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Inonotus rwenzorianus (Basidiomycetes, Hymenochaetales) : an undescribed species from the Rwenzori Mountain range

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Abstract – *Inonotus rwenzorianus* (Basidiomycota, Hymenochaetales) is described as new from the Rwenzori Mountain range, in the Democratic Republic of Congo. The species is above all characterized by the combination of a thick, resupinate basidiomata, small pores, long extra-hymenial setae (setal hyphae) in the hymenophoral trama and the subiculum, abundant, variably shaped hymenial setae, and mostly ellipsoid, slightly thick-walled, very pale yellowish basidiospores. It is compared to *I. pegleri*, which is morphologically the closest species, also occurring in Africa. Its phylogenetic affinities are clearly within the *Inonotus* clade *sensu* Fischer and Wagner, but the relationships within this clade are, for the time being, still poorly resolved.

Africa / Democratic Republic of Congo / Hymenochaetaceae / phylogeny

Résumé – L'espèce *Inonotus rwenzorianus* (Basidiomycètes, Hymenochaetales) est décrite à partir d'une collecte réalisée sur le mont Rwenzori, en République Démocratique du Congo. Cette espèce est surtout caractérisée par des basidiomes résupinés, épais, de petits pores, de longues soies hyphales présentes dans la trame et dans le subiculum, des soies hyméniales abondantes, et des spores ellipsoïdes, hyalines à jaunâtres. Cette espèce est proche de *I. pegleri*. Elle appartient clairement au clade *Inonotus sensu* Wagner et Fischer mais ses affinités dans ce clade sont encore incertaines.

Afrique / République Démocratique du Congo / Hymenochaetaceae / phylogénie

INTRODUCTION

Inonotus P. Karst. has been monographed by Ryvarden (2005). The genus was then, and mostly for pragmatic reasons, considered in a broad sense, although phylogenetic studies (Wagner & Fischer, 2002) revealed that several smaller taxonomic entities could be sorted from the bulk of *Inonotus* species.

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Hundred and one species were then described; a few have been added since then (e.g. Li *et al.*, 2007).

Ten species are currently reported from tropical Africa, half of them so far known only from the area. As far as we have been able to ascertain, none were reported from the Rwenzori mountain range in eastern Congo (Ryvarden, 1978), which remains still poorly explored in what regards its mycota.

During a short collecting trip on the western side of the Rwenzori in the Democratic Republic of Congo, between 800 and 2600 m asl, three *Inonotus* were found, out of which one could not be confidently identified to any known species. It is described below as *Inonotus rwenzorianus* sp. nov. and its phylogenetic affinities are commented.

MATERIAL AND METHODS

Environment of collection site – Collections were made between the Mutsora ICCN–WWF field station (approximately 800 m asl) up to the Kalonga WWF–ICCN chalet area, mainly between 2000 m and 2300 m, with a short excursion up to 2600 m. The main ecosystem there is the mountainous rainforest, dominated by the species *Alangium chinensis*, *Entandrophragma excelsum*, *Celtis africana*, *Neoboutonia macrocalyx*, *Ehretia cymosa*, and *Galiniera coffeoides*.

Morphology – Herbarium specimens are preserved at MUCL (herbarium acronym are from Holmgren *et al.* 1990). Strains used in this study are preserved at BCCM/MUCL. Colors are described according to Kornerup and Wanscher (1981). Specimens were examined in Melzer's reagent, lactic acid Cotton blue (Kirk *et al.,* 2001), and KOH 4%. All microscopic measurements were carried out in Melzer's reagent. In presenting the size range of the microscopic elements, 5% of the measurements were excluded from each end and are given in parentheses, when relevant. $\bar{x}$ = arithmetic mean, R = the ratio of length/width of basidiospores, $\bar{x}_R$ = arithmetic mean of the ratio R.

