

North American Fungi



Volume 6, Number 13, Pages 1-5
Published September 29, 2011

***Inonotus macrosporus* sp. nov. (Fungi: Basidiomycota: Hymenochaetales) on live *Fraxinus nigra* in Wisconsin, United States of America**

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Ginns, J. 2011. *Inonotus macrosporus* sp. nov. (Fungi: Basidiomycota: Hymenochaetales) on live *Fraxinus nigra* in Wisconsin, United States of America. *North American Fungi* 6(13): 1-5. doi: <http://dx.doi.org/10.2509/naf2011.006.013>

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Abstract: A new species of *Inonotus* is proposed because it has a unique combination of morphological characters. No other species of the Hymenochaetales has all of these features (1) a pileate basidioma, (2) a duplex context, (3) large, pigmented, thick-walled basidiospores, (4) tramal setal hyphae, and (5) hymenial setae. The generic placement of the fungus is discussed and several similar species are differentiated from it.

Key words: setae, setal hyphae, polypore taxonomy, *Fraxinus*, *Inonotus*, Basidiomycota, new species.

Introduction: While curating the polypores in the National Mycological Herbarium of Canada (DAOM) a number of presumed novellas were flagged for detailed study. One annual, pileate conk with a brown context was a species of

Inonotus P. Karst. (Fungi: Basidiomycota: Hymenochaetales).

A number of taxonomic studies and floras were consulted in an attempt to identify the conk,

including Pegler's (1964) key to all *Inonotus* species and more recent treatments for the neotropics (Ryvarden 2002), North America (Gilbertson 1976, Gilbertson and Ryvarden 1986), Europe (Ryvarden and Gilbertson 1993), East Africa (Ryvarden and Johansen 1980), East Asia (Núñez and Ryvarden 2000), the Malesian region (Corner 1991), China (Zhao and Zhang 1992, Dai et al. 1997, Cui et al. 2011) and the keys, descriptions and illustrations of 101 species of *Inonotus* by Ryvarden (2005).

In addition, descriptions of the approximately 200 *Inonotus* taxa in Stalpers and Stegehuis (1997) and CABI Bioscience (2005) were compared with the unknown polypore. Finally, because phylogenetic analysis of the DNA sequence data of about 90 species showed *Phellinus* s.l. and *Inonotus* s.l. to be polyphyletic and that several smaller groups are comprised of members of the two taxa side by side (Wagner and Fischer 2002), the key to the world species of *Phellinus* Quél. (Larsen and Cobb-Pouille 1990) was consulted. None of these sources led to a species name and the fungus is proposed as a new species.

Materials and Methods: Morphological features are from a dried basidioma. Color terms that follow Ridgway (1912) begin with capital letters, e.g., Salmon Pink. Microscopic features of the basidioma were examined using 2% (w/v) aqueous potassium hydroxide (KOH) to revive dried shrunken cells, Melzer's Reagent where a blue staining indicates an amyloid reaction and a red-brown staining indicates a dextrinoid reaction, and 0.05% (w/v) cotton blue in lactic acid where wall bluing indicates a cyanophilous reaction. Cyanophilous reactions were evaluated by mounting parts of a basidioma in a drop of KOH on a glass microscope slide, placing a cover slip on the drop, drawing a drop of cotton blue under the cover slip, and examining the tissues 24 h later. The walls of the basidiospores, basidia and hyphae were considered to be cyanophilous if cells that lacked contents had blue walls.

Formulae for these reagents are in Kirk et al. (2001) and Hjortstam et al. (1987). Twenty basidiospores were measured for calculation of sizes as: L = mean spore length (arithmetical mean of all spores), W = mean spore width (arithmetical mean of all spores), Q = quotient of the mean spore length and the mean spore width (L/W ratio). Abbreviations of herbaria, i.e., DAOM and TRTC, follow Thiers (2010).

Results

Inonotus macrosporus Ginns sp. nov.

Figure 1

Mycobank No. 563325
Fructificatio pileata, pileus ferruginosus, pori facies umbrina, pori rotundi, 2–3 per mm, tubi et contextus ferruginosi, systema hypharum monomiticum, hyphae generatoriae 3–11 µm diametro, afibulatae, ferruginosae vel aureae, basidiosporae late ellipsoideae vel subgloboae, (8.4–) 8.8–10 x (6.6–) 6.8–8.0 µm, setae praesentes, 22–37 x 8–10 µm, setales hyphae praesentes, 87–270 x 10–12 µm.

