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Monographic Studies in the Genus Polyporus (Basidiomycotina)

Dirk Krueger
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Dirk Krueger entitled "Monographic Studies in the Genus Polyporus (Basidiomycotina)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.

Dr. Ronald H. Petersen, Major Professor

We have read this dissertation and recommend its acceptance:

Dr. Karen W. Hughes, Dr. Randall S. Small, Dr. Arthur C. Echternacht

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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We have read this dissertation
and recommend its acceptance:

Dr. Karen W. Hughes

Dr. Randall S. Small

Dr. Arthur C. Echternacht

Acceptance for the Council:

Dr. Anne Mayhew

Vice Provost and Dean of Graduate Studies

(Original signatures are on file with official student records.)

Monographic Studies in the Genus *Polyporus* (Basidiomycotina)

A Dissertation

Presented for the

Doctor of Philosophy Degree

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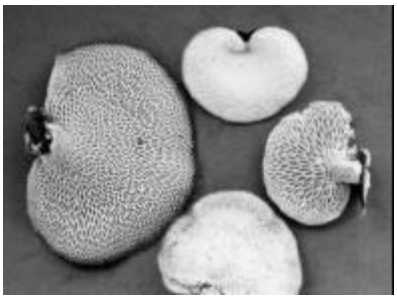
The University of Tennessee, Knoxville, TN, USA



Dirk Krueger

August 2002

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For the most recent years of my university education, I wish to express my deep gratitude to my major professor, Ronald H. Petersen, and committee members Karen W. Hughes, Randy Small, and Sandy Echternacht. Their help, understanding, and support, also in times when I felt quite overwhelmed by tasks, were invaluable. I have learned a lot from you and was allowed to see more of the world. Dr. Leif Ryvarden is thanked for his expertise, advice and hosting me in Oslo. Equally I wish to thank my fellow labmates and contemporary graduate students Ed Grand, J.-K. Jin, Ed Lickey, Juan-Luis Mata, David Sime, Kunsiri Chaw Siripun "Pum", and Shannon Thieken for helping out when in need and having a chat once in a while. Furthermore, I wish to thank everybody working at the Botany Department and core Biology Division for their warmth and friendship, particularily the friendly secretarial staff (Virginia Davis, Eileen Hunley, Eunice Turner), Pat Cox, Susan Farmer, Ken McFarland, Victor Ma, Nadya Psurtseva, and Ed Schilling. Also thanked are the great people at the UT Center for International Education.

Those colleagues and friends who also helped me get to this stage are given my dearest thanks, and these people include Manfred Binder, Raik Elias, Michael Fischer, Antje Gerloff, Eckard Pscheidl, and Markus Scholler. I know of many more, and I certainly thank all of you! Danke! All those are thanked who supported my quest in learning about fungi and nature since early age, including neighbors, teachers at my schools in Germany, and people at clubs and biological stations.

I am indebted to my girlfriend and fellow biologist Gerda's understanding, help, and love through the second half of my stay at Knoxville. Special thanks to that amazing group of people of all walks of life, the Bagelday gang, who have made some weekends a bit relaxing and helped in many ways.

The National Science Foundation is thanked for research support granted to my advisors Ronald H. Petersen and Karen W. Hughes. The Hesler Endowment Fund is thanked for supporting travel.

At last, further people are thanked in the individual parts of this thesis.

ABSTRACT

The aim of this dissertation research was an examination of the homobasidiomycete genus *Polyporus* as currently circumscribed. Emphasis was on addressing the genus by a variety of tools appropriate to modern monographic studies, without producing another worldwide monograph. In addition to morphological examination, which traditionally was the basis of all fungal systematics, mating experiments with monokaryotic cultures, and contemporary phylogenetic tools were used.

For *P. alveolaris*, *P. arcularius*, *P. badius*, *P. brumalis*, *P. ciliatus*, *P. dictyopus*, *P. guianensis*, *P. melanopus*, *P. tubaeformis*, *P. tuberaster*, *P. tricholoma*, *P. umbellatus*, and *P. varius* a tetrapolar mating system was confirmed. Intercollection mate recognition was tested in *P. alveolaris*, *P. arcularius*, *P. brumalis*, *P. ciliatus*, *P. tubaeformis*, *P. tuberaster*, *P. tricholoma*, and *P. varius*. In both *P. varius* and *P. tubaeformis* cultures from European and North American collections were found to be compatible. In *P. tricholoma*, the existence of at least two cryptic biological species entities was found to be contained under the species epithet, whereas in *P. alveolaris* there may also be cryptic species. The type specimen of *P. tricholoma* was studied and one of the *P. tricholoma* cryptic species selected to represent *P. tricholoma* proper.

Sequence analyses of nuclear ribosomal DNA were utilized to elucidate relationships of *Polyporus* and allied genera. With large subunit (nLSU) data, the genus *Polyporus* was found to be non-inclusive under current limits. The infrageneric group *Polyporellus* was transferred into an emended genus *Lentinus*, which traditionally was a genus of gilled fungi. The thelephoroid-hydnoid fungus *Mycobonia flava* was transferred into *Polyporus*.

Investigation of relationships of infrageneric groups in *Polyporus* revealed ITS region rDNA template heterogeneity, supported groups seen in nLSU analyses, and was able to differentiate morphological or biological species. Specific primers and RFLP markers for *P. tricholoma* were evaluated for identification of cryptic species, but were rather successful in uncovering rDNA copy heterogeneity. As a consequence of molecular data and nomenclatural considerations, a new subgenus *Favoliporus* was proposed to absorb infrageneric groups *Favolus* and *Admirabilis*.

Keywords: Aphyllophorales, arthroconidia, classification, dimitic construction, homobasidiomycetes, mating studies, phylogeny, poroid hymenophore, taxonomy, stipitate polypores.

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Image on title page: *Polyporus alveolaris*.

TABLE OF CONTENTS

LIST OF TABLES BY THESIS PART

LIST OF FIGURES BY THESIS PART

PART 1

Overview.

INTRODUCTION

The fungal genus name *Polyporus*, since its proposal in 1729 (Micheli 1729), is approaching 300 years in use. The common ground of what constitutes a *Polyporus* today refers to white-rotting, wood-degrading, pore-bearing fungi with dimitic hyphal construction (generative hyphae + skeleto-ligative hyphae) at maturity of carpophores. Basidiospores are cylindrical, and there are no cystidia in the hymenium. Pore fungi that conform to these characters may include the 32 species recognized in the latest monograph (Nuñez & Ryvarden 1995), plus a few dozen more if genera like *Laccocephalum* and *Pseudofavolus* are included and a handful of species discovered or resurrected since 1995 are accepted. This number of species is significantly less than the thousands of combinations in *Polyporus* described during those 272 years. The genus *Polyporus* has been protected from the taxonomic splitters (compare Overholts 1953, Gilbertson & Ryvarden 1986-1987), especially in North America where some quite old traditions persist. In retrospect, acceptance of "split genera" in the polypores is a continuing slow process, and *Polyporus* was still maintained as a "mega-genus" when other old genera like *Agaricus* and *Boletus* had long been chiseled into smaller segregate genera. Descriptions of *Polyporus* and its history can be found in Nuñez & Ryvarden (1995), Murrill (1903), Overholts (1953), or Nobles (1958).

The doctoral research project presented here utilized methodologies of modern systematics, but was not intended as another monograph *per-se*. Instead, it was designed to examine the recent monograph (Nuñez & Ryvarden 1995). Available material was to be assessed by contemporary molecular biological tools (PCR, RFLP, sequencing), computerized phylogenetic inference, and mating experiments on agar plates.

The goals of the research in particular were to improve knowledge of the phylogenetic status of *Polyporus sensu* Nuñez & Ryvarden (1995) within the homobasidiomycetes, adding to the data combined by Binder & Hibbett (2002). Furthermore, evolutionary relationships of the infrageneric groups outlined by Nuñez & Ryvarden (1995), which are *Polyporus s. str*., *Polyporellus*, *Admirabilis*, *Melanopus*, *Favolus*, and *Dendropolyporus*, was to be addressed.

The mating system of species was to be tested, and intercollection mating tests used to approach the question of whether a morphologically defined species entity was congruent with a biologically defined one. Conclusions from the latter task were restricted in scope because the ability to cross *in-vitro* was judged by the formation of clamp connections/binucleate hyphae. Negative results might only indicate strains to not belong to one biological species (e.g. not "conbiospecific" but cryptic). Barriers involved in speciation in nature may be different.

In essence, this research adheres to comprehensive species criteria beyond a purely morphological (alpha-taxonomical) one. My research did not try to reconstruct the phylogeographic past of *Polyporus* (a much expanded sampling would have been required) or to elucidate the genetic basis of speciation.

As an outcome of this approach, research is presented in a set of separate parts. Laboratory research was almost exclusively performed by myself as a PhD student, but naturally with my major advisors' and labmates' help, guidance, and field-trip-companionship. For consistency, "we" is used throughout when describing research activities and opinions.

A short synopsis of major results and conclusions detailed in the following parts 2-8 of this thesis follows. Materials and methods are described in the appropriate parts.

SYNOPSIS OF THESIS PARTS

Polyporus *& allied genera (see primarily Part 2)*

The nuclear LSU rDNA gene was the tool to infer relationships between infrageneric groups and allied genera, but in the later parts also ITS region rDNA was utilized to "fine-tune" within infrageneric groups. *Polyporus sensu* Nuñez & Ryvarden (1995) was found to be non-inclusive. In the obvious absence of ultimately robust branches across entire phylograms with comprehensive taxon sampling, some important changes to taxonomy are proposed. These include an emendation of *Lentinus*, a historical genus of gilled fungi, to allow recombination of *Polyporus* infrageneric group *Polyporellus* in *Lentinus*. Appropriate recombinations for species are proposed. The "hydnoid" fungus *Mycobonia flava*, in turn, is transferred into *Polyporus* as a subspecies of *Polyporus curtipes*, precipitating an emendation of *Polyporus*.

Infrageneric group Polyporellus *(see Part 3 = Krüger* et al*. 2002, Part 4)*

Using ITS rDNA, an account is given concerning which of the *Polyporellus* sampled may be closest to the type species of *Lentinus*, *L. tigrinus* (Pegler 1983). The *Polyporus tricholoma* complex is that group. Congruence of biological, morphological, and phylogenetic species entities in *P. arcularius*, *P. brumalis*, and *P. ciliatus* was confirmed, as well as a tetrapolar mating system for these three taxa. Collections morphologically resembling *P. ciliatus* (from Ecuador), *P. tricholoma* (from Canada), and cultures/specimens from Canada previously identified as *P. ciliatus* were evaluated by ITS rDNA sequences. As a result, Nuñez & Ryvarden's (1995) suggestion that *P. ciliatus* is absent in North America was supported, and the weakness of morphological characters in identification highlighted. A tentative new species of *Polyporellus* was evaluated, and found likely to be separate from the other *Polyporellus* species investigated.

Polyporus tricholoma *complex (see Part 5)*

In *P.* (*Polyporellus*) *tricholoma*, three interINcompatible groups (cryptic species) tentatively called *P. tricholoma* Group I, II, and "III" were found by intercollection mating experiments. All three exhibited tetrapolar mating systems. Group I and Group II were confirmed by ITS sequence analyses and an attempt was made to quickly distinguish collections by RFLP markers and specific primers. These attempts were less successful because of rDNA template heterogeneity, but a specific primer for Group I was relatively promising. This primer was also tested with a fluorescein label, circumventing the use of ethidium bromide in electrophoresis. A study of the type specimen of *P. tricholoma* was undertaken, and Group II chosen to represent *P. tricholoma* proper. Epitypes and para-epitypes for *P. tricholoma* proper were selected.

Polyporus *infrageneric groups* Melanopus *&* Dendropolyporus *(see Part 6)*

Mating systems were found to be tetrapolar in the group *Melanopus* (*P. badius*, *P. dictyopus*, *P. guianensis*, *P. melanopus*, *P. tubaeformis*, *P. varius*) and *P.* (*Dendropolyporus*) *umbellatus*. Intercollection pairings revealed collections of *P. varius* from North America and

Europe to be conbiospecific. Phylogenetic analyses of ITS rDNA supported the collections of *P. varius* to belong to one species. *Polyporus tubaeformis*, formerly a subspecies of *P. varius*, was found in a closest to *P. badius* and *P. melanopus*. The *Melanopus* group was found non-inclusive, as it was in PART 2. Collections of *P. tubaeformis* from California and Scotland were intercompatible, and both were found to be conspecific with a Norwegian collection by ITS rDNA analysis. Several more collections were reidentified as *P. tubaeformis*, enlarging the known geographical range of the species to western and eastern North America as well as Scotland and northern Germany.

Polyporus *infrageneric group* Polyporus *(see Part 7)*

The *Polyporus s. str.* group was found to be paraphyletic, complementing analyses in PART 2 and PART 6. In addition, the mating system of the type species of *Polyporus*, *P. tuberaster*, was identified as tetrapolar, and collections from central Europe, the Caucasus, and California were partially compatible. As with LSU sequence analyses (PART 2) and in PART 6, *Datronia mollis* was found as closely related to *P. squamosus*.

Polyporus *infrageneric group* Favolus *(see Part 8)*

Polyporus alveolaris was confirmed to exhibit a tetrapolar mating system. An argument for a new subgenus in *Polyporus*, encompassing infrageneric groups *Favolus* and *Admirablis sensu* Nuñez & Ryvarden (1995), is put forward. From this subg. *Favoliporus*, *P. alveolaris* is excluded based on the LSU evidence presented in PART 2.

TERMS & NEW METHODS

New techniques & definitions

The following new primers were developed: Nu-LSU-333-5' (PART 2); NS-7-UTK, ITS 4C (PART 5); and ITS-4B-UTK, PT-I-UTK, PT-II-UTK, PT-III-UTK (PART 5). A method was introduced into fungal systematics in the use of 5'-labeled fluorescent primers (PART 5), circumventing the need to use ethidium bromide in visualization of PCR products in gel electrophoresis. Furthermore, new terminology was coined: "concladic" describes taxa found in one monophyletic clade or relationship group (see below), "conbiospecific" describes taxa belonging to one biological species as judged by experimental mate recognition.

Philosophy of applying the terminology of clades and relationship groups

None of the clade names ("cladonyms") follow the draft PhyloCode (April 2002, http://www.phylocode.org/, Cantino & de Queiroz 2000), which is not yet binding and open for discussion. If the PhyloCode becomes adopted, the new clade names used in this thesis may serve as informal, provisional clade names. Rather, the clade terminology here is inspired by Thomas *et al.* (2002) and Larsson (2002).

The symbol "≠" is proposed to accompany the "/" (clade), meaning a paraphyletic grade = ladder, with no need to repeat the words clade or grade when a symbol is used. In further advancing clade nomenclature the use of ending "– oid" for major clades of Hibbett & Thorn (2001) is not repeated for clades below these major homobasidiomycete clades, following Larsson (2002), thus only /Polyporoid is used. These minor clades are named after one

Linnean taxon (or two, as in /Datronia-Squamosus), with no meaning to endings (Larsson 2002, Thomas *et al*. 2002, as in the PhyloCode draft). The words "core" and "eu" (e.g. Hibbett & Thorn 2001: euagarics, Binder & Hibbett 2002: core euagarics) are taken up as modification in front of the more-inclusive, hierarchically higher cladonym to name a less-inclusive clade, thus "core euagarics" becomes "/core-Euagarics". Use of the modifier "core" requires inclusion of the type of the Linnean system in that clade (e.g. /core-Polyporus contains *P. tuberaster*, the type species of *Polyporus*). Another modifier reserved for information not derived from existing taxon names is an appended secondary name, e.g. "Phaeopodii" in /Tubaeformis-"Phaeopodii". Such secondary clade name may be the forerunner for taxonomic proposals under the ICBN, but endings are meaningless, and the secondary name may as well be in English. After proposing changes in the Linnean system using the secondary clade name, the original clade name may be altered and the older version declared as "syn-cladonym" (e.g. proposing sect. *Phaeopodii* would make /Tubaeformis-"Phaeopodii" a synonym under /Phaeopodii if stated). Newly defined clades are all node-based, with an internal specifier (Art. 11.2 of PhyloCode draft, April 2000), and if necessary, an additional internal apomorphy specifier (Art. 11.2.) and "provisional cladotype" (a specimen that not necessarily is a type specimen according to ICBN). If not specifically introduced as apomorphy specifier, other discussed characters are not meant to define a clade. The node delimiting a newly defined clade is marked with a "node marker" in the tree the clade is defined with. Grades are not node-based, but refer to all taxa included in a labeled grade in a given dendrogram/phylogenetic tree.

According to the PhyloCode draft, species epithets cannot be converted to clade names (Art. 10.1.), nor are double names permitted (Art. 9.2.). Since the clade names here do not follow the PhyloCode draft, there is no statement "*nomen cladi novum*" or "*nomen cladi conversum*" (Art. 9.3.).

In a strict sense, use of phylogenetic terminology requires the tree to have direction, i.e. be rooted, and to be based on a cladistic analysis method, which strictly excludes distance-based analyses such as neighbor-joining (Saitou & Nei 1987) and minimum-evolution (Rzhetsky & Nei 1993). This is the only way to achieve correct identification of apomorphies vs. plesiomorphies, the very basis of cladistics. It is recommended not to use terms such as ancestral, basal, clades, apomorphies, monophyletic etc. for unrooted trees, which quite frequently and unfortunately are called unrooted phylogenies, and may have been used even to define clades with the "/" symbol [Thomas *et al*. (2002) did not indicate if trees were rooted]. One of the conservative approaches in this study was to often not root a tree, and distance methods have been used in analyses. Since relationships require synapomorphies and polarity, the terminology has been altered. The rooting of a tree does not change the number of interior nodes nor the underlying topology of a tree, and thus is only the conscious act of selecting a root and subsequent visualization by pulling out the branch chosen to be the most ancestral.

Based on these considerations, unrooted trees in this thesis are still described as phylogenetic or phenetic, but the terms "monophyletic", "paraphyletic", and "polyphyletic" are replaced by "inclusive" (instead monophyletic) vs. "non-inclusive" (grades). The term "network" widely used for unrooted trees is not applied, as it is mathematically (Hillis *et al*. 1996: 521, Swofford *et al*. 1996: 410) as well as visually incorrect (not a reticulogram as in Hillis *et al*. 1996: 521, Legendre & Makarenkov 2002). Distance-based trees are called dendrograms in this thesis. "Basal", "ancestral", "sister" and other terms with directional meaning are replaced by neutral terms such as "adjacent" or "close". "Clade" is replaced by "relationship group" or by using quotation marks, to differentiate from the infrageneric groups and mating groups in this thesis. Such inclusive relationship groups carry the symbol "/*" when first appearing in a dendrogram, caption, or text, to inform the reader that the cladonym is defined on an unrooted and/or non-cladistic tree. This still allows application of the defined cladonym to clades in

rooted cladistic trees (cladograms). A statement is made if other data in the same PART of the thesis support the relationship group as clade in the strict sense. The term “grade” and symbol “≠” are considered neutral to rooted vs. unrooted trees and the method of phylogenetic reconstruction. Furthermore, trees shown unrooted specify the root that could be chosen based on other data, indicating the potential polarization required for recognition of clades in the strict sense.

Clades are monophyletic, defined in conjunction with rooted cladistic trees, and are understood as a special case of inclusive relationship groups. If shown to be monophyletic, a cladonym initially described for a relationship group in unrooted and/or non-cladistic trees is later used for clades in the strict cladistic sense. Clades and grades defined by other authors/in other publications are used on phylogenetic trees even if they label a part of the tree that is not entirely congruent in content (taxa) or quality (statistical support, is non-inclusive or non-monophyletic) as they serve as a means to refer to these other works. PART 2, for example, uses /Antrodia as label for several disjunct branches. The caption of PART 2 (Fig. 1) points out that the clade name is not coined there, but taken up from different authors (where it was called *Antrodia* clade, as this was before the proposal of the “/” symbol).

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PART 2

Analysis of relationships of *Polyporus* and related genera (Basidiomycotina).

This manuscript is intended for publication, with suggested co-authors Ed Grand, Leif Ryvarden, Karen W. Hughes, and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research.

Running Title: *Polyporus* relationships by sequence analysis.

Abstract: The genus *Polyporus* has seen considerable expansion and contraction over the last 273 years. Here, we evaluate the most recent concepts of the genus with molecular data, and discuss the relationships of the polypores with the agarics. *Lentinus* is emended to include pored species, and species of *Polyporus* infrageneric group *Polyporellus* are transferred to *Lentinus*. We also transfer the non-pored *Mycobonia flava* to *Polyporus*, but accept it as a subspecies of *Polyporus curtipes*.

Keywords: Aphyllophorales, *Datronia*, *Dichomitus*, *Favolus*, molecular systematics, *Mycobonia*, *Lentinus*, LSU rDNA, *Polyporellus*, *Pseudofavolus*.

INTRODUCTION

The original scope of *Polyporus* was of a genus with few species characterized by tough, stipitate basidiomata and poroid hymenophores (Micheli 1729, Adanson 1763). By the time of Fries's sanction of *Polyporus*, the genus contained 130 species (Donk 1960). Overholts (1953) was one of the last authors of major polypore floras to accept the genus in a very wide sense. The most recent monograph by Nuñez & Ryvarden (1995) accepted 32 species within *Polyporus*, arranging them in six infrageneric groups without making recommendations on nomenclatural ranks. Those infrageneric groups were *Dendropolyporus*, *Polyporus s. str.* (= *Squamosus* group), *Polyporellus*, *Melanopus*, *Admirabilis*, and *Favolus*. The breadth of the genus remains in flux as several species have been added or resurrected more recently (Buchanan & Ryvarden 1998, Dai 1996, 1999, Hattori 2000, Popoff & Wright 1998, Thorn 2000).

This paper introduces a framework by which to evaluate this generic circumscription based on large subunit rDNA sequence data. Genera putatively allied with *Polyporus* in the literature (see especially Corner 1984, Nuñez & Ryvarden 1995) have been included in this study. Questions examined are: i) Is *Polyporus sensu* Nuñez & Ryvarden (1995) monophyletic? ii) Is *Favolus* warranted as a separate genus? iii) Is there support for a genus *Royoporus* as conceived by De (1996)? iv) How are the genera *Polyporus*, *Dichomitus*, *Datronia*, *Echinochaete*, *Grifola*, *Lentinus*, *Mycobonia* and *Pseudofavolus* inter-related?

