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Unusual polypore fungi - a taxonomic emendation of *Polyporus* (Basidiomycotina) after ribosomal spacer characters

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R sum — Des travaux antérieurs suggèrent l'existence d'une étroite relation entre l'ADN du gène ribosomal 28S du genre à pores *Polyporus s. str.* et ceux du genre isolé *Pseudofavolus* et du genre *Mycobonia* dépourvus de pores. Nous avons réexaminé la phylogénie en considérant la variabilité des espaceurs (ITS) de l'ADN ribosomal pour prédire les parties de structures transcrites. Les parties de structures secondaires de molécules de pré-rARN prétraitées (ITS2), prédites à partir de neuf séquences sont proposées comme caractère taxonomique rendant compte de la parenté existante entre les trois genres. Nous portons à la correction l'intégration du genre *Mycobonia* au sein du genre *Polyporus*.

Structure consensus / systématique moléculaire / *Datronia* / *Mycobonia* / *Pseudofavolus* / rADN

Abstract —Ribosomal DNA sequences of the 28S large subunit gene have suggested a close relationship between the poroid mushroom genus *Polyporus s. str.*, segregate genus *Pseudofavolus*, and non-poroid *Mycobonia* occurring in the Americas. We re-investigated this phylogeny using the variable ITS spacers of ribosomal DNA and predicted partial transcript structure. Partial secondary structures of pre-processed RNA molecules (ITS2 rRNA), predicted from nine sequences, are described as taxonomic characters indicative of the *Polyporus – Pseudofavolus – Mycobonia* alliance. We therefore emend *Polyporus* to include *Mycobonia.*

Consensus structure / Molecular systematics / *Datronia* / *Mycobonia* / *Pseudofavolus* / rDNA

INTRODUCTION

Polyporus *and related genera*

Polypores are wood-degrading fungal organisms important for the release of carbon contained in lignocellulose. One genus which historically included the majority of polypores is *Polyporus* Adanson: Fr. This near-

cosmopolitan genus contains species which produce fruit bodies with a more or less distinct stipe yet unlike more fleshy mushrooms these fruit bodies quickly become tough due to unique dimitic hyphal construction systems (although these fruit bodies do not persist long in nature). One undoubtedly related genus, *Pseudofavolus* Pat., is distinguished by typically having thinner, shallow-pored fruit bodies and by a predominantly subtropical or tropical distribution. A third genus, *Mycobonia* Pat., shares many characters of fruit body morphology and anatomy with *Pseudofavolus*, yet is generally not considered a polypore genus as its fruit bodies do not have pores. Instead, the underside of the *Mycobonia* fruit body is flat with microscopically small "teeth" (pegs) protruding from a flat surface containing basidia. A fourth and fifth closely allied genus are represented in the main analyses in this paper: *Datronia* Donk has no fruit bodies with a stipe, while its basidiospores and micromorphology are similar to *Polyporus* and another polypore genus, *Trametes* Fr.

The taxonomic delimitation of *Polyporus* remains in flux, with a modern census available in the monograph by Núñez and Ryvarden (1995). During analyses of large subunit (LSU) ribosomal DNA data, and subsequent morphological re-evaluation, Krüger (2002) found evidence to shift generic boundaries involving taxa related to *Polyporus squamosus* (Huds.) Fr. Meanwhile, new ITS rDNA data became available to investigate phylogeny of polyporoid taxa near *P. squamosus*, which allowed for a) deriving a workable emendation of *Polyporus s. str*., and b) infering new identifying morphotaxonomic characters on a level not yet fully explored – the structure of the ITS2 portion of the pre-rRNA transcript.

Selection of taxa for new ITS sequences was informed by previous LSU datasets (Krüger 2002, Krüger & Gargas 2004). These were chosen to address two systematic questions: 1) how is *Polyporus squamosus* related to representatives of the segregate polypore genus *Pseudofavolus*, and non-poroid *Mycobonia*?, and 2) is the resupinate genus *Datronia* Donk more closely related to *Trametes* Fr. or to *Polyporus squamosus*?

Pseudofavolus *and* Mycobonia

Corner (1984: 35) rejected *Pseudofavolus* as a segregate genus, stating that Ryvarden and Johansen's (1980) distinctions of dextrinoid hyphae, dendrohyphidia, large spores, and thin context could all be found in *Polyporus s. str*. (*sensu* Corner). Singer (1949: 269, 284) argued that *Pseudofavolus* and *Mycobonia* are likely related to *Polyporus*. This was criticized by Smith (1963) as having de-emphasized the value of the hymenophore as a taxonomic character. Donk (1964: 294) agreed with Singer. Kreisel (1969: 168-169) synonymized Pleurotaceae Overeem under Polyporaceae Corda (in Polyporales), and also listed *Mycobonia* as a representative genus. Jülich then later (1981: 186) removed *Mycobonia* from Thelephoraceae (see Donk 1957: 83) and proposed that *Mycobonia* should belong to a family Mycoboniaceae (Boreostereaceae Jülich 1982, Bibliotheca Mycologica 85: 357) within the Polyporales. Singer (1986: 163) synonymized Mycoboniaceae with Polyporaceae. Corner (1984: 36) stated that the *Pseudofavolus* group was most closely related to *Mycobonia*. Accepting two species of *Mycobonia* [*M. brunneoleuca* (Berk. & Curtis) Pat., and *M. flava* (Swartz: Fr.) Pat.; compare Reid 1976], Corner stated (1984: 104) that *M. flava* was merely *Pseudofavolus miquelii* (Mont.) Pat. without the pores, but a sectional combination of *Pseudofavolus* and *Mycobonia* in *Polyporus* was not actually

proposed because of the "absurdity" of calling a poreless fungus a polypore (Corner 1984: 102-103).

