Notes on the molecular phylogeny of the 'Polyporellus' group within *Polyporus*: identity of collections from Canada and Ecuador, and relationships with *Lentinus*

D. Krüger[1], K. W. Hughes[2] & R. H. Petersen[2]

[1] Helmholtz-Zentrum für Umweltforschung GmbH – UFZ, Department of Soil Ecology, Theodor-Lieser-Str. 4, D-06120 Halle (Saale), Germany.
Tel.: +49-345-558-5425, e-mail: dirk.krueger@ufz.de.
[2] University of Tennessee, Department of Ecology & Evolutionary Biology, 1416 Circle Dr, Knoxville, TN 37996-1610, USA.

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The basidiomycetous polypore genus *Polyporus s. str.* can be subdivided into several infrageneric groups, one of which is 'Polyporellus'. This publication mainly discusses four widespread species within the 'Polyporellus' group: *Polyporus arcularius, P. brumalis, P. ciliatus*, and *P. tricholoma*. As morphological characters may be ambiguous in identifying similar specimens in this group, this paper expands on an earlier study determining phylogenetic species entities in 'Polyporellus'. A total of 35 additional ITS rDNA (ITS I – 5.8S – ITS II) sequences was inserted into the phylogenetic analysis. The presence of *P. ciliatus* or *P. tricholoma* in temperate North America could not be confirmed by morphology or molecular data. Spore ranges for the three species *P. arcularius* / *P. brumalis* / *P. ciliatus* were found to overlap. Our study indicates a closer relationship of the gilled *Lentinus tigrinus* (= *Polyporus gerdai*) to *P. tricholoma* than to any other of the 'Polyporellus' species investigated.

Keywords: Polypores, Basidiomycota, systematics.

Polypores are a morphological type of mushrooms in reference to usually tough basidiomata with poroid hymenophore, mostly growing on woody substrates as saprobes or pathogens. In modern delimitation, the basidiomycete genus *Polyporus* Adanson 1763: Fr. 1821 emend. D. Krüger in Krüger & Gargas (2004) is characterized by usually forming short-lived basidiomata with stipes, and causing white-rot in wood. Within this genus is an infrageneric group 'Polyporellus', as circumscribed by Nuñez & Ryvarden (1995). We continue to use informal group names following Nuñez & Ryvarden (1995), and not the genus *Polyporellus* established by Karsten (1879) that was never formally described as a subgeneric taxon of *Polyporus*. The phylogeny of the entire genus *Polyporus* and related

genera remains unresolved as of submission of this manuscript (Krüger 2002, Krüger & Gargas 2004, Lutzoni *et al*. 2004).

'Polyporellus' contains three extratropically distributed species frequently encountered across Europe: *Polyporus arcularius* Batsch: Fr., *P. brumalis* Pers.: Fr., and *P. ciliatus* Fr. whereas *Polyporus tricholoma* Mont. is found in tropical and subtropical areas. The phenotypic variability of basidiomata of these species makes identification difficult (Jahn 1969, Krüger 2002: 28). Characters such as hirsute appearance (Bremer 1986, Kreisel 1963) or later appearance of a dark stipe cuticle (Nuñez & Ryvarden 1995: 16) may lead to confusion with the infrageneric group 'Melanopus'. We previously confirmed tetrapolar mating systems for species within 'Polyporellus', confirmed mating barriers between species and assessed phylogenetic relationships between species of this group (Krüger *et al*. 2003, 2004). *Polyporus arcularius, P. brumalis*, and *P. ciliatus*, all originally described from Europe, have also been reported in South America (Popoff & Wright 1998: *P. arcularius* and *P. ciliatus*; Nuñez & Ryvarden 1995). Borges da Silveira & Wright (2002) and Borges da Silveira *et al*. (2003) reported isozyme and mating data in South American 'Polyporellus' but did not include European material. In the past, the name *P. arcularius* was wrongly applied to *P. brumalis*, and *P. brumalis* to *P. ciliatus* (Kreisel 1963).

Here, we address the following problems: (1) What is the identity of several Canadian collections[1] previously identified as *Polyporus ciliatus*? Nuñez and Ryvarden (1995) disputed the existence of *P. ciliatus* in North America, but Hoffmann (1978) reported that a DAOM (Department of Agriculture, Ottawa, Mycology) culture available to us and initially received by him under the name *P. brumalis* yielded monokaryons compatible with *P. ciliatus*. Several additional Canadian collections from DAOM identified as *P. ciliatus* were also examined. (2) Is there a novel species in Canada? Widepored basidiomata of a tentative new species of 'Polyporellus', called *'Polyporus longoporus'* by Mr. Serge Audet, were examined using molecular tools. (3) Is *Polyporus tricholoma*, in the Americas hitherto unknown north of the Caribbean, extant in Canada? A Canadian collection of 'Polyporellus' phenotypically resembling *Polyporus tricholoma* and *P. ciliatus* was examined with molecular tools. (4) Is *P. ciliatus* extant in South America? A collection from Ecuador morphologically resembling *P. ciliatus* as known from Europe was included for molecular identification.

'Polyporellus' as circumscribed by Nuñez & Ryvarden (1995) is the group within *Polyporus* that is closest to *Lentinus* Fr. 1825

[1] Collections are meant to be synonymous to the term specimen or a number of specimens from the same locality.

(Krüger 2002: 28). Based on phylogenetic data and deduced secondary structure of ribosomal RNA *Polyporus* was already emended to include *Lentinus tigrinus* under the new name *Polyporus gerdai* D. Krüger (Krüger & Gargas 2004). The phylogenetic analyses presented here additionally re-examine (5) which species of the poroid 'Polyporellus' taxa is closest to *Lentinus tigrinus* (Bull.: Fr.) Fr., and (6) determine if *P. tricholoma* is an aggregate of morphologically similar, tropical populations of the other 'Polyporellus' species as suggested by Corner (1984: 50). Overall, the analyses here shed light on the geographic range of species in a widely distributed genus which appears to be a non-monophyletic remnant of once much wider taxonomic delimitation. To draw attention to the traditional, morphologically based taxonomy, the name *Lentinus* is used in this paper, which is not a retraction from previous conclusions. Upcoming results from other researchers are expected to lead to an attempt to preserve the two genera, *Lentinus* and *Polyporus*, through efforts of typification and conservation (Scott Redhead, pers. communication).