MATERIALS & METHODS

Collections & microscopy

Collections (see Table 1) were given field book (FB) numbers and annotated. They were identified with the aid of keys furnished by Jülich (1984), Gilbertson & Ryvarden (1986-1987), Ryvarden & Gilbertson (1993-1994), and Nuñez & Ryvarden (1995). Dried specimens were deposited in the University of Tennessee fungal herbarium (TENN). Sections of fungal tissue were mounted in 3% w/v KOH with phloxine dye and observed with phase contrast

Table 1. Fungal specimens and cultures used for phylogenetic 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	Nuñez & Ryvarden (1995) group
FB10848 SBI 2 (TENN 58714)	*Albatrellus* sp.	Costa Rica	RHP	AJ487511 (this study)	
CBS728.85	*Albatrellus syringae* (Parm.) Pouz.	Norway	P. Marstad & D. Kjelsaas	AF393045 Binder & Hibbett (2002)	
DAOM197828	*Antrodia carbonica* (Overholts) Ryv. & Gilbn.	Canada	J. Ginns	AF287844 Hibbett *et al.* (2000)	
GEL 4465	*Antrodia serialis* (Fr.) Donk	Germany	Langer	AJ406519 Langer (unpubl.)	
GEL 4143	*Bjerkandera adusta* (Willd.: Fr.) Karst.	Germany	Langer	AJ406530 Langer (unpubl.)	
DSH93.198	*Coltricia perennis* (L.) Murr.			AF287854 Hibbett *et al.* (2000)	
DAOM211791	*Cryptoporus volvatus* (Pk.) Shear.	Canada	J. Ginns	AF393050 Binder & Hibbett (2002)	
DSH93.182	*Daedaleopsis confragosa* (Bolt.: Fr.) Schroet.			AF393051 Binder & Hibbett (2002)	
GEL 4651	"	Germany	Langer	AJ406547 Langer (unpubl.)	
DAOM211792	*Datronia mollis* (Sommerf.: Fr.) Donk	Canada	J. Ginns	AF393052 Binder & Hibbett (2002)	
O103769 = Ryv38091 (specimen)	*Dichomitus campestris* (Quél.) Doman. & Orli.	Norway	L. Ryvarden	AJ487512 (this study)	

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	Nuñez & Ryvarden (1995) group
Ryv18829 (specimen)	*Dichomitus gunnii* (Berk.) Ryv.	Australia	L. Ryvarden	AJ487513 (this study)	
Ryv33765	*Dichomitus leucoplacus* (Berk.) Ryv.	Zimbabwe	M. Nuñez & L. Ryvarden	Not sequenced, only for illustration.	
MN929 (specimen)	*Dichomitus squalens* (Karst.) Reid	Japan	M. Nuñez	AJ487514 (this study)	
MN247 (specimen)	*Echinochochaete brachyporus* (Mont.) Ryv.	Ecuador	M. Nuñez	AJ487926 (this study)	
DAOM129034	*Fomes fomentarius* (L.: Fr.) Kickx.	Canada	J. Ginns	AF261538 Moncalvo *et al.* (2002)	
BN 2812 (GB)	*Fomitopsis pinicola* (Schwein.: Fr.) Karst.			AF347106 Larsson (2001)	
	Ganoderma lucidum (W. Curt.: Fr.) Karst.			X78776 Moncalvo *et al*. (1995)	
	Ganoderma tsugae Murr.			X78778 Moncalvo *et al*. (1995)	
DAOM137861	*Gloeophyllum sepiarium* (Fr.) Karst.	USA	J. Ginns	AF393059 Binder & Hibbett (2002)	
GEL 3101	*Grifola frondosa* (Dicks.: Fr.) S.F.Gray.	Sweden	Langer	AJ406544 Langer (unpubl.)	
FB8286 SBI 18 (TENN55339)	*Hexagonia hydnoides* (Fr.: Sw.) M. Fidalg.	USA	RHP & KWH	AJ487625 (this study)	
FB10243 (TENN57566) (culture)	*Hexagonia papyracea* Berk.	Costa Rica	RHP	AJ487626 (this study)	

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	Nuñez & Ryvarden (1995) group
PR3728 (KUO)	*Hydnellum gracilipes* (Karst.) Karst.			AY012676 Köljalg & Renvall (2000)	
GEL 2914	*Ischnoderma benzoinum* (Wahlenberg: Fr.) Karst.	Germany	Langer	AJ406543 Langer (unpubl.)	
FB10126 SBI 2 (TENN57346)	*Ischnoderma resinosum* (Fr.) Karst.	USA	DK	AJ487927 (this study)	
Neda C500	*Lentinus squarrosulus* Mont.			AF261563 Moncalvo *et al.* (2002)	
FB11101 SBI 1	*Lentinus tigrinus* (Bull.: Fr.) Fr.			AJ487929 (this study)	
LE(BIN)1305 SBI 1	"	Ukraine		AJ487928 (this study)	
DSH93.181	"			AF135173 Thorn *et al.* (2000)	
FB10125 SBI 17 (TENN57410)	*Lenzites betulina* (Fr.) Fr.	USA	DK	AJ487931 (this study)	
FB10566 (TENN58061) (culture)	*Meripilus giganteus* (Fr.) Karst.	USA	J.K. Jin & DK	AJ487930 (this study)	
FB11281 (TENN59090) (culture)	*Microporellus* sp.	Argentina	E. Albertó	AJ487932 (this study)	
FB11279 (TENN59088) (specimen)	*Mycobonia flava* (Swartz: Fr.) Pat.	Argentina	DK	AJ487933 (this study)	
FB10256 (TENN57579) (culture)	"	Costa Rica	RHP	AJ487934 (this study)	

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	Nuñez & Ryvarden (1995) group
E5252A	*Neolentinus dactyloides* (Cleland) Redhead & Ginns			AF135174 Thorn *et al.* (2000)	
Hallenberg2518 SBI 1 (FCUG) (culture)	*Neolentiporus maculatissimus* (Lloyd) Rajchenb.	Argentina	N. Hallenberg & M. Rajchenberg	AJ487935 (this study)	
FPL5096	*Phaeolus schweinitzii* (Fr.) Pat.			AF287882 Hibbett *et a*l. (2000)	
FB10901 SBI 1 (TENN58384)	*Piptoporus betulinus* (Bull.: Fr.) Karst.	Germany	DK & R. Elias	AJ487936 (this study)	
GEL 5400	"	Germany	Langer	AJ406520 Langer (unpubl.)	
DSH90.36 (DNA extraction received from David Hibbett)	*Polyporus alveolaris* (DC.: Fr.) Bond. & Sing.		D. Hibbett	AJ487937 (this study)	*Favolus*
FB10299 SBI 2 (TENN58370)	*Polyporus arcularius* Batsch: Fr.	Austria	H. Voglmayr	AJ487938 (this study)	*Polyporellus*
FB7883 SBI 2 (TENN53747)	*Polyporus arcularius* Batsch: Fr.	Costa Rica	RHP	AJ487939 (this study)	*Polyporellus*
FB11421 (TENN59126) (culture)	*Polyporus badius* (Pers.) Schwein.	USA	DK & M. Fischer	AJ487940 (this study)	*Melanopus*
FB5085 (TENN59136) (specimen)	"	USA	RHP	AJ487941 (this study)	*Melanopus*
FB10908 SBI 4 (TENN58391)	*Polyporus brumalis* Pers.: Fr.	Germany	DK	AJ487942 (this study)	*Polyporellus*
FB10167 SBI 9 (TENN57698)	*Polyporus ciliatus* Fr.	Denmark	H. Knudsen & RHP	AJ487943 (this study)	*Polyporellus*

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	Nuñez & Ryvarden (1995) group
AAU43758 (specimen)	*Polyporus craterellus* Berk. & Curt.	Ecuador	T. Læssøe	AJ487944 (this study)	*Polyporus*
FB11501 (TENN59385) (specimen)	*Polyporus dictyopus* Mont.	Belize	E. Lickey	AJ487945 (this study)	*Melanopus*
FB11254 SBI 1 (TENN58943)	*Polyporus grammocephalus* Berk.	Paraguay	DK & RHP	AJ487946 (this study)	*Favolus*
FB11288 SBI 1 (TENN59093)	*Polyporus guianensis* Mont.	Argentina	E. Albertó	AJ487947 (this study)	*Melanopus*
FB10921 SBI 8 (TENN58404)	"	Venezuela	T. Iturriaga & L. Ryvarden	AJ487948 (this study)	*Melanopus*
FB10489 SBI 10 (TENN58597)	*Polyporus leprieurii* Mont.	Costa Rica	J. L. Mata & RHP	AJ487949 (this study)	*Melanopus*
CIEFAP19 (culture)	*Polyporus melanopus* (Schwartz) Fr.	Argentina	E. Barroetaveña	AJ487950 (this study)	*Melanopus*
FB11465 (TENN59326) (culture)	*Polyporus melanopus* (Schwartz) Fr.	Austria	H. Voglmayr	AJ487951 (this study)	*Melanopus*
Ryv22750 (specimen)	*Polyporus meridionalis* (A. David) H. Jahn	Italy	K. Hjortstam, K.-H. Larsson & L. Ryvarden	AJ487952 (this study)	*Polyporellus*
FB10295 = Thorn443sp = RGT870610/01 = DAOM198666 (culture)	*Polyporus pseudobetulinus* (Pilát) Thorn, Kotiranta & Niemelä	Canada	G. Thorn	AJ487953 (this study)	*Admirabilis*
FB10298 = Thorn567 = Kotiranta6472 (culture)	"	Finland	H. Kotiranta	AJ487954 (this study)	*Admirabilis*
DAOM198916 (culture)	*Polyporus radicatus* Schwein.	Canada	S. Redhead, *det.* J. Ginns (as *P. lentus*)	AJ487955 (this study)	*Polyporus*

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	Nuñez & Ryvarden (1995) group
FB10968 (TENN58831) (specimen)	*Polyporus radicatus* Schwein.	USA	L. Grand & C. Vernia	AJ487956 (this study)	*Polyporus*
FB9941 (TENN56754) (specimen)	*Polyporus rhizophilus* Pat.	USA	KWH & R. Woodbury	AJ487957 (this study)	*Polyporellus*
FB10923 (TENN58406) (specimen)	*Polyporus* sp.	Costa Rica	I. Lindblad	AJ488105 (this study)	*Favolus*
FPL6846	*Polyporus squamosus* (Huds.) Fr.			AF393069 Binder & Hibbett (2002) AF135181 Thorn *et al.* (2000)	*Polyporus*
FB10831 (TENN58380) (specimen)	"	USA	M. Scholler & DK	AJ488106 (this study)	*Polyporus*
TENN27941 (specimen)	*Polyporus squamosus* (Huds.) Fr.	USA	E. Clebsch, *det.* L. R. Hesler	AJ488107 (this study)	*Polyporus*
GEL 4780	*Polyporus tenuiculus* (Pal. de Beauv.) Fr.	Réunion	Langer	AJ406513 Langer (unpubl.)	*Favolus*
FB10242 (TENN57565) (specimen)	"	Costa Rica	RHP	AJ488108 (this study)	*Favolus*
BD990710-21 (specimen)	"	Korea	Y. W. Lim	AJ488109 (this study)	*Favolus*
FB10226 SBI 7 (TENN57136)	"	USA	RHP	AJ488110 (this study)	*Favolus*
DSMZ-H27 (culture)	*Polyporus tricholoma* Mont.	Australia		AJ488111 (this study)	*Polyporellus*

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	Nuñez & Ryvarden (1995) group
FB10241 SBI 9 (TENN57564)	*Polyporus tricholoma* Mont.	Costa Rica	RHP	AJ488112 (this study)	*Polyporellus*
FB3870 SBI 2 (TENN55844)	"	Mexico	RHP	AJ488113 (this study)	*Polyporellus*
FB4362 SBI 9 (TENN50439)	"	Mexico	RHP	AJ488114 (this study)	*Polyporellus*
FB9579 SBI 1 (TENN56491)	"	Puerto Rico	RHP	AJ488115 (this study)	*Polyporellus*
FB10197 (TENN57727) (specimen)	*Polyporus tuberaster* Jacq.: Fr.	Germany	RHP	AJ488116 (this study)	*Polyporus*
FB10935 SBI 2 (TENN58530)	"	Germany	I. Krisai-Greilhuber	AJ488117 (this study)	*Polyporus*
DAOM7997B	*Polyporus tuberaster* Jacq.: Fr.	Italy	G. Goidanich	AF393070 Binder & Hibbett (2002)	*Polyporus*
Fischer17 = FB5079 SBI 8 (TENN59130)	*Polyporus umbellatus* Fr.	USA	M. Fischer	AJ489167 + AJ489168 = AJ488118 (this study)	*Dendropolyporus*
FB10513 (TENN57743) (culture)	*Polyporus varius* Fr.	Germany	DK	AJ488119 (this study)	*Melanopus*
FB10807 (TENN58216) (culture)	"	Russia	S. Pelchuzhina	AJ488120 (this study)	*Melanopus*
FB10962 SBI 19 (TENN58587)	"	USA	DK	AJ488121 (this study)	*Melanopus*

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	Nuñez & Ryvarden (1995) group
DSH93-195	*Polyporus varius* Fr.			AF393071 Binder & Hibbett (2002)	*Melanopus*
FB11219 (TENN58908) (culture)	*Polyporus virgatus* Berk. & Curt.	Argentina	E. Albertó	AJ488122 (this study)	*Melanopus*
FB11406 SBI 1 (TENN59111) (culture)	"	Argentina	E. Albertó	AJ488123 (this study)	*Melanopus*
FB11221 SBI 1 (TENN58910)	*Pseudofavolus cucculatus* (Mont.) Pat.	Argentina	DK & E. Albertó, *teste* L. Ryvarden	AJ488124 (this study)	
FB8744 SBI 7 (TENN55173) = Ryv *s.n.* (20/7/96) (culture)	"	Mexico	RHP	AJ488125 (this study)	
FB10166 (TENN57697) (culture)	*Pseudotrametes gibbosa* (Pers.) Bond. & Sing.	Denmark	H. Knudsen & RHP	AJ488126 (this study)	
FB10517 (TENN57747) (specimen)	"	Germany	DK	AJ488127 (this study)	
FB10140 SBI 2 (TENN57559)	*Pycnoporus cinnabarinus* (Jacq.: Fr.) Karst.	USA	DK & T. Gräfenhan	AJ488128 (this study)	
FB10198 SBI 5 (TENN57728)	*Trametes hirsuta* (Wulf.: Fr.) Pilát	Germany	DK	AJ488129 (this study)	
GEL 5033	*Trametes versicolor* (L.: Fr.) Pilát	Réunion	Langer	AJ406538 Langer (unpubl.)	
FB11274 SBI 5 (TENN59083)	*Trametes villosa* (Fr.) Kreisel	Argentina	DK/KWH/RHP	AJ488130 (this study)	
FB11262 SBI 1 (TENN59071)	"	Paraguay	KWH, RHP & E. Albertó	AJ488131 (this study)	

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	Nuñez & Ryvarden (1995) group
KEW141	*Tyromyces chioneus* (Fr.) Karst.			AF393080 Binder & Hibbett (2002)	

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

FB = TENN field book number = CulTENN culture collection number.

s.n. = no number

SBI = single basidiospore isolate. If no specification "*leg./det*." the name mentioned in the fourth column is the collector and initial identifier of a collection. All FB and O collections have been seen by DK and compared with recent taxonomic literature.

TENN = Univ. of Tennessee Fungal Herbarium, other herbarium acronyms from Holmgren *et al*. (1981).

at 400 x magnification. Monokaryotic single-basidiospore isolates (SBI) and/or dikaryotic cultures were established (techniques by Gordon & Petersen 1991, Petersen & Hughes 1997). Cultures were stored on agar disks of malt extract agar (MEA: 1.5% w/v Difco malt extract, 2% w/v Difco Bacto-agar) in sterilized water (Burdsall & Dorworth 1994).

DNA extraction

DNA extraction from herbarium specimens or cultures followed techniques described by Krüger *et al*. (2002), using a modified xanthogenate procedure (Tillett & Neilan 2000). If material from broth cultures had a gelatinous consistency and thus an unfavorable weight/volume relation, a centrifugation step (3 min at 6,000 rpm = 2,900 g) and the removal of some liquid preceded the grinding. Grinding was usually carried out in 50 µl TE extraction buffer, but if this alone did not suffice to wet the material (in particular, herbarium specimens), grinding was done after the addition of the xanthogenate buffer. Dissolving the post-precipitation pellet was improved by incubation for 10 min at 50° C.

PCR & sequencing

The 3' end of the nuclear ribosomal large subunit (nLSU) gene was obtained by amplification with primers ITS 5 (White *et al*. 1990) and LR 7 or LROR/LR 7 (both primers from http://www.biology.duke.edu/fungi/mycolab/primers.htm) yielding templates for cycle sequencing. The QBioGene *taq* Polymerase kit (QBioGene, Inc. Carlsbad CA) was used for PCR amplifications. PCR parameters were as follows when using primers LR0R and LR 7 (20 µL reactions): initial denaturation 94° C/3 min, followed by 37 cycles of denaturation 94° C/1 min, annealing 46° C/1 min, extension 72° C/3 min. For ITS 5/LR 7 reactions performed for obtaining templates for both ITS (other studies) and LSU sequencing, these parameters were altered to: initial denaturation 95° C/5 min, 37 cycles of denaturation 95° C/1 min, annealing 46° C/1 min, extension 72° C/4.5 min; final extension 72° C/9 min, storage step at 4° C. For some difficult reactions, a pre-PCR was used (95° C: 5 min/72° C: 5 min - interrupted at 72° C for addition of the polymerase after the hot start method (D'Aquila *et al.* 1991). PCR products were used for sequencing as described by Krüger *et al*. (2002). Ten µl cycle sequencing reactions contained 2 or 3 µl BigDye v. 2.0 reaction mix, 0.32 µl 10 µM primer, and approximately 25 ng template DNA. Sequencing primers were LROR and Nu-LSU333-5' (named according to rules suggested by Gargas & DePriest 1996; CTAAATATTGGCGAGAGAC; T_m = 53.88° C according to calculator at http://www.genosys.com/oligos/). Cleaning was done by adding 10 µl dd H_20, 50 µl 95% ethanol, and 2 µl 3M sodium acetate (pH 5.2) to the cycle sequencing products. DNA was precipitated at room temperature for 20 min. Samples were spun for 20 min at 14,000 rpm = 16,000 g. The supernatant was removed, and the invisible pellet was washed with 190 µl 70% ethanol and re-centrifuged (5 min, 14,000 rpm = 16,000 g). The supernatant alcohol was pipetted off, and the pellet was dried by incubation for 1 min at 90° C.

Sequence data

Sequences were corrected in Chromas v. 1.45 (Technelysium Pty. Ltd., Australia), and assembled using a text editor. Additional sequences were imported from the GenBank database (also listed in Table 1). ClustalX v. 1.64b (Thompson *et al*. 1997) was used for

sequence alignment, followed by manual adjustments in GeneDoc v. 2.6.002 (Nicholas & Nicholas 1997) or BioEdit v. 5.0.9 (Hall 1999).

Neighbor-joining (NJ) analysis (Saitou & Nei 1987) was performed in PHYLIP (Felsenstein 1993) as available for interactive use at the Pasteur Institute website (http://bioweb.pasteur.fr/seqanal/) with a Kimura 2-parameter distance metric (Kimura 1980; alignment was checked to have approximately equal nucleotide frequencies). At the same site we utilized the fastDNAml program (Olsen *et al.* 1994) for assessment of tree topology generated using default maximum-likelihood (ML, Felsenstein 1981) methods. Utilizing ForCon v. 1.0 (Raes & van de Peer 2002), MEGA2 files were created. Using the MEGA2 (Kumar *et al.* 2001) data explorer, invariable sites and gaps were excluded from the data. In MEGA2, NJ analyses with the interior branch test (Nei & Kumar 2000: 168) and bootstrap trees (Felsenstein 1985) were performed with 1,000 pseudoreplicates each. Trees were processed in TreeVIEW v. 1.6.1. (Page 1996) for importing into graphics and text programs, and converted to NEXUS tree blocks with a text editor.

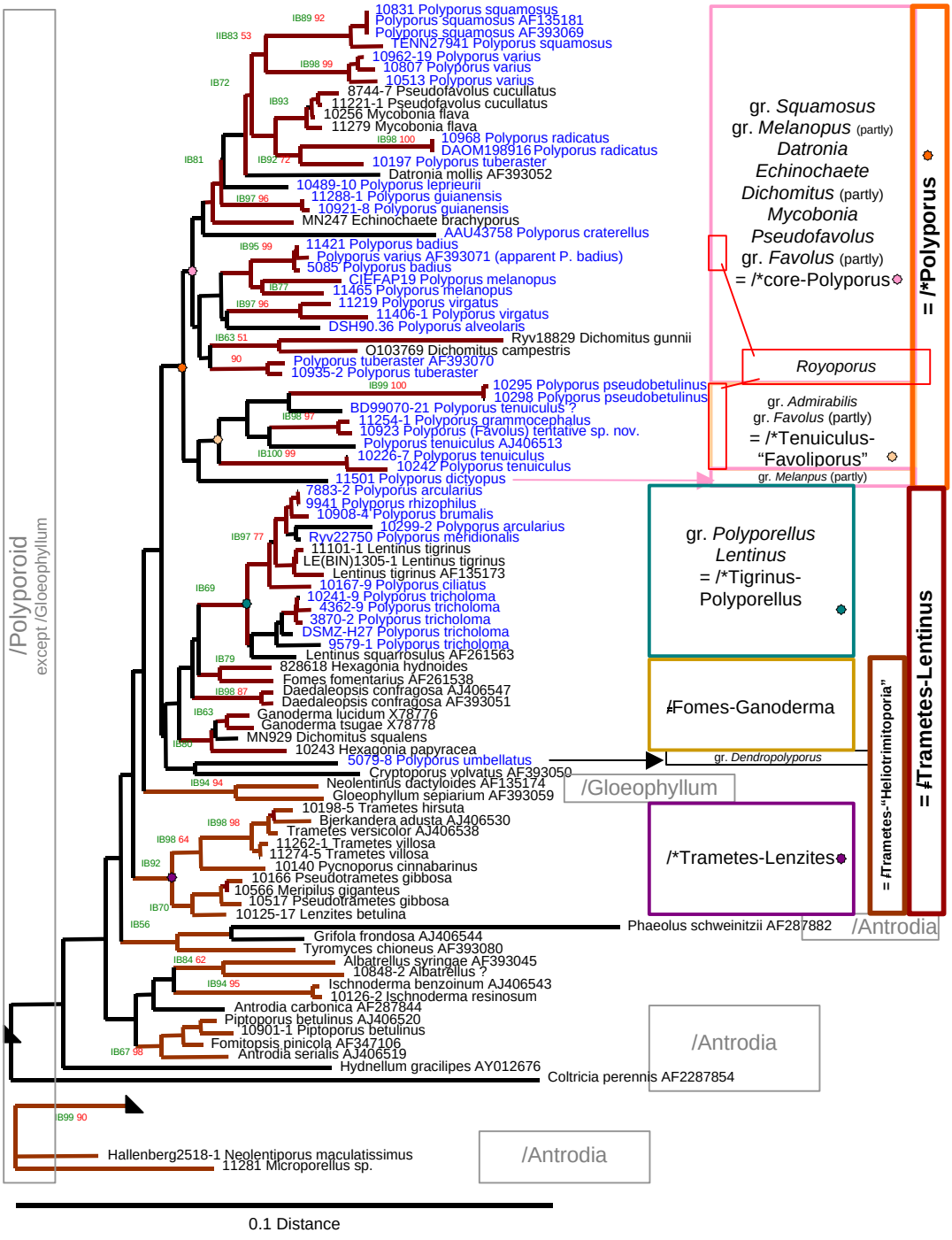
The program package DAMBE v. 4.0.75 (Xia & Xie 2001) was used in calculating a half-deletion jackknife maximum-parsimony (MP) tree (100 pseudoreplicates, Efron & Gong 1983), and it also served in further trimming the data by combining identical phylotypes as prompted to run analyses on the program. PAUP* v. 4.0b8 (Swofford 2001) was used to find the 100 shortest MP trees (parameters were: MAXTREES set to 100 due to computational limitations, TBR swapping, random taxon addition with 10 replicates, 2 trees held at each step during stepwise addition, tree condensing by branches of zero length being allowed to collapse into polytomies, all characters unweighted). PAUP* was unable to perform 10 random taxon addition replicates as 100 trees were found in the first repetition.

TreeVIEW was used to create a constraint NEXUS tree file that forced the monophyly of all taxa considered *Polyporus* by Nuñez & Ryvarden (1995), leaving the *Polyporus* ingroup and the non-*Polyporus* outgroup otherwise unresolved. PAUP* was then used in a second MP analysis to scan the tree-space for MP trees consistent with the constraint, generating another 100 trees. Duplicate trees found in more than one analysis were to be eliminated. All constrained and unconstrained trees in memory were then evaluated using the Kishino-Hasegawa (KH) ML test (Kishino & Hasegawa 1989) with HKY85 model (Hasegawa *et a*l. 1985) and gamma distribution/estimated alpha shape parameter, no sites deemed invariable/no molecular clock. The null hypothesis tested was that the trees are NOT significantly different (= worse in their likelihood). New sequences were prepared for submission to public sequence databases using the feature annotation mode in BioEdit and EMBL's WebIn tool (http://www.ebi.ac.uk/Submissions). Sequence accession numbers are listed in Table 1. Sequence alignments were posted at EMBL with WebInAlign, and are available from EMBL (ftp://ftp.ebi.ac.uk/pub/databases/embl/align/) under accession number ALIGN_000372 (original alignment) and ALIGN_000373 (alignment after exclusion of invariable sites, indels, and duplicate sequences). The convention for referencing clade names follows Moncalvo *et al*. (2002) and Thomas *et al*. (2002), with "/" followed by a name standing for clade, and "/*" symbolizing a newly defined relationship group in connection with a non-cladistic or unrooted analysis. The symbol "≠" stands for paraphyletic grade. Further rules followed in this thesis concerning clades and grades were stated in PART 1. Gr. refers to infrageneric group in *Polyporus*.