We previously noted the striking resemblance between the hexagonal-pored *Pseudofavolus cucullatus* and apparently hydnoid *Mycobonia flava* in basidiocarp coloration, hyphal construction, basidia, and spores (Krüger 2002). The hymenophore of *M. flava* was interpreted as being reduced to a flat surface as in corticioid and thelephoroid fungi, with the pore walls appearing to have become isolated hyphal peg fascicles. Both *P. cucullatus* and *M. flava* had spores and basidia as large as those of *P. squamosus* and other *Polyporus s. str.* (= group *Squamosus*), and *Dichomitus* species. Genetic data also support this interpretation. LSU analyses recovered *Mycobonia* next to *Pseudofavolus*, both nested within *Polyporus s. str*. (Krüger 2002, Krüger & Gargas 2004). *Pseudofavolus*, as well as *Datronia*, were also found allied with *P. squamosus* in phylogenies based on several loci (e.g. Clade 1 of Fig. 3 of Sotome *et al.* 2008).

Scattered septate basidiospores occur in *Pseudofavolus cucullatus* and *Dichomitus leucoplacus* (Berk.) Ryv. (Krüger 2002) similar to those of Buchanan and Ryvarden's (1998) *P. septosporus* and *Dichomitus newhookii* P.K. Buchanan & Ryvarden (Buchanan and Ryvarden 2000).

Datronia

Datronia mollis (Sommerf.: Fr.) Donk (= *Daedalea mollis* Sommerf.: Fr.) has similar spores and hyphal construction as *Polyporus squamosus* but differs in having dark brown, pileate or rarely resupinate basidiomata with dendrohyphidia and cystidioles (Gilbertson and Ryvarden 1986-1987). It appears close to *P. squamosus* / *P. melanopus* in several analyses (Hibbett and Vilgalys 1993; Hibbett and Donoghue 2001; Binder and Hibbett 2002: GenBank **AF393071**; Ko and Jung 2002; Sotome *et al.* 2008). This is remarkable since it was the only species included in this assemblage with pigmented skeletal hyphae giving the context a dark brown color. This led us to sequence the LSU of a slightly reflexed-pileate *Datronia mollis* from Denmark (FB10177, TENN57707, GenBank: **AJ488602**; Krüger 2002). This *D. mollis* LSU sequence was, however, very dissimilar from **AF393071** and appeared close to *Trametes*, where Corner (1989: 18) had placed *D. mollis* based on his finding that *D. mollis* basidiocarps were trimitic rather than dimitic.

Secondary structure

Fungi have notoriously few stable phenotypic characteristics useful for systematics, and robust phylogenetic reconstruction may be constrained by choice of molecular data used. Rokas *et al.* (2003), for instance found, by comparing 106 genes of several related yeast fungi, that inferred phylogenies strongly depend on the gene chosen. Cryptic characters such as secondary structure information could further enhance both systematics of fungi as well as well as understanding of the crucial rRNA molecules.

With nucleic acid folding prediction algorithms available, it is possible to incorporate nucleic acid sequence data to determine nucleotide homology and also to improve alignment strategies. Examination of potential folding also allows inferences of patterns of nucleotide evolution, the targeting of structural motifs for molecular detection and antibiosis, and the specification of new suites of taxonomic characters.

MATERIALS AND METHODS

Collection and culturing

Collected specimens were annotated, given field book numbers (FB) and deposited in TENN (University of Tennessee Fungal Herbarium). Identification followed keys and descriptions by Corner (1984), Gilbertson and Ryvarden (1986-1987), Ryvarden and Gilbertson (1993-1994), and Núñez and Ryvarden (1995). Monokaryotic single-basidiospore isolates (SBIs) were isolated following methods by Gordon and Petersen (1991) and live cultures were stored on disks of malt extract agar (MEA: 1.5% w/v Difco® malt extract, 2% w/v Difco® Bacto-agar; Becton, Dickinson and Company, Franklin Lakes, New Jersey; Nobles 1965) in sterilized water (Burdsall and Dorworth 1994).

