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The basidiomycete genus *Polyporus* – an emendation based on phylogeny and putative secondary structure of ribosomal RNA molecules

With 4 Figures and one Table

Summary

The fungal genus *Polyporus* is an assemblage of white-rotting lignicolous basidiomycetes. It has undergone considerable expansion and contraction over a period of two and three quarter centuries. Current generic circumscription of *Polyporus* has kept the genus non-monophyletic. Species of *Polyporus* infrageneric group *Polyporellus* are closely related to some species of *Lentinus*. We introduce data for ITS2 spacer rRNA secondary structure evolution by quasi-independent comparison with large subunit rRNA phylogeny, and suggest a fraction of primary nuclear rDNA ITS sequence data as novel taxonomic character. A major taxonomic shift is suggested, supported by molecular and morphological characters, and allowing inclusion of species with gilled hymenophores in *Polyporus*. Two new names are proposed: *Polyporus phyllostipes* D.KRÜGER, nom. nov. and *Polyporus gerdai* D.KRÜGER, nom. nov.

Zusammenfassung

Die Gattung *Polyporus* (Basidiomycetes) – eine Emendation auf der Basis von Phylogenie und mutmaßlicher sekundärer Struktur der ribosomalen RNA-Moleküle

Die Pilzgattung *Polyporus*, eine Gruppe Weißfäule erregender holzbewohnender Basidiomyceten, wurde über nahezu drei Jahrhunderte vielfach expandiert und verkleinert. Bei der derzeitigen Gattungsumschreibung von *Polyporus* gilt die Gattung als nichtmonophyletisch. Arten der *Polyporus*-Gruppe *Polyporellus* sind eng verwandt zu einigen *Lentinus*-Arten. Anhand quasi-unabhängigem Vergleich mit der Phylogenie der rRNA der großen Untereinheit (LSU) stellen wir Daten zur Evolution der ITS2 Spacer rRNA vor, und schlagen ein ITS Kern-rDNA-Fragment als taxonomisches Merkmal vor. Unterstützt mit molekularen und morphologischen Daten wird eine grundlegende taxonomische Verschiebung vorgeschlagen, welche Arten mit Lamellen-Hymenophoren in *Polyporus* erlaubt. Zwei neue Namen werden vorgeschlagen: *Polyporus phyllostipes* D.KRÜGER, nom. nov. und *Polyporus gerdai* D.KRÜGER, nom. nov.

1 Introduction

One of the more conspicuous genera of pore fungi, *Polyporus* (Basidiomycotina) has been in use since 1729 (MICHELI 1729). The name represents a basidiomycete alliance with a history of periodic contraction and expansion. When ADANSON (1763) took up MICHELI'S name *Polyporus*, he omitted reference to a figure (Pl. 71, Fig. 1) later selected as the iconotype for the *Polyporus* generic type species *P. tuberaster* by DONK (1960: 261). Circa 1812, PAULET (Icon. Champ.) used the name *Polyporus* in seven binomials, with *P. ulmi* PAULET the first name mentioned.

FRIES (1821: 341), in sanctioning *Polyporus* as "*Polyporus* MICHELI p. 129", applied the name to almost all polypores, leaving *Fistulina* BULL. and *Daedalea* PERS. separate. Published by NÚÑEZ & RYVARDEN (1995), the latest

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monographic treatment contained only 32 species. They were arranged in six infrageneric groups that were not given ranks. Those groups were *Dendropolyporus*, *Polyporus* s. str. (= *Squamosus* group), *Polyporellus*, *Melanopus*, *Admirabilis*, and *Favolus*. Several species have been added or resurrected more recently (BUCHANAN & RYVARDEN 1998; DAI 1996, 1999; HATTORI 2000; POPOFF & WRIGHT 1998; THORN 2000).

The following lectotypifications have been proposed: i) MURRILL (1903) first selected a lectotype for *Polyporus* (MICHELI) PAULET: PAULET'S first species, *P. ulmi* PAULET [Icon. Champ.: Pl. 13 (1793) – DONK (1960: 261) gave the date as 1812–1835]. MURRILL (1903) regarded *P. ulmi* as synonymous with *P. squamosus* HUDS.: FR. and *P. caudicinus* SCOP.: FR. Admittedly using the then fashionable "first-species-rule" which has been judged mechanical (ICBN St. Louis Code by GREUTER et al. 2000: 12 Art. 9.2 and 16 Rec. 9A.2.), this lectotypification is not tenable. ii) CLEMENTS & SHEAR (1931: 347) selected *P. brumalis* (PERS.) FR. as type of *Polyporus* (MICH.) FR. They chose the type "from the best known or more important species ... in order to avoid change and ensuing confusion as far as possible" [CLEMENTS & SHEAR (1931: 15); an argument also used by REDHEAD & GINNS (1985) to defend a CLEMENTS & SHEAR typification of *Lentinus* (see below)]. *Polyporus brumalis* is the sixth species in FRIES (1821: 348) of "*Polyporus* MICHEL." B. *Microporus* Trib. I. *Mesopus*. CLEMENTS & SHEAR (l. c.) referred to *Polyporus* as "*Polyporus* (MICHELI) FR. Epicr. 427 (1838)", where one finds FRIES himself referring to "S. M. p. 341" (i.e. FRIES 1821: 341) and giving *P. brumalis* as twelfth species (Epicris. Syst. Mycol.: 430). Thus, CLEMENTS & SHEAR certainly did not use mechanical means of selecting types, making their proposed lectotype the first acceptable type for *Polyporus*. iii) DONK (Meded. Nederl. Mycol. Ver. 22: 124–126, 1933) instead selected *P. tuberaster* as a species common to MICHELI'S, PAULET'S, and FRIES' sense of *Polyporus* (see DONK 1960: 262). DONK (1960: 263) dismissed CLEMENTS & SHEAR'S lectotypification, as he considered *P. brumalis* as only "doubtfully represented" among species illustrated by MICHELI. Dismissal of the valid 1931 lectotypification based on this argument is not supported by the ICBN (GREUTER et al. 2000: 12 Art. 9.2 and 16 Rec. 9A.2.), as any species included by FRIES in the sanctioning volumes may be selected as type. iv) Without expressed justification, CUNNINGHAM (1948: 1) proposed *P. arcularius* (BATSCH) FR. as type, but this is later than CLEMENTS & SHEAR'S proposal. The species names *Polyporus arcularius*, *P. brumalis*, and *P. ciliatus* have been misapplied in the past (KREISEL 1963), perhaps having led to *P. arcularius* being stated as type by CUNNINGHAM (1948).