RESULTS

A distance-based phenetic analysis with a data set of 754 unambiguously aligned characters from 95 LSU DNA sequences produced the unrooted dendrogram shown as Fig. 1. The major relationship groups ("clades", but unrooted distance-based analyses precludes

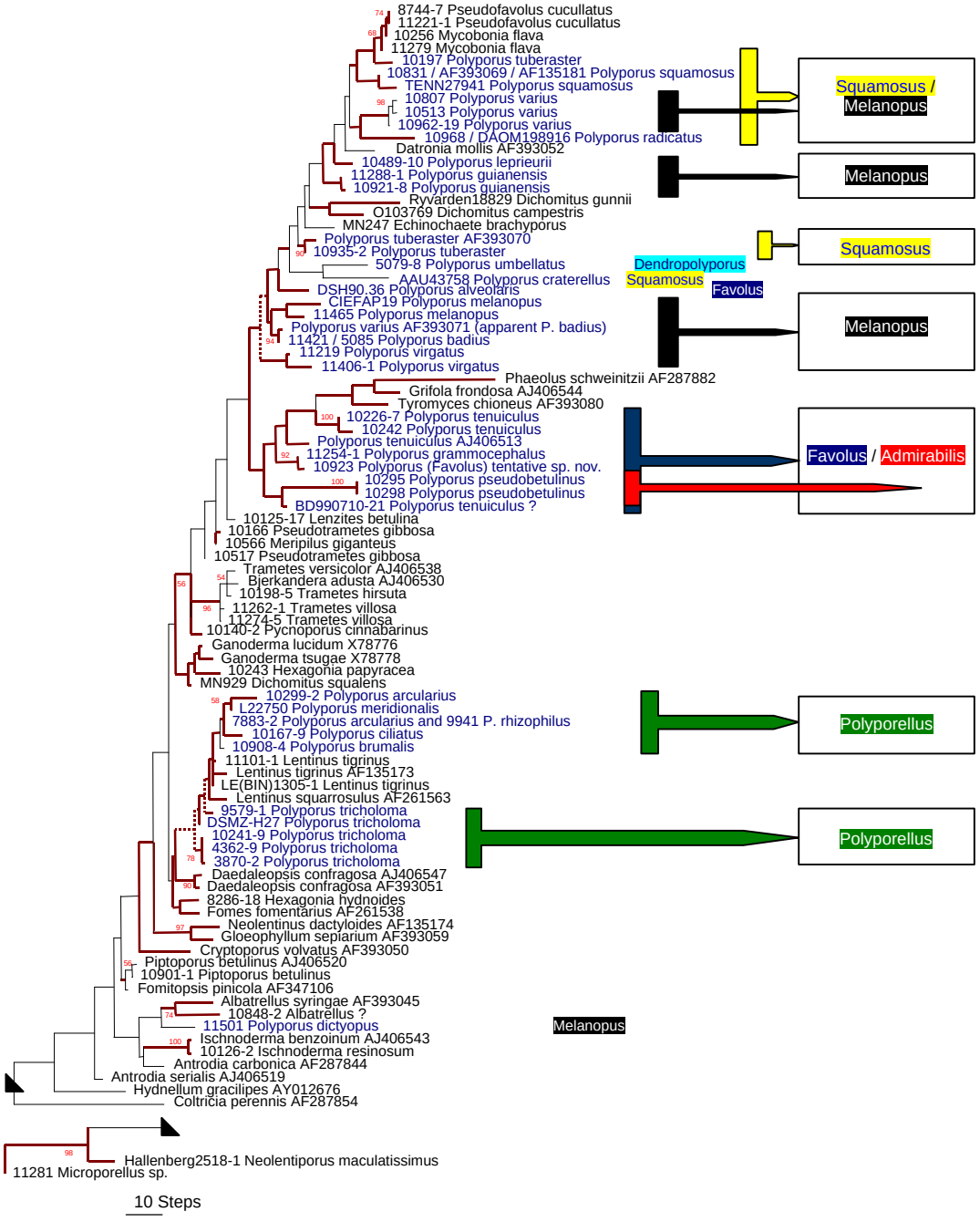
Figure 1. (next page) PHYLIP (Pasteur Server) Kimura 2-parameter NJ dendrogram for polypore LSU data, unrooted [/Antrodia taxa qualify as outgroup following sister-relationship to other taxa in /Polyporoid in Hibbett & Donoghue (2001)]. Brown branches represent topology recovered in MEGA2 Kimura 2-parameter NJ bootstrap tree. Values on branches stem from NJ analyses in MEGA2 with 1,000 pseudoreplicates (IB = Interior Branch Test support, without the IB = MEGA2 bootstrap support). Branch taxon labels in blue shade = species included in *Polyporus* (Nuñez & Ryvarden 1995). Sequences imported from GenBank are given with accession number. SBI number given after dash following our FB number, with the FB acronym omitted. Gray cladonyms adopted from Hibbett & Donoghue (2001). gr. = infrageneric group of *Polyporus*. For new cladonyms defined (node-based) in connection to this dendrogram, a colored node marker is inserted atop the node delimiting the relationship group or clade, and repeated next to the name. The tree is unrooted and thus technically does not allow the use of polarized phylogenetic terminology like clades and apomorphy, however, rooting would be possible on /Antrodia, e.g. *Neolentiporus*, included in Hibbett & Donoghue (2001), where /Antrodia was part of a sister clade to *Polyporus*.


/Polyporoid
except /Gloeophyllum
10831 Polyporus squamosus
Polyporus squamosus AF135181
Polyporus squamosus AF393069
TENN27941 Polyporus squamosus
10962-19 Polyporus varius
10807 Polyporus varius
10513 Polyporus varius
8744-7 Pseudofavolus cucullatus
11221-1 Pseudofavolus cucullatus
10256 Mycobonia flava
11279 Mycobonia flava
10968 Polyporus radicatus
DAOM198916 Polyporus radicatus
10197 Polyporus tuberaster
Datronia mollis AF393052
10489-10 Polyporus leprieurii
11288-1 Polyporus guianensis
10921-8 Polyporus guianensis
MN247 Echinochaete brachyporus
AAU43758 Polyporus craterellus
11421 Polyporus badius
Polyporus varius AF393071 (apparent P. badius)
5085 Polyporus badius
CIEFAP19 Polyporus melanopus
11465 Polyporus melanopus
11219 Polyporus virgatus
11406-1 Polyporus virgatus
DSH90.36 Polyporus alveolaris
Ryv18829 Dichomitus gunnii
Q103769 Dichomitus campestris
Polyporus tuberaster AF393070
10935-2 Polyporus tuberaster
10295 Polyporus pseudobetulinus
10298 Polyporus pseudobetulinus
BD99070-21 Polyporus tenuiculus ?
11254-1 Polyporus grammocephalus
10923 Polyporus (Favolus) tentative sp. nov.
Polyporus tenuiculus AJ406513
10226-7 Polyporus tenuiculus
10242 Polyporus tenuiculus
11501 Polyporus dictyopus
7883-2 Polyporus arcularius
9941 Polyporus rhizophilus
10908-4 Polyporus brumalis
10299-2 Polyporus arcularius
Ryv22750 Polyporus meridionalis
11101-1 Lentinus tigrinus
LE(BIN)1305-1 Lentinus tigrinus
Lentinus tigrinus AF135173
10167-9 Polyporus ciliatus
10241-9 Polyporus tricholoma
4362-9 Polyporus tricholoma
3870-2 Polyporus tricholoma
DSMZ-H27 Polyporus tricholoma
9579-1 Polyporus tricholoma
Lentinus squarrosulus AF261563
828618 Hexagonia hydnoides
Fomes fomentarius AF261538
Daedaleopsis confragosa AJ406547
Daedaleopsis confragosa AF393051
Ganoderma lucidum X78776
Ganoderma tsugae X78778
MN929 Dichomitus squalens
10243 Hexagonia papyracea
5079-8 Polyporus umbellatus
Cryptoporus volvatus AF393050
Neolentinus dactyloides AF135174
Gloeophyllum sepiarium AF393059
10198-5 Trametes hirsuta
Bjerkandera adusta AJ406530
Trametes versicolor AJ406538
11262-1 Trametes villosa
11274-5 Trametes villosa
10140 Pycnoporus cinnabarinus
10166 Pseudotrametes gibbosa
10566 Meripilus giganteus
10517 Pseudotrametes gibbosa
10125-17 Lenzites betulina
Phaeolus schweinitzii AF287882
Grifola frondosa AJ406544
Tyromyces chioneus AF393080
Albatrellus syringae AF393045
10848-2 Albatrellus ?
Ischnoderma benzoinum AJ406543
10126-2 Ischnoderma resinosum
Antrodia carbonica AF287844
Piptoporus betulinus AJ406520
10901-1 Piptoporus betulinus
Fomitopsis pinicola AF347106
Antrodia serialis AJ406519
Hydnellum gracilipes AY012676
Coltricia perennis AF2287854
Hallenberg2518-1 Neolentiporus maculatissimus
11281 Microporellus sp.
0.1 Distance
gr. Squamosus
gr. Melanopus (partly)
Datronia
Echinochaete
Dichomitus (partly)
Mycobonia
Pseudofavolus
gr. Favolus (partly)
= /*core-Polyporus
Royoporus
gr. Admirabilis
gr. Favolus (partly)
= /*Tenuiculus-
"Favoliporus"
gr. Melanpus (partly)
= /*Polyporus
gr. Polyporellus
Lentinus
= /*Tigrinus-
Polyporellus
≠Fomes-Ganoderma
gr. Dendropolyporus
/Gloeophyllum
/*Trametes-Lenzites
= ≠Trametes-"Heliotrimitoporia"
= ≠Trametes-Lentinus
/Antrodia
/Antrodia
/Antrodia

application of clades in the strict sense) are well-supported but deeper nodes have poor support. The lack of deep node support was also reported in recent publications on homobasidiomycete evolution (Hibbett & Thorn 2001, Binder & Hibbett 2002, Moncalvo *et al.* 2002). An obvious feature is that taxa of the /Polyporoid as defined by Hibbett & Thorn (2001) are adjacent to each other, separate from representatives of /Antrodia (Hibbett & Donoghue 2001) (from *Microporellus sp*. to *Phaeolus schweinitzii*, containing species of Grifolales Jülich 1981, Fomitopsidaceae Jülich 1981, Ischnodermataceae Jülich 1981). There is no identifiable /Phlebia (Hibbett & Donoghue 2001) as appropriate taxa were not included with the exception of *Bjerkandera adusta*. *Bjerkandera adusta* resides in a relationship group /*Trametes-Lenzites defined in Fig. 1 [node-based, internal specifier according to April 2000 PhyloCode draft (www.phylocode.org): *Trametes hirsuta* (Wulf.: Fr. 1821): Pilát 1939, internal apomorphy specifier: trimitic hyphal construction]. The /Gloeophyllum (Hibbett & Donoghue 2001) is intercalated with low support between trametoid taxa and all relationship groups containing *Polyporus spp*. The gr. *Polyporellus* of *Polyporus*, alongside *Lentinus*, is here the terminus of a branch containing *Hexagonia*, *Ganoderma*, *Dichomitus pro parte*, *Fomes*, and *Daedaleopsis* (Ganodermatales Jülich 1981, Fomitaceae Jülich 1981, Daedaleaceae Jülich 1981, Polyporaceae Corda 1839 *p.p.* = ≠Fomes-Ganoderma). *Polyporus* (*Dendropolyporus*) *umbellatus* is found outside *Polyporus*, but with low branch support. It must be remarked that approximately 80 bases of the 28S rDNA gene were never recovered in sequencing of this *P. umbellatus* collection (AJ489167 + missing data + AJ489168 = combined sequence AJ488118). /Gloeophyllum (Gloeophyllaceae Jülich 1981), /Tigrinus-Polyporellus (defined below), ≠Fomes-Ganoderma, gr. *Dendropolyporus* + *Cryptoporus* (Cryptoporaceae Jülich 1981), and /Trametes-Lenzites (incl. *Meripilus* of Meripilaceae Jülich 1981) together form ≠Trametes-Lentinus (newly defined grade, without /Tigrinus-Polyporellus = ≠Trametes-"Heliotrimitoporia", newly defined grade; paraphyletic - named solely for convenience). The gr. *Favolus*, alongside a member of the gr. *Admirabilis*, is nested within the bulk of *Polyporus*, and these contain representatives or candidates for the clampless genus *Royoporus* as proposed by De (1996). Further up, as a related group to /*Tenuiculus-"Favoliporus" [newly defined relationship group, node-based, internal specifier *Polyporus tenuiculus* (Pal. de Beauv. 1806) Fr. 1821] is an agglomerate of the grs. *Polyporus* and *Melanopus* of Nuñez & Ryvarden (1995). This relationship group, called /*core-Polyporus [newly defined relationship group (node-based, internal specifier *Polyporus tuberaster* Jacq. 1796: Fr. 1821, including the non-type specimen = provisional cladotype FB10935), forming /*Polyporus (newly defined relationship group, node-based, internal specifier *Polyporus tuberaster* Jacq. 1796: Fr. 1821, including the non-type specimen = provisional cladotype FB10935) with /Tenuiculus-"Favoliporus"], also contains *P.* (*Favolus*) *alveolaris*, and the genera *Dichomitus pro parte*, *Pseudofavolus*, *Echinochaete* (seen as related to *Polyporus* by Nuñez & Ryvarden 1995), and *Mycobonia* (see Corner 1984). In addition, this large assemblage harbors a sequence of *Datronia mollis* already found as close to representatives of the grs. *Melanopus* and *Squamosus* in prior analyses (e.g. Hibbett & Donoghue 2001). Our *Datronia mollis* LSU sequence (AJ488602) differed significantly [not so in ITS, where it did appear close to *P. squamosus* (PARTS 6, 7)].

MP analysis in PAUP* was based on a shortened data set containing 188 variable characters (133 of which PAUP* recognized as parsimony-informative) with NTAX now only 89 as analyzed in DAMBE. Identical phylotypes were combined and taxon labels accordingly altered. Figure 2 shows the most-parsimonious tree selected by the method described above. Constrained topologies with enforced monophyly of *Polyporus* could not be considered as the best trees as all 100 trees were 792 steps in length (CI = 0.324, RI = 0.635), 30 steps longer than unconstrained MP trees. With KH likelihood of – *ln* L = 3799.02412 to 3805.86115 and *P* = 0.0016 to 0.0036, these trees were significantly worse than unconstrained trees and therefore dendrograms in agreement with monophyly of *Polyporus* were rejected. The selected best tree was an unconstrained MP tree of – *ln* L = 3704.27580 (as all unconstrained trees found in one tree island: 762 steps, CI = 0.337, RI = 0.665). The other 99 unconstrained trees produced scores of – *ln* L 3717.31889 for the most unlikely tree (*P* = 0.1368) and *P*

Figure 2. (next page) PAUP* best MP tree by KH ML test (762 steps, CI = 0.337, RI = 0.655, RC = 0.221, -*ln* L 3704.27580), unrooted. Shaded branches represent topology recovered by fastDNAml. Dotted branches = partly recovered (different branching order toward tips). DAMBE MP half-deletion jackknife values (100 pseudoreplicates) near branches. Branch taxon labels in blue shade = species included in *Polyporus* (Nuñez & Ryvarden 1995). Sequences imported from GenBank are given with accession number. SBI number given after dash following our FB number, with the FB acronym omitted.


8744-7 Pseudofavolus cucullatus
11221-1 Pseudofavolus cucullatus
10256 Mycobonia flava
11279 Mycobonia flava
10197 Polyporus tuberaster
10831 / AF393069 / AF135181 Polyporus squamosus
TENN27941 Polyporus squamosus
10807 Polyporus varius
10513 Polyporus varius
10962-19 Polyporus varius
10968 / DAOM198916 Polyporus radicatus
Datronia mollis AF393052
10489-10 Polyporus leprieurii
11288-1 Polyporus guianensis
10921-8 Polyporus guianensis
Ryvarden18829 Dichomitus gunnii
O103769 Dichomitus campestris
MN247 Echinochaete brachyporus
Polyporus tuberaster AF393070
10935-2 Polyporus tuberaster
5079-8 Polyporus umbellatus
AAU43758 Polyporus craterellus
DSH90.36 Polyporus alveolaris
CIEFAP19 Polyporus melanopus
11465 Polyporus melanopus
Polyporus varius AF393071 (apparent P. badius)
11421 / 5085 Polyporus badius
11219 Polyporus virgatus
11406-1 Polyporus virgatus
Phaeolus schweinitzii AF287882
Grifola frondosa AJ406544
Tyromyces chioneus AF393080
10226-7 Polyporus tenuiculus
10242 Polyporus tenuiculus
Polyporus tenuiculus AJ406513
11254-1 Polyporus grammocephalus
10923 Polyporus (Favolus) tentative sp. nov.
10295 Polyporus pseudobetulinus
10298 Polyporus pseudobetulinus
BD990710-21 Polyporus tenuiculus ?
10125-17 Lenzites betulina
10166 Pseudotrametes gibbosa
10566 Meripilus giganteus
10517 Pseudotrametes gibbosa
Trametes versicolor AJ406538
Bjerkandera adusta AJ406530
10198-5 Trametes hirsuta
11262-1 Trametes villosa
11274-5 Trametes villosa
10140-2 Pycnoporus cinnabarinus
Ganoderma lucidum X78776
Ganoderma tsugae X78778
10243 Hexagonia papyracea
MN929 Dichomitus squalens
10299-2 Polyporus arcularius
L22750 Polyporus meridionalis
7883-2 Polyporus arcularius and 9941 P. rhizophilus
10167-9 Polyporus ciliatus
10908-4 Polyporus brumalis
11101-1 Lentinus tigrinus
Lentinus tigrinus AF135173
LE(BIN)1305-1 Lentinus tigrinus
Lentinus squarrosulus AF261563
9579-1 Polyporus tricholoma
DSMZ-H27 Polyporus tricholoma
10241-9 Polyporus tricholoma
4362-9 Polyporus tricholoma
3870-2 Polyporus tricholoma
Daedaleopsis confragosa AJ406547
Daedaleopsis confragosa AF393051
8286-18 Hexagonia hydnoides
Fomes fomentarius AF261538
Neolentinus dactyloides AF135174
Gloeophyllum sepiarium AF393059
Cryptoporus volvatus AF393050
Piptoporus betulinus AJ406520
10901-1 Piptoporus betulinus
Fomitopsis pinicola AF347106
Albatrellus syringae AF393045
10848-2 Albatrellus ?
11501 Polyporus dictyopus
Ischnoderma benzoinum AJ406543
10126-2 Ischnoderma resinosum
Antrodia carbonica AF287844
Antrodia serialis AJ406519
Hydnellum gracilipes AY012676
Coltricia perennis AF287854
Hallenberg2518-1 Neolentiporus maculatissimus
11281 Microporellus sp.
10 Steps
Squamosus / Melanopus
Melanopus
Squamosus
Dendropolyporus
Squamosus
Favolus
Melanopus
Favolus / Admirabilis
Polyporellus
Polyporellus
Melanopus

reaches 0.4265 in the next-probable tree of – *ln* L = 3709.77744. There is no guarantee that the best MP tree was found in our analysis, and the data set seems to be highly homoplasious. The nearest-likely trees only differ in some branch positions towards groups around representatives of /Antrodia.

When distance analyses and step-parsimony-based analyses were compared, there were several major differences. Unexpected was the weakly supported location of *P. dictyopus* near /Antrodia (Hibbett & Thorn 2001), next to *Albatrellus* in our Fig. 2. In Hibbett & Donoghue (2001), *Albatrellus* was found removed from the /Antrodia and basal in the /Polyporoid. No conclusion can be drawn from using one sequence of *P. dictyopus*, a widespread tropical taxon harboring many synonyms. With NJ (Fig. 1), *Phaeolus*/*Grifola*/*Tyromyces* were separate from /Polyporus and ≠Trametes-Lentinus. In MP (Fig. 2), these three are located within *Polyporus* infrageneric grs. *Favolus*/*Admirabilis*. Branch support is low. Our *Dendropolyporus*/*Cryptoporus* relationship group in NJ fragments in MP, with *P. umbellatus* appearing next to *P. craterellus*, *and Cryptoporus volvatus* appearing between the /Antrodia and /Gloeophyllum as defined by Hibbett & Thorn (2001). Notably, in Hibbett & Donoghue (2001), the /Gloeophyllum is very distant from any other polypore included in our study. With these exceptions, the different analyses converge on similar topologies.

Misidentifications & potential new taxa

The sequence AF393071 (DSH93.195: Binder & Hibbett 2002, as *P. varius*) both in NJ and MP clusters with our *P. badius* sequences (99% identity vs. 92% identity with our *P. varius* sequences as calculated by BioEdit). We have not seen their specimen whose DNA does not cluster with DNA from our collections of *P. varius* (which were identified after mating studies and morphology). There is a possibility that their sequence actually stems from a *P. badius*.

DAOM198916, deposited as *Polyporus lentus*, is *P. radicatus* by its DNA sequence (also ITS region, see PART 7).

The collection FB10923 is a shattered, small-pored, flabelliform, isabelline-colored polypore unfortunately lacking fertile elements, but perhaps allied with *P.* (*Favolus*) *grammocephalus*. *Polyporus grammocephalus* is another small-pored tropical *Favolus*, which, however, lacks such colors.

DISCUSSION & TAXONOMY

Evolution of Polyporus *and other polypores*

Generally, mycologists have long suspected a close relationship between certain *Polyporus* and *Lentinus s. str*. taxa (Singer 1986: 163-166). Kühner (1928) described similarities between *Polyporus* and *Lentinus* (Schaeff.: Fr.) Bres. The close relationship of *Lentinus* and *Polyporus* was highlighted again by Nuss (1980): just as agarics, basidiomata are unable to engulf large foreign bodies (grass, twigs), already noted by Benedix (1959). Corner (1984: 38) suggested that *Lentinus*, *Polyporus*, and *Echinochaete* evolved in parallel from an ancestor that also led to *Ganoderma*/*Amauroderma*. In our analyses (Figs. 1, 2) *Ganoderma* indeed is included in the unnamed clustering (≠Trametes-Lentinus + /Polyporus) that contains all *Polyporus*. Patouillard (1900) transferred *Favolus* to the Agaricales but molecular data suggest that *Favolus* should taxonomically remain with the polypores. Van Overeem (1924, 1925) suspected a *Polyporus–Pleurotus* (Agaricales) relationship. Even if not included here, we know from papers like Hibbett & Thorn (2001) and from searches on GenBank (BLAST-n:

Altschul *et al*. 1997) that *Pleurotus* is in the /Euagarics, together with other polypore-like fungi (*Fistulina*, *Schizophyllum*), but not *Polyporus s. str*.

Our phylogenetic reconstructions reject the hypothesis that *Polyporus* as currently circumscribed, with or without gr. *Favolus*, is an inclusive genus. This conclusion is based on a relatively wide selection across the different infrageneric groups. Recent analyses touching on the topic (Hibbett & Donoghue 2001, Binder & Hibbett 2002) lacked this depth of resolution and sampling. The /Polyporoid was only weakly supported as monophyletic, and did not address the monophyly of *Polyporus* as circumscribed above.

/Gloeophyllum has not previously (Hibbett & Donoghue 2001) been found phylogenetically close to other genera of polypores achieving trimitic construction, where they appear in our Fig. 1, but notably based on much fewer characters than in Hibbett & Donoghue (2001). Except for *Bjerkandera adusta*, *Meripilus giganteus*, *Polyporus umbellatus* (note: low support for inclusion of *P. umbellatus*), and perhaps *Neolentinus* and *Heliocybe* (Redhead & Ginns 1985), all taxa in the grade called ≠Trametes-"Heliotrimitoporia" are trimitic (Gilbertson & Ryvarden 1986-1987), which was not resolved before, and here also only partly resolved in a grade. The "helio" in the grade name refers to observations that *Heliocybe sulcata* (Berk.) Redhead & Ginns (in the /Gloeophyllum of Hibbett & Donoghue 2001), *Gloeophyllum sepiarium*, and *Neolentinus lepideus* (close to *Neolentinus dactyloides*, Redhead & Ginns 1985: 358) all tend to be found fruiting on extremely desiccated, sun-exposed conifer wood (DK observations, Redhead & Ginns 1985), where they cause a brown-rot (Gilbertson & Ryvarden 1986-1987). Perhaps the extreme xeric conditions endured by these fungi are mastered with special enzymes or osmolytes. Both *N. lepideus* and *G. sepiarium* often have serrate gills (DK observation). If ≠Trametes-"Heliotrimitoporia" is indicating a natural relationship, then trimicity has been lost in case of *Meripilus* and *Neolentinus*/*Heliocybe*. *Gloeophyllum* was indeed found ancestral to *Neolentinus*/*Heliocybe* in Hibbett & Donoghue (2001). Inclusion of *Ganoderma* in ≠Trametes-Lentinus indicates that Ganodermatales Jülich 1981 and Ganodermataceae (Donk) Donk 1948 may be superfluous in polypore systematics.