Specimens examined, cultures and sequences available

SBI cultures are maintained in the University of Tennessee Fungal Culture Collection (CulTENN) under FB numbers. *Datronia mollis* (Sommerf.: Fr.) Donk: DENMARK. STORSTRØMS AMT: Fakse-Leestrup. Probably on *Fagus*, 19 May 1999. *Ronald H. Petersen FB10177*. TENN57707. SBI 1: **AF516557**. *Mycobonia flava* (Sw.: Fr.) Pat.: ARGENTINA. MISIONES: Wanda, Uruguai Provincial Park, 25°51'29"S / 54°10'08"W. 26 May 2001. *Dirk Krüger FB11279*. TENN59088. **AY513571**. COSTA RICA. PUNTARENAS: Sta. Elena, Road to Biological Reserve, 10°20'32"N / 84°47'55"W. On fence posts, 17 Mar 1999. *Ronald H. Petersen FB10256*. TENN57579. Specimen sequence: **AY513569**. SBI 1 **AY513570**. *Polyporus squamosus* (Huds.) Fr.: RUSSIA. BELGOROD. *Nadia Psurtseva 50*. TENN59384. SBI 1: **AF516572**. USA. MICHIGAN: Albion, Albion College Nature Center, 42°15'N / 84°45'W. On *Ulmus*, 30 May 2001. *Ed Grand FB11420*. TENN59125. **AF516573** (from specimen). *Pseudofavolus cucullatus* (Mont.) Pat.: ARGENTINA. MISIONES: Puerto Iguazú, Iguazú National Park, Isla San Martín, 25°40'55"S / 54°26'50"W. On hardwood stick, 24 May 2001. *Dirk Krüger & Edgardo Albertó FB11221*. teste Leif Ryvarden. TENN58910. SBI 1: **AF516600**. MEXICO. NAYARIT: San Juan, Vulcan San Juan, 21°28'N / 105°00'W. On standing hardwood stem, 20 Jul 1996. *Ronald H. Petersen FB8744*. TENN55173. SBI 7: **AF516601**. *Trametes hirsuta* (Wulfen: Fr.) Pilát: GERMANY. MECKLENBURG-VORPOMMERN: Neubrandenburg-Broda, Brodaer Holz Forest at Belvedere, 53°33'N / 13°16'E. On *Fagus* limb, 24 May 1999. *Dirk Krüger FB10198*. TENN57728. SBI 5: **AF516556**.

Sequence data generation

Nucleic acid extraction followed Krüger *et al.* (2003) or Krüger *et al.* (2004). The nuclear ITS rDNA (ITS1 – 5.8S – ITS2) was initially amplified using primers ITS 1F / ITS 4B (Gardes and Bruns 1993) or ITS 5 (White *et al.* 1990) / LR 7 (www.biology.duke.edu/ fungi/ mycolab/primers.htm). For some difficult isolates, primers NS7UTK and ITS 4C (Krüger *et al.* 2004) were used for ITS region amplification.

PCR products were cleaned with the Amicon Microcon PCR Centrifugal Filter Device (Millipore Co., Billerica, Massachusetts) before sequencing, using the following protocol: first centrifugation step 5 min at 1300 g, second spin step

1000 g, with 30 µl bi-distilled water used for DNA elution. Primers NS7UTK or ITS 5, ITS 3 and / or ITS 2 (White *et al.* 1990) were used for sequencing. Cycle-sequencing was done with 2.5 µl ABI PRISM BigDye Terminator (v 2.0; Applied Biosystems, Inc., Foster City, California) per 10 µl reaction, following manufacturer's instructions using approximately 25 ng DNA template (Hoefer DyNA Quant 200 fluorometer; Amersham Biosciences Co., Piscataway, New Jersey). Each BigDye cycle-sequencing product was cleaned using 50 µl 95% v/v ethyl alcohol (room temperature), 2 µl 3M sodium acetate, and 10 µl bi-distilled water. After 20 min precipitation at room temperature, tubes were centrifuged 20 min at 16000 g, and the supernatant removed. The resultant pellet was rinsed with 190 µl 70% v/v cold ethyl alcohol. Following a second centrifugation (16000 g, 5 min), the supernatant was again removed, and the tube placed for 1 min in a heating block at 90 C.

Sequence alignment

Sequences obtained from ABI electropherogram files were corrected in Chromas v. 1.45 (Technelysium Pty. Ltd., Tewantin, Qeensland, Australia), and assembled and aligned using BioEdit v. 5.0.9 (Hall 1999) and ClustalX v. 1.64b (Thompson *et al.* 1997). Alignments as well as individual sequence data are available from the public databases (see Specimens Examined above; EMBL ALIGN_000654). A new dataset of LSU was also compiled and analyzed to serve as upfront visualization of relationships. From sequences similar by BLAST-n searches, alignable data with taxonomic overlap to the ITS data were kept. Alignment was done using QAlign2 Panta Rhei (Sammeth *et al.* 2006) with the T-Coffee algorithm (Notredame *et al.* 2000).

ITS2 secondary structure prediction

Secondary structure of the ITS spacers (assumed DNA barcode, Nielsen *et al.* 2009, Hughes *et al.* 2009, Seifert 2009) in pre-rRNA is important for correct ribosome assembly (Lalev and Nazar 2001, Coleman 2009) and their presence in environmental RNA can suggest current activity (Anderson & Parkin 2007). ITS rDNA sequences are under evolutionary constraints distinct from standard models of primary sequence evolution. ITS2 rDNA boundaries were determined by comparison to sequences of *Saccharomyces cerevisiae* (5.8S rDNA region from Rubin 1973, LSU rDNA region from Bayev *et al.* 1981). Structure prediction for the ITS2 transcript was performed by Mfold version 3.1 (www.bioinfo.rpi.edu/applications/mfold/; Zuker 2003) using free energy calculations (default conditions: linear RNA sequence, folding temperature of 37 C, 20% sub-optimality, upper bound of 50 foldings, no limit to the maximum distance between paired bases, no constraint information, maximum number of nucleotides in a bulge or loop=30, maximum asymmetry of an interior/bulge loop=30). From the initially predicted foldings – including the available ITS2 primary sequence in its entirety – those most closely resembling the conserved pairing regions (stems) hypothesized for plants, green algae (Mai and Coleman 1997), and various fungi (Krüger & Gargas 2004, Krüger & Gargas 2008) were saved. We then repeated the foldings with shorter stretches of primary sequence data to obtain only the first two of these major stem-loop structures, termed P1 and P2, choosing those that did not conflict with the initial foldings.