DONK'S selection of *P. tuberaster* was followed by BONDARTSEV & SINGER (1941: 58), IMAZEKI (1943: 61), KREISEL (1960), and RYVARDEN (1991). RYVARDEN (1991) erred in attributing the choice of *P. tuberaster* to MURRILL (1903). After pointing out that the correct type must be *P. brumalis* (NÚÑEZ 1993), NÚÑEZ & RYVARDEN (1995: 7) opted for *P. tuberaster* to stabilize use of the genus name *Polyporus*, resulting in a renaming of unranked infrageneric groups between 1993 and 1995. NÚÑEZ & RYVARDEN (1995) stated that MURRILL (1903) selected *P. caudicinus* (SCOP.) FR. *Polyporus caudicinus* [1903; ut "*P. caudicinus* (SCOP.)" (= *Boletus squamosus* HUDS. = *Polyporus squamosus* HUDS.: FR.)] is a misapplication of *Polyporus caudicinus* [J. SCHAEFFER "fig. 131–132" (but Fung. Bav. Palat. Nasc. Icones. 4: 86 is 1774) ex SCOPOLI, Fl. Carniol. ed. 2, 2: 469 (1772)] J. SCHROETER 1888 [= *Boletus imbricatus* BULL. 1788 = *Laetiporus sulphureus* (BULL. 1787) MURRILL 1920]; the CBS Aphyllophorales database at www.cbs.knaw.nl/aphyllo/database.html lists *P. caudicinus* MURRILL, J. Mycol. 9: 89 (1903) as an illegitimate name *non P. caudicinus* (SCOP.) J. SCHROETER 1888.

Reference of *P. tuberaster* as type was based on three arguments: i) *P. tuberaster* was a once cultivated organism deserving name stability (KREISEL 1960), ii) rejection of automatic first-name-rule typification (see NÚÑEZ 1993; NÚÑEZ & RYVARDEN 1995), and iii) stability of the use of the name *Polyporus*. CORNER (1984: 11) challenged *P. tuberaster* as *Polyporus* lectotype, instead opting for *P. squamosus* HUDS.: FR., reporting that *P. squamosus* had the typical hyphal construction of *Polyporus* s.str., and that *P. tuberaster*

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was poorly known. These taxonomic opinions cannot disqualify *P. brumalis* as first selected lectotype. DONK (1960: 261) suggested that *P. tuberaster* and *P. squamosus* might be conspecific, which has been refuted by mating studies by NÚÑEZ (1995) and molecular data (HIBBETT & DONOGHUE 1995; KRÜGER 2002: e.g. 25, 150; KO & JUNG 2002a).

The close relationship of *P.* (*Polyporellus*) *arcularius* (Polyporaceae CORDA 1839) and the gilled mushrooms of *Lentinus* subgen. *Lentinus* (Lentinaceae JÜLICH 1981) was confirmed by HIBBETT & VILGALYS (1993), HIBBETT & DONOGHUE (1995), and KO & JUNG (2002a), although such positional proximity within the weakly resolved / Polyporoid [the "/" indicates a monophyletic clade, as in BAUM et al. (1998), MONCALVO et al. (2002) and THOMAS et al. (2002)] was not seen in HIBBETT & DONOGHUE'S (2001) consensus tree. HIBBETT & VILGALYS (1995) presented evidence for *Lentinus* being derived from polypores, as postulated by PEGLER (1983: 11). KO & JUNG (2002a) suggested that *Polyporus sensu* NÚÑEZ & RYVARDEN (1995) was not monophyletic based on mitochondrial rDNA sequences. So far, no taxonomic arrangements to reflect this inferred kinship of *Lentinus* and *Polyporus* have been proposed.

The aim of this study was i) to investigate *Lentinus* and *Polyporus* species relationship, as evidenced by nuclear large subunit DNA data; ii) to compare the putative secondary structure of spacer nuclear ribosomal RNA and define a consensus structure for taxonomic characters independent of large subunit primary sequence, and iii) to derive a practicable taxonomic and nomenclatural conclusion for *Lentinus* and *Polyporus*.

2 Materials and methods

Collections and microscopy

Collections (see Table 1) were given field book numbers and annotated. Specimens were deposited in the University of Tennessee fungal herbarium (TENN). Identification was done using keys furnished by JÜLICH (1984), GILBERTSON & RYVARDEN (1986–1987), RYVARDEN & GILBERTSON (1993–1994), and NÚÑEZ & RYVARDEN (1995).

DNA extraction

DNA was extracted from herbarium specimens or cultures following protocols described by KRÜGER et al. (2003, 2004), using a modified xanthogenate-based procedure (TILLETT & NEILAN 2000). With culture material, a short centrifugation step and the removal of some liquid was done before proceeding to grinding. Mechanical cell disruption was carried out in 50 µl TE extraction buffer or after the addition of the xanthogenate buffer.

PCR and sequencing

Amplification of the nuclear ribosomal large subunit (nLSU) gene was conducted with primers ITS 5 (WHITE et al. 1990) and LR 7 or LROR/LR 7 (www.biology.duke.edu/ fungi/mycolab/primers.htm). PCR parameters for the primer pair LROR and LR 7 (20 µL reactions) were as follows: initial denaturation 94 °C for 3 min, followed by 37 cycles of denaturation 94 °C for 1 min, annealing 46 °C for 1 min, extension 72 °C for 3 min, with adjustments when amplifying with primers ITS 5 and LR 7. Subsequent LROR and Nu-LSU333-5′ (CTAAATATTGGCGAGAGAC; T_m = 53.88 °C after calculation at www.genosys.com) cycle sequencing reactions (10 µl) contained 2 or 3 µl BigDye v. 2.0 reaction mix, 0.32 µl 10 µl primer, and approximately 25 ng template DNA. Excess primers and nucleotides were removed by an ethanol precipitation protocol: adding 10 µl 1 dd H_2O, 50 µl 95% ethanol, and 2 µl 3 M sodium acetate (pH 5.2) to the cycle sequencing products; precipitation at room temperature for 20 min. Next, samples were spun for 20 min (16,000 g). Supernatant was removed, and the pellet was washed with 190 µl 70% ethanol and recentrifuged (5 min, 16,000 g). The supernatant alcohol was pipetted off, and the pellet was dried by incubation for 1 min at 90 °C. Sequencing reactions were analyzed on Perkin-Elmer automated sequencers at the University of Tennessee sequencing facility.

Phylogenetic analyses

Sequences obtained with electropherograms were corrected in Chromas v. 1.45 (Technelysium Pty. Ltd., Australia), and assembled using a text editor. One additional sequence was imported from GenBank (X78776, MONCALVO et al. 1995). ClustalX v. 1.64b (THOMPSON et al. 1997) and BioEdit v. 5.0.9 (HALL 1999) were used in alignment. The data used here represent a subset of a dataset in KRÜGER (2002), containing 24 taxa and 724 aligned characters. All trees were *a-priori* rooted with Hallenberg 2518-1 *Neolentiporus maculatissimus*, and processed in TREEVIEW v. 1.6.1. (PAGE 1996).