Gr. Dendropolyporus, Grifola, Meripilus

The positions of *Grifola frondosa* and *P.* (*Dendropolyporus*) *umbellatus* in our analyses were not resolved. Our analyses indicate *Meripilus giganteus* to be near groups of trametoid polypores (/Trametes-Lenzites), whereas in Hibbett & Donoghue (2001) *M. giganteus* appears basal in the /Polyporoid, together with *Panus rudis*. Bondartsev & Singer (1941) united *Grifola frondosa* and *Meripilus giganteus* in *Polypilus* Karst., forming part of Scutigeraceae with *Boletopsis*, *Bondarzewia*, and *Scutiger* (= *Albatrellus*). They saw *Polypilus* as related to Clavariaceae and Cantharellaceae. Bondartsev (1953) included *G. frondosa*, *P. umbellatus*, and *M. giganteus* as *Polypilus* species. Donk (1974: 226-227) argued for separation of the three aforementioned species, but was doubtful about the inclusion of *P. umbellatus* in *Polyporus*. Jülich (Jülich 1981: 397) then resolved the problem by elevating *Polyporus* subg. *Dendropolyporus* Pouzar [Folia Geobot. Phytotax. 1: 360 (1966)], to genus rank, which was not taken up by the Ryvarden school (Ryvarden 1991: 137). *Meripilus giganteus* and *Grifola frondosa* were placed in Grifolales (Grifolaceae Jülich 1981 and Meripilaceae Jülich 1981, Jülich 1981: 227-229). The available GenBank *Grifola frondosa* LSU sequence (AJ406544) tends to cluster with *Phaeolus* and *Tyromyces* near Hibbett & Thorn's (2001) /Antrodia. Apparently, *Grifola frondosa*, *Polyporus umbellatus*, and *Meripilus giganteus* are not congeneric. The phylogenetic analyses of *Grifola* and *Polyporus umbellatus* (ITS region and ß-tubulin) in the dissertation of Shen (2001) must be regarded as unsatisfactory, as there seemed to be *a-priori* assumption about a close relationship of taxa once included in *Grifola* and now placed in *Polyporus* (*Dendropolyporus*), *Meripilus*, and *Grifola*. Shen excluded his

one *Meripilus* sequence from his data due to unalignability, and based his analysis on many *Grifola* isolates and just one *P. umbellatus* culture from ATCC, and with Shen *et al*. (2002) omitted both *M. giganteus* and *P. umbellatus* in presented phylogenies. We do not see evidence for uniting the large multipileate polypores, including *P. umbellatus*, especially considered for the purpose of this study, in one genus. There is no molecular evidence, however, to determine the position of *P. umbellatus* within or outside *Polyporus*.

Gr. Polyporellus

The close relationship of *P.* (*Polyporellus*) *arcularius* (Polyporaceae Corda 1839) and *Lentinus* subg. *Lentinus* (Lentinaceae 1981) first was confirmed on rDNA data by Hibbett &Vilgalys (1993) and Hibbett & Donoghue (1995), although such positional proximity within the weakly resolved /Polyporoid 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). The inflated generative hyphae as depicted by Pegler (1983, e.g. p. 47 for *L. tigrinus*) for *Lentinus s. str.* (subg. *Lentinus*, section *Tigrini*), and also found prominently in many young specimens of *Polyporellus* by us appear to be a unifying character for a *Lentinus–Polyporellus* alliance [= /*Tigrinus-Polyporellus as in Fig. 1, (newly defined relationship group, node-based, internal specifier *Lentinus tigrinus* [Bull. 1781: Fr. 1821] Fr. 1828*,* internal apomorphy specifier = inflated generative hyphae] 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* & *Dendropolyporus* groups) and *Laccocephalum* McAlp. & Tepper (Nuñez 1995, Nuñez & Ryvarden 1995). It remains to be investigated whether the formation of sclerotia arose independently in the *Lentinus*/*Polyporellus* alliance and in other polypores.

Polyporus rhizophilus has been noticed as similar to *P. arcularius* (Jahn 1969), and our analyses (Figs. 1 and 2) confirm this. There, FB9941 *P. rhizophilus* is most related to the Costa Rican *P. arcularius* (FB7883).

Based on our analyses with increased taxon sampling with LSU data and ITS region data (PART 4), we conclude that the group of *Polyporus s. l.* segregated by Karsten (1880) as *Polyporellus*, in the circumscription used by Nuñez & Ryvarden (1995), should be united with *Lentinus*. The other groups of *Polyporus* do not share such molecular phylogenetic proximity.

Polyporellus spp. differ from other *Polyporus spp*. in spore sizes (*Polyporus* gr. *Squamosus* spores are longer, see Nuñez & Ryvarden 1995) and existence of widely inflated generative hyphae. We found these both in tropical specimens of *P. tricholoma* and specimens fruited on sawdust (separate studies). 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 in gr. *Polyporellus* one can find irregularily-shaped clamp connections that also are branching points. Other features typical for gr. *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* encountered by us developed reddish or ochraceous stains. These sometimes impede 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).

Not all species listed under *Polyporellus* by its original author (Karsten 1882) were kept in *Polyporellus* by Nuñez & Ryvarden (1995). Karsten included polypores that are now in the *Melanopus* group [*P. varius* incl. *P. varius* (= *P. elegans*, *P. leptocephalus*, *P. nummularis*)], *P.*

melanopus, *P. picipes* (= *P. badius*) or *Squamosus* group [*P. squamosus* (= *Favolus boucheanus*, *P. infundibuliformis, P. rostkovii*].

Lectotypification of *Polyporus* (Cunningham 1948, Donk 1960, Kreisel 1960, Nuñez 1993, Nuñez & Ryvarden 1995) has been discussed since the first typification in the early 20th century (Murrill 1903). *Polyporus arcularius*, *P. brumalis*, *P. squamosus*, and *P. tuberaster* all have been suggested. If one does not opt for *P. arcularius* or *P. brumalis*, and we follow here the monographic work of Nuñez & Ryvarden (1995), the type species of *Polyporus* is not in gr. *Polyporellus*. Both *P. arcularius* and *P. brumalis* have been confused in the past (Kreisel 1963). Nowadays, *Polyporus tuberaster* is usually accepted as the type species of *Polyporus* (Imazeki 1943, Donk 1960, Kreisel 1960, Nuñez & Ryvarden 1995). This was based on two arguments: i) *P. tuberaster* was a once cultivated organism deserving name stability (Kreisel 1960) or ii) rejection of a perhaps automatic first-name-rule typification (see Nuñez 1993, Nuñez & Ryvarden 1995). Acceptance of *P. tuberaster* as the type allows us to retain the name *Polyporus.* As *Lentinus* (Fries 1825) is the older name, *Polyporellus* would be placed in synonymy, and *Lentinus* Fr. is hereby emended to allow inclusion of mushrooms with poroid hymenophores. *Lentinus* is accepted with type species *L. tigrinus* (Redhead & Ginns 1985), in section *Tigrini* of subg. *Lentinus*, with which *Polyporellus* most likely is closely allied.

Lentinus Fr. 1825 (Fries, Syst. Orb. Veg.: 77) *emend*. Krüger

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

We here propose the following new combinations of *Polyporellus* species in Nuñez & Ryvarden (1995) or published thereafter:

Lentinus arcularius (Batsch: Fr.) Krüger, *comb. nov*.
BASIONYM: *Boletus arcularius* Batsch 1783, Elench. Fung.: 97

≡ *Polyporus arcularius* Batsch: Fr.

Lentinus brumalis (Pers.: Fr.) Krüger, *comb. nov*.
BASIONYM: *Boletus brumalis* Pers. 1794, Neues Mag. Bot. 1: 107

≡ *Polyporus brumalis* Pers.: Fr.

Lentinus corylinus (Mauri) Krüger, *comb. nov*.
BASIONYM: *Polyporus corylinus* Mauri 1818, Giorn. Arcadico di Roma 54: 3

Lentinus meridionalis (A. David) Krüger, *comb. nov*.
BASIONYM: *Leucoporus meridionalis* A. David 1972, Bull. Soc. Mycol. Fr. 88: 301

≡ *P. meridionalis* (A. David) Jahn

Lentinus mongolicus (Pilát) Krüger, *comb. nov*.
BASIONYM: *Polyporellus arcularius* var. *mongolicus* Pilát 1940, Ann. Mycol. 38: 69

≡ *P. mongolicus* (Pilát) Y.-C. Dai 1996, Ann. Bot. Fennici 33: 154.
HOLOTYPUS: the specimen defined by Dai (1996) for *P. mongolicus* (PRM808923, not seen). Kreisel (1963) already hinted on this being another good species.

Lentinus gerdai Krüger, *nom. nov*.
= *P. tricholoma* Mont.

As discussed by Krüger (PART 5), synonyms and varieties may not easily be assigned to *P. tricholoma* proper. Therefore, the need to circumvent confusion with the established *Lentinus tricholoma* Berk. & Cooke 1877, in Journ. Linn. Soc. Bot. 15: 374, according to Pegler (1983: 37) a synonym of *Lentinus swartzii* Berk., leads us to propose a new species epithet. It is in honor of the major supporter of DK's life as a graduate student.
HOLOTYPUS: **Cuba:** Ramon de la Sagra *s.n.* (PC!), EPITYPUS: **USA:** Puerto Rico: Luquillo National Forest, El Toro Recreational Trailhead, 11 June 1998, KWH FB9579 (TENN56491); and PARA-EPITYPES: see PART 5.

Lentinus rhizophilus (Pat.) Krüger, *comb. nov*.
BASIONYM: *Polyporus rhizophilus* Pat. 1894, Journ. Bot. 8: 219

Polyporus ciliatus Fr. requires further research before any nomenclatural action can be taken. This is to prevent a later homonym of *Lentinus ciliatus* Lév. 1844, in Ann. Sci. Nat. Bot. Ser. 3, 2: 175. Perhaps *Polyporus lepideus* Fries (1831, Epicris. Syst. Mycol.: 430) qualifies as a priorable name to be transferred, if *P. ciliatus* f. *lepideus* of *stat. nov*. in Kreisel (1963) can be a synonym of *P. ciliatus*, albeit unmentioned in Nuñez & Ryvarden (1995). However, also the epithet *lepideus* is taken in *Lentinus* (Pegler 1983). Alternatively, if *Polyporus vernalis* Fries (1874, Hymen. Europ: 527) is indeed a good synonym, *Lentinus vernalis* (Fr.) will have to be the recombined name.

Gr. Favolus*, gr.* Admirabilis*,* Royoporus

Favolus Fries (1828, Elenchus 1: 44), inspired from Palisot de Beauvois's *Favolus* (1805, Fl. Oware Benin Afriq. 1: 1), was integrated into *Polyporus* by Nuñez & Ryvarden (1995). Previously, Ryvarden & Johansen (1980) retained it as a separate genus, noting that in addition to elongated pores, *Favolus* was distinct in that it lacked clamps, and skeletal hyphae were weakly branched. *Favolus* Fr. has been typified with *Daedalea brasiliensis* Fr. [= *Favolus brasiliensis* (Fr.) Fr., which Nuñez & Ryvarden (1995) synonymized under *Polyporus tenuiculus non tenuicaulis*]. *Favolus* Pal. de Beauv. is typified by *Favolus hirtus* (according to Ryvarden 1991), a species of *Hexagona* Poll. *sensu* Fr. (Fries: *ut Hexagonia*, Donk 1960: 210-211, 224-227) not studied by us nor included in Nuñez & Ryvarden (1995).

Corner (1984: 35) treated *Favolus* within *Polyporus* and argued that the rank of subgenus was not required. Nuñez & Ryvarden (1995) mentioned scattered clamps (confirmed by this study) so the character "lack of clamps" is not absolute for *Favolus*. *Favolus alveolaris* (= *Hexagonia mori*) exhibits numerous clamps. Donk (1960: 214) and Kreisel (1963) noted its perceived affinity to *P. arcularius*. Our *Favolus* LSU phylotypes, except for that of *P. alveolaris*, cluster together with low bootstrap support in both distance- and parsimony-based analyses. *Polyporus* (*Admirabilis*) *pseudobetulinus* also falls into /Tenuiculus-"Favoliporus" in our analyses. Our LSU molecular data do not confirm the suggested affinity of *Favolus spp*. and *P. arcularius*. Within gr. *Admirabilis*, we were able to sample *P. pseudobetulinus*, also reported as lacking clamps (Thorn *et al*. 1990, we have seen specimens at O and also did not find clamp connections).

Royoporus has been proposed for simple-septate polypores, based on *Favolus spathulatus* [= *Favolus grammocephalus* (according to Nuñez & Ryvarden 1995)]. *Favolus grammocephalus* is included in our study. *Favolus spathulatus* is the monotype of *Royoporus* (De 1996). Later, *P. badius* of the *Melanopus* group was transferred (De 1997) as second species to *Royoporus*. Based on the lack of clamps, *P. pseudobetulinus* would be a candidate for transfer to *Royoporus* (Thorn, pers. comm.), or if lack of clamps really holds true for *Favolus*, then it could become a *Favolus* (Thorn *et al*. 1990). However, the reliability of the

lack of clamps as a generic character may be doubtful. Donk (1974: 226) referred to Nobles (1965: 1101) who stated that also in *Meripilus,* the lack of clamps is not absolute, at least in cultures, and Ryvarden & Gilbertson (1993-1994: 396) depicted rare clamp connections in *M. giganteus*, which usually does not form clamps. Corner (1984: 20, 35) gave the absence of clamps no more value than to distinguish species and varieties and also discussed the withering of generative hyphae and occurrence of secondary simple septa as a problem in *Polyporus* systematics. Recently, a number of taxa with clampless hyphae have been shown to have clamps (see De 1996 for a discussion), and *Favolus spathulatus*, *Polyporus pseudobetulinus*, and *P. badius* may be the only truly clampless taxa (Corner 1984, Thorn *et al*. 1990, De 1996), but Nuñez (1993) mentions few clamps in *P. pseudobetulinus*. Based on our data (Figs. 1, 2), the members and candidates of *Royoporus* do not appear in an inclusive relationship group or "clade", so we do not find support for the generic concept of *Royoporus*. A word of caution, however, may be that the *P. grammocephalus* specimen used in this study was very fine-pored, unlike the collection De (1996) used to typify *Royoporus*. In description and in De's Fig. 2, elongated pores are evident, leaving open to investigation whether *Royoporus spathulatus* = *P. grammocephalus*.

The NJ-tree (Fig. 1) features *P. dictyopus* adjacent to *Favolus* but with low support. The sequence is outside *Favolus* in the MP tree (Fig. 2). However, the basidiomata bear some resemblance to *P.* (*Favolus*) *grammocephalus* in that they are thin and have small pores.

Taken together, the taxon *Favolus* Pal. de Beauv. *sensu* Fr. appears non-monophyletic unless *P. alveolaris* is excluded and *P. pseudobetulinus* is included. If the lack of clamps is not critical to systematic placement as mentioned above, it may be feasable to emend *Favolus* to allow more robust extratropical taxa. However, other species of gr. *Admirabilis* mentioned by Nuñez & Ryvarden (1995) and Thorn (2000) must be evaluated as well. For *Polyporus* systematics, *Favolus* should remain subordinate to *Polyporus*, but unless many more tropical *Favolus* taxa are analyzed we would not recommend this with certainty. If retained in *Polyporus*, *Favolus spathulatus* would have to be called *P. moluccensis* if not in synonymy under *P. grammocephalus* (discussed by De 1996).

Gr. Melanopus*, gr.* Squamosus*,* Datronia*,* Echinochaete*,* Dichomitus

Species of gr. *Melanopus* (= *Varius* group in Nuñez 1993) and gr. *Polyporus* (= *Squamosus* group in Nuñez 1993) of Nuñez & Ryvarden (1995) cluster together in our analyses as a major component of /core-Polyporus, albeit with low backbone support (bootstrap and jackknife less than 50%). Both infrageneric groups are not inclusive. Together, basidiomata are characterized by possession of a stipe that has a blackish, velvety or cracked cuticle already when young (except for *P. tubaeformis*). The blackened stipe of gr. *Melanopus sensu* Nuñez & Ryvarden, however, can also be found in aged gr. *Polyporellus spp*. and at the distal stipe attachment point of flabelliform *Favolus* and *Pseudofavolus* fruit bodies.

Corner (1984: 72) was of the opinion that *P. badius*, *P. dictyopus*, *P. blanchettianus,* and *P. melanopus* were merely varieties of one variable species. Our analyses, however, clearly separate *P. badius, P. dictyopus* and *P. melanopus* into distinct relationship groups. *Polyporus blanchettianus* was synonymized by Nuñez & Ryvarden (1995) under *P. dictyopus*, and could not be sequenced successfully. Our analyses cluster *Polyporus* whose basidiomatal caps have the ability to form dark chestnut to violaceous, blackish brown colors (*P. badius*, *P. melanopus*, *P. virgatus*) in topological proximity on phylogenetic trees. Notably, *P. varius* lacks this ability, and rather has the yellow-brown cap colors also seen in the genera *Mycobonia* and *Pseudofavolus* as well as *P. squamosus*, *P. radicatus*, and *P. tuberaster*.

Species of grs. *Melanopus* and *Polyporus* of Nuñez & Ryvarden (1995) were previously treated partly in *Favolus sensu* Fr., in *Polyporellus*, or Quélet's segregates *Leucoporus* (Quél. 1886, Ench. Fung.: 165) and *Cerioporus* (Quél. 1886, Ench. Fung.: 167). For example, *P. squamosus* could be treated as a *Favolus* because of the widely elongated pores, or as a *Melanopus* because of the blackish stipe. Our analyses best correspond to a clustering of mostly large-spored "polypores" (*Datronia*, *Pseudofavolus*, *Mycobonia*, *Squamosus* group – but includes also small-spored *P. varius*). In several of these, DK has observed occasional apparently septate basidiospores similar to those of Buchanan & Ryvarden's (1998) *P. septosporus* and *Dichomitus newhookii* (Buchanan & Ryvarden 2000). Figure 3 A shows spores of *Ps. cucullatus* (incl. septate spores). No septa were observed in *Mycobonia flava* (Fig. 3 B), but in *Dichomitus leucoplacus* again septate spores occured (Fig. 3 C). Strangely, *P. tuberaster* of gr. *Squamosus* appears to have dissimilar LSU phylotypes not correlated by ITS data and mating studies (PART 7) which may call for a more thorough mating study investigation.

Datronia mollis, which is similar in spores and hyphal construction to *Polyporus squamosus* but differs in having grayish brown, resupinate basidiomata with dendrohyphidia and cystidioles (Gilbertson & Ryvarden 1986-1987), appeared close to *P. squamosus*/*P. melanopus* in several analyses (Hibbett & Vilgalys 1993, Binder & Hibbett 2002: GenBank AF393071). This led us to sequence the LSU of *Datronia mollis* from Denmark (FB10177, TENN57707, GenBank: AJ488602). The latter LSU sequence was, however, very dissimilar and appeared close to *Trametes*, where Corner (1989: 18) mentioned *D. mollis* based on his

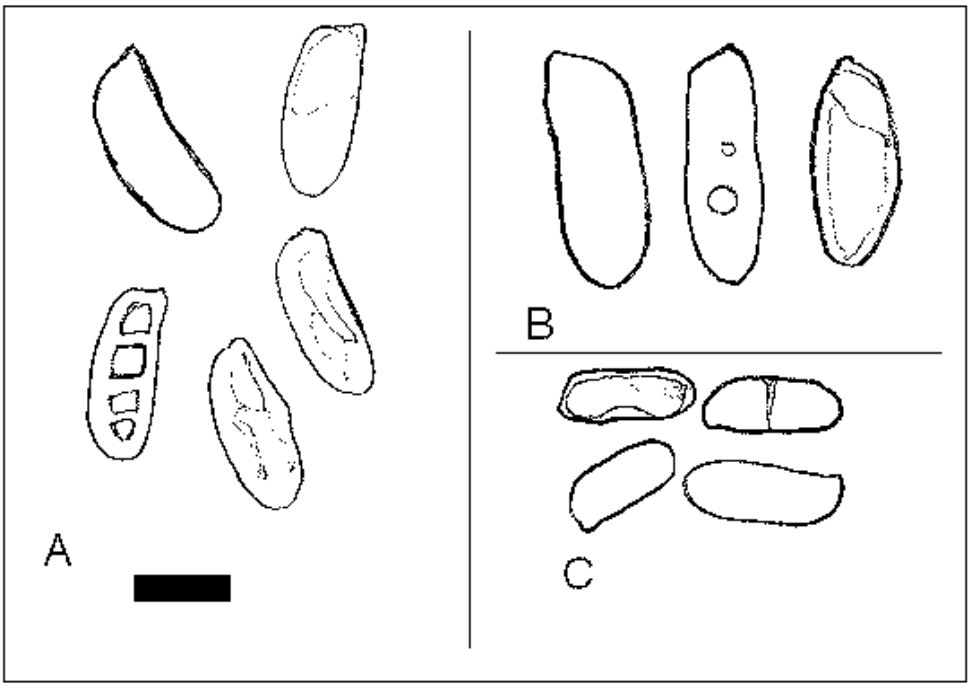


Figure 3. Basidiospores of three taxa allied with *Polyporus*.
A: *Pseudofavolus cucullatus* Ryv *s.n.* "20/7/96" (Mexico), aseptate and septate basidiospores. **B**: *Mycobonia flava* FB11279 (Argentina), basidiospores. **C**: *Dichomitus leucoplacus* Ryv33765 (Zimbabwe), aseptate and septate basidiospores. Scale bar: 10 µm.

finding of it being trimitic rather than dimitic. The ITS sequence of our collection FB10177, however, easily aligned with *Polyporus* and appears basal to *P. squamosus* (PARTS 6, 7). If this can be confirmed, *Datronia mollis* (Sommerf.: Fr.) Donk (= *Daedalea mollis* Sommerf.: Fr.) 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 (apparently a *Tyromyces*, see Cunningham 1948, also known as *Leptoporus mollis* (Pers.) Quél., 1886, Ench. Fung.: 176)]. At this time, we cannot explain why our LSU sequence differs so much from AF393071. However, we also note the topological distance in dendrograms containing *P. tuberaster* (FB10197 distant from FB10935 and AF393070), which is not supported by mating studies (of course, without including AF393070), and not supported by ITS sequence data to be published separately.

Dichomitus is typified by *D. squalens* (Karst.) Reid. This species fruits on conifers and has a hyphal construction that is different from other resupinate species like *D. campestris* in that its skeleto-ligative hyphae are much more branched (Gilbertson & Ryvarden 1986-1987: 238). Ryvarden & Johansen (1980) stated that *Dichomitus* was close to *Antrodia*. In discussing culture morphology by Nobles (1965) and potential affinity to *Polyporus* and *P. pseudobetulinus* in particular, Thorn *et al*. (1990) concluded that *Dichomitus* should be separate from *Polyporus* and potentially allied with *Trametes*. *Dichomitus campestris* was thought to be a resupinate derivate of *P. squamosus,* even having marginal remnants of the black stipe (Ryvarden 1991). Ryvarden (1991) and Nuñez (1993) suggested that *D. campestris* and the tropical *Dichomitus spp*. might be close to *Polyporus s. str*. Nuñez (1993) indicated a relationship of *D. squalens* with *Ganoderma* and *Amauroderma*.