Materials and Methods

The collections investigated in this study are listed in Table 1. Maintenance of cultures and collections followed procedures described by Krüger *et al.* (2003). In addition, we have calculated spore ranges for collections identified as *Polyporus arcularius, P. brumalis*, or *P. ciliatus* within this study and/or Krüger *et al.* (2003). Average spore sizes (length, width, Q = length divided by width), minimum and maximum, and standard deviations were calculated with Microsoft Excel. Only collections identified to species by at least two criteria (mating studies, morphology, or sequence data) here and in Krüger *et al.* (2003) were used for calculation of these ranges.

Extraction of total nucleic acids followed methods described by Krüger *et al.* (2003) or Krüger (2002: 19). PCR of the ribosomal ITS – region (internal transcribed spacer: ITS I – 5.8S – ITS II nuclear rDNA) and sequencing were as described by Krüger *et al.* (2003), or Krüger (2002: 77).

Sequence correction and alignment followed procedures from Krüger *et al.* (2003). MEGA2 files were created with the program ForCon v. 1.0 (Raes & van de Peer 2002). Modeltest v. 3.06 (Posada and Crandall 1998), MrModeltest (Nylander 2004), and PAUP* v. 4.0b10 (Swofford 2001) were used for suitable models of evolution. Minimum-evolution (ME) distance analysis was performed in MEGA2 (Kumar *et al.* 2001), with 100 bootstrap resamplings, closest-neighbor-interchange swapping level 2, pairwise deletion of gaps, Tamura-Nei model with gamma shape parameter 0.59 esti-

mated by Modeltest. Treefinder (Jobb *et al.* 2004), and MrBayes 3 (Ronquist and Huelsenbeck 2003) were used for maximum-likelihood (ML) and Bayesian (BI) analyses under the Modeltest parameters. In Treefinder, 100 ML bootstrap pseudoreplicates were performed. Bayesian analysis was done in MrBayes3 under the following parameters: ngen=1000000 samplefreq=100 burnin=10000 contype=allcompat ngammacat=6 mcmcp and 6 MCMC chains.

DAMBE v. 4.0.75 (Xia & Xie 2001) was used to prune identical sequences (and hence represent identical sequences only once), and data were further reduced in the MEGA2 data explorer by removing parsimony-uninformative and gap characters. Resulting shortened data were analysed in maximum-parsimony (MP) in DAMBE (1000 jackknife pseudoreplicates).

A ME ratchet (Nixon 1999, Sikes & Lewis 2001, Vos 2003) was implemented in PAUP*, using 100 each of 25%, 50%, and 75% deletion faststep jackknife pseudoreplicates each for multiple hill-climbing starting points for the first of 100 iterations (hs status=no nrep=1 swap=TBR start=current steepest=yes). The ME ratchet is an application of the ratchet reweighting and search principle from MP and ML into ME. Subsequently, 3 best trees from all 99 following iterations performed with a neighbor-joining starter tree, nearest-neighbor-interchange branch swapping and 500 seconds time limit were saved. Resulting PAUP* ME trees form the jackknife and the ratchet were compared using the SH-RELL test (Kishino *et al.* 1990, Shimodaira & Hasegawa 1999) in ML under the model suggested by Modeltest, as well as compared with constrainted-analysis (Table 2) ME trees.

Trees were processed in TreeView v. 1.6.1. (Page 1996) and Mesquite (Maddison & Maddison 2003) and imported into graphics and text programs. The constraints were created using TreeView.

Described pruning of data to the most informational content, use of ME faststep and ratchet methods in PAUP*, and use of the faster DAMBE MP and MEGA2 ME algorithms were needed due to the high level of positional instability within the major clades, which results in large amounts of equiparsimonious trees in MP analysis. Conversely, Treefinder is among the fastest programs for ML analysis.

Results

The aligned ITS region rDNA sequences contained 660 nucleotide sites including gaps [available from the public databases under accession number ALIGN_000375 (ftp://ftp.ebi.ac.uk/pub/databases/embl/align/)]. A total of 166 sites were variable. Of the 99 parsimony-informative sites 64 sites were retained by MEGA2. All trees

Tab. 1. – Fungal specimens and cultures investigated.

Identifying number	Fungal species and authors	Country of origin	Collectors and identifiers	GenBank number
FB10672 hseq	*Lentinus tigrinus* (Bull.:Fr.) Fr.	Austria: Niederösterreich	H. Voglmayr & I. Krisai-Greilhuber	AF516517
FB9770 [from LE(BIN)0861]	*Lentinus tigrinus* (Bull.:Fr.) Fr.	Mongolia		SBI 5: AF516518
FB8937 (TENN55557) hseq	*Lentinus tigrinus* (Bull.:Fr.) Fr.	Russia: Krasnodar Region	RHP *et al.*	AF516519
FB9093 (TENN54918) hseq	*Lentinus tigrinus* (Bull.:Fr.) Fr.	USA: Louisiana	RHP	AF516520
FB10832 (TENN58433) hseq	Lentinus tigrinus (Bull.:Fr.) Fr.	USA: Texas	D. Lewis	AF516521
DSH92.144 (Hibbett & Donoghue 1995) DNA extraction only	*P. arcularius* Batsch: Fr.		D. Hibbett	AB070860
VT959 (Hibbett & Vilgalys 1993) DNA extraction only	*P. arcularius* Batsch: Fr.			AB070862
FB10299 (TENN58370)	*P. arcularius* Batsch: Fr.	Austria: Niederösterreich	H. Voglmayr	SBI 2: AB070865, SBI 4: AB070866
CBS 222.91= RGT830522/01	*P. arcularius* Batsch: Fr.	Canada: Ontario	G. Thorn	AF516522
CBS 223.91 = RGT830522/01	*P. arcularius* Batsch: Fr.	Canada: Ontario	G. Thorn	AB070858
CBS 224.91= RGT830522/01	*P. arcularius* Batsch: Fr.	Canada: Ontario	G. Thorn	AF516523
FB4124 (TENN50834)	*P. arcularius* Batsch: Fr.	China: Guizhou	RHP	SBI 1: AB070863, SBI 2: AB070864
FB7883 (TENN53747)	*P. arcularius* Batsch: Fr.	Costa Rica: San Jose	RHP	SBI 2: AF516524
SBUG-M1244 (fruited for obtaining specimen/spores) (TENN58529, 58569, 58588)	*P. arcularius* Batsch: Fr.	Germany: Mecklenburg-Vorpommern	R. Bütow	AB070861
DAOM94067 = PRE52	*P. arcularius* Batsch: Fr.	South Africa	P. Talbot	AB070859
FB10929 (TENN58412)	*P. arcularius* Batsch: Fr.	USA: Tennessee	H. Voglmayr	SBI 1: AB070867, SBI 2: AB070868

Tab. 1. – continued.