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Table 1
Fungal material used for phylogenetic and Mfold analyses

Strain numbers and/or herbarium voucher numbers if known	Fungal species and authors	Country of origin	Names of collectors and identifiers	GenBank number and study	NÚÑEZ & RYVARDEN (1995) group
	Ganoderma tsugae MURRILL			X78778 MONCALVO et al. (1995)	
FB10126 SBI 2 (TENN57346)	*Ischnoderma resinosum* (WAHLENB.: FR.) P.KARST.	USA	DK	AJ487927 (this study)	
FB11101 SBI 1	*Lentinus tigrinus* (BULL.: FR.) FR.			AJ487929 (this study)	
FB9770 SBI 5 (culture ex spore print ex culture LE(BIN)0861)	*Lentinus tigrinus* (BULL.: FR.) FR.	Mongolia		ITS sequence AF516518 (KRÜGER et al. 2004)	
FB10125 SBI 17 (TENN57410)	*Lenzites betulina* (FR.) FR.	USA	DK	AJ487931 (this study)	
FB11279(TENN59088) (specimen)	*Mycobonia flava* (SW.: FR.) PAT.	Argentina	DK	AJ487933 (this study)	
Hallenberg2518 SBI 1 (FCUG) (culture)	*Neolentiporus maculatissimus* (LLOYD) RAJCHENB.	Argentina	N. Hallenberg & M. Rajchenberg	AJ487935 (this study)	
FB10901 SBI 1 (TENN58384)	*Piptoporus betulinus* (BULL.: FR.) P.KARST.	Germany	DK & R. Elias	AJ487936 (this study)	
DSH90.36 (DNA extraction received from David Hibbett)	*Polyporus alveolaris* (DC.: FR.) BONDARTSEV & SINGER		D. Hibbett	AJ487937 (this study)	*Favolus*
FB10299 SBI 2 (TENN58370)	*Polyporus arcularius* BATSCH: FR.	Austria	H. Voglmayr	AJ487938 (this study)	*Polyporellus*
FB10299 SBI 2 (TENN58370)	*Polyporus arcularius* BATSCH: FR.	Austria	H. Voglmayr	ITS sequence AB070865 (KRÜGER et al. 2003)	*Polyporellus*
FB5085(TENN59136) (specimen)	*Polyporus badius* (PERS.) SCHWEIN.	USA	RHP	AJ487941 (this study)	*Melanopus*
FB10908 SBI 4 (TENN58391)	*Polyporus brumalis* PERS.: FR.	Germany	DK	AJ487942 (this study)	*Polyporellus*
FB10908 SBI 4 (TENN58391)	*Polyporus brumalis* PERS.: FR.	Germany	DK	ITS sequence AB070876 (KRÜGER et al. 2003)	*Polyporellus*
FB10167 SBI 9 (TENN57698)	*Polyporus ciliatus* FR.	Denmark	H. Knudsen & R. H. Petersen	AJ487943 (this study)	*Polyporellus*
FB10167 SBI 10 (TENN57698)	*Polyporus ciliatus* FR.	Denmark	H. Knudsen & R. H. Petersen	ITS sequence AB070883 (KRÜGER et al. 2003)	*Polyporellus*
FB11254 SBI 1 (TENN58943)	*Polyporus grammocephalus* BERK.	Paraguay	DK & R. H. Petersen	AJ487946 (this study)	*Favolus*
FB10921 SBI 8 (TENN58404)	*Polyporus guianensis* MONT.	Venezuela	T. Iturriaga & L. Ryvarden	AJ487948 (this study)	*Melanopus*
FB10489 SBI 10 (TENN58597)	*Polyporus leprieurii* MONT.	Costa Rica	J. L. Mata & R. H. Petersen	AJ487949 (this study)	*Melanopus*

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Table 1 (continued)

Strain numbers and/or herbarium voucher numbers if known	Fungal species and authors	Country of origin	Names of collectors and identifiers	GenBank number and study	NÚÑEZ & RYVARDEN (1995) group
FB11465 (TENN59326) (culture)	*Polyporus melanopus* (PERS.) FR.	Austria	H. Voglmayr	AJ487951 (this study)	*Melanopus*
FB10298 = Thorn567 = Kotiranta6472 (culture)	*Polyporus pseudobetulinus* (MURASHK. ex PILÁT) THORN, KOTIR. & NIEMELÄ	Finland	H. Kotiranta	AJ487954 (this study)	*Admirabilis*
FB10831(TENN58380) (specimen)	*Polyporus squamosus* (HUDS.) FR.	USA	M. Scholler & DK	AJ488106 (this study)	*Polyporus*
FB9579 SBI 1 (TENN56491)	*Polyporus tricholoma* MONT.	Puerto Rico	R. H. Petersen	AJ488115 (this study)	*Polyporellus*
FB9579 SBI 1 (TENN56491)	*Polyporus tricholoma* MONT.	Puerto Rico	R. H. Petersen	ITS sequence AF516553 (KRÜGER et al. 2004)	*Polyporellus*
FB10197 (TENN57727) (specimen)	*Polyporus tuberaster* JACQ.: FR.	Germany	R. H. Petersen	AJ488116 (this study)	*Polyporus*
FB10962 SBI 19 (TENN58587)	*Polyporus varius* FR.	USA	DK	AJ488121 (this study)	*Melanopus*
FB11219 (TENN58908) (culture)	*Polyporus virgatus* BERK. & M.A.CURTIS	Argentina	E. Albertó	AJ488122 (this study)	*Melanopus*
FB8744 SBI 7 (TENN55173) = Ryv *s.n.* (20/7/96) (culture)	*Pseudofavolus cucullatus* (MONT.) PAT.	Mexico	R. H. Petersen	AJ488125 (this study)	
FB10198 SBI 5 (TENN57728)	*Trametes hirsuta* (WULFEN: FR.) PILÁT	Germany	DK	AJ488129 (this study)	
FB10198 SBI 5 (TENN57728)	*Trametes hirsuta* (WULFEN: FR.) PILÁT	Germany	DK	ITS sequence AF516556 (KRÜGER et al. 2004)	

FB = TENN field book number = CulTENN culture collection number.
SBI = single basidiospore isolate.
TENN = Univ. of Tennessee Fungal Herbarium, other herbarium acronyms from HOLMGREN et al. (1981)

Maximum parsimony

The program SEPAL (SALISBURY 2001) was used for a maximum-parsimony analysis with jackknife resampling procedure (EFRON & GONG 1983; 100 pseudoreplicates, 20% deletion, 50% majority rule). In addition, branch support was estimated by a calculation of decay indices (BREMER 1994). PAUP* v. 4.0b10 was also used to TBR-swap on the decay tree of SEPAL. All most-parsimonious trees (MPTs) found in this round, together with the SEPAL-generated tree, were combined into one 50% majority rule tree and displayed with indicated bipartition frequencies over 50% achieved in the jackknife analysis. All trees were also compared in their minimum-evolution (ME)-score, under the Kimura-2 parameter model.