Our DNA analyses confirm a close proximity of *D. squalens* to *Ganoderma* and *Trametes* in both NJ and MP analyses, although not well-supported. The other two sampled taxa, *D. campestris* and *D. gunnii*, are positioned near one of *P. tuberaster* sequences, in the larger /core-Polyporus. Neither appeared near the two included *Antrodia* sequences. We extrapolate that *Dichomitus* is indeed paraphyletic, and suggest using *Polyporus campestris* (Quél.) Krüger, *comb. nov*. (BASIONYM: *Trametes campestris* Quél. 1885, Champ. Jura Vosges 1: 271) unless this creates a later homonym. *Dichomitus gunnii* is not a resupinate fungus, but forms large, thick basidiomata with hexagonal pores, somewhat reminescent of *Daedalea quercina* in its robustness. *Dichomitus gunnii* (Berk.) Ryv. (= *Hexagonia gunnii* Berk. 1841, Ann. Mag. Nat. Hist. 7: 452), cannot be recombined into *Polyporus* without changing the epithet as there is also *Polyporus gunnii* [Berkeley 1860, Fl. Tasmaniae 2: 253 [= *Tyromyces campylus* (Berk.) Ryv. 1984, Mycotaxon 20: 333, see Cunningham 1965, Ryvarden 1984]. According to the CBS database maintained by J. Stalpers, Berkeley proposed a superfluous name, and *Polyporus vesparius* Berk. (1839, Ann. Mag. Nat. Hist. 3: 323) is the older name for *P. gunnii* Berk. 1860. Other species of *Dichomitus* require investigation before making taxonomic proposals.

Echinochaete (Echinochaetaceae Jülich 1981) has been seen as closely allied to, but not included in *Polyporus* by Corner (1984: 113) and Nuñez & Ryvarden (1995). Corner (1984: 113) argued for even closer ties to *Lentinus*, which our molecular data analyses do not support, but leave room for great improvement in *Lentinus* sampling as we have only *Lentinus* subg. *Lentinus* included. The one sampled species indeed appears nested within the large relationship group of grs. *Melanopus*/*Squamosus* (i.e. /core-Polyporus). *Echinochaete brachyporus* also has been shown (Petersen & Cifuentes-Blanco 1998) to have culture morphologies and a tetrapolar mating system unrelated to flat-barrage reactions as reported in *Polyporellus* (Hoffmann 1978). *Echinochaete* has setal elements which may have evolved from inflated skeleto-ligative hyphae as depicted in Nuñez & Ryvarden (1995: 13) for *P. diabolicus* (= *P. dictyopus*), a character which earlier led to the proposal of the genus name *Atroporus* (Ryvarden (1973). We were unable to sequence members of the segregate genus

Laccocephalum, also considered as closely related to *Polyporus* (Nuñez & Ryvarden 1995). Ryvarden (1991) did not subscribe to *Laccocephalum* being a separate genus.

Pseudofavolus, Mycobonia

Corner (1984: 35) rejected *Pseudofavolus* as a necessary segregate genus, stating that Ryvarden & Johansen's (1980) distinctions of dextrinoid hyphae, dendrohyphidia, large spores, and thin context could all be found in *Polyporus s. str.* (*sensu* Corner). Our analyses show *Pseudofavolus cucullatus* as nested within /core-Polyporus, with moderate support in the distance-based analyses (Fig. 1). In both phylogenetic trees (Figs. 1, 2), *Pseudofavolus* is close to *Mycobonia* and to *Polyporus tuberaster* FB10197.

Singer (1949: 269, 284) along with the inclusion of Polyporaceae in Agaricales, stated 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 (1981: 186) removed *Mycobonia* from Thelephoraceae (see Donk 1957: 83) and proposed Mycoboniaceae within the Polyporales. Singer (1986: 163) synonymized Mycoboniacae with Polyporaceae. Corner (1984: 36) stated that the *Pseudofavolus* group was most closely related to *Mycobonia*. Accepting two species of *Mycobonia* (*M. brunneoleuca* and *M. flava*), he stated (Corner 1984: 104) that *M. flava* was merely *Pseudofavolus miquelii* without the pores. 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). Unfortunately, Nuñez & Ryvarden (1995: 18) mistakenly reported *Mycobonia* as being poroid agarics.

We also found the striking resemblance of the hexagonal-pored *Pseudofavolus cucullatus* and apparently hydnoid *Mycobonia flava* in coloration, hyphal construction, basidia, and spores. The hymenophore of *M. flava* is reduced to a flat surface as in corticioid and thelephoroid fungi, and the pore walls appear to have become isolated hyphal peg fascicles. Both *P. cucullatus* and *M. flava* have spores and basidia as large as those of *P. squamosus* and other *Polyporus s. str.* (= gr. *Squamosus*) and *Dichomitus* species. Our analyses always recovered *Mycobonia* next to *Pseudofavolus*, both nested within *Polyporus s*. *str*., and BioEdit calculated the LSU sequences to be 98–99% similar to each other. Scattered septate spores occurred in *Pseudofavolus cucullatus* (Fig. 3A), as are in *Polyporus septosporus*. The latter appears to us to be morphologically similar to *P. squamosus/P. udus,* but could not be sequenced.

The taxonomy of *Mycobonia* was also discussed by Martin (1939), where *M. brunneoleuca* was conceived as synonymous with *M. flava*. Corner (1984: 103-104) kept the two species apart. 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*. As we do not know *Pseudofavolus miquelii*, we opt not to reject *Ps. cucullatus*. Therefore, we must 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 touching on *Mycobonia brunneoleuca*, which we do not know, we propose the transfer of *Mycobonia flava* as a subspecies to *P. curtipes*.

Polyporus Adans.: Fr. *emend*. Krüger
Accepted as circumscribed by Nuñez & Ryvarden (1995), 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 Krüger, *comb. nov.*
BASIONYM: *Peziza flavus* 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.

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PART 3

Studies in *Polyporus* subg. *Polyporellus*: on congruence of three biological, morphological and phylogenetic species.

The following article was written by Dirk Krüger with proofreading by Ronald H. Petersen and Karen W. Hughes and has been accepted for publication. It is reproduced here with formatting required by the Graduate School at the University of Tennessee, and minor editing, e.g. correction of previously undetected errors in the submitted manuscript. The title would have been more appropriately indicated *Polyporellus* unranked infrageneric group. The addendum (following as PART 5) includes data collected on the same topic since submission of the manuscript. The alignment has since been posted as ALIGN_000386, retrievable from ftp://ftp.ebi.ac.uk/pub/databases/embl/align/.

Reference:
Krüger D, Hughes KW, Petersen RH. 2002. Studies in *Polyporus* subgenus *Polyporellus*. In: Laflamme G, Bérubé JA (Eds). Proceedings of the 10th International IUFRO Conference on Root and Butt Rots, September 17-19th 2001, Québec City QC. Canadian Forest Service Information Report. In press.

Abstract: The white-rotting polypore genus *Polyporus* comprises several infrageneric groups or subgenera, including *Polyporellus*, which in turn contains several similar morphological species. Crosses using monokaryotic single-spore isolates of *Polyporus arcularius*, *P. brumalis*, and *P. ciliatus* obtained from different geographic locations failed to uncover cryptic biological species. ITS rDNA sequences provided additional information on the phylogeny of these closely related members of *Polyporellus*.

Keywords: Basidiomycotina, sexual compatibility, molecular systematics, ribosomal DNA.

INTRODUCTION

The group *Polyporellus* contains, in part, three morphotaxa widespread in the Northern Hemisphere: *P. arcularius*, *P. brumalis*, and *P. ciliatus* (Nuñez & Ryvarden 1995). *P. arcularius* is nearly cosmopolitan, but does not extend into the boreal realm. For example, in Germany there is a northern limit reported by Conrad *et al*. (1995). *P. brumalis* is circumhemispheric in Eurasia and North America, and *P. ciliatus* has been reported from temperate Eurasia. All three species have also been reported from South America (Popoff & Wright 1998 for *P. arcularius* and *P. ciliatus*, Nuñez & Ryvarden 1995: by virtue of accepting synonyms to *P. brumalis* and *P. ciliatus* from Spegazzini).

Spanish and Costa Rican collections of *P. arcularius* have previously been shown to belong to the same intercompatibility group (Nuñez 1993). Likewise, Hoffmann (1978) reported collections of *P. ciliatus* from Europe and North America to belong to one interfertility group (Hoffmann used both clamp connections and formation of fruit bodies as indicator of compatibility) and collections of *P. brumalis* from Europe, North America, and India to form another interfertility group. There has been nomenclatural confusion in *P. arcularius*, *P. brumalis*, and *P. ciliatus*, and names have been misapplied (see Jahn 1969; Kreisel 1963). Based on mating study results, Hoffmann (1978) assigned two German and one Canadian collection obtained as *P. brumalis* to *P. ciliatus*. For Hoffmann (1978) all three taxa (with one strain of *P. arcularius* included) were mutually INcompatibile.

A particular question is whether *Polyporus ciliatus* occurs in North America, as claimed by Hoffmann (1978) and herbarium labels at DAOM, or in South America, as indicated by

Popoff & Wright (1998). Nuñez & Ryvarden (1995) regarded *P. ciliatus* as unknown from North America. We were also interested in addressing the phylogeny of *Polyporellus*, and whether different morphospecies correspond to single biological and phylogenetic species entities.

Here we report current progress in our research on *Polyporellus* as we collect the initial framework of data to answer the above problems, including an assessment of the suitability of the use of nuclear ITS rDNA data for the questions of interest.

MATERIALS & METHODS

Specimens, establishment & maintenance of cultures

Our own collections were assigned field book numbers, annotated, dried for preservation, and accessioned into TENN (Holmgren *et al*. 1981). Phase contrast microscopic observations were undertaken after squash mounting of herbarium specimens in 3% w/v KOH and phloxine dye, at 400 x magnification. Identification based on morphological characters was accomplished with the aid of keys by Gilbertson & Ryvarden (1986-1987), Ryvarden & Gilbertson (1993-1994), and Nuñez & Ryvarden (1995). In some cases, colleagues furnished spore prints from which cultures were obtained.

Techniques for establishing monokaryotic single-basidiospore isolates (SBIs) were described by Gordon & Petersen (1991). Dikaryon cultures were established for a number of collections as described by Petersen & Hughes (1997). Monokaryon and dikaryon cultures were stored on agar disks of malt extract agar (MEA: 1.5% w/v Difco malt extract, 2% w/v Difco Bacto-agar, Nobles 1965) in sterile water in microvials (Burdsall & Dorworth 1994). Occasional bacterial contamination was overcome by passage through MEA plates supplemented with 1.54 mM chloramphenicol (Calbiochem 220551) and 10.67 µM streptomycin sulfate (Sigma S0890).

In order to obtain fruit bodies for herbarium specimens and/or monokaryon cultures, fruiting of dikaryon cultures on *Fagus*, *Acer*, *Juglans*, or *Betula* wood chips followed the procedure of Psurtseva & Mnoukhina (1998). Additional dikaryotic cultures were obtained from culture collections, and specimens or DNA extractions from other sources below. The following list of specimens, ordered alphabetically by country of origin, is read as: COUNTRY. Infracountry. Notes. *Collector* and *date.* **Field book number** or other number obtained elsewhere **/ TENN number** or other collection number if available **(GenBank number, with SBI number if applicable)**. Other acronyms: CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. DAOM = "Department of Agriculture, Ottawa, Mycology" (National Mycological Herbarium, Ottawa, Canada). DSH = D. S. Hibbett collection number. DSMZ-H = cultures used by Hoffmann (1978) and kept at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. O = University of Oslo herbarium, Oslo, Norway. SBUG-M = "Sektion Biologie Univ. Greifswald - Myzelpilze" (Univ. Greifswald, Germany, fungal culture collection). VT = "Virginia Tech".

P. arcularius Batsch: Fr.
AUSTRIA. Niederösterreich: Kaltenleutgeben. On *Fagus*. *H. Voglmayr Apr 04 1999*. **10299/TENN58370 (**SBI2: **AB070865**, SBI4: **AB070866)**. / CANADA. Ontario: Rondeau Prov. Park. On *Tilia*. *R. G. Thorn May 22 1983*. **CBS 223.91/RGT830522/01 (AB070858)**. / COSTA RICA. Cartago: km 66 of Interamerican Highway. *R. Petersen Jul 01 1998*. **9473/TENN56447**. / CHINA. Guizhou. *R. Petersen Aug 31 1991*. **4124/TENN50834 (**SBI1: **AB070863**, SBI2: **AB070864)**. / GERMANY. Mecklenburg-Vorpommern: Usedom. On *Fagus*. *R. Bütow Jun*

1997. **SBUG-M1244** (fruited for obtaining specimen/spores) **/TENN58529**, **58569**, **58588 (AB070861)**. / SOUTH AFRICA. Kruisfountein. On *Olea*. *P. Talbot Nov 1955*. **DAOM 94067 (from PRE52) (AB070859)**. / USA. Florida: Welaka. On wood chips. *E. Lickey Mar 19 2001*. **10975/TENN58890**. / USA. Louisiana: Baton Rouge. *D. Sime May 21 1997*. **9076/TENN54876**. / USA. Louisiana: Lafayette. *R. Petersen May 23 1997*. **9101/TENN54925**. / USA. Louisiana: West Feliciana. *E. Lickey May 26 1997*. **9214/TENN55948**. / USA. Tennessee: Tremont. *H. Voglmayr Apr 02 2000*. **10929/TENN58412 (**SBI1: **AB070867**, SBI2: **AB070868)**. / USA. Tennessee: Knoxville. *H. Voglmayr Apr 04 2000*. **10930/TENN58438**. / USA. Texas. On *Pinus*. *J. L. Mata Jun 10 2000*. **10477/TENN58540**. / USA. Texas. *A. Methven Jun 10 2000*. **10693/TENN58779**. / Extraction from D. S. Hibbett (Hibbett & Donoghue 1995). **DSH92.144 (AB070860)**. / Extraction from D. S. Hibbett (Hibbett & Vilgalys 1993). **VT959 (AB070862)**.

P. brumalis Pers.: Fr.
AUSTRIA. *I. Krisai-Greilhuber Sep 28 1996*. **8037/TENN55596**. / AUSTRIA. *I. Krisai-Greilhuber Jun 10 1996*. **8038/TENN55597**. / AUSTRIA. Niederösterreich: Muckendorf. *I. Krisai-Greilhuber & H. Voglmayr Oct 04 1998.* **10123/TENN57347**. / AUSTRIA. Oberösterreich: St. Willibald. On *Betula*. *H. Voglmayr Oct 23 1999*. **10666/TENN58382**. / AUSTRIA. Oberösterreich: Kirchschlag. On *Sorbus*. *H. Voglmayr Nov 01 1999.* **10667/TENN58383**. / CANADA. Quebec: Kingsmere. *J. W. Groves Oct 31 1955*. **DAOM 31983 (AB070869)**. / CANADA. Quebec: Kingsmere. *J. W. Groves Oct 31 1955*. **DSMZ-H17 (from DAOM 31983) (AB070870)**. / DENMARK. Roskilde Amt: Lellinge. On *Fagus*. *H. Knudsen May 18 1999*. **10169/TENN57700 (**SBI1: **AB070872**, SBI3: **AB070873)**. / DENMARK. Storstrøms Amt: Fakse. On *Fraxinus*. *R. Petersen May 19 1999*. **10178/TENN57708**. / GERMANY. Mecklenburg-Vorpommern: Malchow. On *Fagus*. *D. Krüger May 09 1999*. **10147/TENN57678**. / GERMANY. Mecklenburg-Vorpommern: Neustrelitz. On *Fagus*. *D. Krüger Dec 28 1999*. **10908/TENN58391 (**SBI4: **AB070876**, SBI5: **AB070877)**. / NORWAY. Oslo. On *Betula*. *D. Krüger Mar 25 2000*. **10917/TENN58400**. / NORWAY. Telemark: Grasdalen. On *Sorbus*. *A.-E. Torkelsen May 23 1972*. **O92301 (AB070871)**. / RUSSIA. On *Alnus*. *R. Petersen Sep 21 1996*. **8971/TENN55631**. / USA. Alaska: Anchorage. On *Alnus*. *R. Petersen Sep 09 1995*. **7992/TENN53984** (was identified as *P. ciliatus*). / USA. Alaska: Anchorage. On *Alnus*. *R. Petersen Sep 11 1995*. **8122/TENN53936** (was identified as *P. ciliatus*). / USA. Tennessee. On *Fagus*. *K. McFarland Nov 07 1999*. **10665/TENN58381 (**SBI1: **AB070874**, SBI2: **AB070875)**. / USA. West Virginia. *A. Kovalenko Sep 30 2000*. **10964/TENN58828**. / USA. West Virginia. *A. Kovalenko Sep 30 2000*. **10965/TENN58827**.

P. ciliatus Fr.
AUSTRIA. Niederösterreich: Hainburg. On *Populus*. *I. Krisai-Greilhuber & H. Voglmayr Apr 29 1999*. **10300/TENN58371**. / DENMARK. Ribe Amt: Billund. *J. Vesterholl May 14 1999*. **10507/TENN57737**. / DENMARK. Roskilde Amt: Lellinge. *R. Petersen, H. Knudsen & D. Krüger May 18 1999*. **10165/TENN57696**. / DENMARK. Roskilde Amt: Lellinge. On *Acer*. *H. Knudsen & R. Petersen May 18 1999*. **10167/TENN57698 (**SBI9: **AB070882,** SBI10: **AB070883)**. / DENMARK. Roskilde Amt: Lellinge. On *Fagus*. *H. Knudsen & D. Krüger May 18 1999*. **10168/TENN57699**. / FINLAND. Etelä-Häme: Evo. *R. Petersen Sep 13 1994*. **7480** (fruited for obtaining spores to make up for lost monokaryons) **/TENN53639, 58441, 58823 (**SBI2: **AB070880**, SBI3: **AB070881)**. / GERMANY. Baden-Württemberg: Tübingen. On *Betula*. *D. Krüger May 31 1999*. **10521/TENN57751**. / GERMANY. Mecklenburg-Vorpommern: Malchow. On *Quercus*. *D. Krüger May 05 1999*. **10149/TENN57680**. / GERMANY. Mecklenburg-Vorpommern: Zislow. On buried *Betula* wood. *D. Krüger May 05 1999*. **10151/TENN57682**. / GERMANY. Mecklenburg-Vorpommern: Neustrelitz. On *Fagus*. *D. Krüger May 13 1999*. **10156/TENN57687**. / GERMANY. Mecklenburg-Vorpommern: Greifswald. On *Fagus*. *M. Scholler & D. Krüger May 22 1999*. **10181/TENN57711**. / GERMANY. Mecklenburg-Vorpommern: Carpin, Nature Reserve Serrahn. *R. Petersen May 25 1999*. **10318/TENN57966**. / GERMANY. Mecklenburg-Vorpommern: Zwenzow, Krummer See. *D. Krüger May 26 1999*. **10320/TENN57968**. / GERMANY. Mecklenburg-Vorpommern: Carpin,

Nature Reserve Serrahn. On *Fagus*. *D. Krüger May 25 1999*. **10508/TENN57738**. / SWEDEN. Uppland: Tarnby Lund. On *Betula*. *R. Petersen Sep 04 1994*. **7257/TENN53619 (**SBI2: **AB070878**, SBI7: **AB070879)**.

P. tricholoma Mont. (included in phylogeny)
COSTA RICA. Heredia: Chilimate. On buried wood. *R. Petersen Mar 13 1999*. **10240/TENN57563 (AB070885)**. / COSTA RICA. Heredia: Chilimate. *R. Petersen Mar 13 1999*. **10241/TENN57564 (AB070888)**. / MEXICO. Chiapas. Small dead angiosperm branchlets. *R. Petersen Oct 18 1997.* **3870/TENN55844 (AB070884)**. / USA. Puerto Rico: Palmer. Hardwood log. *R. Petersen Jun 09 1998*. **9568/TENN56481 (AB070887)**. / USA. Puerto Rico: Palmer. Hardwood log. *R. Petersen Jun 12 1998*. **9591/TENN56503 (AB070886)**.

P. alveolaris (DC.: Fr.) Bondartsev & Singer (*Favolus* group; outgroup in phylogeny)
Extraction from D. S. Hibbett (Hibbett & Vilgalys 1993). **DSH 90.36 (AB070828)**.

Mating experiments

Self-cross pairings for determination of mating types and tester strains were made among 12 randomly selected monokaryotic SBIs of at least one collection per species. Tester strains are SBIs with known mating type assigned for later testing of new arrivals. Subtester strains are auxiliary tester monokaryons selected later from collections of a different geographic origin, without prior knowledge of the actual mating type of the SBI. The technique for self-crosses was described by Petersen (1992). For intercollection pairings, four SBIs (randomly selected, or testers/subtesters when assigned) were paired with four SBIs (randomly selected, or testers/subtesters when assigned) from other collections in this study, thus having 4 plates per pairing.

DNA extraction

DNA was extracted using a CTAB method (either modified from Carlson *et al*. 1991, see Hughes *et al*. 1999; or modified from Doyle & Doyle 1987; Zolan & Pukkila 1986; see Krüger *et al*. 2001), SDS-based method (Lee & Taylor 1990), or more recently, with a xanthogenate/SDS miniprep protocol modified from Tillett & Neilan (2000). All methods gave approximately equivalent results and were not always effective. In the CTAB methods, 10 to 50 mg of herbarium material, or less of hyphal material scraped off MEA plates, was placed in a 1.5 ml reaction tube with 0.5 ml prewarmed (65° C) CTAB extraction buffer (0.1M Tris, 0.2 M Na_2EDTA, 1.5 M NaCl, 55 mM CTAB = hexadecyltrimethylammonium bromide, Sigma H5882). The material was incubated for 30 to 60 min at 65° C in a 1.5 ml tube and shaken occasionally before being ground with a sterilized plastic mini pestle (Kontes Pellet Pestle®, Kontes 749520).

For tough carpophores of herbarium specimens, grinding was supported with sterile sand, and the material repeatedly frozen and heated (3 cycles of 10 min at -80° C/10 min at 65° C (Vrålstad *et al*. 2000). Here, a 3% w/v SDS (sodium dodecyl sulfate, Sigma L4509), 1% w/v mercaptoethanol extraction buffer (500 µl, 65° C) was used instead of CTAB extraction buffer.

With either extraction procedure, approximately the same volume of 24:1 chloroform:isoamyl alcohol was added, the mixture vortexed briefly, and spun at 12,000 rpm for

four min. The upper, clear phase was transferred to a new 1.5 ml reaction tube and mixed with an equal volume of isopropyl alcohol (4° C). The reaction tube contents were mixed, refrigerated (4° C) for one hour, and centrifuged for four min at 13,000 rpm. The resulting pellet was rinsed twice in 70% v/v ethyl alcohol, air-dried, and resuspended in 100 µl TE buffer (10 mM TrisHCl, 1 mM Na_2EDTA; pH 8.0).

DNAs were also extracted from fungal tissue using a modified xanthogenate protocol (Tillett & Neilan 2000). Fresh material was soaked for several weeks at 4° C in CTAB/sodium azide preparations (after Rogstad 1992: 6 M NaCl, 3 mM NaN_3, 41.1 mM CTAB). Alternatively, fresh or herbarium material was stored in SDS buffer (50 mM Tris/HCl, 50 mM Na_2EDTA, 10% w/v SDS, pH 7.2). Ten to 50 mg of such material was ground in 50 µl TE buffer with a small amount of sterile sand in a 1.5 ml microfuge tube as described above. For material grown 2-8 weeks at room temperature in malt extract (ME) broth (ca. 10 ml 1.5% w/v Difco malt extract, in baby food jar), 250-500 mg of material was filtered, blotted dry, then ground. After addition of 50 µl TE extraction buffer, a minipestle mounted on a drill was used to grind the material. Following grinding, 750 µl of potassium ethyl xanthogenate buffer (100 mM Tris/HCl pH 7.2, 20 mM Na_2EDTA pH 8.0, 1% w/v SDS, 800 mM ammonium acetate, 1% w/v $C_3H_5KOS_2$ = potassium ethyl xanthogenate: Fluka 60045) was added. The tube contents were vortexed, and incubated at 70° C for 60 min, with occasional vortexing. After a final, vigorous vortex for 10 sec, samples were placed on ice for 30-60 min, and then centrifuged at 14,000 rpm for 10 min. The supernatant was recovered, and DNA precipitated with 750 µl isopropyl alcohol (80% v/v, 4° C), and spun at 10,000 rpm for 10 min. The alcohol was aspirated, and the pellet was washed with 250 µl 95% v/v cold ethyl alcohol, spun again at 10,000 rpm for 10 min. Remaining alcohol was then pipetted off, removed with a piece of paper towel, and evaporated 2 min at 70° C on a heating block. The pellet was then resuspended in 50 µl TE buffer supplemented with 2 µl RNAse Plus (5 Prime - 3 Prime, Inc., now Eppendorf-5 Prime, Inc.), and incubated 10 min at 50° C.