Identifying number	Fungal species and authors	Country of origin	Collectors and identifiers	GenBank number
DAOM155905 hseq, also dikaryon culture sequenced	*P. brumalis* Pers.: Fr.	Canada: Ontario	J. Ammirati & J. Ginns (as P. ciliatus)	AF516525
DAOM72515 hseq, also dikaryon culture sequenced	*P. brumalis* Pers.: Fr.	Canada: Ontario	M. Pantidou [revised by J. Ginns 1980 as *P. ciliatus* after Hoffmann (1978)]	AF516526
DSMZ-H5 (from DAOM72515) [culture 5 of Hoffmann (1978)]	*P. brumalis* Pers.: Fr.	Canada: Ontario	M. Pantidou	AF516527
Audet's *P.* cf. *tricholoma s.n.* hseq	*P. brumalis* Pers.: Fr.	Canada: Quebec	Received from S. Audet	AF516528
DAOM31983 culture sequenced and hseq	*P. brumalis* Pers.: Fr.	Canada: Quebec	J. W. Groves	AB070869
DSMZ-H17 (from DAOM31983) [culture 17 of Hoffmann (1978)]	*P. brumalis* Pers.: Fr.	Canada: Quebec	J. W. Groves	AB070870
FB10169 (TENN57700)	*P. brumalis* Pers.: Fr.	Denmark: Roskilde Amt	H. Knudsen	SBI 1: AB070872, SBI 3: AB070873
FB10178 (TENN57708)	*P. brumalis* Pers.: Fr.	Denmark: Storstrøms Amt	RHP	AF516529
DK0083 (TENN57748) hseq	*P. brumalis* Pers.: Fr.	Germany: Mecklenburg-Vorpommern	DK	AF516530
FB10147 (TENN57678)	*P. brumalis* Pers.: Fr.	Germany: Mecklenburg-Vorpommern	DK	AF516531
FB10908 (TENN58391)	*P. brumalis* Pers.: Fr.	Germany: Mecklenburg-Vorpommern	DK	SBI 4: AB070876, SBI 5: AB070877
DSMZ-H21 = FRI285 [culture 21 of Hoffmann (1978)]	*P. brumalis* Pers.: Fr.	India		AF516532

Tab. 1. – continued.

Identifying number	Fungal species and authors	Country of origin	Collectors and identifiers	GenBank number
NG980201/3 (SNU) hseq	*P. brumalis* Pers.: Fr.	Korea	Y.-W. Lim (as *P. melanopus*)	AF516533
O92393 = Ryv31528 hseq	*P. brumalis* Pers.: Fr.	Norway: Finnmark	L. Ryvarden	AF516534
O92301 hseq	*P. brumalis* Pers.: Fr.	Norway: Telemark	A.-E. Torkelsen	AB070871
DSMZ-H20 = FPRL 174a [culture 20 of Hoffmann (1978)]	*P. brumalis* Pers.: Fr.	UK: England		AF516535
DSMZ-H18 [from ATCC9385 = Overholts 24800 [culture 18 of Hoffmann (1978)]	*P. brumalis* Pers.: Fr.		L. O. Overholts, deposited by J. W. Sinden	AF516536
FB10665 (TENN58381)	*P. brumalis* Pers.: Fr.	USA: Tennessee	K. McFarland	SBI 1: AB070874, SBI 2: AB070875
FB10167 (TENN57698)	*P. ciliatus* Fr.	Denmark: Roskilde Amt	H. Knudsen & RHP	SBI 9: AB070882, SBI 10: AB070883
FB7480 (fruited for obtaining spores to make up for lost monokaryons) (TENN53639, 58441, 58823)	*P. ciliatus* Fr.	Finland: Etelä-Häme	RHP	SBI 2: AB070880, SBI 3: AB070881
DSMZ-H24 [culture 24 of Hoffmann (1978)]	*P. ciliatus* Fr.	France		AF516537
DSMZ-H25 [culture 25 of Hoffmann (1978)]	*P. ciliatus* Fr.	France		AF518752 (short fragment, not used in phylogeny)
FB7257 (TENN53619)	*P. ciliatus* Fr.	Sweden: Uppland	RHP	SBI 2: AB070878, SBI 7: AB070879
O: Bernicchia 5647 hseq	*P. corylinus* Mauri	Italy: Rome	Cherubini, det. A. Bernicchia	AF516538

Tab. 1. – continued.

Identifying number	Fungal species and authors	Country of origin	Collectors and identifiers	GenBank number
Audet's *'P. longoporus' s.n.* hseq	*P. sp.* (labeled as *P. longoporus nom. prov.* by S. Audet)	Canada: Ontario	Y. Lamoureux, received from S. Audet	AF516539
DSMZ-H27 (from Forest Products Lab Melbourne) [culture 27 *'P. brumalis* ?' of Hoffmann (1978)]	*P. tricholoma* Mont. complex	Australia: Queensland		AF516540
FB10240 (TENN57563)	*P. tricholoma* Mont. complex	Costa Rica: Heredia	RHP	SBI 1: AB070885, SBI 2: AF516543
FB10241 (TENN57564)	*P. tricholoma* Mont. complex	Costa Rica: Heredia	RHP	SBI 9: AF516541, SBI 10: AF516542
FB10258 (TENN57581)	*P. tricholoma* Mont. complex	Costa Rica: Puntarenas	RHP	SBI 2: AF516546
FB10920 (TENN58403)	*P. tricholoma* Mont. complex	Costa Rica: Puntarenas	E. Navarro	SBI 1: AF516547
FB9909 (TENN56537)	*P. tricholoma* Mont. complex	Costa Rica: Puntarenas	RHP	Dikaryon: AJ132939, SBI 2: AF516544
AAU44971 = TENN59383 hseq	*P. tricholoma* Mont. complex	Ecuador: Napo	T. Læssøe (as *P.* cf. *ciliatus*)	AF516548
FB3870 (TENN55844)	*P. tricholoma* Mont. complex	Mexico: Chiapas	RHP	Dikaryon: AJ132940; SBI 2: AB070884 = AF516549
TENN18091 hseq	*P. tricholoma* Mont. complex	Mexico: Chiapas	A. J. Sharpe, *det.* J. A. Stevenson	AF516550
FB4362 (TENN50439)	*P. tricholoma* Mont. complex	Mexico: Tabasco	RHP	SBI 9: AF516551
FB9568 (TENN56481)	*P. tricholoma* Mont. complex	USA: Puerto Rico	RHP	Dikaryon: AJ132942, SBI 1: AB070887 = AF516552

Tab. 1. – continued.