Bayesian posterior probabilities

Bayesian likelihood analyses were performed in MrBayes (HUELSENBECK & RONQUIST 2001), including parameter optimization on a general-time-reversible (GTR) likelihood model of sequence evolution with gamma-shaped distribution of rate heterogeneity. The analysis settings were (after doing three independent analyses to decide on settings): 100,000 generations with a random starting tree, burn-in threshold of 10,000 trees, sampling frequency of 500. Six search chains of Markov Chain Monte Carlo were run in parallel. PAUP* v. 4.0b10 (SWOFFORD 2001) was used to examine the sampled trees from Bayesian analysis and to calculate an 80% majority rule consensus tree. Bipartitions with over 60% support are indicated in Fig. 2.

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Puzzle likelihood

Puzzle tree-reconstruction (STRIMMER & VON HAESELER 1996; STRIMMER et al. 1997) was run assuming a gamma-distributed rate heterogeneity (eight categories; approximated shape parameter, nucleotide frequencies, and transition/transversion bias estimated from the data), HKY model (HASEGAWA et al. 1985), neighbour-joining tree as starting tree.

Likelihood-based tests

The Kishino-Hasegawa (KISHINO & HASEGAWA 1989) and Templeton (TEMPLETON 1983) tests implemented in PAUP* were utilized to compare all parsimony trees from SEPAL and PAUP*, as well as the Bayesian likelihood consensus tree and the Puzzle likelihood tree, in the significance of parsimony length differences. As the compared trees do not include a most-likely tree, these statistical tests do not fall under the circumstances described as improper by GOLDMAN et al. (2000).

Displaying trees

Trees were prepared for publication with TREEVIEW v. 1.6.1. (PAGE 1996) and data and trees were archived in TreeBASE (Study Number S1113).

ITS2 secondary structure generation

Secondary structure of rRNA spacers is important in ribosome assembly and thus must be under evolutionary constraints (LALEV & NAZAR 2001). ITS2 rDNA boundaries were distinguished by comparison to published sequences for *Saccharomyces cerevisiae*; the 5.8S DNA region to RUBIN (1973), and the LSU rDNA region to BAYEV et al. (1981). Folding for ITS2 rRNA transcript was performed by Mfold version 3.1 (ZUKER 2003) using free energy calculations (default conditions: linear RNA sequence, folding temperature of 37 °C, 20% suboptimality, upper bound of 50 foldings, no limit to the maximum distance between paired bases, maximum number of nucleotides in a bulge or loop = 30, maximum asymmetry of an interior/bulge loop = 30). From the initially predicted foldings, those most closely resembling the four conserved pairing regions (stems) hypothesized for plants and green algae (MAI & COLEMAN 1997), dinoflagellates (GOTTSCHLING & PLÖTNER 2004) and various unrelated fungi (GARGAS & KRÜGER unpublished) were carefully scrutinized. 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 hereafter. This was an iterative process stopped once full ITS2 rRNA and partial ITS2 rRNA foldings inferred identical structures for the P1 through P2 part of ITS2.

Comparing putative ITS2 structure with LSU phylogeny

The 5′ half of the 28S large subunit rRNA of the pre-rRNA transcript that is encoded by the data used for phylogenetic analyses is assumed to not interact on the secondary structural level with the ITS2 spacer pre-rRNA segment. Thus, we assume that the sequences analyzed here for phylogeny and for putative secondary structure are largely independent. This allows the postulating of hypothetical events in ITS structure evolution when assuming the correctness of the phylogenetic trajectory indicated by LSU data.

Nomenclature

For nomenclature questions, we consulted the current ICBN Code (GREUTER et al. 2000, www.bgbm.fu-berlin.de/iapt/ nomenclature/code/ SaintLouis/ 0000St.Luistitle.htm). Synonymy was checked with various taxonomic literature cited throughout, as well as using online databases (www.indexfungorum.org; STALPERS 2004).

3 Results

Modeltest

The general-time-reversible (GTR + G + I, – ln L = 2,966.429) model was selected as best by Modeltest (POSADA & CRANDALL 1998), but in Puzzle we chose to use the computationally less demanding HKY model (– ln L = 3,109.048) for likelihood assessments among available models.

Maximum parsimony

The data included 74 variable but parsimony-uninformative characters and 94 parsimony-informative characters. Swapping on the SEPAL tree (174,560 TBR rearrangements) generated another 38 most-parsimonious trees (MPT) in PAUP*. Each had a length of 384 steps (207 to 624 steps possible), CI = 0.539, and RC = 0.310. The other parsimony trees scored with: 405 steps / CI = 0.511 / RC = 0.268 / ME-score = 0.52888 (SEPAL), and 421 steps / CI = 0.492 / RC = 0.268 / ME-score = 0.53952 (SEPAL jackknife: 20% deletion).

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Bayesian posterior probabilities

180 trees were used to compute the 80% majority rule consensus tree of the Bayesian analysis. The consensus tree had the following parameters: 405 steps / CI = 0.511 / RC = 0.268 / ME-score = 0.53994. It was identical to the SEPAL tree in parsimony parameters.

Puzzle likelihood

10,626 quartets (19.4% unresolved) were analyzed, 1,888 site patterns found, and the shape parameter estimated as alpha = 0.12. The estimated transition/transversion ratio was 3.51 and the estimated pyrimidine transition/purine transition ratio was 0.62. After thirteen iterations, likelihood converged on $-\ln L = 3{,}009.90$. The Puzzle tree scored (in PAUP*) with 416 steps / CI = 0.498 / RC = 0.248 / ME-score = 53,461.

Likelihood-based tests

The likelihood-based tests applied to nucleotide character change steps rejected all non-parsimonious trees.

Tree depiction

Shown herein are the following trees: the Puzzle tree (Fig. 1) with Puzzle bipartition supports, the Bayesian consensus (Fig. 2 left) with branching supports over 50 indicated, and one of the MPT (indicated as best in the likelihood-based tests) with decay indices and jackknife bipartition supports (Fig. 2 right).

Secondary structure

Each of the sequences used resulted in only one structure (Figs. 3, 4) deduced with standard Mfold settings. Note that the placement of the basal base pair of the major stem-loops, and the size of the joiner between them, depends on the extent of sequence data pasted into Mfold.

4 Conclusions

Observed and inferred phenotypic characters

The inflated generative hyphae as depicted by PEGLER (1983: e.g. p. 47 for *L. tigrinus*) for *Lentinus s. str.* (subgen. *Lentinus*, section *Tigrini*), and also found prominently in many young specimens of *Polyporellus* by us (KRÜGER 2002) appear to be a unifying character for a *Lentinus–Polyporellus* alliance perhaps in addition to hyphal pegs as suggested by HIBBETT & VILGALYS (1993). PEGLER (1983: 5) mentioned the sclerotia/pseudosclerotia of *Lentinus*, which are also found in *Polyporus* (*Squamosus* and *Dendropolyporus* groups) and *Laccocephalum* MCALP. & TEPPER (NÚÑEZ 1995; NÚÑEZ & RYVARDEN 1995). It remains to be determined whether the formation of sclerotia arose independently in the *Lentinus/Polyporellus* alliance and in other polypores.