PCR & sequencing

DNA selected for sequencing was primarily monokaryotic in origin. For tester and subtester isolates we attempted to sequence two monokaryons for each collection. The nuclear ribosomal ITS I – 5.8S – ITS II region was amplified with primers ITS 1F and ITS 4B (Gardes & Bruns 1993). A 50 µl reaction contained 1 X buffer supplied by manufacturer [includes $MgCl_2$ (EPWMG) in case of QBioGene polymerase kit, or separate 3 mM $MgCl_2$], 0.8 mM each dNTP, 0.2 ng/µl of bovine serum albumin (Sigma A7906), 0.2 mM primer each, 1.1 units *Taq* Polymerase (QBioGene EPTQA023; for difficult reactions: TaKaRa ExTaq ^{TM} kit used after manufacturer's instructions, PanVera). Parameters were as follows: initial denaturation 94° C/3 min, followed by 35 cycles of denaturation 94° C/1 min, annealing 52° C/1 min, extension 72° C/1 min. The final extension was 72° C/3 min, followed by an indefinite storage step at 4° C. PCR products were electrophoresed in a 1.5% w/v agarose/TBE gel. In spite of secondary bands, amplified DNA product was not excised, but used after cleaning with a Microcon-PCR device kit (Millipore), according to manufacturer's instructions. This seemed appropriate because disappearance after treatment with restriction enzymes *Bfi*I (MBI Fermentas) and *Nci*I (New England Biolabs) in *Polyporus tricholoma* proved those bands to be due to secondary structure. Internal primers ITS 5 and ITS 3 (White *et al.* 1990) were used as sequencing primers. Cycle-sequencing was performed with the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin-Elmer), following manufacturer's instructions and using approximately 200 ng DNA template.

Sequence data

Sequences were corrected using Chromas v. 1.45 (Conor McCarthy; Griffith University, Southport, Australia) for viewing and manipulating ABI electropherograms and Programmer's File Editor v. 0.07.002 (Alan Phillips, Lancaster University, UK). Sequence alignment was done with ClustalX 1.64b (Thompson *et al*. 1997), followed by visual confirmation and manual optimization. The neighbor-joining (Saitou & Nei 1987) algorithm as implemented in ClustalX was used for initial phylogenetic analysis (ClustalX by default produces a tree with each alignment). A NEXUS file (Maddison *et al*. 1997) was manually generated for use in PAUP*4.0b8 (Swofford 2001). Gaps were coded as missing after trying alternative treatment modes. Heuristic searches were performed both in 20% deletion jackknife (Efron & Gong 1983) and bootstrap (Felsenstein 1985) resampling analyses (TBR swapping, MAXTREES set to 1,000, 100 random taxon addition repeats per resampling pseudoreplicate). TreeVIEW 1.5 (Page 1996) was used to view and manipulate trees. Sequences were submitted to EMBL/GenBank/DDBJ databases using the DDBJ Sakura submission system.

RESULTS

Mating studies & culture morphology

We confirmed a tetrapolar mating system for *P. arcularius* (Vandendries 1936a, Hoffmann 1978), *P. brumalis* (Vandendries 1936b, Hoffmann 1978) and *P. ciliatus* (Hoffmann 1978, Petersen *et al*. 1997). We selected the following tester strains for later research*: P. arcularius* field book number 10299: A_1B_1 (SBI1), A_1B_2 (SBI4), A_2B_1 (SBI3), A_2B_2 (SBI2); *P. brumalis* field book number 10908: A_1B_1 (SBI1), A_1B_2 (SBI4), A_2B_1 (SBI8), A_2B_2 (SBI5); and *P. ciliatus* field book number 10167: A_1B_1 (SBI11), A_1B_2 (SBI9), A_2B_1 (SBI10), A_2B_2 (SBI12). Results of the intercollection pairing experiments are shown in Fig. 1.

Cultures of newly isolated *Polyporellus* SBIs tended to form carpophore primordia on MEA plates and in ME broth (also observed by Hoffmann 1978), a phenomenon that seems to disappear after one passage through storage in water vials. Brown crusts were formed, but were less apparent than in the *Melanopus* group. Conidiogenesis occured, but was only occasionally oserved, unlike in the *Squamosus* and *Melanopus* groups, where most or all aerial hyphae were sometimes converted to arthroconidia. The "Border Zone" mentioned by Hoffmann was not distinguished by us from barrage-like non-self contact zones. Several SBIs of *P. arcularius* field book number 10929 and most of *P. brumalis* field book number 8971 tended to form "halos" around the inoculation block where hyphae lay appressed on the agar surface, and more aerial growth beyond the halo. "Barrage" and "flat" reactions were not useful in assigning mating types, as mentioned by Petersen *et al*. (1997), and as explained by the control through mating-type independent genes *bfI*, *bfII*, and *bi* (Hoffmann 1978). Several older SBIs of *P. ciliatus* (used in Petersen *et al.* 1997) appeared to have lost viability in the course of repetitive work with them over several years (few aerial hyphae, only thin repent hyphae on wetted agar surface), resulting in replacement of tester stocks.

1A

P. arcularius

Collection		1	2	3	4	5	6	7	8	9	10	11
China	1 (4124)		25	25	50	100	50	100	100	100	100	100
Austria	2 (10299)			75	0	50	50	75	100	75	50	25
Germany	3 (SBUG-M1244)				50	100	100	75	75	x	x	x
Costa Rica	4 (9473)					100	100	100	75	x	x	x
USA	5 (9214)						100	100	100	x	x	x
USA	6 (9076)							100	100	x	x	x
USA	7 (10930)								100	x	x	x
USA	8 (10929)									100	50	100
USA	9 (10477)										x	x
USA	10 (10693)											x
USA	11 (10975)											

P. brumalis

Collection		16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
16 (8122)	USA																
15 (7992)	USA	75															
14 (10965)	USA	x	x														
13 (10964)	USA	x	x	x													
12 (10665)	USA	75	100	100	100												
11 (10178)	Denmark	x	x	x	x	100											
10 (10169)	Denmark	x	x	100	100	75	50										
9 (10917)	Norway	x	x	x	x	100	100	75									
8 (10908)	Germany	100	50	100	100	100	75	0	100								
7 (10147)	Germany	x	x	x	x	100	x	100	x	100							
6 (10667)	Austria	x	x	x	x	100	100	25	100	100	x						
5 (10666)	Austria	x	x	x	x	100	100	50	100	100	x	75					
4 (10123)	Austria	x	x	x	x	100	100	50	100	100	x	75	100				
3 (8038)	Austria	x	x	x	x	75	x	100	x	50	x	x	x	x			
2 (8037)	Austria	x	x	x	x	100	100	75	100	75	x	100	100	75	x		
1 (8971)	Russia	x	x	x	x	100	x	100	x	50	x	x	x	x	x	x	

Figure 1. Schematic overview of mating compatibility. **A.** *Polyporus arcularius* (upper triangle) and *P. brumalis* (lower triangle). Major geographic regions separated by broader cell boundaries. Numbers on upper and lower end of image correspond to the collections (field book numbers) left and right-hand. X = not tested. 100 = 4/4 pairings form clamps, 75 = 3/4, 50 = 2/4, 25 = 1/4, 0 = 0/4. Continued on next page.

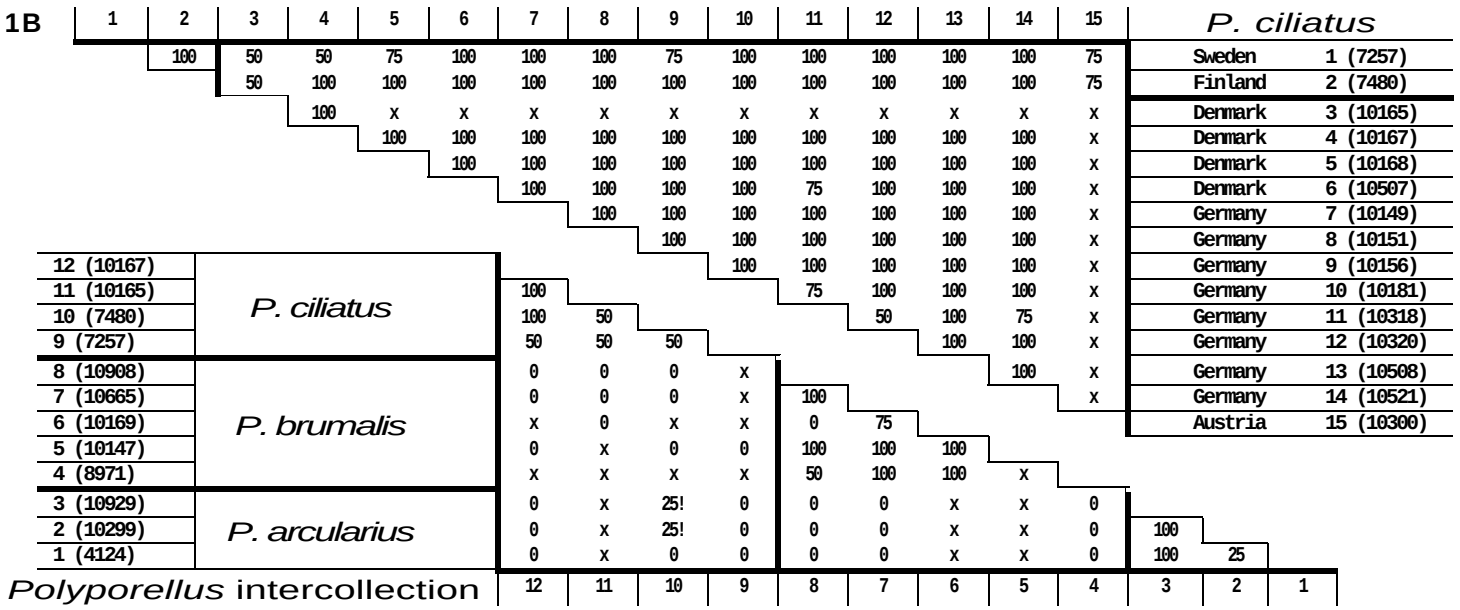

Figure 1. Continued. Schematic overview of mating compatibility. **B.** *Polyporus ciliatus* (upper triangle) and cross-species within three taxa (lower triangle). Major geographic regions separated by broader cell boundaries. Numbers on upper and lower end of image correspond to the collections (field book numbers) left and right-hand. X = not tested. 100 = 4/4 pairings form clamps, 75 = 3/4, 50 = 2/4, 25 = 1/4, 0 = 0/4. ! = unexpected formation of clamps, confirmed by a repetition.

Molecular phylogeny

Sequence analyses were based on 610 aligned characters, of which 462 were stable, 75 variable but parsimony-uninformative, and 73 parsimony-informative. In the ITS 1, ITS 2 and 5.8S downstream (= 3') end regions, numerous ambiguous positions were found, but were left as ambiguous base codes in the analyses reported here. It is not yet known how much of the ambiguity stems from PCR artifacts or to what extent the rDNA repeat may not be completely homogenized.

Results of the neighbor-joining (NJ) function in ClustalX performed with 1,000 bootstrap repeats are given in Fig. 2. NJ bootstrap support (out of 1,000 trees, only if above 500) is given next to nodes. This dendrogram was rooted with *P. alveolaris*, and also gives the PAUP* MP jackknife and bootstrap supports for major nodes, combining results from MP and NJ analyses. Nodes not showing PAUP* heuristic support values were either not recovered at all in majority consensus of trees found by these parsimony methods, or were recovered at a percentage lower than 50. *P. brumalis* consisted of two NJ-clades (low NJ bootstrap support) in a trichotomy with *P. arcularius*, but was found as one single clade in PAUP* resampling analyses. This clade itself is collapsed, but supported with 99% jackknife value vs. 89% bootstrap value. In general, jackknife values are higher than bootstrap values. With high resampling support values, *P. arcularius*, *P. brumalis*, and *P. ciliatus* clades are consistently recovered with the different cladistic methods (NJ: bootstrap, MP heuristic: bootstrap, jackknife) employed.

A PAUP* bootstrap analysis with 100 pseudoreplicates was unable to perform more than one random taxon addition repeat (instead of 100 intended) in each pseudoreplicate as the buffer of 1,000 trees was reached within few sec. The 50% majority rule consensus scored with CI = 0.840, HI = 0.160, RI = 0.890, RC = 0.747. The 100 pseudoreplicates jackknife resampling analysis on PAUP*, based on 20% deletion (122 actual characters) also could not run the intended 100 random taxon addition replicates for the same reason as mentioned for the bootstrap. Its 50% majority rule dendrogram had following scores: CI = 0.687, HI = 0.313, RI = 0.900, RC = 0.767.

Initially we tried performing the analysis with GAPMODE = newstate. Here we reduced gaps longer than one character to one position. Several gap-carrying positions with unclear homology were excluded, and in one case those were treated as missing data. All these analyses converge on the same topology of above major clades in a majority rule consensus of 1,000 heuristic bootstrap analysis trees in PAUP*. Therefore GAPMODE = newstate was not used for time-consuming resampling analyses.

CONCLUSIONS

Morphology

The three morphospecies may be successfully separated using keys and descriptions by Gilbertson & Ryvarden (1986-1987), Ryvarden & Gilbertson (1993-1994), and Nuñez & Ryvarden (1995), although we experienced some difficulty with aged carpophores. When in doubt, availability of mating experiments allowed assignment to the species.

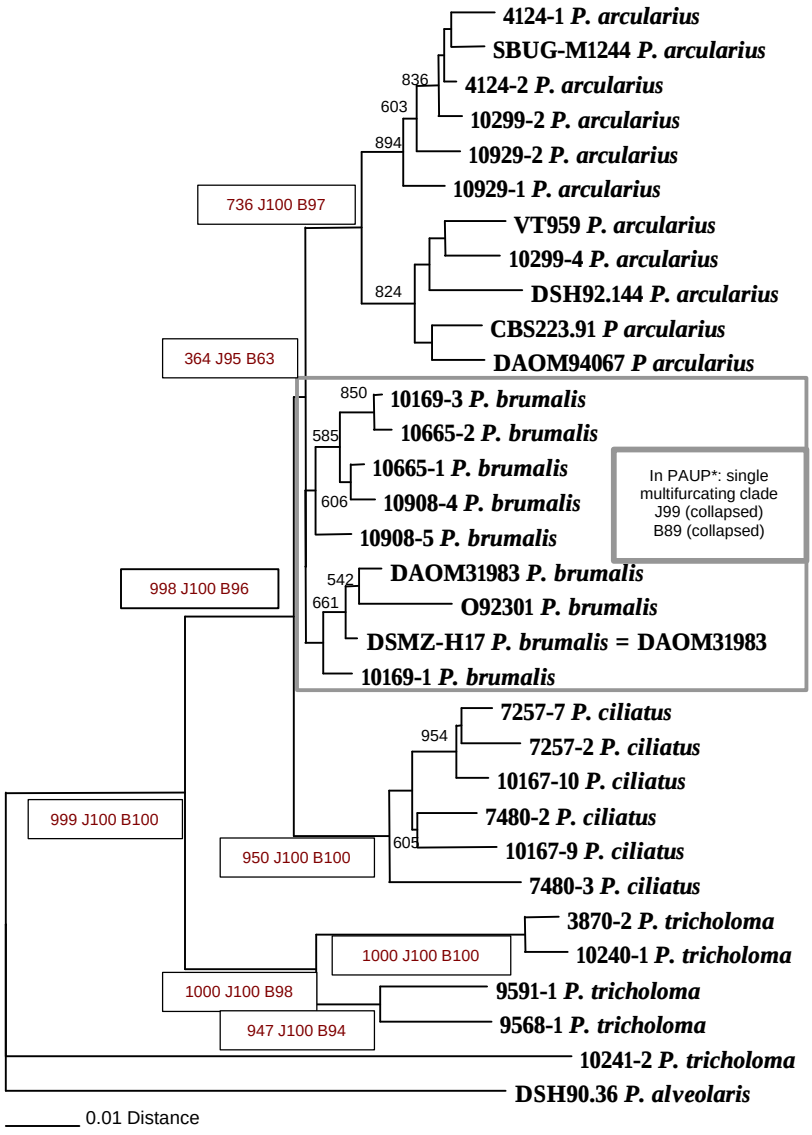

Figure 2. ClustalX NJ dendrogram. SBI number following field book number for sequenced monokaryons is given (e.g. 9591-1: 1 denotes SBI1 of field book number 9591). NJ bootstrap support (out of 1,000) is given at left of the node. Boxes at major nodes contain following values: first number: ClustalX NJ bootstrap support, second number: parsimony analysis using jackknife resampling: jackknife (J) support as a percentage, third number: parsimony analysis using bootstrap resampling: bootstrap (B) support as a percentage. Gray box around *P. brumalis* indicates two NJ clades collapsing to one single, polytomous clade in PAUP* parsimony resampling analyses.

Compatibility

Polyporus arcularius, *P. brumalis*, and *P. ciliatus* were each shown to have a tetrapolar mating system. Each morphospecies is congruent with one biological species by virtue of clamp formation in mate recognition. Two exceptional formations of clamps in the contact zone of plates with *P. arcularius* X *P. ciliatus* (Fig. 1B, note "!") were confirmed in a repetition. Aside from this, no other compatibility was detected across morphospecies boundaries.

Molecular phylogenetic reconstruction

In *P. arcularius*/*brumalis*/*ciliatus* we found that phylogenetic species confirmed the biological species entities. The major phylogenetic groups are recovered with NJ/MP, jackknife, and bootstrap analyses. *P. arcularius* and *P. brumalis* appear to be sister taxa, with *P. ciliatus* further removed in a position between ancestral *P. tricholoma and P. arcularius/brumalis*. Both *P. ciliatus* and *P. tricholoma* have smaller pores and more apparent cilia than *P. arcularius*/*brumalis*, which may mirror this relationship. We also conclude that ITS rDNA may be suitable to assign specimens/cultures to phylogenetic clades, but it is too laden with gaps, homoplasy, and ambiguity to resolve phylogeographic groups within these major clades.

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PART 4

Notes on *Polyporus* group *Polyporellus*: identity of collections from Canada and Ecuador and relation of *Lentinus*.

(Addendum to PART 3)

This manuscript is intended for publication, with suggested co-authors Karen W. Hughes and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research.

Running Title: *Polyporellus-Lentinus* molecular phylogeny.

Abstract: The polypore genus *Polyporus s. str*. can be subdivided into several infrageneric groups, one of which is *Polyporellus*. This publication mainly focuses on three species within the *Polyporellus* group, *P. arcularius*, *P. brumalis*, and *P. ciliatus*. As morphological characters may be misleading in identifying related species of this group, this paper expands on a previously published study determining phylogenetic *Polyporellus* species entities. A total of 35 additional ITS rDNA (ITS I – 5.8S – ITS II) sequences was inserted into the phylogenetic analysis of *Polyporellus*, aiding in correct identification. The presence of *P. ciliatus* or *P. tricholoma* in temperate North America could not be confirmed by morphology or molecular data. Spore measurements in relationship to the phylogenetic species are also discussed. Spore ranges for the three species *P. arcularius*, *P. brumalis*, and *P. ciliatus* were found to be overlapping. Our study indicates a closer relationship of the gilled *Lentinus tigrinus* to *P. tricholoma* than to any other of the *Polyporellus* species investigated.

Keywords: Aphyllophorales, Basidiomycotina, molecular phylogeny, ITS, ribosomal DNA.

INTRODUCTION

Within the genus *Polyporus*, the infrageneric group *Polyporellus*, as circumscribed by Nuñez & Ryvarden (1995), contains three widely distributed species (among others): *Polyporus arcularius*, *P. brumalis*, and *P. ciliatus*. The phenotypic variability of these species makes identification difficult (see PART 2, Jahn 1969), and characters such as hirsute appearance (Kreisel 1963, Bremer 1986) or later appearance of a dark stipe cuticle (Nuñez & Ryvarden 1995: 16) may lead to confusion with the infrageneric group *Melanopus*. We have previously confirmed tetrapolar mating systems for species within *Polyporellus*, confirmed mating barriers between species and determined phylogenetic relationships between and within species of this group (Krüger *et al*. 2002). *Polyporus arcularius*, *P. brumalis*, and *P. ciliatus,* all originally described from Europe, have also been assumed to be present in South America (Popoff & Wright 1998: *P. arcularius* and *P. ciliatus*, Nuñez & Ryvarden 1995). In the past, the name *P. arcularius* was wrongly applied to *P. brumalis*, and *P. brumalis* for *P. ciliatus* (Kreisel 1963).

In this paper we address the following problems: 1) Identity of several Canadian collections previously determined as *Polyporus ciliatus*. Nuñez & Ryvarden (1995) disputed the existence of *P. ciliatus* in North America, but Hoffmann (1978) reported that a DAOM culture available to us and initially identified as *P. brumalis* yielded monokaryons compatible with *P. ciliatus*. Several additional Canadian collections from DAOM identified as *P. ciliatus* were also examined. 2) Basidiomata of a tentative new species of *Polyporellus* from Canada with wide pores were examined using molecular tools. 3) A Canadian collection of *Polyporellus* phenotypically resembling *P. tricholoma* and *P. ciliatus* was examined with molecular tools. 4) An Ecuador collection morphologically resembling *P. ciliatus* as known from Europe was included for molecular identification. 5) Of the genus *Polyporus*, the infrageneric group *Polyporellus* as circumscribed by Nuñez & Ryvarden (1995) is the one that is closest to *Lentinus* (PART 2). The phylogenetic analyses presented here additionally determine which of the *Polyporellus* taxa is closest to *Lentinus tigrinus*, and whether *P.*

tricholoma is only an aggregate of tropical populations of the other *Polyporellus* species as suggested by Corner (1984: 50).

MATERIALS & METHODS

Collections utilized in this study are listed in Table 1. Maintenance of cultures and collections followed procedures described by Krüger *et al.* (2002) and PART 2. In addition, we have calculated spore measurement ranges for collections identified as *P. arcularius*, *P. brumalis*, or *P. ciliatus* within this study and/or Krüger *et al*. (2002). 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 ITS sequence) here and in Krüger *et al.* (2002) were used for calculation of these ranges.

Extraction of total nucleic acids followed methods described by Krüger *et al*. (2002) or PART 2. PCR of the ribosomal ITS - region (ITS I – 5.8S – ITS II nuclear rDNA) and sequencing were as described by Krüger *et al*. (2002), or PART 5 for most recent additions to our sequence database.

Sequence correction and alignment followed procedures from Krüger *et al*. (2002). Neighbor-joining (NJ) analysis (Saitou & Nei 1987) was performed in PHYLIP v. 3.57 (Felsenstein 1993) utilizing the Kimura 2-parameter distance model (Kimura 1980), after checking nucleotides to be approximately equal in frequency. MEGA2 files were created with the program ForCon v. 1.0 (Raes & van de Peer 2002). In MEGA2 (Kumar *et al.* 2001), interior branch tests (Nei & Kumar 2000: 168) using 1,000 pseudoreplicates were performed for a statistical evaluation of branch support in NJ. Trees were processed in TreeVIEW v. 1.6.1. (Page 1996) and imported into graphics and text programs.

With the MEGA2 sequence data explorer, only sites recognized by this program as parsimony-informative were retained. A half-deletion jackknife maximum-parsimony (MP) tree (100 pseudoreplicates, Efron & Gong 1983) was calculated in DAMBE v. 4.0.75 (Xia & Xie 2001). This program also further reduced data by combining identical sequences (mandatory to run DAMBE). The resulting data set was later used in PAUP* (Swofford 2001) analyses, as we wanted PAUP* to evaluate alternative phylogenetic hypothesis.

PAUP* v. 4.0b8 was utilized to find the 100 shortest MP trees (parameters were MAXTREES set to 100 due to computational limitations, TBR swapping, random taxon addition with 10 replicates, all characters unweighted, one tree held at each step, branches collapsed into polytomies if maximum branch length zero). PAUP* was unable to perform 10 random taxon addition replicates as 100 trees were found in the first repetition. The program was thus instructed to keep no more than 100 trees shorter than or equal to the tree of shortest tree length found in a series of initial runs. TreeVIEW and a text editor were used to create constraint NEXUS tree files to enforce alternative topologies reflecting different systematic hypotheses and collection identifications. PAUP* was then used to search for MP trees compatible with these hypotheses. It was also used to evaluate unconstrained and constrained trees using the Kishino-Hasegawa maximum-likelihood (ML) test (Kishino & Hasegawa 1989) with both the F84 (see Kishino & Hasegawa 1989) and HKY85 model (Hasegawa *et al.* 1985) and gamma distribution/estimated alpha shape parameter, no sites deemed invariable/no molecular clock. In each case, the null hypothesis tested was that the constrained trees were NOT significantly different and thus worse in their likelihood.