Identifying number	Fungal species and authors	Country of origin	Collectors and identifiers	GenBank number
FB9579 (TENN56491); EPITYPE as discussed in Krüger *et al.* (2004).	*P. tricholoma* Mont. complex	USA: Puerto Rico	KWH	SBI 1: AF516553, SBI 2: AF516554
FB9591 (TENN56503)	*P. tricholoma* Mont. complex	USA: Puerto Rico	RHP	Dikaryon: AJ132941, SBI 1: AB070886 = AF516555
FB10198 (TENN57728)	*Trametes hirsuta* (Wulf.:Fr.) Pilát	Germany: Mecklenburg-Vorpommern	DK	SBI 5: AF516556

DK = Dirk Krüger. KWH = Karen W. Hughes. RHP = Ronald H. Petersen.

TENN = Univ. of Tennessee Fungal Herbarium; herbarium acronyms (also AAU, O, SNU) from Holmgren *et al.* (1981). Culture collections: ATCC = American Type Culture Collection, DAOM = Department of Agriculture, Ottawa, Mycology, DSMZ = Cultures from Hoffmann (1978) kept at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Braunschweig (original specimens not seen), FPRL (now NCWRF) = United Kingdom Forest Products Research Laboratory, FRI = Forest Research Institute Dehradun (Indian Council of Forestry Research & Education), LE(BIN) = Culture Collection of Basidiomycetes of the Komarov Botanical Institute St. Peterburg, PRE (now PPRI) = National Botanical Institute Pretoria, SBUG-M = Sektion Biologie der Universität Greifswald – Myzelpilze.

FB = Field book number used in TENN (acronym FB omitted in phylogenetic trees) = usually identical to CulTENN (University of Tennessee Fungal Culture Collection) number but distinct from TENN number. hseq = Sequence from herbarium specimen (all other sequences are from cultures). All AB GenBank accession numbers are from Krüger *et al.* (2003). All TENN and O collections have been seen by DK and compared with recent taxonomic literature.

s.n. = no number. SBI = Single basidiospore isolate.

Tab. 2. – Statistical values of ME (minimum-evolution) trees compared with ML (maximum-likelihood) test methods.

Hypothesis	ME score	Range – *ln* L GTR	*P**	Supported by
Unconstrained ME ratchet	0.53614 (tree 232) – 0.77836 (tree 504)	2449.62783 – 3048.6247 incl. best tree overall 268	0.05–1.00	
Constraint 1				
P. tricholoma (incl. AAU44971 *'P. cf. ciliatus'*) is an unresolved clade sister to *Lentinus tigrinus*.	0.53505	2465.07635	0.995 not significantly different	MP (Fig. 1), ML, BI (Fig. 2), ME (Fig. 3)
Constraint 2				
Lentinus tigrinus sister to 'Polyporellus' (all pore-bearing *Polyporus* taxa in the dataset), all other topology unresolved.	0.55907	2469.76423	0.981 not significantly different	MP (Fig. 1), ML, BI (Fig. 2), ME (Fig. 3)
Constraint 3				
Bernicchia 5647 is phylogenetically conspecific ('concladic') with *P. ciliatus* as previous preliminary analyses found it nested within *P. ciliatus*.	0.54621	2470.57444	0.976 not significantly different	Maybe (because basal position): MP (Fig. 1), ML, BI (Fig. 2), ME (Fig. 3)
Constraint 4				
Audet's *'P. longoporus'* is concladic with *P. ciliatus*, as in preliminary analyses it appeared nested within or basal to it.	0.55420	2486.70023	0.842 not significantly different	Maybe (because basal position): MP (Fig. 1), ML, BI (Fig. 2), ME (Fig. 3)

Tab. 2. – continued.

Hypothesis	ME score	Range – *ln* L GTR	*P**	Supported by
Constraint 5				
Audet's *'P. longoporus'* is concladic with *P. arcularius* because the elongated pores indicate morphological similarity to the latter.	0.54942; note: these trees result in *'P. longoporus'* in a trichotomy between *P. arcularius* and *P. ciliatus* + *P. brumalis*	2474.82125	0.955 not significantly different	not found
Constraint 6				
Audet's *'P.* cf. *tricholoma'* is conspecific with *P. ciliatus*. It was deemed too far away from *P. tricholoma* clades in preliminary analyses to be considered as related to *P. tricholoma*.	0.56945	2523.47848	0.393 different	not found
Constraint 7				
AAU44971 *'P.* cf. *ciliatus'* is conspecific with *P. ciliatus* based on its morphological similarity.	0.60215	2546.06439	0.228 different	not found

* Approximate probability of getting a more extreme test statistic under the null hypothesis of no difference between two trees (two-tailed t-test).

depicted were rooted with *Trametes hirsuta* (Wulf.: Fr.) Pilát. The model of evolution for the 660 nucleotide data obtained from Modeltest was GTR+G+I (Lset Base=(0.2203 0.2436 0.2349) Nst=6 Rmat=(1.1195 3.5278 3.4817 0.8250 3.5278) Rates=gamma Shape= 0.5971 Pinvar=0.4265).

Results of the DAMBE MP analysis are given in Fig. 1. Only jackknife values above 50 % (500) are indicated. Within the major clades, support is low and the position of the tip branches is variable, albeit within *P. tricholoma*, high support values are also encountered.