Polyporellus spp. differ from other *Polyporus* spp. in spore sizes (*Polyporus* group *Squamosus* spores are longer, see NÚÑEZ & RYVARDEN 1995) and existence of widely inflated generative hyphae, especially tropical specimens of *P. tricholoma*, and specimens fruited on sawdust (KRÜGER et al. 2003). Central lengths of skeleto-binding hyphae also can take the inflated form, with a varying width of lumen in the wider part. Abrupt changes from generative to skeletal hyphae or vice versa can be seen at a clamp connection. Sometimes one can find irregularily-shaped clamp connections that also are branching points. Other features typical for group *Polyporellus* may be monokaryotic fruiting (HOFFMANN 1978), and the sometimes fuzzy hirsute appearance of entire fruit bodies or caps (KREISEL 1963; BREMER 1986). Other basidiomata of *P. brumalis* and *P. ciliatus* develop reddish or ochraceous stains, occasionally impeding easy recognition in the field. Fuzzy and ciliate pilear surface and margin also can be found in species of *Lentinus* (e.g. compare PEGLER 1983: 30, 32, 38).

Mfold inference of ITS2 structures was influenced by ambiguity codes in the sequences of *Polyporus arcularius, P. brumalis*, and *P. tricholoma*. In particular, ambiguity lead to additional loops (e.g. ambiguity replaced by boxed N, see Fig. 3). We could not judge whether there were non-orthologous rDNA or non-functional divergent copies (KO & JUNG 2002b; KRÜGER et al. 2004; RAZAFIMANDIMBISON et al. 2004), or whether PCR or sequencing artifacts led to the ambiguity. We drew binding indicators across the loops generated by the nucleotide ambiguity (Fig. 3). ITS hy-

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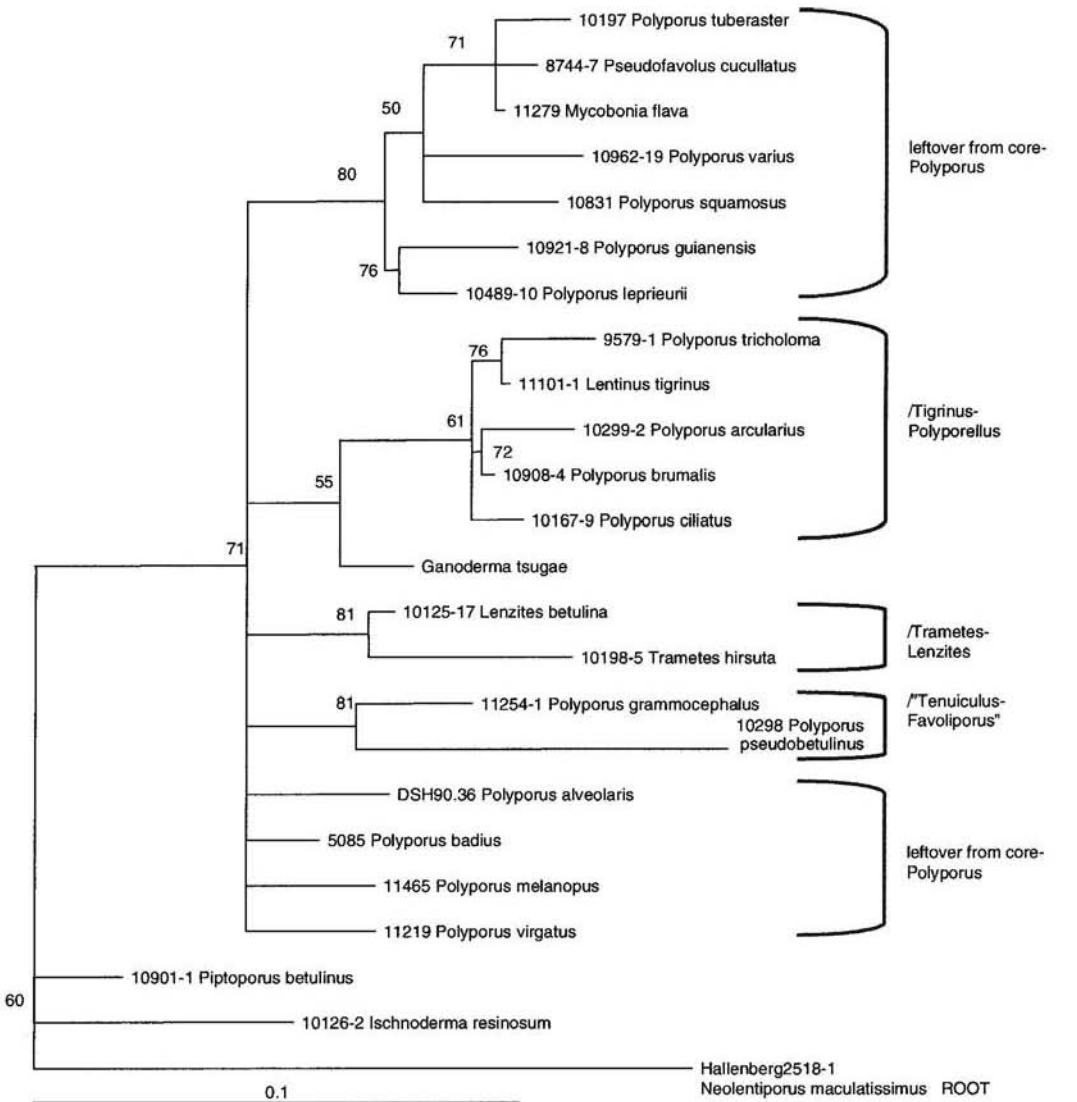

Fig. 1
Puzzle phylogram, 24 taxa. Support for branching next to nodes. Parsimony scores: 416 steps / CI = 0.498 / RC = 0.248

pervariability due to microsatellites has recently found renewed interest in fungal molecular phylogenetics (MIADLIKOWSKA et al. 2003; DEN BAKKER et al. 2004), and there is urgent need to re-evaluate secondary structure and concerted evolution of rDNA repeats. As multi-gene phylogenies are accumulating, refining the models of sequence evolution of non-protein coding sequences, e.g. involving secondary structure information (e.g. SMITH et al. 2003), appears feasible. This is also necessary in light of lack of resolution in single-gene phylogenies (ROKAS et al. 2003) and finding break points of chimerics in environmental rDNA PCR clone libraries (HUGENHOLTZ & HUBER 2003).