Table 1. Fungal specimens and cultures investigated.

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
FB10672	*Lentinus tigrinus* (Bull.: Fr.) Fr.	Austria: Niederösterreich	H. Voglmayr & I. Krisai-Greilhuber	AF516517
FB9770 [from LE(BIN)0861]	"	Mongolia		SBI 5: AF516518
FB8937 (TENN55557)	"	Russia: Krasnodar Region	RHP *et al.*	AF516519
FB9093 (TENN54918)	"	USA: Louisiana	RHP	AF516520
FB10832 (TENN58433)	"	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	"			AB070862
FB10299 (TENN58370)	"	Austria: Nieder-österreich	H. Voglmayr	SBI 2: AB070865, SBI 4: AB070866
CBS 222.91= RGT830522/01	"	Canada: Ontario	G. Thorn	AF516522
CBS 223.91 = RGT830522/01	"	Canada: Ontario	G. Thorn	AB070858
CBS 224.91= RGT830522/01	"	Canada: Ontario	G. Thorn	AF516523
FB4124 (TENN50834)	"	China: Guizhou	RHP	SBI 1: AB070863, SBI 2: AB070864
FB7883 (TENN53747)	"	Costa Rica: San Jose	RHP	SBI 2: AF516524
SBUG-M1244 (fruited for obtaining specimen/spores) (TENN58529, 58569, 58588)	"	Germany: Mecklenburg-Vorp.	R. Bütow	AB070861
DAOM94067 = PRE52	"	South Africa	P. Talbot	AB070859
FB10929 (TENN58412)	"	USA: Tennessee	H. Voglmayr	SBI 1: AB070867, SBI 2: AB070868
DAOM155905 hseq, also dikaryon sequenced	*P. brumalis* Pers.: Fr.	Canada: Ontario	J. Ammirati & J. Ginns (as *P. ciliatus*)	AF516525

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
DAOM72515 hseq, also dikaryon 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)]	"	Canada: Ontario	M. Pantidou	AF516527
Audet's *P. cf. tricholoma s.n.* hseq	"	Canada: Quebec	Received from S. Audet	AF516528
DAOM31983 also hseq	"	Canada: Quebec	J. W. Groves	AB070869
DSMZ-H17 (from DAOM31983) [culture 17 of Hoffmann (1978)]	"	Canada: Quebec	J. W. Groves	AB070870
FB10169 (TENN57700)	"	Denmark: Roskilde Amt	H. Knudsen	SBI 1: AB070872, SBI 3: AB070873
FB10178 (TENN57708)	"	Denmark: Storstrøms Amt	RHP	AF516529
DK0083 (TENN57748) hseq	"	Germany: Mecklenburg-Vorp.	DK	AF516530
FB10147 (TENN57678)	"	Germany: Mecklenburg-Vorp.	DK	AF516531
FB10908 (TENN58391)	"	Germany: Mecklenburg-Vorp.	DK	SBI 4: AB070876, SBI 5: AB070877
DSMZ-H21 = FRI285 [culture 21 of Hoffmann (1978), no specimen seen]	"	India		AF516532
NG980201/3 (SNU) hseq	"	Korea	Y.-W. Lim (as *P. melanopus*)	AF516533
O92393 = Ryv31528 hseq	"	Norway: Finnmark	L. Ryvarden	AF516534
O92301 hseq	"	Norway: Telemark	A.-E. Torkelsen	AB070871
DSMZ-H20 = FPRL 174a [culture 20 of Hoffmann (1978), no specimen seen]	"	UK: England		AF516535

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
DSMZ-H18 [from ATCC9385 = Overholts 24800 [culture 18 of Hoffmann (1978), no specimen seen]	*P. brumalis* Pers.: Fr.		L. O. Overholts, deposited by J. W. Sinden	AF516536
FB10665 (TENN58381)	"	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)	"	Finland: Etelä-Häme	RHP	SBI 2: AB070880, SBI 3: AB070881
DSMZ-H24 [culture 24 of Hoffmann (1978), no specimen seen]	"	France		AF516537
DSMZ-H25 [culture 25 of Hoffmann (1978), no specimen seen]	"	France		AF518752 (short fragment, not used in phylogeny)
FB7257 (TENN53619)	"	Sweden: Uppland	RHP	SBI 2: AB070878, SBI 7: AB070879
Bernicchia 5647 hseq	*P. corylinus* Mauri	Italy: Rome	Cherubini, *det.* A. Bernicchia	AF516538
Audet's *"P. longoporus" s.n.* hseq	*P. sp.* (labeled as *P. longoporus nom. prov*. by S. Audet), *P. longiporus* would be better name	Canada: Ontario	Y. Lamoureux, received from S. Audet	AF516539
DSMZ-H27 (from FPL Melbourne) [culture 27 "*P. brumalis ?*" of Hoffmann (1978), no specimen seen]	*P. tricholoma* Mont. complex	Australia: Queensland		AF516540
FB10240 (TENN57563)	"	Costa Rica: Heredia	RHP	SBI 1: AB070885, SBI 2: AF516543

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
FB10241 (TENN57564)	*P. tricholoma* Mont. complex	Costa Rica: Heredia	RHP	SBI 9: AF516541, SBI 10: AF516542
FB10258 (TENN57581)	"	Costa Rica: Puntarenas	RHP	SBI 2: AF516546
FB10920 (TENN58403)	"	Costa Rica: Puntarenas	E. Navarro	SBI 1: AF516547
FB9909 (TENN56537)	"	Costa Rica: Puntarenas	RHP	Dikaryon: AJ132939, SBI 2: AF516544
AAU44971 = TENN59383 hseq	"	Ecuador: Napo	T. Læssøe (as *P. cf. ciliatus*)	AF516548
FB3870 (TENN55844)	"	Mexico: Chiapas	RHP	Dikaryon: AJ132940; SBI 2: AB070884 = AF516549
TENN18091 hseq	"	Mexico: Chiapas	A. J. Sharpe, *det.* J. A. Stevenson	AF516550
FB4362 (TENN50439)	"	Mexico: Tabasco	RHP	SBI 9: AF516551
FB9568 (TENN56481)	"	USA: Puerto Rico	RHP	Dikaryon: AJ132942, SBI 1: AB070887 = AF516552
FB9579 (TENN56491); EPITYPE as discussed in PARTS 2, 5.	"	USA: Puerto Rico	KWH	SBI 1: AF516553, SBI 2: AF516554
FB9591 (TENN56503)	"	USA: Puerto Rico	RHP	Dikaryon: AJ132941, SBI 1: AB070886 = AF516555
FB10198 (TENN57728)	*Trametes hirsuta* (Wulf.: Fr.) Pilát	Germany: Mecklenburg-Vorp.	DK	SBI 5: AF516556

Table 1. Continued (footnotes).

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

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

FB = TENN field book number (acronym FB omitted in phylogenetic trees) = CulTENN culture collection number. hseq = sequence from herbarium specimen (all others sequences from cultures). All AB accession numbers are from Krüger *et al*. (2002).

s.n. = no number

SBI = single basidiospore isolate. If no specification "*leg.*/*det*." the name mentioned in the fourth column is the collector and initial identifier of a collection. All FB and O collections have been seen by DK and compared with recent taxonomic literature.

TENN = Univ. of Tennessee Fungal Herbarium, other herbarium acronyms from Holmgren *et al*. (1981).

The constraints were created after seeing the trees of above analyses, to evaluate several alternative topologies. PAUP* searched for the 100 shortest trees compatible with each of the constraints. Branches were allowed to collapse if branch length of zero occurred. All trees selected as best by Kishino-Hasegawa ML tests with F84 and HKY models (both on MP tree length and likelihood) were combined into a single NEXUS TREE block for a second round of direct ML comparison, with exclusion of any duplicate trees. In these analyses, trees were again evaluated against each other with both ML models.

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/)]. 166 sites were variable in total. Of the 64 sites retained as parsimony-informative by MEGA2 (99 including gap-containing sites) and also used by DAMBE, 62 were considered parsimony-informative by PAUP*, indicating difference in recognition of informative sites between MEGA2 and PAUP*. All trees depicted were rooted on *Trametes hirsuta*.

Results of the initial PHYLIP NJ analysis are depicted in Fig. 1. It also contains superimposed interior branch test values, based on 1,000 pseudoreplicates in MEGA2. Furthermore, DAMBE's MP-jackknife support (100 pseudoreplicates) values for branches appearing in DAMBE analyses are given. As previously shown by Krüger *et al.* (2002), there is good support for congruence between different methods of analysis for the major clades corresponding to morphological and biological species. Within the major clades, support is low and the position of the tip branches is variable (only values above 50 are indicated unless specifically discussed below).

The shortest 100 trees found by PAUP* on the trimmed data set with 64 sites (ALIGN_000376), without topological constraints, were 129 steps long and all from one tree island. PAUP* was, after initial test runs, instructed to retain only trees of a maximum length of 131 steps (NCHUCK = 100 CHUCKSCORE = 131). Statistical parameters for these trees were CI = 0.628, RI = 0.915 and thus RC = 0.575.

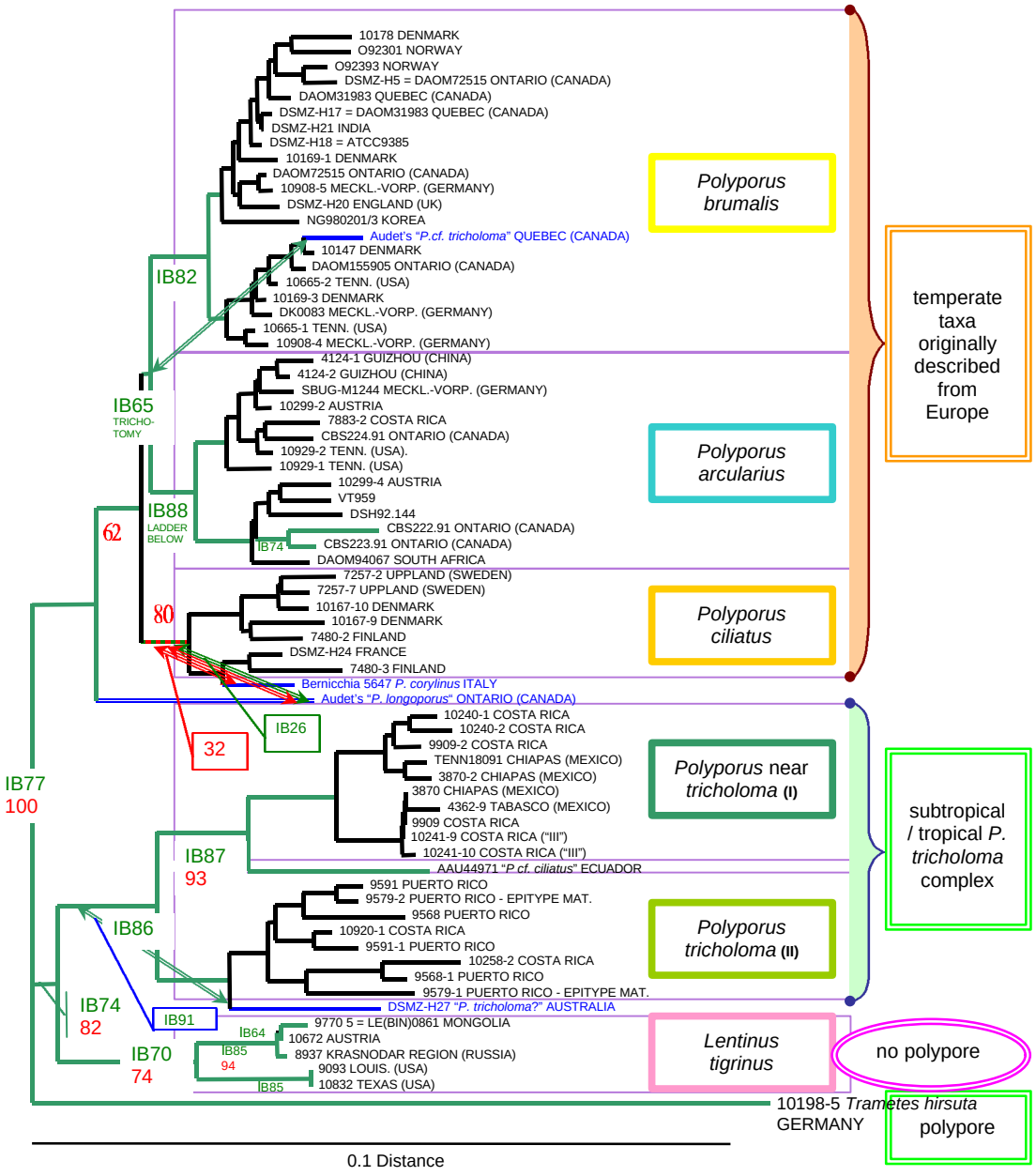


Figure 1. PHYLIP Kimura 2-parameter NJ dendrogram rooted with *Trametes hirsuta*. MEGA2 Kimura 2-parameter NJ interior branch test support "IB" (1,000 pseudoreplicates, in green) and DAMBE MP half-deletion jackknife support (100 pseudoreplicates, in red) superimposed. Green-shaded branches are contopological in MEGA2 and PHYLIP. Only values above 50 shown unless discussed in the text. Arrows mark branch insertion changes between analyses (red = in bootstrap, green = in IB). A designation like "9770-5" refers to TENN fieldbook number FB9770 SBI 5.

We created the following topological constraints (with the other major clades being collapsed to polytomies): Constraint 1 forced *Lentinus tigrinus* to be the sister clade to all *P. tricholoma* collections, including the "*P. ciliatus* look-alike" AAU44971 shown to be close to *P. tricholoma* (PART 5). Constraint 2 forced *Lentinus tigrinus* to be the sister to the *Polyporellus* clade, and not only to *P. tricholoma*. Constraint 3 forced the collection Bernicchia 5647 to be phylogenetically conspecific ("concladic") with *P. ciliatus*, as previous preliminary analyses found it nested within *P. ciliatus*. Constraint 4 forced *"P. longoporus"* to be concladic with *P. ciliatus*, as in preliminary analyses it appeared nested within or basal to it. Constraint 5 forced *"P. longoporus"* to be concladic with *P. arcularius*, because the elongated pores indicate morphological similarity to the latter. Constraint 6 forced "*P. cf. tricholoma*" to be concladic with *P. ciliatus*. It was deemed too far away from *P. tricholoma* clades in preliminary analyses to be considered as related to *P. tricholoma*. Constraint 7 was for AAU44971 "*P. cf. ciliatus*" to be concladic with *P. ciliatus* based on its morphological similarity (PART 5).

Thirty-three unique trees were kept for direct ML-comparison. The best tree selected by both F84 and HKY models (Fig. 2) was an unconstrained tree. Table 2 shows the ML test results and indices of compared trees. In Fig. 2, *P. ciliatus* and *P. brumalis* switched positions when compared to Fig. 1. Based on statistics, the hypothesis that collection AAU44971 from Ecuador was phylogenetically concladic with the temperate taxon *P. ciliatus* was rejected. Trees consistent with such constraint always depicted AAU44971 basal to *P. ciliatus* and on a very long branch. Such high number of autapomorphic character changes is not a parsimonious explanation (see below for morphological data). The Canadian collection "*P. cf. tricholoma*" (see Table 1) that resembles a thin small *P. tricholoma* is not conspecific with either of the included biological species of *P. tricholoma*, but related to *P. brumalis* and *P. arcularius*. The hypothesis (constraint 5) that "*P. longoporus*" is concladic with *P. arcularius* was not statistically rejected, which may correlate to the unstable position at the base of *P. ciliatus* in Fig. 1. The other hypotheses (constraints 1, 2, 3, 4) cannot be statistically rejected.

Topologies of all analyses graphically presented in Figs. 1 and 2 converge on six major clades [*P. arcularius*, *P. brumalis*, *P. ciliatus*, *P. tricholoma* (II), *P.* near *tricholoma* (I), *L. tigrinus*] with more flexible positions for the lesser sampled Canadian "*P. cf. tricholoma*", *P. corylinus*, "*P. longoporus*", and DSMZ-H27 *P. tricholoma* from Australia (see PART 5). Specifically notable is that in Fig. 1 (MEGA2), the Canadian "*P. cf. tricholoma*" was found in a moderately low-supported trichotomy with *P. brumalis* and *P. arcularius* while in the PHYLIP NJ analysis, it was found within the *P. brumalis* clade. In the same MEGA2 analysis, the *P. corylinus* nested within *P. ciliatus* (PHYLIP) becomes basal to *P. ciliatus*, with low support. Also in MEGA2 analysis, DSMZ-H27 leaves the basal position related to *P. tricholoma* II (*P. tricholoma* proper) for a basal position to all of the *P. tricholoma* complex, with high support (interior branch test = 91). With the DAMBE MP jackknife analysis, "*P. longoporus*" moves from a position basal to the temperate *Polyporellus* to one directly basal to *P. ciliatus* (jackknife value 80). Here *P. corylinus* moves out of the *P. ciliatus* clade to be basal to both *"P. longoporus"* and *P. ciliatus*, but with a low jackknife support of 32. Both Fig. 1 and 2 indicate the *P. tricholoma* complex/*Lentinus tigrinus* as sister in a trichotomy with both the *Trametes* outgroup and all other *Polyporellus* taxa.

Morphological notes

Spore ranges for the *P. tricholoma* complex are reported in PART 5 and Fig. 3 plots approximate spore measurements for the three other major species, *P. arcularius*, *P. brumalis*, and *P. ciliatus*. For *P. ciliatus*, 171 spores of 18 collections positively identified by Krüger *et al.* (2002) or here were measured. They ranged from (5.0 –) 6.827 (– 9.0) µm X (1.5 –) 1.924 (– 3)

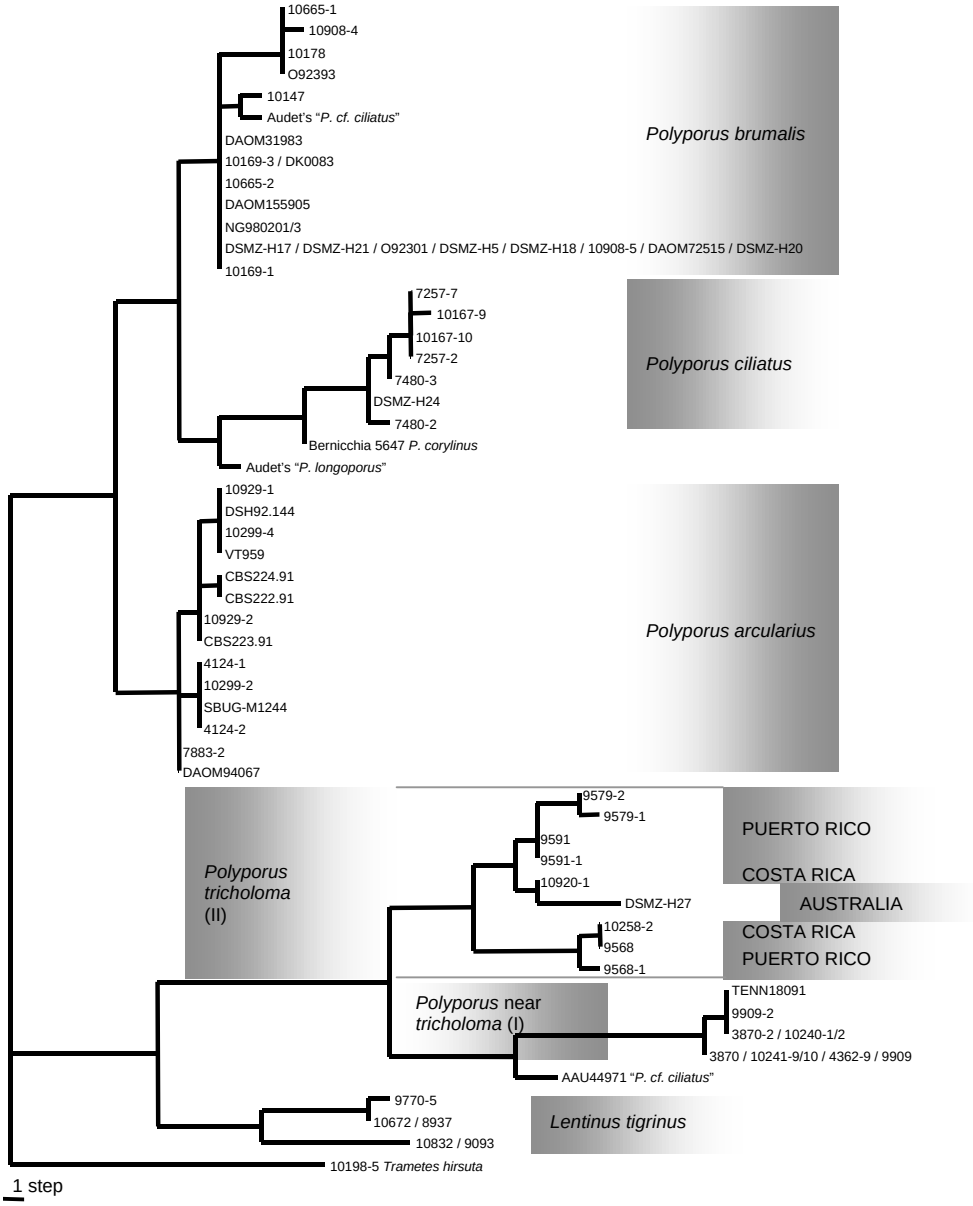
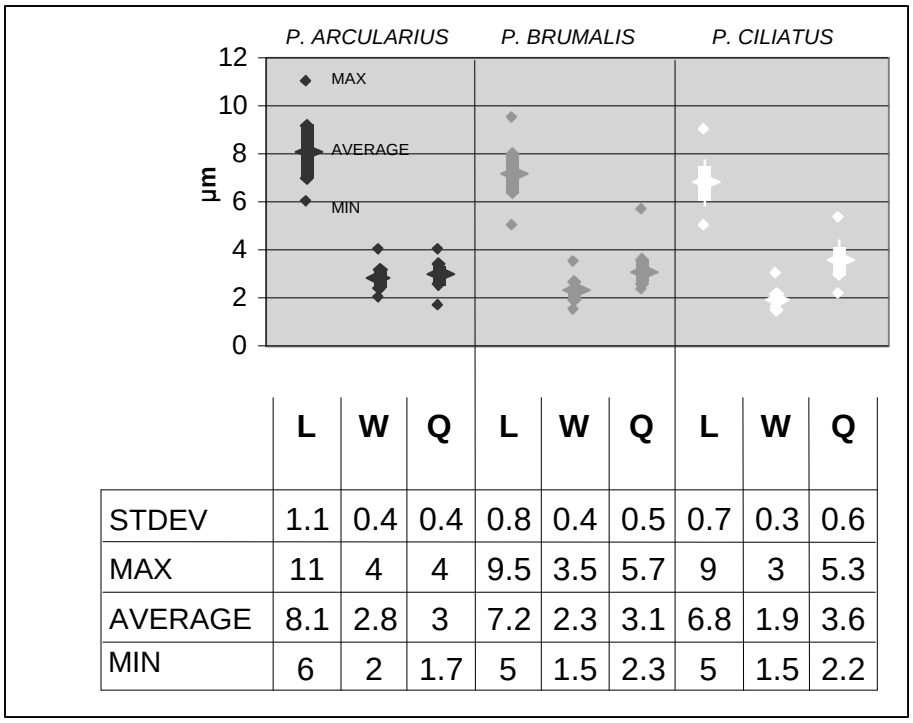


Figure 2. PAUP* best of 100 equally most-parsimonious unconstrained MP trees, rooted with *Trametes hirsuta*, selected by ML tests of constrained and unconstrained trees: 129 steps, CI = 0.628, RI = 0.915, RC = 0.575, HI = 0.372, - *ln* L 652.90180 (HKY), - *ln* L 652.88965 (F84). Based on a trimmed dataset with identical nucleotide phylotypes combined. For the *P. tricholoma* complex, some geographic locations indicated. A designation like "9770-5" refers to TENN fieldbook number FB9770 SBI 5.