Phylogeny of total ITS rDNA data

For the nine investigated ITS sequences, we determined model parameters using jModeltest software (Posada 2008). In MEGA4 (Kumar 2008), we analyzed the data under minimum evolution (ME) as optimality criterion with 1000 replicates of interior branch support testing with the following parameters: gaps completely deleted, keeping 1000 trees during search level 2, closest-neighbor-interchange, Maximum Composite Likelihood model with gamma shape = 0.2620.

Maximum likelihood (ML) analysis was performed in fastDNAml (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=fastdnaml; Olsen *et al.* 1994, Transition/transversion parameter = 1.526738) with base frequencies estimated by jModeltest (A = 0.2348, C = 0.2171, G = 0.2301, T = 0.3180).

Phylogeny of LSU rDNA data

Sequence evolution models were predetermined using JModeltest and incorporated in PAUP* (Swofford 2001) as Lset base=(0.2210 0.2188 0.2363 0.3238) nst=6 rmat=(2.1536 8.9077 2.1536 1.0000 8.9077 1.0000) rates=gamma shape=0.2310 ncat=4 pinvar=0; where we performed ML bootstrapping with 100 pseudoreplicates. In addition, MEGA4 was used for a maximum parsimony (MP) analysis with 1000 bootstrap pseudoreplicates.

RESULTS AND DISCUSSION

LSU phylogeny

The phylogenetic reconstruction (Fig. 1) using ML and MP is in agreement with previous studies where *Polyporus* was not monophyletic. There is no meaningful support for something directly corresponding a Clade 1 *sensu* Sotome *et al.* (core-*Polyporus*) here, though specific taxa previously affiliated do so again. The close relationship of *Datronia* with *Polyporus squamosus*, and of *Pseudofavolus* with *Mycobonia*, is confirmed again with 99 and 80% bootstrap support, respectively, in MP as well as in ML tree topology.

Secondary structures and recoding

Mfold generated one structure each for the nine sequences when only the region containing the first two major stem-loops were parsed into the program (Tab. 1). The only exception was *Polyporus squamosus* FB11420, where three alternative structures were generated. Only the third structure was similar to that of the other sequences; two had much enlarged loops dissimilar from the others. As only FB11420 contained ambiguity codes, these were recognized as responsible for generating the alternative foldings. All predicted and accepted partial ITS2 rRNA structures are shown in Fig. 2, which also indicates the approximate position of recoded parts of predicted structure. Individual character states were then compiled using the predicted and accepted structures (Fig. 2). Various phylogenetic analyses on the nine sequences (not shown) using UPGMA or least-squares neighbor-joining approaches in PAUP* yielded phylogenies not in conflict with the nucleotide phylogenies.

Table 1. Overview of structures generated with mfold.

Sequence source, accession number	*Number of predicted structures (initial dG in brackets)*
Datronia mollis **AF516557**	1 (-23.3 kcal/mole)
Mycobonia flava **AY513569**	1 (-29.3 kcal/mole)
Mycobonia flava **AY513570**	1 (-29.3 kcal/mole)
Mycobonia flava **AY513571**	1 (-30.1 kcal/mole)
Polyporus squamosus **AF516572**	1 (-23.3 kcal/mole)
Polyporus squamosus **AF516573**	3 (-16.2 kcal/mole, -16.2 kcal/mole, -16.1 kcal/mole). The third corresponds best to the folding of the other sequences.
Pseudofavolus cucullatus **AF516600**	1 (-29.8 kcal/mole)
Pseudofavolus cucullatus **AF516601**	1 (-27.6 kcal/mole)
Trametes hirsuta **AF516556**	1 (-28.2 kcal/mole)

Total ITS rDNA data

/Datronia-Squamosus and a /Pseudofavolus-Mycobonia are sister clades. Both the dikaryon and monokaryon (SBI 1) sequence of CulTENN 10256 *Mycobonia flava* from one tip basal to the remainder of /Pseudofavolus-Mycobonia (Fig. 3 far left).

The ME consensus tree has identical topology with the ML tree introduced above (Fig. 3 left). Two groups, *Pseudofavolus* + *Mycobonia* versus *Polyporus squamosus* + *Datronia*, are highly supported with ME interior branch support values of 99%.

Consensus structures

Four consensus structures are introduced as taxonomic characters (Fig. 4). Consensus structure A corresponds to the taxonomic entities contained in /Pseudofavolus-Mycobonia, B is a taxonomic character for *Pseudofavolus cucullatus*, C for *Mycobonia flava*, and D for /Datronia-Squamosus.

Character evolution

The consensus structures defined are not unambiguous for inferring unequivocal polarized changes. As an example, we discuss here two of the recoded characters that change within a subset of the phylogenetic ITS tree containing *Mycobonia flava* and *Pseudofavolus cucullatus* (Fig. 4). The topology of this tree is used here as the assumed true phylogeny of the taxa (Fig. 1). Under this assumption, it can be postulated that sometime before or after the split leading to the *Mycobonia* specimens 10256 and 11279, event I must have happened. That event may have been a resizing mutation that affected the extent of an internal loop of P1 (character 1). It is most parsimonious to assume that this event was synapomorphous for 11279 *Mycobonia*, as well as for *Pseudofavolus*.