Holotypus: On living *Fraxinus nigra* Marsh., USA: Wisconsin: Adams Co.: 20 miles (33.3 kms) N of Wisconsin Dell, 20 August 1965, R.F. Cain, TRTC 43317 (DAOM 241473).

Etymology: 'macrosporus' refers to the comparatively large size of the basidiospores.

Basidioma annual, solitary, dimidiate, appanate, 10 cm in depth from margin to substrate, 2.7 cm thick, lightweight, **pileus surface** finely hirsute, red-brown (near Kaiser Brown), margin brownish yellow (near Ochraceous-Tawny but brighter yellow), broadly rounded, obtuse. In vertical section, **context** 2 cm thick, dull, soft, fragile, duplex, the upper layer brownish yellow (Ochraceous-Tawny), distinct, 2-3 mm thick, the darker core yellowish brown (near Sudan Brown), 1.8 cm thick, context separated from the tubes by a thin, pale yellow line. **Tubes** pale brownish yellow (near Clay

Color), 7 mm deep, **dissepiments** thin, **pores** 2-3 per mm, round to angular, **pore mouths** uneven but not fimbriate, brown with a slight dark red tint (near Auburn).

Hyphal system monomitic. Tissues staining red then darkening in KOH. Hyphae with simple septa, walls acyanophilous, neither amyloid nor dextrinoid. Fascicles on the pileus surface straight, densely packed, 80–110 μm diam, composed of vertically oriented, parallel, (2–) 4–8 μm diam hyphae with walls thin, pale yellow, and some terminal segments with yellow brown contents. **Context hyphae** parallel, essentially horizontally arranged, radiating, infrequently branched, 4–11 μm diam, walls hyaline or yellow to reddish brown, thin to 0.5 μm thick. **Tramal hyphae** infrequently branched, 3–5 μm diam, walls pale yellow-brown, thin. **Setal hyphae** confined to the trama, 87–270 \times 10–12 μm , most acicular, i.e., cylindrical except for a pointed apex, rarely branched, some with a slightly broadened, lance-shaped apex up to 100 μm long,

narrowed to 5 μm diam at the base, walls red brown, thickened. **Setae** subulate to ventricose, typically 22–37 \times 8–10 μm , most terminal, some intercalary, projecting up to 10 μm , principally in the hymenium, some originating adjacent to subhymenium, a few with a distinct stem up to 100 μm long and 4 μm diam, walls red brown, thickened. **Basidia** broadly ellipsoid to broadly clavate, 11–16 \times 7–9 μm , walls hyaline, thin, sterigmata 2–4, each 4–5 μm long. **Basidiospores** broadly ellipsoid to subglobose, (8.4–) 8.8–10.0 \times (6.6–) 6.8–8.0 μm ($n = 20$), $L = 9.3$, $W = 7.3$, $Q = 1.3$, walls pale yellow to reddish brown, smooth, thickened, acyanophilous, neither amyloid nor dextrinoid.

Discussion: The specimen is a fragmented slice of a dried basidioma. Thus its width, and colors and texture when fresh are unknown. Presumably a part of the basidioma is at TRTC but it has not been available for study. Nevertheless, the specimen has a combination of distinctive features not found in any of the several hundred