Sequencing – The DNA was extracted from freshly collected mycelium from pure culture grown in liquid malt at 25°C in the dark. Extractions were carried out using the QIAGEN Dneasy plant Mini Kit (QIAGEN Inc.) and later purified with Geneclean® III kit (Q-Biogene), following the manufacturer's recommendations. The primer pairs LROR-LR6 (White *et al.,* 1990) were used to amplify the 5'end of the nr LSU DNA regions. Successful PCR reactions resulted in a single band observed on a 0.8% agarose gel, corresponding to approximately 1200 bps. Polymerase chain reaction products were cleaned using the QIAquick® PCR purification kit (250) (QIAGEN Inc.), following the manufacturer's protocol. Sequencing reactions were performed using CEQ DTCS Quick Start Kit® (Beckman Coulter), according to the manufacturer's recommendations, with the primers LROR, LR3, LR3R, LR5 (biology.duke.edu/fungi/mycology/primers). Nucleotide sequences were determined with a CEQ 2000 XL capillary automated sequencer (Beckman Coulter).

Phylogenetic analysis – Initially, nucleotide sequences were automatically aligned with Clustal X for Macintosh (version 1.5b), then manually adjusted as necessary with the editor in PAUP* (version 4.0b10). The final data set includes 24 sequences (24 taxa) and 896 characters, including gaps. 716 characters are constant and 82 are parsimony informative.

Phylogenetic analysis of the aligned sequences was performed using the maximum parsimony method of PAUP* version 4.0b10 (Swofford, 2002) with gaps treated as fifth base. The most parsimonious trees were identified using a heuristic searches with random addition sequence (1000), and further evaluated by bootstrap analysis, retaining clades compatible with the 50% majority-rules in the bootstrap consensus tree. Analysis conditions were: tree bisection addition branch swapping (TBR), starting tree obtained via stepwise addition, steepest descent not in effect, MulTrees effective. *Inocutis tamaricis* (Pat.) Fiasson & Niemelä AF311021 was used as outgroup.

Sequences generated at MUCL (available at MUCL) – Inonotus henanensis Juan Li & Y.C. Dai MUCL 47790; *I. micantissimus* (Rick) Rajchenb. MUCL 46035; *I. ochroporus* (van der Byl) Pegler MUCL 49987; *I. rickii* (Pat.) Reid MUCL 45529; *I. rwenzorianus* Balezi & Decock MUCL 51007; *Inonotus sp*. MUCL 44666, MUCL 45517, MUCL 47834, MUCL 49252; *Phellinus undulatus* MUCL 44139.

RESULTS

The LSU-based phylogenic inference under the parsimony hypothesis yielded 90 most parsimonious trees, 328 steps in length, CI = 0.665, RI = 0.628. These 90 trees present the same overall topologies, most of the variations lying within the terminal clade relationships. The phylogeny confidently resolved a clade (bootstrap value 86%) containing *I. rwenzorianus*, and other species from tropical/subtropical areas (with the exception of *I. nidus pici*, that is a temperate taxa). All the species of this clade, but *Ph. pachyphloeus*, have resupinate basidiomata.

TAXONOMY

***Inonotus rwenzorianus* Balezi et Decock *sp. nov*.** Figs. 2–6

Mycobank: MB 513171

Fructificatio resupinata, ad 20 mm crassa; pororum facies umbrina, pori rotundi, 6–7 per mm; subiculum tenuissimum, luteo brunneum; tubi atrocinnamomei vel cinereo-brunnei, ad 20 mm crassi; systema hypharum monomiticum; hyphae generatoriae hyalinae ad pallide luteae, afibulatae ; hyphae setales et setae presentes; hyphae setales in trama inclusae, 135–240 µm longae (8 = 173 µm); setae in hymenio inclusae, pallide ferrugineo-fuscae, conicae vel ventricosae, apice acutae, rectae, (10–)13–20(–25) × (4.0–)4.5–8.0(–10.0) µm, 8 = 16.9 × 6.2 µm; basidiosporae ellipsoideae, crassitunicatae, non-dextrinoidae, hyalinae ad pallide aureae, (6.5–)6.5–8.5(–8.8) × (4.3–)4.3–5.5(–5.5) µm, 8 = 7.5 × 5.0 µm.