Operating under the same parsimony criterion, a silent mutation (event II) may have been synonymous with the change of a single nucleotide somewhere before or after the splitting of 10256 and 11279. This nucleotide change on character 9 in the P2 terminal loop was sequentially followed by an event III. Event III, operating alongside the speciation event leading to *Pseudofavolus*, has a known directional polarity (from *Mycobonia*, to *Pseudofavolus*). This polarity means more information than we have about events I and II. However, such event III could have had two different parsimonious

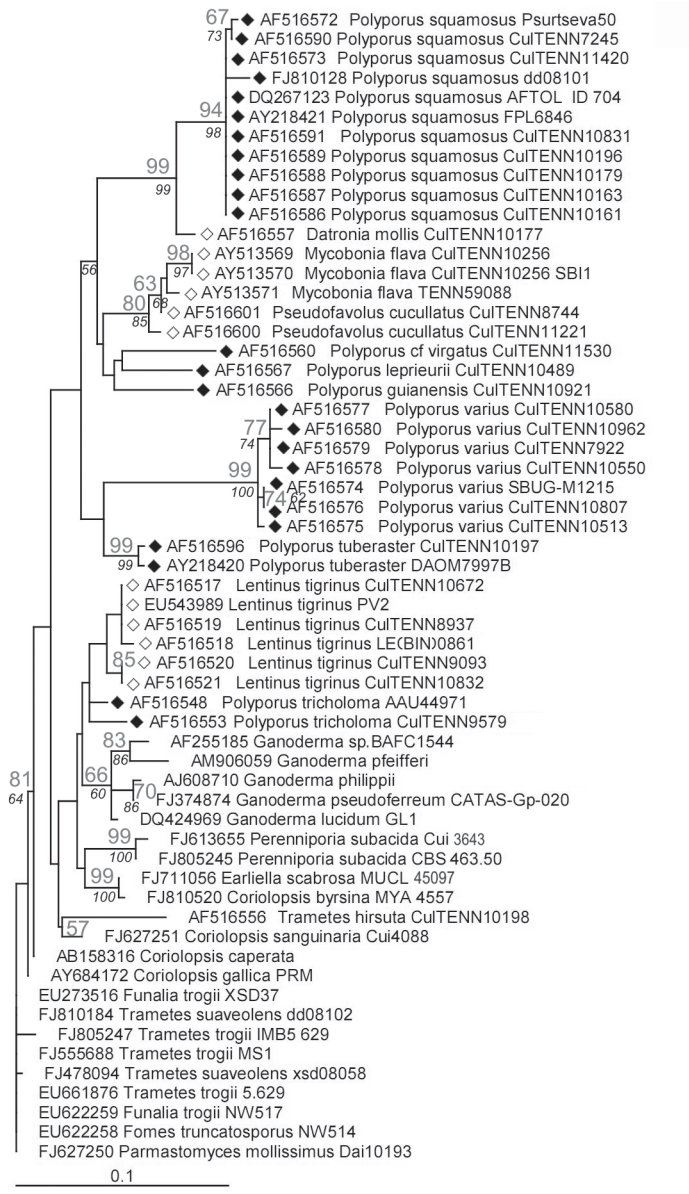

Fig. 1. Nuclear LSU phylogeny of *Polyporus* and allies with the root placed on the lowerhand assemblage involving *Trametes*. Topology of maximum likelihood analysis, with bootstrap support from 100 pseudoreplicates (in italics), and maximum parsimony bootstrap support from 1000 pseudoreplicates. Filled diamonds = *Polyporus*, hollow diamonds = affiliated genera.

modus operandi. One would entail a second silent mutation, the other one would entail at least two additional mutations. A larger sampling of rDNA sequence data may reveal a more detailed picture of hypothetical events of secondary structure changes.