The bootstrap consensus from the ML analysis in Treefinder determined the tree topology as shown in Fig. 2. As in MP, the *P. tricholoma* aggregate is the best-resolved part of the tree, while the other major clades show little internal resolution. BI results are congruent with the high support values given for the major clades in Fig. 2.

All of MP, ME (e.g. Fig. 3, a simple ME analysis), ML, and Bayesian analyses support a closer relationship of *Lentinus tigrinus* to the *P. tricholoma* aggregate than to the three major temperate taxa *P. arcularius, P. brumalis,* and *P. ciliatus*. To further evaluate statistically the reliability of this hypothesis and others alluded to above, we created topological constraints with unaffected clades being collapsed to polytomies.

A total of 620 trees were evaluated after the unconstrained ME ratchet and constrained ME analyses. The best tree selected by ML methods was an unconstrained tree found during the ME ratchet. Table 2 summarizes the ML test results and indices of compared trees. Constraints 1 and 2 forcing *Lentinus tigrinus* to be the sister clade to all *Polyporus tricholoma* or even all 'Polyporellus' collections yield statistically sound phylogenetic trees not significantly different from the best of unconstrained trees. Constraint 3 forced *P. corylinus* to be 'concladic' with *P. ciliatus*, which was found to be statistically sound and is the result in all unconstrained analyses. With a lesser P value, *'P. longoporus'* can be supported as concladic to *P. ciliatus* (Constraint 4), while the alternative Constraint 5 with *'P. longoporus'* to be concladic with *P. arcularius* is more supported. In all unconstrained analyses, this collection appears basal to *P. ciliatus,* with *P. corylinus* the next relative towards the *P. ciliatus* clade tip. A low P value was found on analysis with Constraint 6, suggesting Canadian *P.* cf. *tricholoma'* is unlikely close to *P. ciliatus*. Unconstrained analyses always position this collection in *P. brumalis*. Constraint 7 (Ecuadorian AAU44971 *'P.* cf. *ciliatus'*) received even lesser probability of actually supporting conspecifity with *P. ciliatus*. All of the unconstrained analyses under any of the optimality criteria actually placed this collection into the *P. tricholoma* complex.

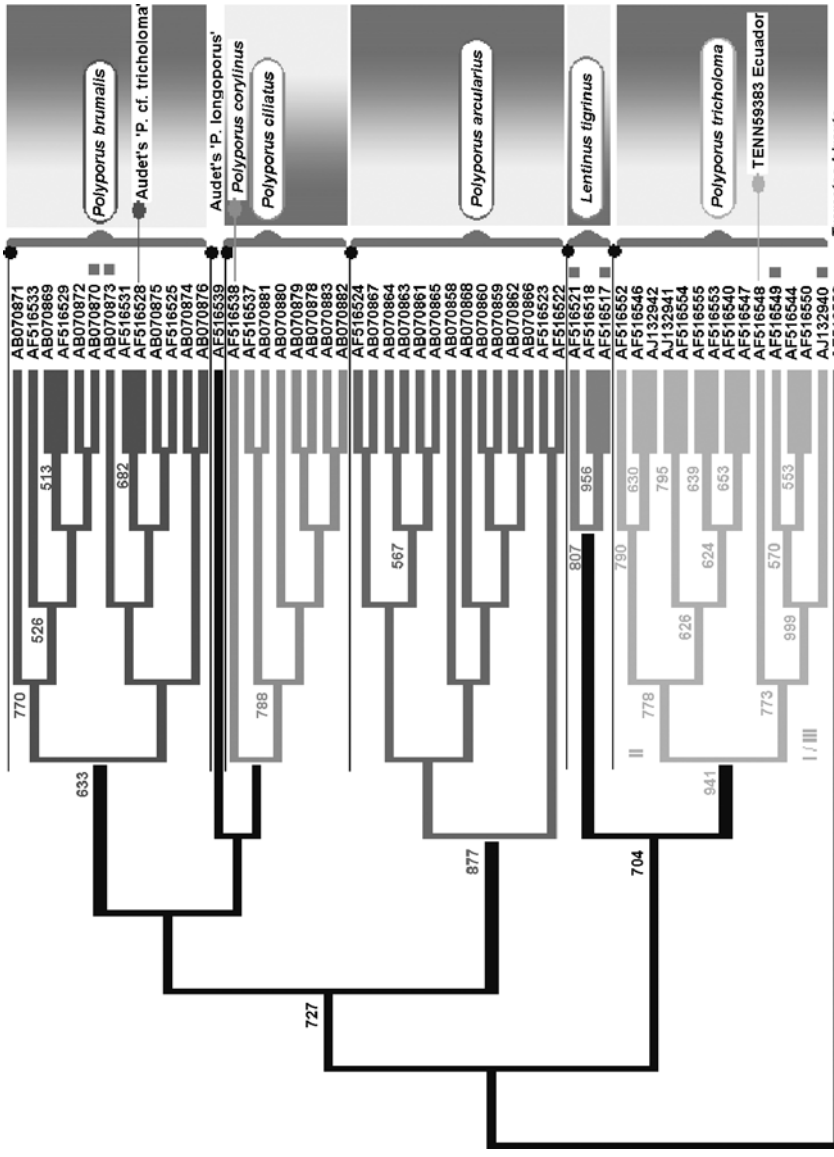


Fig. 1. DAMBE MP (maximum-parsimony) dendrogram rooted with *Trametes hirsuta,* Mesquite style. 1000 jackknife pseudoreplicates, only values above 500 indicated. Collapsed tips mark sequences with identical parsimony-informative characters.