HOLOTYPUS: Democratic Republic of Congo, North Kivu, Virunga National Park, Rwenzori Mountain Range, areas of the WWF/ICCN Kalonga altitude chalet, 00°33,961'N – 29°81,795'E, about 2160 m asl, on a dead hanging branch (20 cm diam) of an unidentified angiosperm, 03–05 Feb. 2008, *C. Decock*,

A. Balezi, *G. Bashonga*, and *F. Monya, CO-08-20*, deposited at MUCL as MUCL 51007 (culture ex-holotype deposited at BCCM/MUCL as MUCL 51007)

Basidiomata resupinate, cushion-shaped, separable, especially when dried, reaching 400 mm long, 100 mm wide, up to 20 mm thick at the centre, with a general soft corky consistency when fresh, corky to brittle when dried; ***margin*** absent; ***pores surface*** greyish brown (6E[3–4], brownish beige); ***pores*** small, 5–7/mm, round to irregular, collapsed on drying; ***dissepiments*** entire, thin to thick; ***subiculum*** reduced to thin layer, about 100 µm thick, light golden brown, contrasting with the tube layer; ***tube layer*** single, up to 20 mm thick, light brown, brown (6[D–E][7–8], light brown, raw sienna, rust brown to cocoa brown) or grayish brown (7E[3–4], greyish brown to fawn), with a corky consistency when fresh, drying hard and fragile; ***hyphal system*** monomitic in both the subiculum and hymenophoral trama; ***generative hyphae*** simple septate, hyaline, yellowish, to pale yellowish brown, thin- to slightly thick-walled, few branched, loosely mixed in the subiculum, more compact in hymenophoral trama, 2–4 µm wide; ***extra-hymenial setae*** (***setal hyphae***) present both in the *hymenophoral trama* and *the subiculum* (and the wood just beneath); *in the hymenophoral trama*, embedded into and parallel to the trama, narrowly lanceolate, straight, very thick-walled, brown, 135–240 µm long ($\bar{x}$ = 173 µm), gradually widening from 3.5–5.0 µm wide at the basal septum to 6.5–9.5(–10) µm wide ($\bar{x}$ = 8.0 µm) at the widest part near the apex; *in the subiculum*, pale yellowish brown, only slightly thick-walled, and then collapsing on drying, up to 14 µm wide; ***hymenial setae*** numerous, directly projecting through the hymenium or arising from an hyphal-like stalk parallel to the tramal hyphae then abruptly bent into the hymenium, variable in shape, conical to commonly, symmetrically or unilaterally, ventricose, straight or slightly curved, the apex acute and straight, (10–)13–20(–25) × (4.0–)4.5–8.0(–10.0) µm, $\bar{x}$ = 16.9 × 6.2 µm; ***basidia*** subglobose, with a small stalk-like basal part, with four sterigmata; ***cystidioles*** present, cylindrical, hyphal-like, or with the base somewhat enlarged, bulbous; ***basidiospores*** (hyaline) to pale yellowish, slightly thick-walled, often with a large gutta, mostly ellipsoid, occasionally broadly ellipsoid or slightly amygdaloidal, not dextrinoid, cyanophilous, (6.5–)6.5–8.5(–8.8) × (4.3–)4.3–5.5 (–5.5) µm, $\bar{x}$ = 7.5 × 5.0 µm, R = (1.3–)1.4–1.6(–1.7), $\bar{x}_R$ = 1.5; *chlamydospores* absent;

substrate: so far known from dead, hanging branch;
type of rot: white rot;
cultural features: undescribed;
sexuality: unknown;
habitat: Afro-mountainous humid forest;
distribution: known from the Rwenzori in RDC.