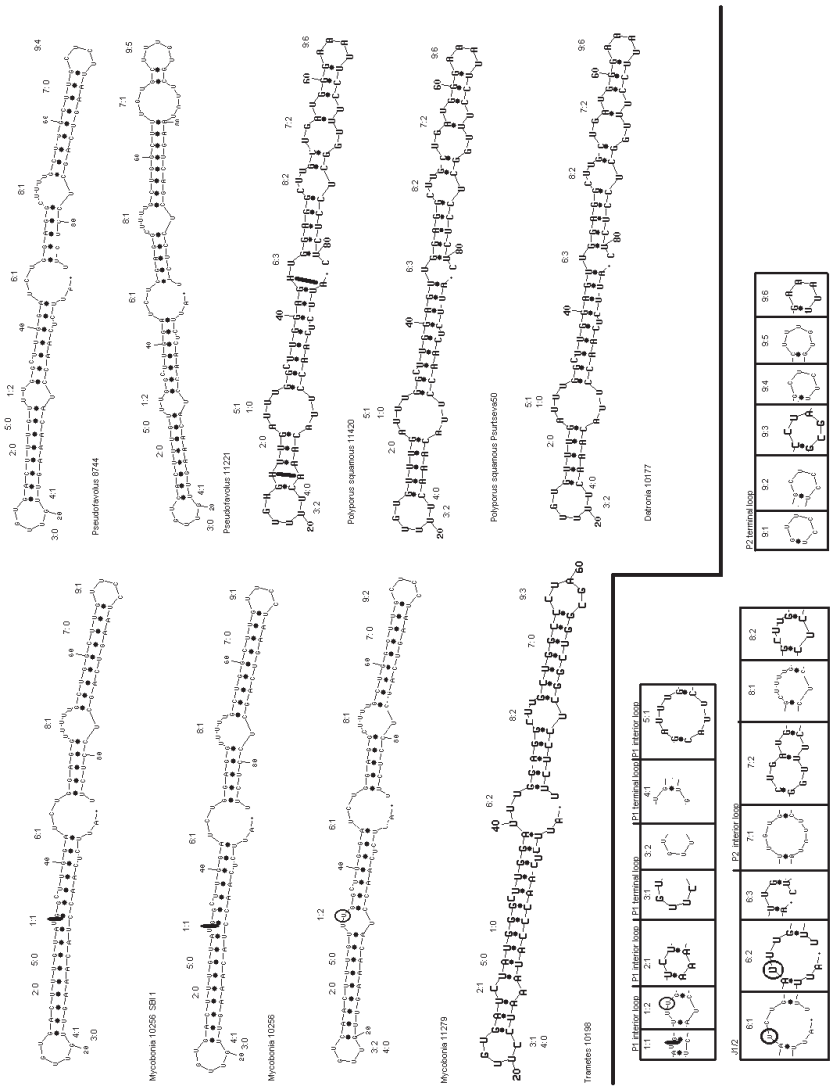

Fig. 2. Mfold-generated secondary structure of ITS2 P1 through P2 (compiled screen shots). Structure altered by inclusion of IUPAC ambiguity codes, here generally equaled with N, is highlighted by addition of the non-conflicting dsRNA bond as a bold line, see *P. squamosus* 11420 structure. Certain indels are highlighted with circles. The character states of recoded parts of the structure are marked, and compiled on the bottom of the figure.

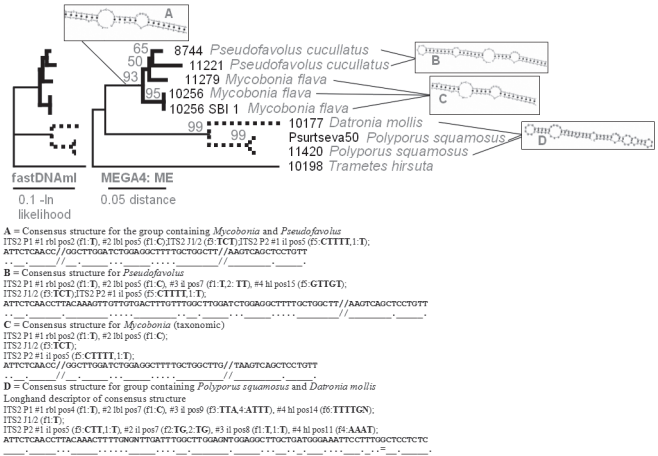

Fig. 3. fastDNAml maximum likelihood phylogram (far left), MEGA4 minimum evolution phenogram with interior branch support (left). Bolded part of dendrograms correspond to /Pseudofavolus-Mycobonia, broken lines indicate /Datronia-Squamosus. Consensus structures mapped onto the dendrogram. Below: detailed descriptors for consensus structures (Krüger & Gargas 2008; note: f5:CTTTT,1:T denotes an internal loop with 5 free bases on the first side from 5', and one free base on the opposite side) and underline annotation (underlined transcribed nucleotides are in dsRNA). "//" indicates omissions (primary sequence data not included in consensus or otherwise discussed structure). Scale bar = distance.

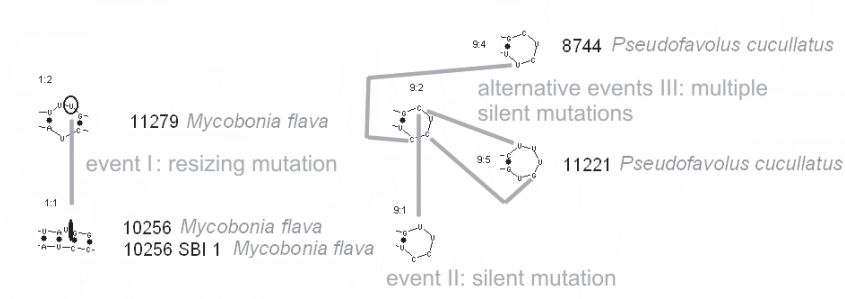

Fig. 4. Hypothetical mutational events that changed the drawn secondary structure.

Conclusion: Linnean taxonomy

The taxonomy of *Mycobonia* was discussed by Martin (1939), where *M. brunneoleuca* was conceived as synonymous with *M. flava*. Reid (1976) and Corner (1984: 103-104) kept the two species separate. Corner (1984: 90) transferred *Pseudofavolus cucullatus* (Mont.) Patouillard (1900, Essai Tax.: 81) as a variety under *Polyporus miquelii* Mont. This avoided the issue of *Polyporus cucullatus* Berk. & Curtis [1872, Notices of North American Fungi. Grevillea I (4): no. 134] occupying the epithet in *Polyporus* (see Ryvarden 1991). *Favolus cucullatus* Montagne 1842 (Ann. Sci. Nat. Ser. II Vol. 2 17: 125), basionym of *Pseudofavolus cucullatus*, would effectively create a homonym in *Polyporus* even though the species epithet otherwise commands priority.

We are of the opinion that *Pseudofavolus* should be included in *Polyporus*. We opt not to reject *Ps. cucullatus* as a valid species. Therefore, we opt for *Polyporus curtipes* [Ryvarden 1991, Gen. Polypores: 213 (based on *Favolus curtipes* Berk. & Curtis 1849, Dec. Fungi XXIII: 222)] as the valid name for *Ps. cucullatus* in *Polyporus* (Ryvarden 1991). Without including *Mycobonia brunneoleuca*, which we were unable to investigate, we propose the transfer of *Mycobonia flava* as a subspecies under *P. curtipes*.