Taking the LSU phylogeny as trajectory for the evolution of inferred ITS2 rRNA foldings,

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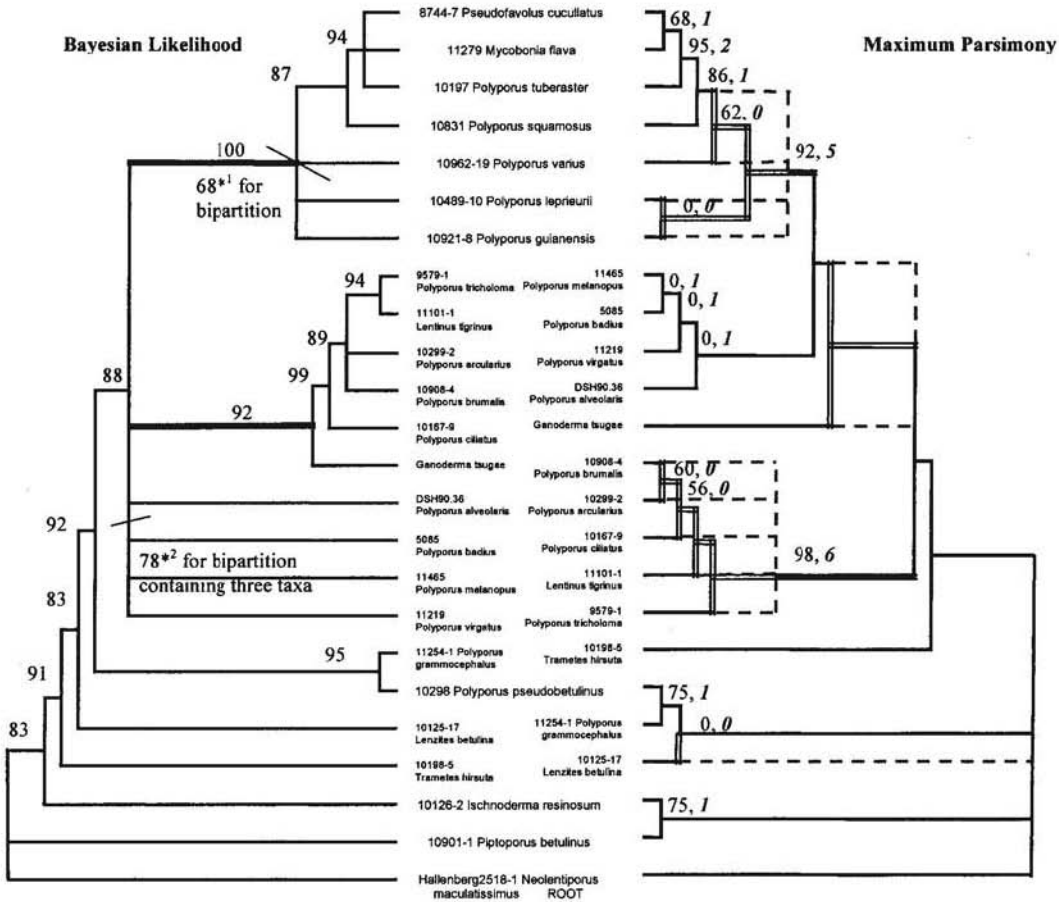

Fig. 2
Left: Bayesian dendrogram (80% majority rule consensus), 24 taxa. Support for branching next to nodes. *1: *Polyporus leprieurii / P. guianensis* vs. above-located taxa split in bipartition, with 68% support for *Polyporus leprieurii / P. guianensis* clade. *2: *Polyporus badius / P. melanopus / P. virgatus* supported as a distinct clade with 78% support. Parsimony scores: 405 steps / CI = 0.511 / RC = 0.268. Right: Parsimony dendrogram, 24 taxa. One most-parsimonious tree. Jackknife support for branching next to nodes. Decay indices in italics, decay phylogram topology indicated by dashed lines. Parsimony scores: 384 steps / CI = 0.539 / RC = 0.310. (Double lines = topology not found in decay phylogram.)

one can formulate patterns of character evolution (Fig. 4, based on Puzzle tree Fig. 1) within / *Tigrinus-Polyporellus*. Starting from an unspecified common ancestor of both *Trametes* and /*Tigrinus-Polyporellus*, a two-sided interior loop subterminally located in the first major stem-loop of ITS2 (labeled P1) at the base of boxed element 1 disappeared somewhere basal to the included *Polyporellus* and *Lentinus* taxa (hypothetical event **a**). Event **b**, T/C and G/C point mutations for element 2 of *P. tricholoma*, allows for a structural synapomorphy. The enlargement of the terminal loop of P2 (element 3) appears to be related to a hypothetical insertion of an A in the coding DNA in the ancestor of *P. ciliatus* (event **c**). Event **d** appears to include several point mutations affecting the primary sequence within element 3 which cannot accurately be placed on a branch. The one structural element that seems consistent with the suggested phylogeny is the loss of the interior loop associated with event a, allow-

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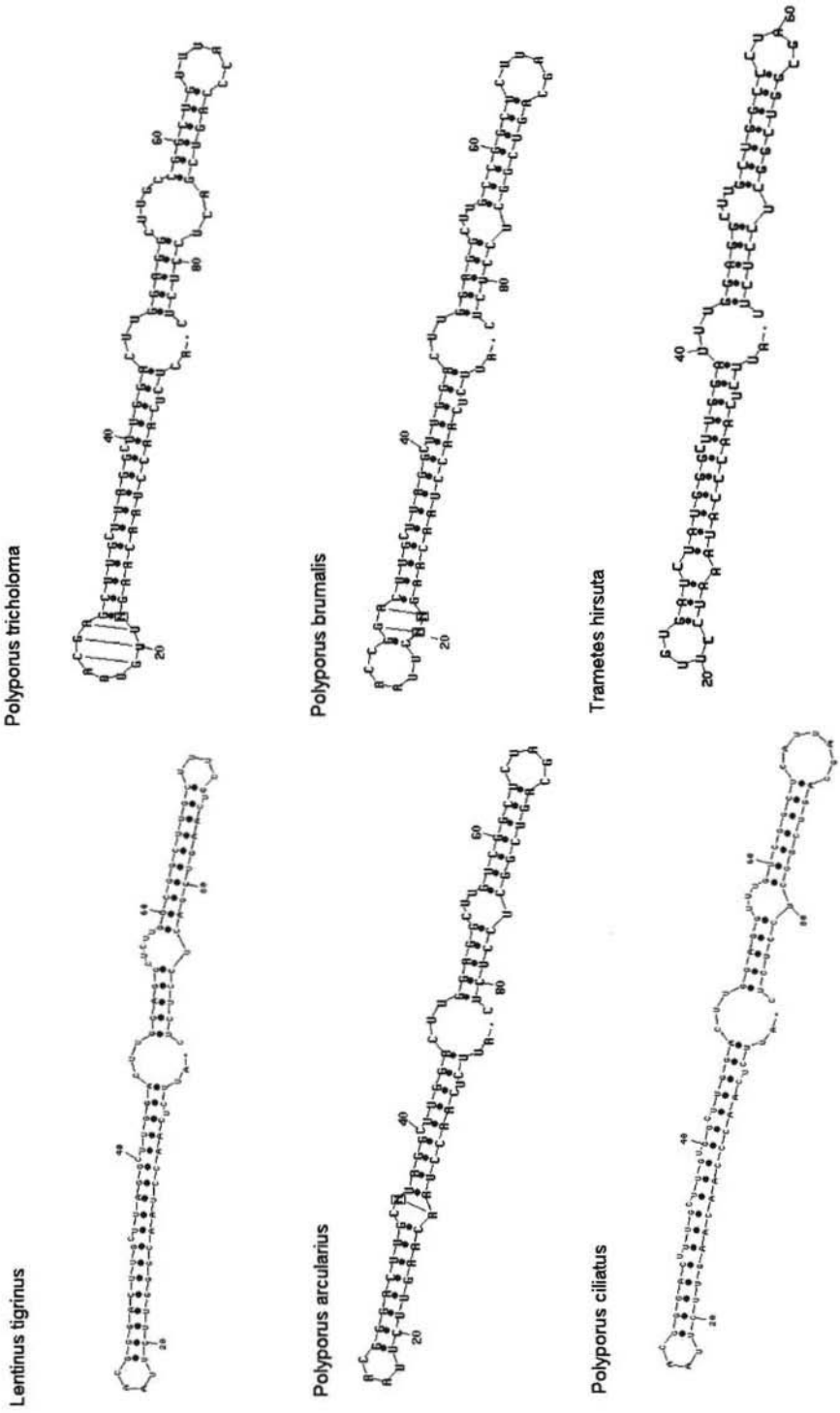