DISCUSSION

The species is distinct by the combination of a thick (to 20 mm), resupinate basidiomata, small pores (6–7/mm), presence of both extra-hymenial (setal hyphae) and hymenial setae (Figs. 2–3), the former long and narrowly lanceolate, the latter variable in shape, often ventricose, and mostly ellipsoid basidiospores (Fig. 6), averaging 7.5 × 5.0 µm. Extra-hymenial setae are also present in the subiculum and in the wood just beneath; they are there slightly

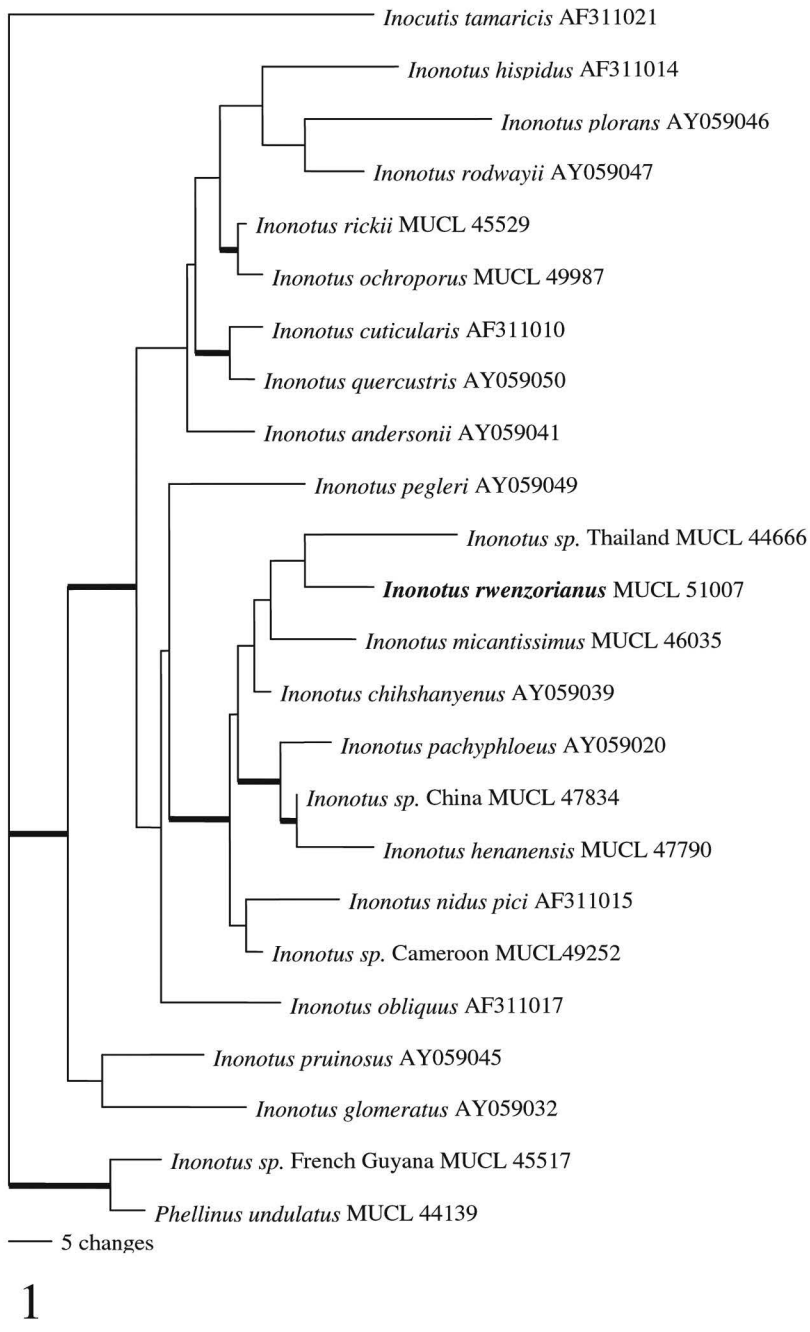
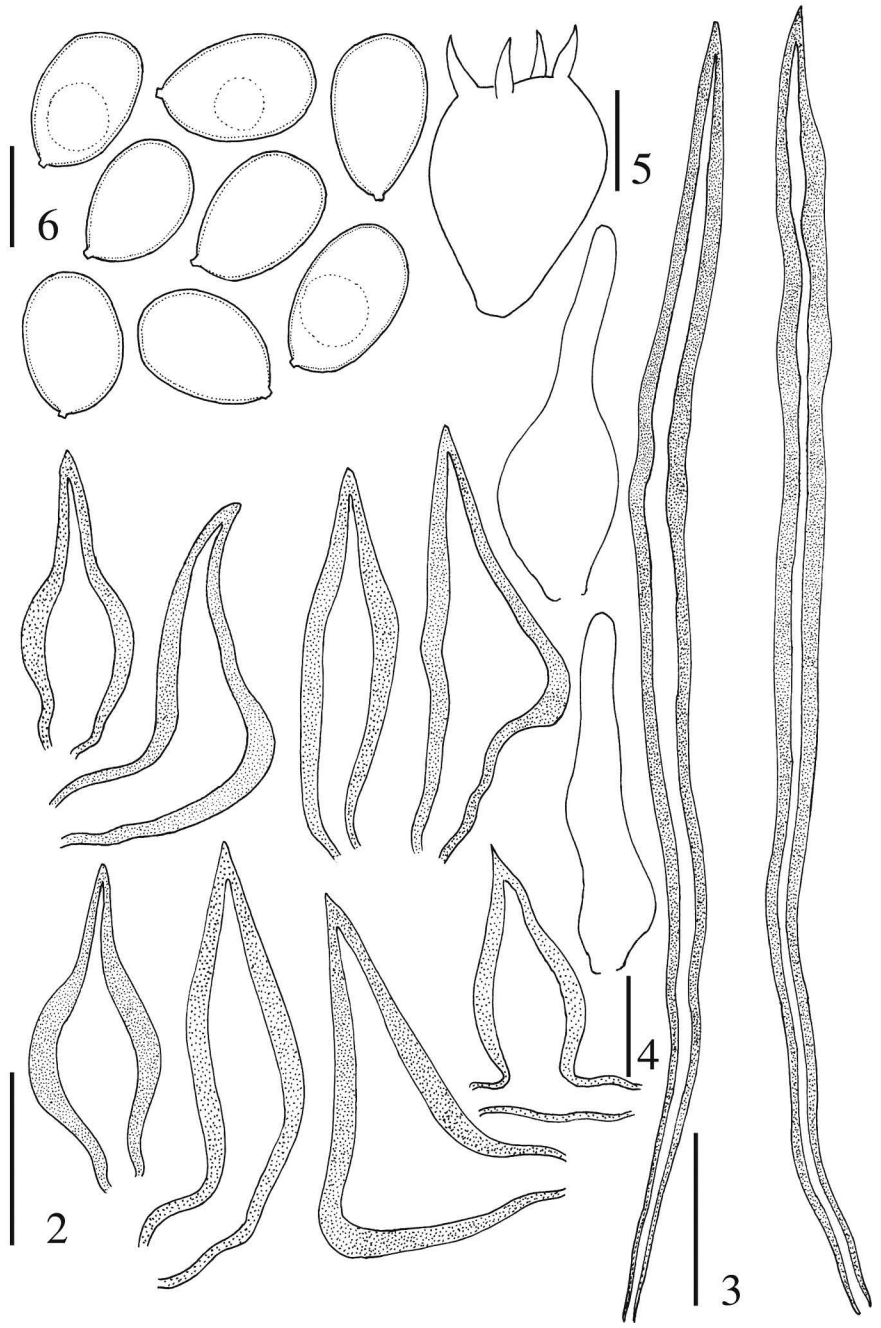