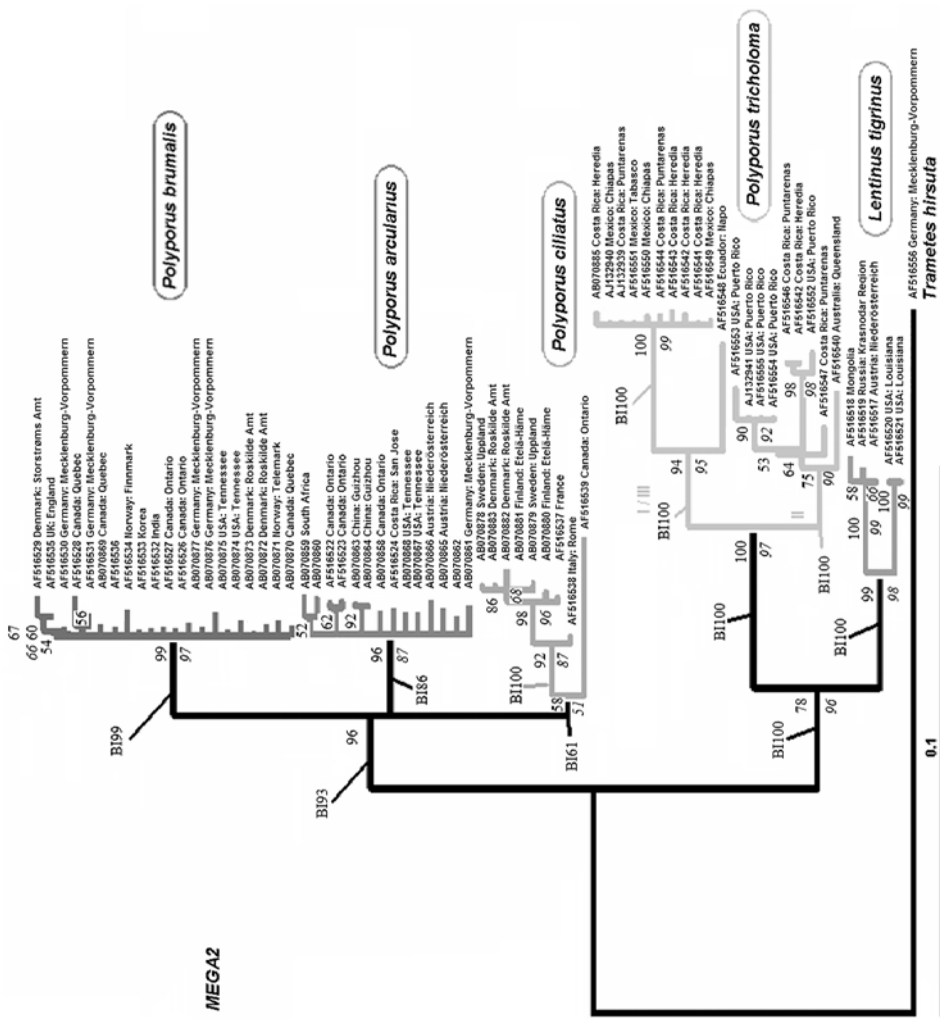


Fig. 2. Treefinder ML phylogram, rooted with *Trametes hirsuta*, superimposed MEGA2 ME bootstrap support values (100 pseudoreplicates, minimum 50% support) and Bayesian (BI) support for major clades.

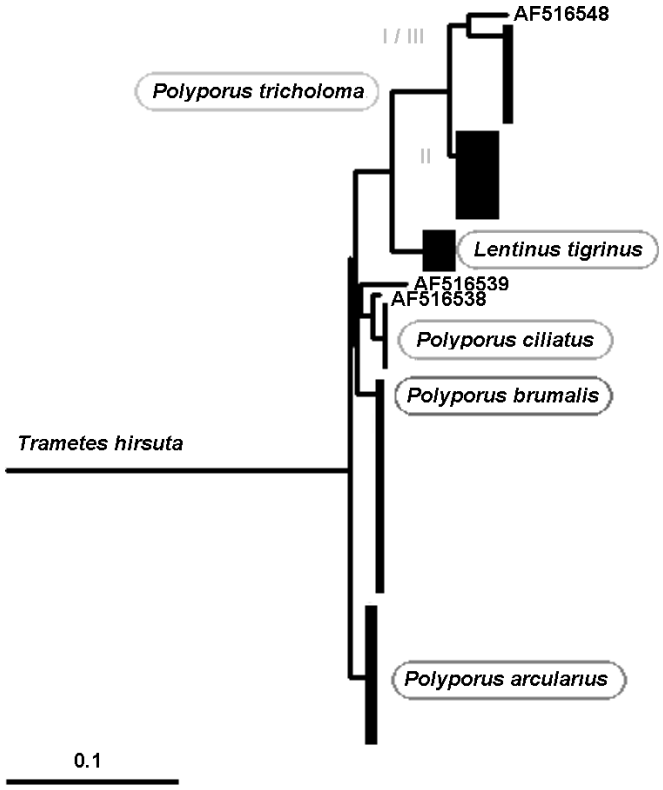


Fig. 3. PAUP* simple heuristic ME analysis. Major clades collapsed, rooted with *Trametes hirsuta.*

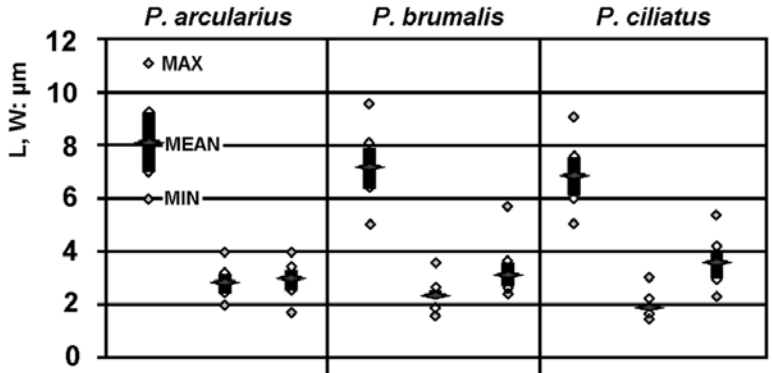
Regarding the search strategy we note that the ME ratchet resulted in trees becoming less and less robust under ML criteria across the progressing iterations, while the ME score across the search remained unstable.

Morphological notes

Spore ranges for the *Polyporus tricholoma* complex were reported in Krüger *et al.* (2004), and Fig. 4 plots approximate spore measurements for the three other major 'Polyporellus' species, *P. arcularius, P. brumalis,* and *P. ciliatus*. For *P. ciliatus,* 171 spores from 18 collections positively identified here or in Krüger *et al.* (2003) were measured. They ranged from (5.0–)[2] 6.827 (–9.0) µm x (1.5–) 1.924 (–3) µm, Q (2.167–) 3.635 (–5.333), indicating narrowly cylindrical

[2] Extreme measures in brackets.

spores. For 238 spores from 24 of 30 collections of *Polyporus brumalis* measurements were (5–) 7.166 (–9.5) µm x (1.5–) 2.315 (–3.5) µm, Q (2.286–) 3.150 (–5.667). Excluding FB7883, we took spore measurements of 110 *Polyporus arcularius* spores derived from 11 of 21 available collections. The *P. arcularius* values were as follows: (6.0–) 8.095 (–11.0) µm x (2.0–) 2.763 (–4.0) µm, Q (1.714–) 2.961 (–4.000). Ten FB7883 spores measured (6.0–) 8.440 (–9.0) µm x (2.0–) 3.000 (–3.5) µm, Q (2.170–) 2.810 (–3.200).