Fig. 3
Six secondary structures of the first two major pinloops (lefthand: P1, righthand: P2) of ITS2 rRNA transcripts as inferred by Mfold

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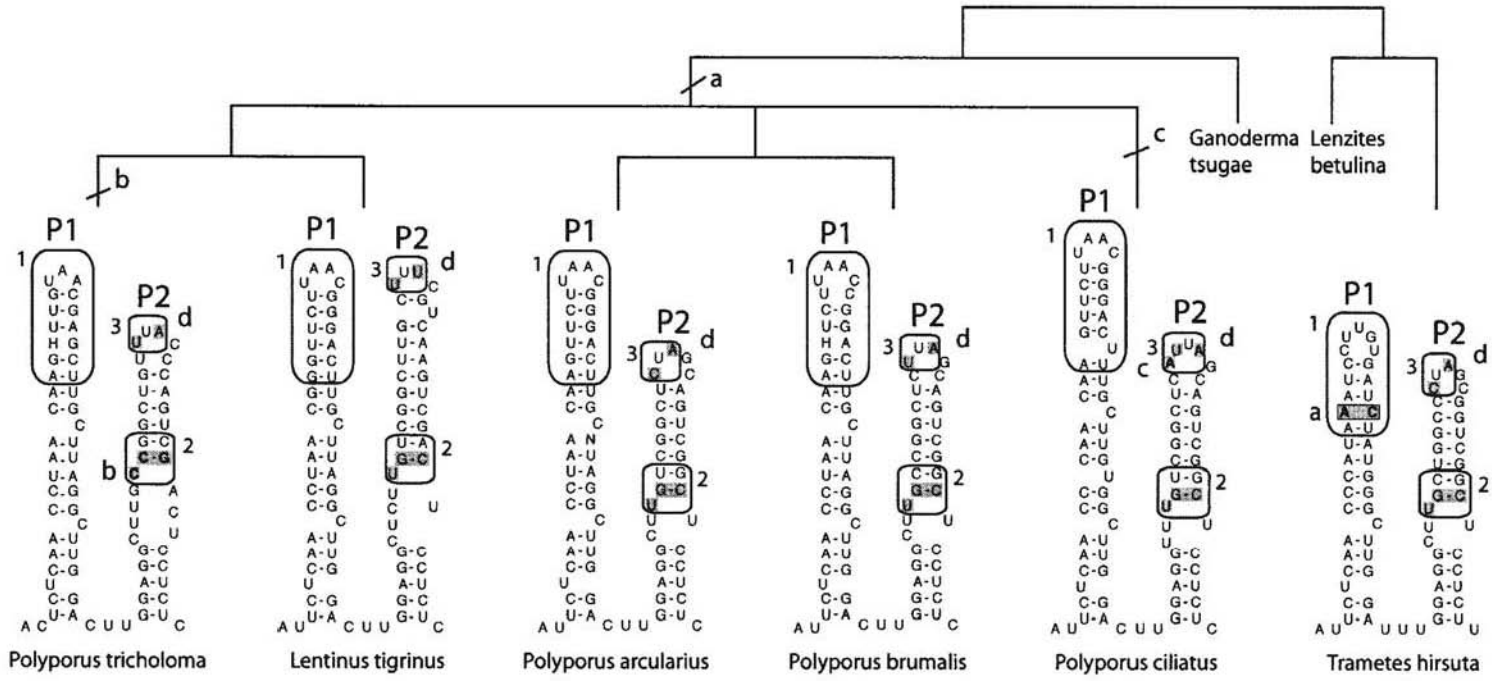

Fig. 4
Derived putative diagnostic structures of ITS2 rRNA (P1, P2) for six taxa of */Tigrinus-Polyporellus* and one outgroup (*Trametes*) with deduced hypothetical events based on a takeout (partial phylogeny) of Fig. 1. The three discussed example structure elements are labeled 1, 2, and 3 (boxed). Hypothesized events labeled on internodes, explained here at the DNA level. Event a = Loss of two-sided internal loop within element 1. Event b = potential switching of T versus C and G versus C in DNA coding for element 2 in *P. tricholoma*. Event c = insertion of an A enlarging the terminal loop in element 3 of *P. ciliatus*. Event d = point mutations in DNA encoding terminal loop in element 3, no defined internodal position of event. Nucleotides affected by these events highlighted in shaded boxes

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potheses contains the majority of included/core-*Polyporus* taxa: Puzzle: 80%, Bayesian: 100%, 92% parsimony jackknife support, decay index of 5. Publications based on results in KRÜGER (2002) are underway to address further problems of *Polyporus* evolution and systematics.

Descriptions

***Polyporus* ADANSON (Fam. Pl. 2: 10. 1763): FRIES, Syst. Mycol. 1: 341 (1821) emend. D.KRÜGER**

Genus accepted as circumscribed by NÚÑEZ & RYVARDEN (1995), but including stipitate, wood-decaying fungi with dimitic hyphal construction [generative and skeleto-binding hyphae, both can be inflated as in PEGLER (1983: 47)], lamelloid hymenophore, often ciliate cap margin. No decision is made about the concept of Lentinaceae JÜLICH 1981 vs. Polyporaceae CORDA 1839.