Polyporus Adanson (Fam. Pl. 2: 10. 1763): Fries, Syst. Mycol. 1: 341 (1821) emend. D. Krüger

Accepted as circumscribed by Núñez and Ryvarden (1995) and emended by Krüger and Gargas (2004), but including fungi with smooth hymenophore containing sterile hyphal peg fascicles. Polyporaceae Corda 1839 is thus also emended to include fungi with these characters, placing Mycoboniaceae Jülich 1981 in synonymy.

Polyporus curtipes ssp. flavus (Swartz: Fr.) D. Krüger, comb. nov. (**MycoBank No. MB 511673**)

BASIONYM: *Peziza flava* Swartz 1788, Prod.: 150: Fr., 1823 Systema 2: 161.
≡ *Hydnum flavus* (Swartz: Fr.) Berk. 1842, Ann. Mag. Nat Hist. I 10: 380.
≡ *Bonia flava* (Swartz: Fr.) Pat. 1892, Bull. Soc. Myc. Fr. 8: 49.
≡ *Mycobonia flava* (Swartz: Fr.) Pat. 1894, Bull. Soc. Myc. Fr. 10: 77.

As we believe a close relationship of *Polyporus* and *Datronia* is evidenced by the shown putative ITS2 structure, *Datronia mollis* should again be known as *Polyporus sommerfeldtii* Karsten (1882, Bidr. Känned. Nat. Folk. Finl. 37: 53) to avoid the homonymy of *Polyporus mollis* (Sommerf.) Karsten (1876, Bidr. Känned. Nat. Folk Finl. 25: 280) with *Polyporus mollis* (Pers.) Fr. [1821, Syst. Mycol. 1: 360 (also known as *Leptoporus mollis* (Pers.) Quél., 1886, Ench. Fung.: 176)].

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REFERENCES

ANDERSON I.C. & PARKIN P.I., 2007 — Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. *Journal of Microbiological Methods* 68: 248-253.

BAYEV A., GEORGIEV O.I., HADJIOLOV A.A., NIKOLAEV N., SKRYABIN K.G., & ZAKHARYEV V.M., 1981 — The structure of the yeast ribosomal RNA genes. 3. Precise mapping of the 18 S and 25 S rRNA genes and structure of the adjacent regions. *Nucleic Acids Research* 9: 789-799.

BINDER M. & HIBBETT D.S., 2002 — Higher-level phylogenetic relationships of homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. *Molecular Phylogenetics and Evolution* 22: 76-90.

BUCHANAN P.K. & RYVARDEN L., 1998 — New Zealand polypore fungi (Aphyllophorales): three new species and a new record. *New Zealand Journal of Botany* 36: 219-231.

BUCHANAN P.K. & RYVARDEN L., 2000 — New Zealand polypore fungi (Aphyllophorales): six new species and a redetermination. *New Zealand Journal of Botany* 38: 251-263.

BURDSALL H.H. & DORWORTH E.B., 1994 — Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water in cryovials. *Mycologia* 86: 275-280.
COLEMAN A.W., 2009 — Is there a molecular key to the level of "biological species" in eukaryotes? A DNA guide. *Molecular Phylogenetics and Evolution* 50: 197-203.
CORNER E.J.H., 1984 — Ad Polyporaceas II. *Polyporus, Mycobonia, Echinochaete. Beihefte zur Nova Hedwigia* 78: 1-129.
CORNER E.J.H., 1989 — Ad Polyporaceas V. *Beihefte zur Nova Hedwigia* 96: 1-218.
DONK M.A., 1957 — The generic names proposed for Hymenomycetes VII. "Thelephoraceae" (continuation). *Taxon* 6: 68-85.
DONK M.A., 1964 — A conspectus of the families of Aphyllophorales. *Persoonia* 3: 199-324.
GARDES M. & BRUNS T.D., 1993 — ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.
GILBERTSON R.L. & RYVARDEN L., 1986-1987 — *North American Polypores*. 2 volumes. Oslo, Norway, Fungiflora.
GORDON S.A. & PETERSEN R.H., 1991 — Mating systems in *Marasmius*. *Mycotaxon* 41: 377-386.
HALL T.A., 1999 — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
HIBBETT D.S. & DONOGHUE M.J., 2001 — Analysis of character correlations among wood decay mechanisms, mating systems, substrate ranges in Homobasidiomycetes. *Systematic Biology* 50: 215-241.
HIBBETT D.S. & VILGALYS R., 1993 — Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Systematic Botany* 18: 409-433.
HUGHES K.W, PETERSEN R.H., & LICKEY E.B., 2009 — Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. *New Phytologist*. DOI: 10.1111/j.1469-8137.2009.02802.x.
JÜLICH W., 1981 — Higher taxa of Basidiomycetes. *Bibliotheca Mycologica* 85. Vaduz, Liechtenstein, J. Cramer.
KO K.S. & JUNG H.S., 2002 — Phylogenetic evaluation of *Polyporus s.str.* based on molecular sequences. *Mycotaxon* 82: 315-322.
KREADER C.A., 1996 — Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology* 62: 1102-1106.
KREISEL H., 1969 — *Grundzüge eines natürlichen Systems der Pilze*. Lehre, Germany, J. Cramer.
KRÜGER D., 2002 — Monographic studies in the genus *Polyporus* (Basidiomycotina). Ph.D. thesis. Knoxville TN, University of Tennessee. Available from http://etd.utk.edu/2002/KruegerDirk.pdf.
KRÜGER D., HUGHES K.W., & PETERSEN R.H., 2002 — Studies in *Polyporus* subg. *Polyporellus*: on congruence of three biological, morphological and phylogenetic species. *In:* Laflamme G., Bérubé J.A., & Bussières G. (eds.), *Root and Butt Rots of Forest Trees. Proceedings of the 10th International IUFRO Working Party 7.02.01, September 16-22, 2001.* Québec City, Québec, Canadian Forest Service.
KRÜGER D., HUGHES K.W., & PETERSEN R.H., 2004 — The tropical *Polyporus tricholoma* (Polyporaceae) - taxonomy, phylogeny, and the development of methods to detect cryptic species. *Mycological Progress* 3: 65-80.
KRÜGER D. & GARGAS A., 2004 — The basidiomycete genus *Polyporus* – an emendation based on phylogeny and putative secondary structure of ribosomal RNA molecules. *Feddes Repertorium* 115: 530-546.
KRÜGER D. & GARGAS A., 2008 — Secondary structure of ITS2 rRNA provides taxonomic characters for systematic studies – a case in Lycoperdaceae (Basidiomycota). *Mycol Research* 112: 316-330.
KUMAR S., DUDLEY J., NEI M., & TAMURA K., 2008 — MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9: 299-306.
LALEV A.I. & NAZAR R.N., 2001 — A chaperone for ribosome maturation. *Journal of Biological Chemistry* 276: 16655-16659.
MAI J.C. & COLEMAN A.W., 1997 — The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution* 44: 258-271.
MARTIN G.W., 1939 — New or noteworthy fungi from Panama and Colombia. III. *Mycologia* 31: 239-249.
NILSSON R.H., RYBERG M., ABARENKOV K., SJÖKVIST E., & KRISTIANSSON E., 2009 — The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*. DOI: 10.1111/j.1574-6968.2009.01618.x.
NOBLES M.K., 1965 — Identification of cultures of wood-inhabiting Hymenomycetes. *Canadian Journal of Botany* 43: 1097-1139.