Fig. 1. Phylogenetic relationships of *Inonotus rwenzorianus* as inferred by Parsimony analysis of a partial LSU database. One of the 90 MPTs (328 steps in length, CI = 0.665, RI = 0.628). Branch in bold are supported by a bootstrap value > 75%.



Figs. 2–6. *Inonotus rwenzorianus*, holotype. 2. Hymenial setae (scale bar = 10 µm); 3. Extrahymenial setae (setal hyphae in the hymenophoral tama) (scale bar = 20 µm); 4. Basidia; 5. Cystidioles; 6. Basidiospores (4–6, scale bar = 5 µm).

thick-walled and collapsed on drying. They are also formed (abundantly) within the mycelium mat in pure culture on malt agar.

Inonotus pegleri Ryvarden (Ryvarden, 1975, 2005; Ryvarden & Johansen, 1980) from eastern tropical Africa is, morphologically, the most closely related species. It shares most of the macro- and microscopic features of *I. rwenzorianus* but differs in having larger pores (4–5/mm *versus* 6–7/mm), rare hymenial setae, larger setal hyphae (up to 25 µm wide *versus* 6.5–9.5 µm wide), and globose and smaller basidiospores (6–7 µm in diam. *versus* 6.5–8.5 × 4.3–5.5 µm) (Li *et al.,* 2007; Ryvarden, 1975, 2005; Ryvarden & Johansen, 1980). *Inonotus henanensis* is also comparable, differing in having globose and smaller basidiospores (Li *et al.,* 2007).

Among the other species with both extra-hymenial and hymenial setae, *I. chihshanyenus*, so far known from Taiwan only, differs by a much thinner basidiomata (up to 2.5 mm thick) and larger pores (1–3/mm) (Chang & Chou, 1998). *Inonotus micantissimus* has characteristic large, sub-globose basidiospores, 10–13 × 8–12 µm (Rajchenberg, 1987; Ryvarden, 2005), and is, in all probability, endemic to the Neotropics.

In a phylogenetic perspective, both *I. pegleri* and *I. henanensis* are phylogenetically distinct from *I. rwenzorianus* (Fig. 1). They all belong to the *Inonotus* clade *sensu* Wagner and Fischer (2002). However, still, the phylogenetic inferences based on DNA sequence from the 5'end of the nuclear ribosomal LSU poorly resolved the relationships within this clade (Fig. 1). A more detailed molecular revision of the clade including more species and based on ITS or protein-coding gene sequences, could, hopefully, resolved more confidently the species relationships.

Other species from the *Inonotus* clade from the Rwenzori.

Two other species of *Inonotus sensu* Wagner and Fischer (2002) were found locally, *viz*. *I. ochroporus* and *I. pachyphloeus* (Pat.) T. Wagner & M. Fisch. They have both extra- (setal hyphae) and hymenial setae, but are pileate (Ryvarden, 2005).

Inonotus ochroporus[3] was collected twice, at about 2160 m, at the base of living trunks of *Celtis* sp. (Ulmaceae) and *Croton* sp. (Euphorbiaceae). Both collections are fertile basidiomata, with few contextual setal hyphae near the base of the basidiomata and on the pileus, and lack chlamydospores. Phylogenetically, the species appears closely related to *I. rickii* (Pat.) Pat.

Inonotus pachyphloeus[4] was collected near the ICCM Mutsora station, at approximately 800 m, on a fallen log of *Ficus vallis-choudae*.

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3 ***Specimens examined***: Democratic Republic of Congo, North Kivu, Virunga National Park, Rwenzori Mountain Range, areas of the WWF/ICCN Kalonga altitude chalet, 00°33,961'N – 29°81,795'E, about 2160 m asl, base of a living trunk of *Celtis* sp., 03–05 Feb. 2008, *C. Decock*, *A. Balezi*, *G. Bashonga*, and *F. Monya*, *CO-08-14,* deposited at MUCL as MUCL 49987 (culture ex- as MUCL 49987); same data, base of a living trunk of *Croton* sp., *CO-08-22*, deposited at MUCL as MUCL 51900.

4 ***Specimen examined***: Democratic Republic of Congo, North Kivu, Virunga National Park, Rwenzori Mountain Range, areas of the WWF/ICCN Mutsora station, about 800 m asl, dead fallen branch of *Ficus vallis-choudae*, 02 Feb. 2008, *C. Decock*, *A. Balezi*, *G. Bashonga*, and *F. Monya*, *CO-08-05*, deposited at MUCL as MUCL 49982 (culture ex- as MUCL 49982).

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