	P. arcularius			P. brumalis			P. ciliatus		
	L	W	Q	L	W	Q	L	W	Q
STDEV	1.1	0.4	0.4	0.8	0.4	0.5	0.7	0.3	0.6
MAX	11	4	4	9.5	3.5	5.7	9	3	5.3
AVERAGE	8.1	2.8	3	7.2	2.3	3.1	6.8	1.9	3.6
MIN	6	2	1.7	5	1.5	2.3	5	1.5	2.2

Fig. 4. Box plot of spore size ranges in 'Polyporellus'. L = length, W = width, Q = L divided by W, STDEV = standard deviation, MAX = maximum size, MEAN = arithmetic mean, MIN = minimum size. Values plotted are rounded in the table below.

These spore sizes correspond well with those reported in the monograph by Nuñez & Ryvarden (1995), but we measured some smaller and larger spores, widening the range mostly upwards. The averages, however, were within the ranges given by Nuñez & Ryvarden. *Polyporus arcularius* has the largest spores, followed by *P. brumalis*, and then *P. ciliatus,* which also has the narrowest spores. This may correlate with usual pore size, but this can be variable.

Another noticeable feature reported by Hoffmann (1978) and observed here was the production of apparently monokaryotic fruiting bodies. *Polyporus ciliatus* and *P. brumalis* tended to form elongated stipes on the agar surfaces. One of Hoffmann's original dikaryon cultures (DSMZ-H25) also formed such a stipe when grown in liquid medium for DNA extraction.

Discussion

Specimens re-identified: We received two Canadian voucher specimens and cultures, DAOM155905 and DAOM72515, filed as *Polyporus ciliatus*. The latter, originally identified as *P. brumalis*, was re-identified only after Hoffmann (1978) reported that the fruited dikaryotic culture generated monokaryons compatible with *P. ciliatus*. We also received Hoffmann's original culture, kept for a few decades at DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). However, all sequences ultimately derived from DAOM72515 appeared in the *P. brumalis* clade (Figs. 1, 2). This also holds true for DAOM155905, the other strain[3] received as *P. ciliatus*. Based on sequence data alone, these identifications can be corrected to *P. brumalis*, and the specimens agree with *P. brumalis* sensu Nuñez & Ryvarden (1995). Hoffmann's report of compatibility may be based on the occasional ability of members of the 'Polyporellus' group to hybridize, as also mentioned by Krüger *et al.* (2003). So far, we see no evidence of *P. ciliatus* being present in North America, confirming the suggestion by Nuñez & Ryvarden (1995).

The Canadian collection received from Mr. Audet as *Polyporus* cf. *tricholoma* resembles *P. tricholoma* or *P. ciliatus* in small pores, thin and ciliate fruiting body. Similarity notwithstanding, by sequence data it is not identical to *P. tricholoma* or *P. ciliatus*, but is related to *P. brumalis* and *P. arcularius*, and possibly conspecific with the former. This may result from past hybridization, or otherwise may reflect support for Corner's (1984: 50) suggestion that *P. tricholoma* is an agglomerate of aberrant specimens of several *Polyporus* species. Morphology can be misleading and no mating studies could be conducted, as there was no culture available. The collection AAU44971, resembling *P. ciliatus* in macromorphology and spore statistics, is closely related to the *P. tricholoma* groups discussed in Krüger (2002: 92 ff). These results cast some doubt on the validity of basidiome identification in this group based on macromorphology or micromorphology of basidiomata.

[3] The use of the term strain here indicates a culture officially maintained in a culture collection.

Perhaps due to darkening with age, a Korean collection (NG980201/3) was received as *Polyporus melanopus* Mont. ('Melanopus' group of *Polyporus*, Nuñez & Ryvarden 1995), but sequence and spore statistics identified it as *P. brumalis*. We have previously corrected misidentified Danish and Austrian collections of *P. brumalis* and *P. ciliatus* collections in the course of mating studies (Krüger *et al*. 2003), and there also, either hirsute fruiting body surfaces or darkened stipes/pores of aged fruiting bodies caused difficulties with identification. These basidiomata were sometimes named *P. melanopus* by previous collectors. Notably, DAOM155905, identified as *P. ciliatus*, had orange pores, suggesting *P. ciliatus* f. *ciliatus* and not *P. ciliatus* f. *lepideus* in the sense of Kreisel (1963). Aged fruiting bodies and herbarium specimens may lose the snow-white appearance of pores Kreisel attributed to *P. ciliatus* f. *lepideus*, which was compatible with *P. ciliatus s. str.* (Hoffmann 1978). One finds pore surfaces with greyish, reddish, brown or cream shades in both *P. brumalis* and *P. ciliatus*.

No definitive judgment has been made about *'Polyporus longoporus'* except that it appears not to be conspecific with *P. arcularius*. The Mediterranean *P. corylinus* may or may not be conspecific with *P. ciliatus* while its DNA implicates it is as closely related to the latter.

Nuclear LSU (large subunit) rDNA data showed *Lentinus tigrinus* to be nested between *Polyporus tricholoma* and the other 'Polyporellus' taxa (Krüger 2002: 22, 25). In this paper, ITS sequences show the closer relationship of gilled *L. tigrinus* to the *P. tricholoma* complex than to other 'Polyporellus'. The lack of more taxa of poroid 'Polyporellus' as well as *Lentinus* in the data matrix might account for the trichotomy at the base of the ingroup, and calls for wider sampling of both. *Lentinus* for us contains gilled 'Polyporellus' pending transferral into a wider *Polyporus* as forecasted from Krüger & Gargas (2004).