The first author (DK) herewith proposes the following new combinations of *Lentinus* subgen. *Lentinus* species:

***Polyporus phyllostipes* D.KRÜGER, nom. nov.**

≡ (replacing the priorable but unavailable nomenclatural synonym) *Agaricus crinitus* Linnaeus, Sp. Pl., ed. 2: 1644 (1763).
≡ *Lentinus crinitus* (L.) FR., Syst. Orb. Veg.: 77 (1825).
non Polyporus crinitus SPRENG., K. Svenska Vet.-Akad. Handl. 1820: 51 (1820).
non Agaricus crinitus BERTERO, K. Svenska Vet.-Akad. Handl. 1820: 20 (1820).
non Agaricus crinitus SCHWEIN., Schr. Naturforsch. Ges. Leipzig 1: 89 (1822).
≡ *Panus crinitus* (L.) SINGER, Lilloa 22: 275 (1951).

Etymology: referring to gills and stipe.

Justification

The need to circumvent creation of an illegitimate later homonym to *P. crinitus* SPRENG. leads to the proposal of a new species epithet.

Agaricus crinitus L. was adopted and hence sanctioned by FRIES (Syst. Mycol. 1: 175, 1821), and subsequently transferred to *Lentinus* in the index of a sanctioning volume (FRIES, Syst. Orb. Veg.: 77, 1825; FRIES, Index Syst. Mycol.: 107, 1832). This was under exclusion of 1) *Agaricus crinitis* BERTERO, K. Svenska Vet. Akad. Handl.: 20 (1820) = *Lentinus bertieri* (FR. 1821) FR. 1825, 2) *Agaricus crinitis* SCHWEIN., Schr. Naturforsch. Ges. Leipzig 1: 89 (1822) = *Lentinus strigosus* (SCHWEIN. 1822) FR. 1825, and 3) *Polyporus crinitis* SPRENG. [= *Boletus hydnoides* SW. 1806 *sensu* FRIES (*ut Polyporus hydnoides*), Syst. Mycol. 1: 362 (1821) ≡ *Hexagonia* (*ut Hexagona*) *hydnoides* (SW. 1806) M.FIDALGO 1968].

Following the arguments expressed in the CBS Aphyllophorales database (STALPERS 2004), *Polyporus crinitus* SPRENG. 1820 is a legitimate name sanctioned by use in FRIES in Syst. Mycol. 1 (1821) (GREUTER et al. 2000: Art. 13.1d), as is *Agaricus crinitus* L. Both names are protected against any potential earlier homonyms and synonyms (GREUTER et al. 2000: Art. 15.1). For the purpose of the nomenclatural process described here, *Polyporus crinitus* SPRENG. is protected against any new combination. *Polyporus crinitus* based on a different type, such as *Agaricus crinitus* L. In other words, according to Article 15.2. (GREUTER et al. 2000), the epithet *crinitus* is unavailable for use in *Polyporus* because of the sanctioned name *P. crinitus* SPRENG. We assume that *P. crinitus* SPRENG. and *A. crinitus* L. are different from each other, hence *Polyporus crinitus* SPRENG. is not priorable to a potential *Polyporus* combination with *A. crinitus* L. as basionym. The epithets *crinitus* in *P. crinitus* and *A. crinitus* will still be available for use in other combinations.

***Polyporus gerdai* D.KRÜGER, nom. nov.**

≡ (replacing the priorable but unavailable nomenclatural synonym) *Agaricus tigrinus* BULLIARD, Hist. Champ. France: Pl. 70 (1781).
≡ *Lentinus tigrinus* (BULL.) FR., Syst. Orb. Veg.: 78 (1825). *non Agaricus tigrinus* SCHAEFFER, Fung. Bav. Palat. Nasc. Icones 1: Tab. 89 (1762) ≡ *Tricholoma tigrinum* (SCHAEFFER) FR., Hyménomycètes: 118 (1874) ≡ *Pleurotus tigrinus* (BULL.) KÜHNER, Bull. Mensuel Société Linnéenne Lyon, Numéro Spécial 49: 895 (1980).
non Polyporus tigrinus E.ROSTRUP, Bot. Tidsskr. 24: 359 (1902) ≡ *Polystictus tigrinus* (E.ROSTR.) SACC. & D.SACC. (Syll. Fung. 17: 128; 1905) = current name *Microporellus obovatus* (JUNGHUHN 1838, Verh. Batav. Genootsch. 17 – Praemissa in Floram Cryptogamicam Javae Insulae:

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65) RYVARDEN 1972, Norw. J. Bot. 19: 232 – see RYVARDEN & JOHANSEN, A Preliminary Polypore Flora of East Africa: 427 (1980) for synonymy.

non Polyporus tigrinus PERS., Mycol. Eur. 2: 54 (1825) = *Polyporus squamosus* [HUDSON, Fl. Anglica ed. 2: 626 (1778) FR., Syst. Mycol.: 343, 1821], see FRIES (Index Syst. Mycol.: 148, 1832; Epicris. Syst. Mycol. 1: 73, 1838) = older name *Polyporus juglandis* (J. SCHAEFFER, Icones 2: Tab. 101, Fig. 102, Fung. Bav. Palat. Nasc. Icones 4: 75; 1774; *ut iuglandis*) PERS., Mycol. Eur. 2: 38 (1825), see FRIES (Epicris. Syst. Mycol. 1: 73, 1838) = *Boletus cellulosus* LIGHTFOOT, Fl. Scot.: 1032 (1778) *non Boletus cellulosus* MÜLLER, Fl. Danica: Tab. 716-1 (1777).

≡ *Omphalia tigrina* (BULL.) GRAY, Natural Arrangement of British Plants (London) 1: 613 (1821).

≡ *Pocillaria tigrina* (BULL.) KUNTZE, Revisio Gen. Plant. Vol. 2: 866 (1891).

≡ *Lentodium tigrinum* (BULL.) EARLE, Bull. New York Bot. Gard. 5: 434 (1909).

≡ *Panus tigrinus* (BULL.) SINGER, Lilloa 22: 275 (1951).

Justification

The avoidance of an illegitimate later homonym leads to the proposal of a new species epithet. It is in honor of the major supporter of DK's life as a graduate student.

Lentinus tigrinus (BULL.) FR., Syst. Orb. Veg. (Lundae) 1: 78 (1825), was based on *Agaricus tigrinus* BULL., 1781, and appears in a part of a sanctioning volume, FRIES' Index Syst. Mycol. (1832). PERSOON'S name *Polyporus tigrinus* is considered legitimate and FRIES' synonymy (Epicris. Syst. Mycol. 1: 73) is accepted, and hence the name *P. tigrinus* E.ROSTR. is illegitimate, as would be any new combination with a different taxonomic type. Thus, the epithet *tigrinus* as in *Agaricus tigrinus* BULL. is unavailable in *Polyporus*.

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