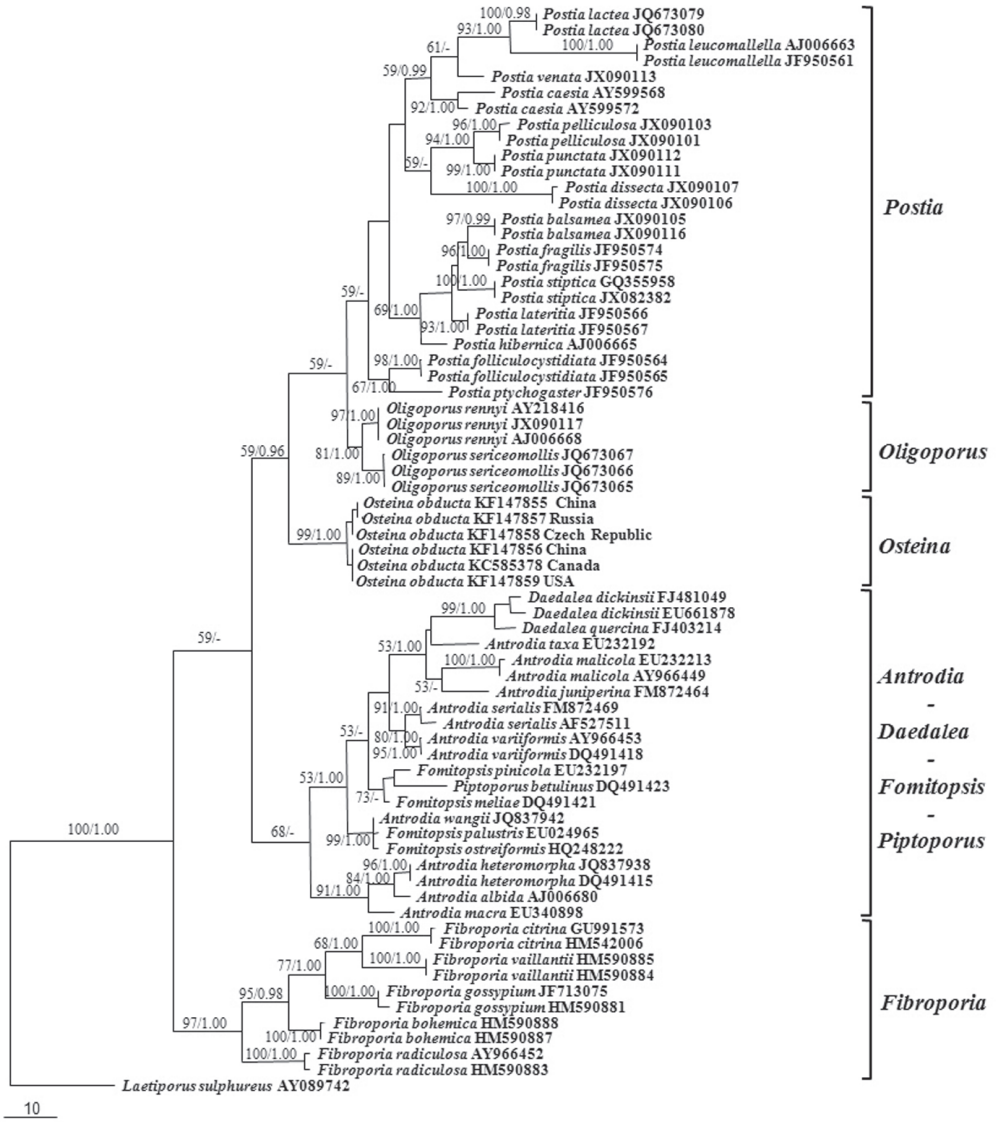

Figure 2. Strict consensus tree illustrating the phylogeny of *Osteina obducta* and related species generated by Maximum Parsimony based on ITS sequences. Branches are labeled with parsimony bootstrap proportions (before the slash markers) higher than 50% and Bayesian posterior probabilities (after the slash markers) more than 0.95.

Osteina share a monomitic hyphal structure with *Postia* P. Karst. (*Oligoporus*), but the former has a bone hard fruiting body when it is dry, and its hyphae are strongly thick-walled and sometimes inflated. *Osteina obducta* can therefore be distinguished from *Postia*. *Fibroporia* Parmasto differs from *Osteina* in its resupinate fruiting body, a dimitic (although generative hyphae are dominant) hyphal structure, and thick-walled basidiospores.

Osteina obducta is a relatively common polypore in natual forests of Northeast and Southwest China [31], mainly in old growh forests of *Larix*, but occasionally on other gymnosperm wood, e.g. *Picea* and *Pinus*, rarely on angiosperm wood. The fungus mostly grows on roots or rotten wood underground, so that it seems to be a terrestrial species. *Osteina obducta* has not been found in the larch plantations in China although larches are planted commonly in the studied area. The fungus was reported on *Tsuga* in Japan [28] and on *Larix* in Russian Far East [32]. *Osteina obducta* has a wide distribution in Central Europe mostly on dead gymnosperm, especially on *Larix* [4]; while it is common on *Pseudotsuga menziesii* in North America [2].

Phylogenetically, *Postia* and *Oligoporus* clustered together with a low support (MP = 59%, BPP lower than 0.95; Figure 2), then grouped with *Osteina*. The six samples of *Osteina obducta* from different countries in northern hemisphere formed a strong supported monophyletic clade and represented a separate genus. Thus, based on the morphological characters, ecological habitats and molecular phylogeny, *Osteina obducta* should be considered as the valid name of the species rather than *Oligoporus obductus*.

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