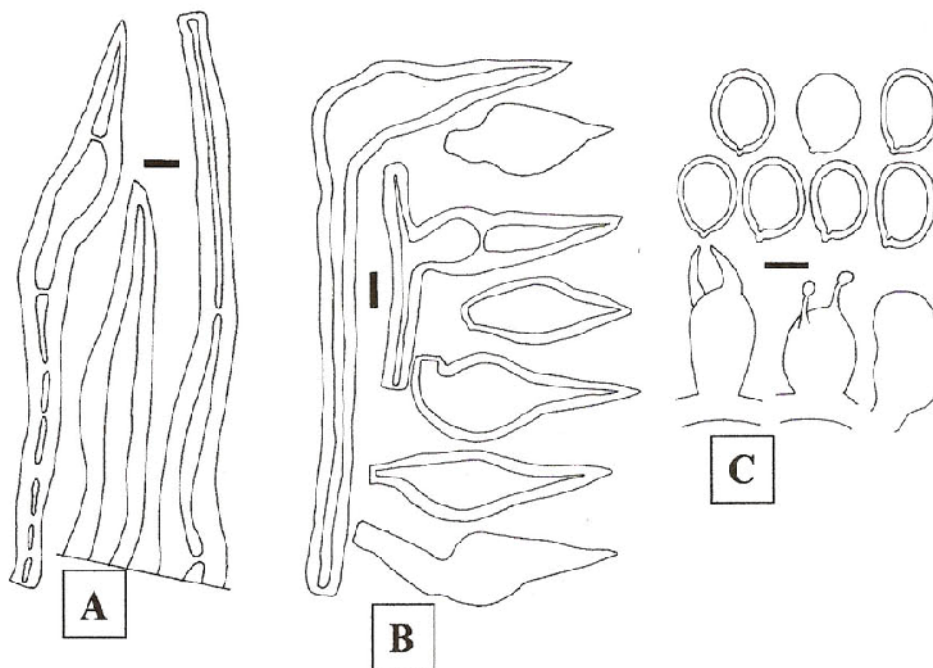


Figure 1. *Inonotus macrosporus* microscopic features. (A) Setal hyphae from the trama. (B) Setae from the hymenium and subhymenium. (C) Basidiospores and basidia. From TRTC 43317. Scale bars equal 5 μm .

species of *Inonotus s.l.* and *Phellinus s.l.* The group of features that characterize *I. macrosporus* are a pileate basidioma with a duplex context, large, pigmented, thick-walled basidiospores, setal hyphae that are confined to the trama, and setae in the hymenium. Although relatively few sterigmate basidia were seen, most were two-sterigmate and it is uncertain whether this is the norm. Only 2 of the 23 North American species of *Inonotus* are reported to have both 2- and 4-spored basidia (Gilbertson and Ryvardeen 1986).

Although the host, *Fraxinus nigra*, is relatively common in the Great Lakes region, only one collection of *I. macrosporus* is known and it had been labeled as *I. hispidus* (Bull.:Fr.) P. Karst. Additional specimens may be in herbaria mislabeled as *I. hispidus*.

The occurrence of *I. macrosporus* on a live tree raises the question whether it is parasitic or saprotrophic on the dead tissue of the heartwood. A surprisingly high 74% of the 23 species of *Inonotus* in North America occur on living trees; only 5 are considered pathogenic (Gilbertson and Ryvardeen 1986).

Wagner and Fischer (2002:1010) used nuc-18S rDNA sequence data to analyse the phylogenetic relationships between nearly 90 species of *Inonotus* and *Phellinus*. The species were distributed among several genera. Following their generic circumscriptions, *I. macrosporus* is placed in the much reduced *Inonotus s.s.* because it is the only genus that is characterized, in part, by a combination of pigmented basidiospores, hymenial setae and setal hyphae.

In Ryvardeen's (2005) synopsis of the genus *Inonotus* there are several species that share some of the characteristic features, such as pileate habit, duplex context, large, pigmented basidiospores, and the presence of setae in the hymenium and setal hyphae in the trama, of *I. macrosporus*. Two species have relatively large

basidiospores. *Inonotus hispidus*, type species of the genus, with basidiospores 8–11 x 6–8 µm and brown walls, lacks a duplex context, setae and setal hyphae. *Inonotus pacificus* Ryvardeen with basidiospores 9–12 x 7–8 µm lacks a duplex context and hymenial setae. Five species (*I. hemmisii* Gilb. & Ryvardeen, *I. ochroporus* (Van der Byl) Pegler, *I. patouillardii* (Rick) Pat., *I. rickii* (Pat.) D.A. Reid, and *I. roadwayii* D.A. Reid) with pileate basidiomata, setal hyphae, setae and pigmented basidiospores differ in having shorter setae (12–28 x 4–9 µm) and smaller basidiospores (6–9 x 4–7 µm).

Acknowledgments: R. Greg Thorn, University of Western Ontario, constructively criticized a draft of the manuscript. Two reviewers provided constructive comments that further improved the manuscript. The Directors and staff at Agriculture and Agri-Food Canada's Pacific Agriculture Research Centre, Summerland, British Columbia and the National Mycological Herbarium (DAOM), Ottawa, Ontario are thanked for their cooperation. The Latin description was reviewed by Owen Ewald, C. May Marston Assistant Professor of Classics & LCL Chair, Seattle Pacific University

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