NOTREDAME C., HIGGINS D., & HERINGA J., 2000 — T-Coffee: A novel method for multiple sequence alignments. *Journal of Molecular Biology* 302: 205-217.
NÚÑEZ M. & RYVARDEN L. 1995 — *Polyporus* (Basidiomycotina) and related genera. *Synopsis Fungorum* 10: 1-85.
OLSEN G.J., MATSUDA H., HAGSTROM R., & OVERBEEK R., 1994 — fastDNAml: A tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Computer Applications in the Biosciences* 10: 41-48.
POSADA D., 2008 — jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.
REID D.A., 1976 — Notes on polypores. 2. *Memoirs of the New York Botanical Garden* 28: 179-198.
ROKAS A., WILLIAMS B.L., KING N., &. CARROLL S.B., 2003 — Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798-804.
RUBIN G.M., 1973 — The nucleotide sequence of *Saccharomyces cerevisiae* 5.8 S ribosomal ribonucleic acid. *Journal of Biological Chemistry* 248: 3860-3875.
RYVARDEN L., 1991 — *Genera of Polypores*. Nomenclature and Taxonomy. Synopsis Fungorum 5. Oslo, Norway, Fungiflora.
RYVARDEN L. & GILBERTSON R.L., 1993-1994 — *European Polypores*. 2 volumes. Oslo, Norway, Fungiflora.
RYVARDEN L. & JOHANSEN I., 1980 — *A preliminary polypore flora of East Africa*. Oslo, Norway, Fungiflora.
SAMMETH M., GRIEBEL T., TILLE F., & STOYE J., 2006 — Panta rhei (QAlign2): an open graphical environment for sequence analysis. *Bioinformatics* 22: 889-890.
SEIFERT K.A., 2009 — Progress towards DNA barcoding of fungi. *Molecular Ecology Resources* 9(Suppl. 1): 83-89.
SINGER R., 1949 [1951] — *The Agaricales (mushrooms) in modern taxonomy*. Lilloa 22. Tucuman, Argentina.
SINGER R., 1986 — *The Agaricales in modern taxonomy*. Königstein, Germany, Koeltz Scientific Books.
SMITH A.H., 1963 — Review on "The Agaricales in Modern Taxonomy Ed. 2" by Rolf Singer. *Mycologia* 55: 691-697.
SOTOME K., HATTORI T., OTA Y., TO-ANUN C., SALLEH B., & KAKISHIMA M., 2008 — Phylogenetic relationships of *Polyporus* and morphologically allied genera. *Mycologia* 100: 603-615.
SWOFFORD D.L., 2001 — PAUP* 4.0. Phylogenetic analysis using parsimony (* and other methods). Beta version 4.0b10. Sunderland MA, Sinauer Associates, Inc.
THOMPSON J.D., GIBSON T.J., PLEWNIAK F., JEANMOUGIN F., & HIGGINS D.G., 1997 — The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876-4882.
WHITE T.J., BRUNS T., LEE S., & TAYLOR J.W., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis M.A., Gelfand D.H., Sninsky J.J., & White T.J. (eds.), *PCR Protocols: A Guide to Methods and Applications*. San Diego CA, Academic Press, pp. 315-322.
ZUKER M., 2003 — Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* 31: 3406-3415.