Overlapping spore ranges are of limited taxonomic value. Spore statistics were included here to link with the keys published so far, allowing later comparison with material from outside the recorded geographic range. The value of macromorphology can be limited by variable pore sizes, decoloration of aged specimens, varying degrees of hirsute surfaces and formation of dark stipe cuticles. This multi-technical approach may yet lead to further discovery of evolutionary and phylogeographic patterns in polypores. The addition of ITS rDNA data is a powerful identification tool if restricted to the use of unambiguously alignable sequence lengths. Use of further sequence data may help resolve outstanding issues of phylogenetic history of polypores, if there is any one detectable common signal that can trace a real and dichotomous phylogeny (Bapteste *et al*. 2005). Per-

haps concatenated gene sequences increasingly available in the phylogenomics era will lead to strong support for the one or the other phylogeny applicable to a new delimitation of the genus *Polyporus* and its component taxa. Limited sequence data such as those of the rDNA cluster alone may have indicative value for the practicing taxonomist and forest pathologist.

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References

Bapteste E., Susko E., Leigh J., Macleod D., Charlebois R. L., Doolittle W. F. (2005) Do orthologous gene phylogenies really support tree-thinking? *BMC Evolutionary Biology* **5**: 33.

Borges da Silveira R. M., Wright J. E. (2002) *Polyporus s. str.* in southern South America: mating tests. *Mycological Research* **106**: 1323–1330.

Borges da Silveira R. M., Saidman B. O., Wright J. E. (2003) *Polyporus s. str.* in southern South America: isoenzyme analysis. *Mycological Research* **107**: 597–608.

Bremer K. (1986) Eine Bildungsabweichung bei *Polyporus brumalis*. *Boletus* **10**: 9–10.

Corner E. J. H. (1984) *Ad Polyporaceas II. Polyporus, Mycobonia,* and *Echinochaete. Nova Hedwigia,* Beiheft **78**: 1–129.

Efron B., Gong G. (1983) A leisurely look at the bootstrap, the jackknife, and cross-validation. *American Statistician* **37**: 36–48.

Felsenstein J. (1993) PHYLIP (Phylogeny Inference Package). Vers. 3.57c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Hasegawa M., Kishino H., Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**: 160–174.

Hibbett D. S., Vilgalys R. (1993) Phylogenetic relationships of Lentinus (Basidiomycota) inferred from molecular and morphological characters. *Systematic Botany* **18**: 409–433.

Hibbett D. S., Donoghue M. J. (1995) Progress toward a phylogenetic classification of the *Polyporaceae* through parsimony analysis of mitochondrial ribosomal DNA sequences. *Canadian Journal of Botany* **73** (Supplement 1): S853–S861.

Hoffmann P. (1978) *Genetische Grundlagen der Artbildung in der Gattung Polyporus*. *Bibliotheca Mycologica* **65**, J. Cramer, Vaduz.

Holmgren P. K., Keuken W., Schofield E. K. (1981) Index Herbariorum. Part I, the Herbaria of the world. Bohn, Scheltema and Holkema, Antwerpen.

Jahn H. (1969) Die Gattung *Polyporus s. str.* in Mitteleuropa. *Schweizerische Zeitschrift für Pilzkunde* **47**: 218–227.

Jobb G., von Haeseler A., Strimmer K. (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology* **4**: 18.

Karsten P. A. (1879) Symbolae ad mycologiam fennicam (*Polyporaceae*). *Meddelanden af Societas pro Fauna et Flora Fennica* **5**: 37–40.

Kimura M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.

Kishino H., Hasegawa M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topology from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* **29**: 170–179.

Kishino H., Miyata T., Hasegawa M. (1990) Maximum likelihood inference of protein phylogeny, and the origin of chloroplasts. *Journal of Molecular Evolution* **31**: 151–160.

Kreisel H. (1963) Über *Polyporus brumalis* und verwandte Arten. *Feddes Repertorium* **68**: 129–138.

Krüger D. (2002) *Monographic Studies in the Genus Polyporus*. Doctoral dissertation. The University of Tennessee, Knoxville. Available electronically through the portal etd.utk.edu (not dated).

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., Hughes K. W., Petersen R. H. (2003) Studies in *Polyporus* subg. *Polyporellus:* on congruence of three biological, morphological and phylogenetic species. In: Proceedings of the 10th International IUFRO Working Party 7.02.01, Québec City, Canada, 16–22 September, 2001. (eds. Laflamme G., Bérubé J. A., Bussières, G.), Canadian Forest Service, Québec City: 14–23.

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.

Kumar S., Tamura K., Jakobsen I. B., Nei M. (2001) MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Tempe.

Lutzoni F. *et al*. (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.

Maddison W. P., Maddison D. R. (2003) Mesquite: a modular system for evolutionary analysis. Vers. 1.0; mesquiteproject.org (not dated).

Nei M., Kumar S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, Oxford.

Nixon K. C. (1999) The parsimony ratchet – a new method for rapid parsimony analysis. *Cladistics* **15**: 407–414.

Nuñez M., Ryvarden L. (1995) *Polyporus* (Basidiomycotina) and related genera. *Synopsis Fungorum* **10**: 1–85.

Nylander J. A. A. (2004) MrModeltest Vers 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Page R. D. M. (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

Popoff O. F., Wright J. E. (1998) Fungi of Paraguay. 1. Preliminary checklist of wood-inhabiting polypores (Aphyllophorales, Basidiomycota). *Mycotaxon* **67**: 323–340.

Posada D., Crandall K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Raes J., Van de Peer Y. (2002) ForCon vers. 1.0 for Windows. Distributed by the authors. University of Antwerpen, Antwerpen.

Ronquist F., Huelsenbeck J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Saitou N., Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.

Sikes D. S., Lewis P. O. (2001) PAUPRat: PAUP implementation of the parsimony ratchet. Beta software vers. 1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs.

Shimodaira H., Hasegawa M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.

Swofford D. L. (2001) PAUP* 4.0 Phylogenetic analysis using parsimony (* and other methods). Beta vers. 4.0b10. Sinauer Associates, Sunderland.

Vos R. A. (2003) Accelerated likelihood surface exploration: The likelihood ratchet. *Systematic Biology* **52**: 368–373.

Xia X., Xie Z. (2001) DAMBE: Data analysis in molecular biology and evolution. *Journal of Heredity* **92**: 371–373.

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