Table 2. Statistical values of equiparsimonious MP trees compared with ML test methods, using the Kishino-Hasegawa test with Hasegawa-Kishino-Yano (HKY) model or Felsenstein 1984 (F84) model implemented in PAUP*.

Hypothesis	**Length, CI, RI**	**Range – *ln* L HKY (F84)**	**Range *P** HKY (F84)**
unconstrained	129, 0.628, 0.915	652.89947 - 652.90750 (652.88965 - 653.07702)	1 - 0.4236 (1 - 0.4243) - same tree selected as best, though with F84 two trees equally probable
constraint 1 *P. tricholoma* (incl. AAU44971 "*P. cf. ciliatus*") is an unresolved clade sister to *Lentinus tigrinus*	131, 0.618, 0.912	657.40425 - 659.25182 (657.36225 - 659.21034)	0.3408 - 0.1938 (0.3428 - 0.1953) not significantly different
constraint 2 *Lentinus tigrinus* sister to *Polyporellus*, all other topology unresolved	132, 0.614, 0.910	659.66458, 659.48672 (659.43748, 659.60771)	0.2115 - 0.1802 (0.2144 - 0.1824) not significantly different
constraint 3 Bernicchia 5647 is conspecific with *P. ciliatus*	132, 0.614, 0.910	659.96278 - 661.80831 659.91384 - 661.76329)	0.1846 - 0.1031 (0.1864 - 0.1046) not significantly different
constraint 4 Audet's *"P. longoporus"* is conspecific with *P. ciliatus*	132, 0.614, 0.910	659.95224 - 661.79874 (659.90994 - 661.75686)	0.1833 - 0.1020 (0.1852 - 0.1038) not significantly different
constraint 5 Audet's *"P. longoporus"* is conspecific with *P. arcularius*	134, 0.604, 0.906	663.27712 - 665.12328 (663.20856 - 665.05534)	0.0809 - 0.0444* (0.0806 - 0.0443*) partly significantly different (worse)
constraint 6 Audet's "*P. cf. tricholoma*" is conspecific with *P. ciliatus*	137, 0.591, 0.901	all 678.86279 (678.75784 - 678.75785)	all 0.0044* (all 0.0044*) significantly different (worse)
constraint 7 AAU44971 "*P. cf. ciliatus*" is conspecific with *P. ciliatus*	149, 0.544, 0.880	696.90164 - 696.90755 (696.76561 - 696.80150)	all 0.0004* (all 0.0004*) significantly different (worse)

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



	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

Figure 3. Box plot of spore size ranges in *Polyporellus*. L = length, W = width, Q = L divided by W, STDEV = standard deviation, MAX = largest size, AVERAGE = arithmetic mean, MIN = smallest size. Values plotted are rounded in the table below.

µm, Q (2.167 –) 3.635 (– 5.333), indicating very slender spores. Standard deviations were 0.748, 0.339, and 0.626. *P. brumalis* spores were (238 spores, 24 collections of 30 available) (5 –) 7.166 (– 9.5) µm X (1.5 –) 2.315 (– 3.5) µm, Q (2.286 –) 3.150 (– 5.667), standard deviations 0.840, 0.375, 0.496.

Excluding FB7883, we took spore measurements for 110 *P. 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), standard deviations 1.093, 0.399, 0.408. 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).

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

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

DISCUSSION & TAXONOMY

We received two Canadian voucher specimens and cultures, DAOM155905 and DAOM72515, identified as *P. ciliatus*. The latter was only re-identified from *P. brumalis* 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. All of these DAOM72515-based sequences bear the signature of *P. brumalis* (not considering slight differences that stem from sequencing errors and non-homogenized rDNA copies), and in all our analyses appeared in the *P. brumalis* clade (Figs. 1, 2). This also holds true for DAOM155905, the other strain received as *P. ciliatus*. Based on sequence data alone, 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 occasional ability of members of the *Polyporellus* group to hybridize as also mentioned by Krüger *et al.* (2002). So far, we see no evidence of *P. ciliatus* being extant in North America, confirming the suggestion by Nuñez & Ryvarden (1995).

The collection received from Mr. Audet (*P. cf. tricholoma*) that resembles *P. tricholoma* or *P. ciliatus* (small pores, thin, ciliate fruit body) is, based on the phylogenetic analyses, not identical to *P. tricholoma* nor *P. ciliatus*, but related to *P. brumalis* and *P. arcularius*, if not conspecific with *P. brumalis*. This may again have to do with a past hybridization, or otherwise may reflect support for Corner's (1984: 50) suggestion that *P. tricholoma* is an agglomerate of several reduced *Polyporus* species. Morphology is misleading and no mating studies could be conducted, as there was no culture available. On the other hand, the collection AAU44971, resembling *P. ciliatus* by its macromorphology and spore statistics, is closely related to the *P. tricholoma* groups discussed in PART 5. These results cast some doubt about the validity of basidiome identification in this group based on macromorphology or micromorphology and calls for more extensive sampling of collections, in particular those called *P. arcularius*, *P. brumalis*, and *P. ciliatus* in the Americas.

Perhaps due to darkening of color with age, a Korean collection (NG980201/3) was received as *P. melanopus* (*Melanopus* group of *Polyporus*, Nuñez & Ryvarden 1995), but sequence and spore statistics identified the collection as *P. brumalis*. We also corrected misidentified *P. brumalis* and *P. ciliatus* collections from Europe in the course of mating studies by Krüger *et al*. (2002), and there also, either hirsute fruit body surfaces or darkened stipes/pores of aged fruit bodies caused difficulties with identification. These basidiomata were often classified as *P. melanopus* by collectors. Notably, DAOM155905, identified as *P. ciliatus*, had orange colored pores, which points toward *P. ciliatus* f. *ciliatus* and not *P. ciliatus* f. *lepideus* in the sense of Kreisel (1963). However, aged fruit bodies and, equally, herbarium specimens may lose the snow-white appearance of pores Kreisel attributed to *P. ciliatus* f. *lepideus* (which was intersterile with *P. ciliatus s. str*.; see Hoffmann 1978). One finds pore surfaces with grayish, reddish, brown or cream shades in both *P. brumalis* and *P. ciliatus*.

No definitive judgement has been made about "*P. longoporus*" other than that it appears not to be conspecific with *P. arcularius*. The Mediterranean *P. corylinus* may or may not be conspecific with *P. ciliatus*.

Nuclear LSU rDNA data showed *Lentinus tigrinus* to be nested between *P. tricholoma* and the other *Polyporellus* (PART 1). In this paper, ITS sequences show the closer relationship of gilled *L. tigrinus* to the *P. tricholoma* complex than to other *Polyporellus*. No phylogeographic signal was seen in our ITS rDNA phylogenies presented here to infer more details about the evolution of *Polyporellus* and *Lentinus*.

With the spore range data, one can, to a certain extent, identify species, but there is overlap. Spore statistics were included here to have some equal footing with the keys published so far. Macromorphology can be obscured by varying pore sizes, decoloration of aged specimens, varying degrees of hirsute surfaces and formation of dark stipe cuticles. Biological species may be obscured by occasional hybridization, and rDNA sequence phylogeny by template heterogeneity (PART 5). So even if we know the correct use of names, we may be misled in identification regardless of the species concept being used. However, this multitechnical approach may lead to further discovery of evolutionary and phylogeographic patterns in polypores. Generally, the addition of ITS rDNA data is a powerful tool to establish or exclude conspecificity of a collection if only unambiguously alignable sequence lengths are used.

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PART 5

Polyporus tricholoma in the Mesoamerican tropics.

This manuscript is intended for publication, with suggested co-authors Karen W. Hughes and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research, using some preliminary mating study data done by RHP.

Running Title: *Polyporus tricholoma* cryptic species.

Abstract: The stipitate pore-bearing fungus *Polyporus tricholoma* (*Polyporus* infrageneric group *Polyporellus*) is commonly reported from Central America and more rarely from the palaeotropics. Collections from Puerto Rico, Mexico, and Costa Rica did not differ morphologically, but their monokaryotic cultures revealed the existence of interINcompatibility groups. These putative cryptic species exist sympatrically in Costa Rica. Molecular evidence supported the existence of a close relative in Australia. Sequencing, restriction enzyme digestion, and specific primers targeting the ITS region of nuclear ribosomal DNA (ITS I - 5.8S - ITS II) could only partly be used for detecting intercompatibility groups. Heterogeneity within the rDNA repeat was observed.

Keywords: Aphyllophorales, Basidiomycotina, fluorescein, mating types, molecular systematics, ITS region rDNA, RFLP, sibling taxa.

INTRODUCTION

The white-rotting, centrally stipitate polypore, *Polyporus tricholoma* Mont., is commonly found in the neotropics of Central America (Murrill 1915, Overholts 1953, Dennis 1970, Carranza-Morse 1992), but has less commonly been reported from the old world tropics (Nuñez 1993, Nuñez & Ryvarden 1995), India (Roy & De 1977), Nigeria (Ryvarden & Johansen 1980), and Borneo and New Guinea (Corner 1984). *Polyporus tricholoma* was originally described by Montagne (1837) from Cuba, and has been placed in the unranked infrageneric group *Polyporellus* by Nuñez & Ryvarden (1995), who also furnished a list of synonyms.

Corner (1984) reported five varieties from South America and SE Asia, suggesting that *P. tricholoma* is an aggregate of reduced forms of several species. A recent description of the ciliate-margined *P. tricholoma* was furnished by Gilbertson & Ryvarden (1987). The species has previously been reported as heterothallic and tetrapolar (Roy & De 1977, David & Rajchenberg 1985).

For this study, we investigated the mating compatibility and morphology of collections from Puerto Rico, Costa Rica, and Mexico. We attempted to correlate nuclear ITS-based dendrograms [including a collection tentatively identified as *P. cf. ciliatus* (AAU44971), in PART 4 found related to *P. tricholoma*] with mating studies and morphological data, and to design specific primers for the screening of material available to us.

MATERIALS & METHODS

Specimens, establishment & maintenance of cultures

New collections were assigned field book numbers, annotated, dried for preservation, and deposited in TENN (Holmgren *et al*. 1981). Identification was accomplished with the aid of keys and descriptions by Gilbertson & Ryvarden (1986-1987), Ryvarden & Gilbertson (1993-1994), and Nuñez & Ryvarden (1995).

Techniques for establishing monokaryotic single-basidiospore isolates (SBIs) were described by Gordon & Petersen (1991). Dikaryotic cultures were established for a number of collections as described by Petersen & Hughes (1997). Cultures were stored on agar disks of malt extract agar (MEA: 1.5% w/v Difco malt extract, 2% w/v Difco Bacto-agar, Nobles 1965) in sterilized water (Burdsall & Dorworth 1994). In several cases, colleagues furnished spore prints from which cultures were obtained. Micromorphological analyses of spores, basidia, and hyphal construction were undertaken. Sections of fungal tissue were mounted in 3% w/v KOH with phloxine dye and observed with phase contrast at 400 x magnification.

Collections

Abbreviations used below to describe collections are:
s.n. = no number. TENN = Univ. of Tennessee Fungal Herbarium, other herbarium acronyms after Holmgren *et al.* (1981). FB = TENN field book number = CulTENN Univ. of Tennessee Fungal Culture Collection number (the acronym FB is omitted for simplicity in phylogenetic trees). DK = Dirk Krüger (has seen specimens where available). KWH = Karen W. Hughes. RHP = Ronald H. Petersen.
DSMZ-H = cultures used by Hoffmann (1978) and kept at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.
GB = GenBank accession number. hseq = sequence from herbarium specimen. **X** = no sequence available.

P. tricholoma Mont. (cryptic species identity known by mating studies and/or sequencing): **Costa Rica:** Alajuela: Fortuna, Arenal Observatory Lodge, 14 Mar. 1999, J.-L. Mata, R. Halling & RHP FB10048 (TENN57617, **X**); Heredia: Chilimate, 13 Mar. 1999, RHP 10240 (TENN57563, SBI 1 GB: AB070885, SBI 2 GB: AF516543); Heredia: Chilimate, 13 Mar. 1999, RHP FB10241 (TENN57564, SBI 2 GB: AB070888, SBI 9 GB: AF516541, SBI 10 GB: AF516542); Puntarenas: La Amistad Pacifico, 16 Mar. 1999, E. Navarro FB10920 (TENN58403, SBI 1 GB: AF516547); Puntarenas: Road to Sta. Elena Biological Reserve, 17 Mar. 1999, RHP FB10258 (TENN57581, SBI 1 GB: AF516545, SBI 2 GB: AF516546); Puntarenas: Road to Sta. Elena Biological Reserve, 17 Mar. 1999, RHP FB10261 (TENN57584, **X**); Puntarenas: San Vito, 07 Apr. 1998, RHP FB9909 (TENN56537, dikaryon GB: AJ132939, SBI 2 GB: AF516544); **Mexico:** Chiapas: Cacoahatan, 18 Oct. 1997, RHP FB3870 (TENN55844, dikaryon GB: AJ132940; SBI 2 GB: AB070884 = AF516549); Chiapas: Mapastepec, 01 Nov. 1945, A. J. Sharpe 4372 (TENN18091, GB: AF516550 hseq), *det*. J. A. Stevenson; Mexico: vicinity of San Fancisco de Oxototilpa, 05 July 1996, RHP FB8420 (TENN55246, **X**); Tabasco: Crutas Cocona Town Park, 21 Oct. 1997, RHP FB4362 (TENN50439, SBI 9 GB: AF516551); Xilitan: San Luis Potosi, 21 Apr. 1946, A. J. Sharpe 5902, *det*. W. A. Murrill (TENN17522, GB: AF518753, hseq); **USA:** Puerto Rico: Luquillo National Forest, El Toro Recreational Trailhead, 11 June 1998, KWH FB9579 (TENN56491, SBI 1 GB: AF516553, SBI 2 GB: AF516554); Puerto Rico: Luquillo National Forest, El Verde Biological

Station, 09 June 1998, E. Lickey FB9286 (TENN56364, **X**); Puerto Rico: Luquillo National Forest, El Verde Biological Station, 09 June 1998, RHP FB9568 (TENN56481, dikaryon GB: AJ132942, SBI 1 GB: AB070887 = AF516552); Puerto Rico: Luquillo National Forest, El Yunque, Big Tree Trail, 12 June 1998, RHP FB9591 (TENN56503, dikaryon GB: AJ132941, SBI 1 GB: AB070886 = AF516555)

P. cf. tricholoma:
Australia: Queensland: Kenilworth. "DSMZ-H27" (culture 27 of Hoffmann 1978, no specimen seen, dikaryon GB: AF516540); **Belize:** E. Lickey FB11536 (TENN59417, **X**); **Costa Rica:** Puntarenas: National Park Corcovado, 29 Mar. 2000, E. Fletes FB10919 (TENN58402, X); **Cuba:** Ramon de la Sagra *s. n*. (PC!, HOLOTYPE, see Montagne 1837, **X**); Habana: Calabazar, 02 Oct. 1968, H. Kreisel (GFW, duplicate of HAJB 00341, **X**); **Ecuador:** Napo: 10 July 1983, T. Læssøe: AAU44971 (TENN59383, received from L. Ryvarden, duplicate in AAU, GB: AF516548, hseq); **Guyana:** Leprieur 949 (PC!, **X**); **Mexico:** Oaxaca: Niltepec, 02 Apr. 1946, A. J. Sharpe 5746, *det*. W. A. Murrill (TENN17531, **X**); **USA:** Puerto Rico: Luquillo National Forest, El Yunque, Big Tree Trail, 12 June 1998, J. Jin FB9585 (TENN56497, **X**)

P. arcularius Batsch: Fr.:
Austria: Niederösterreich: Kaltenleutgeben, on *Fagus*, 04 Apr. 1999, H. Voglmayr FB10299 (TENN58370, SBI 2 GB: AB070865); **China:** Guizhou: 31 Aug. 1991, RHP FB4124 (TENN50834, SBI 1 GB: AB070863); **USA:** Tennessee: Tremont, 02 Apr. 2000, H. Voglmayr FB10929 (TENN58412, SBI 1 GB: AB070867, SBI 2 GB: AB070868)

P. brumalis Pers.: Fr.:
Denmark: Roskilde Amt: Lellinge, on *Fagus*, 18 May 1999, H. Knudsen FB10169 (TENN57700, SBI 3 GB: AB070873); **Germany:** Mecklenburg-Vorpommern: Neustrelitz, on *Fagus*, 28 Dec. 1999, DK FB10908 (TENN58391, SBI 4 GB: AB070876); **USA:** Tennessee: on *Fagus*, 07 Nov. 1999. K. McFarland FB10665 (TENN58381, SBI 2 GB: AB070875)

P. ciliatus Fr.:
Denmark: Roskilde Amt: Lellinge, on *Acer*, 18 May 1999, H. Knudsen & RHP FB10167 (TENN57698, SBI 10 GB: AB070883); **Finland:** Etelä-Häme: Evo, 13 Sep. 1994, RHP FB7480 (fruited for obtaining spores to make up for lost monokaryons; method by Psurtseva & Mnoukhina 1998) (TENN53639/58441/58823, SBI 2 GB: AB070880); **Sweden:** Uppland: Tarnby Lund, on *Betula*, 04 Sep. 1994, RHP FB7257 (TENN53619, SBI 2 GB: AB070878)

L. tigrinus (Bull.: Fr) Fr.:
Mongolia: FB9770 [ex culture LE(BIN)0861, SBI 5 GB: AF516518]

Mating experiments

Self-cross pairings for elucidation of mating types and tester single-basidiospore isolates (SBIs) were made among randomly selected monokaryotic SBIs of at least one collection per species. Tester SBIs are monokaryon cultures with known mating types and were used for later testing of newly available collections. Subtester SBIs are additional tester SBIs selected later from collections of a different geographic origin, without prior knowledge of the actual mating type of the SBI. The technique for self-crosses followed Petersen (1992). For intercollection pairings, four SBIs (randomly selected, or testers/subtesters when assigned) were paired with four SBIs from other collections in this study.

Molecular biology

DNA sequencing and RFLPs were used to pursue the question whether *P. tricholoma* can be further differentiated by intersterility group (from here on, called “Group” to differentiate from infrageneric group) or geographic origin, and to gather information on the relationship to other members of *Polyporellus*. Sequences generated earlier (Krüger *et al*. 2002) were augmented with sequences of additional collections.

DNA was extracted following a CTAB method (modified from Hughes *et al*. 1999; or modified from Zolan & Pukkila 1986, Doyle & Doyle 1987), an SDS-based method (Lee & Taylor 1990), or more recently, with a xanthogenate/SDS miniprep protocol adopted from Tillett & Neilan (2000). These methods gave approximately equivalent results, although they were not always effective. The xanthogenate method was quicker and allowed a higher sample throughput, and was generally used since initial work (Krüger *et al*. 2002).

Treatment prior to DNA extraction, grinding

In the CTAB methods, up to 50 mg of herbarium material, or a lesser weight of hyphae scraped off agar plates, was placed in a 1.5 ml tube with 0.5 ml 65◦ C CTAB extraction buffer (0.1M Tris, 0.2 M Na_2EDTA, 1.5 M NaCl, 55 mM CTAB = hexadecyltrimethylammonium bromide, Sigma H5882). The material was incubated for 30 to 60 min at 65◦ C and vortexed occasionally before being ground with a sterilized mini pestle (Kontes Pellet Pestle, Kontes 749520).

For tough herbarium specimens, grinding was supported with sterilized sand, and the material subjected to alternating freezing and heating (3 cycles of 10 min at -80◦ C/10 min at 65◦ C; Vrålstad *et al*. 2000). Here, a 3% w/v SDS (sodium dodecyl sulfate, Sigma L4509), 1% w/v mercaptoethanol extraction buffer (500 µl, 65◦ C) replaced the CTAB extraction buffer.

Cell-debris removal

With any procedure above, approximately the same volume of 24:1 (v:v) chloroform:isoamyl alcohol was added, this mixture vortexed briefly, and centrifuged at 11,750 g for 4 min. The upper phase was transferred to a new 1.5 ml reaction tube and combined with an equal volume of isopropanol at 4◦ C. This mixture was refrigerated (4◦ C) for one hour, and centrifuged for four min at 13,800 g. The resulting pellet was rinsed twice in 70% v/v ethanol, air-dried, and suspended in 100 µl TE buffer (10 mM TrisHCl, 1 mM Na_2EDTA; pH 8.0).

Xanthogenate protocol

Extraction was mainly performed with a modified xanthogenate protocol (Tillett & Neilan 2000). Fresh material was kept for several weeks at 4◦ C in CTAB/sodium azide preparations (after Rogstad 1992: 6 M NaCl, 3 mM NaN_3, 41.1 mM CTAB). Alternatively, fresh or herbarium material was stored in an SDS buffer (50 mM Tris/HCl, 50 mM Na_2EDTA, 10% w/v SDS, pH 7.2). Cultures were grown 2-8 weeks at room temperature in malt extract (ME) broth (ca. 10 ml 1.5% w/v Difco malt extract, in baby food jar).

Ten to 50 mg of material was crushed in 50 µl TE buffer with a small amount of sterile sand. Cultures from ME broth were prepared by filtering 250-500 mg material, and blotting on a paper towel. If growth was slow and resulting tiny hyphal clumps tended to be lost through

the filtering cheese cloth, the fungal material was pipetted out of the jar, transferred directly to a tube, centrifuged, and the medium pipetted off. After adding 50 µl TE extraction buffer, a minipestle mounted on a drill was utilized to grind the material.

Following grinding, 750 µl of potassium ethyl xanthogenate buffer (100 mM Tris/HCl pH 7.2, 20 mM Na_2EDTA pH 8.0, 1% w/v SDS, 800 mM ammonium acetate, 1% w/v $C_3H_5KOS_2$ = potassium ethyl xanthogenate: Fluka 60045) was added. In case of dry herbarium specimens taken directly to DNA extraction, grinding was done after addition of the xanthogenate buffer. The tube contents were mixed and incubated at 70° C for 60 min, with occasional vortexing. After a final vortex for 10 sec, samples were placed on ice for 30-60 min, and then spun at 16,000 g for 10 min. The supernatant was recovered, and nucleic acids precipitated with 750 µl isopropanol (80% v/v, 4° C), and spun at 8,100 g for 10 min. The alcohol was aspirated, and the pellet was rinsed with 250 µl 95% v/v cold ethanol, spun again at 8,100 g for 10 min. The new alcohol was then removed by pipetting, and evaporated 2 min at 70° C on a heating block. The pellet was resuspended in 50 µl TE buffer and incubated 10 min at 50° C. We found it not necessary to add RNAse.

PCR & sequencing

The nuclear ITS rDNA (ITS I - 5.8S - ITS II) was initially amplified using primers ITS 1F/ITS 4B (Gardes & Bruns 1993) or ITS 5 (White *et al.* 1990)/LR 7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm), see Krüger *et al*. (2002) for PCR parameters. After encountering amplification problems with those primer pairs in several taxa of *Polyporus*, we designed new primers NS-7-UTK (GAGGCAATAACAGGTCTGTGAT, T_m = 61.86° C) and ITS 4C (GAGCTYWTCCCGCTTCAC, T_m = 62.50° C) for ITS region amplification (T_m: denaturation temperature according to http://www.genosys.com/cgi-win/oligo_calconly.exe). A 20 µl reaction contained 1 X buffer supplied by manufacturer (buffer EPWMG already supplemented with $MgCl_2$ in case of QBioGene polymerase kit EPTQA023, or separate 3 mM $MgCl_2$), 0.8 mM each dNTP, 0.2 ng/µl of bovine serum albumin (Sigma A7906; Kreader 1996), 0.2 mM each primer, 1.1 units *Taq* Polymerase (QBioGene EPTQA023). PCR cycling parameters for NS-7-UTK/ITS 4C differed as they were optimized for T_m and amplicon length, and 37 cycles of amplification run. Parameters were: initial denaturation 94° C/4 min, denaturation 94° C/1 min - annealing 57° C 1 min, extension 72° C 2 min, final extension of 72° C/3 min.

PCR products were electrophoresed in a 1.5% w/v agarose/TBE gel. PCR products bound for sequencing were cleaned with the Amicon Microcon PCR Centrifugal Filter Device (Millipore UFC7PC250), according to manufacturer's instructions, but with following amendments: first centrifugation step 5 min at 1,300 g, second spin step 1,000 g, with 30 µl instead of 20 µl bi-distilled water for washing off the DNA. Primers NS-7-UTK or ITS 5, ITS 3 and/or ITS 2 (White *et al.* 1990) were used as sequencing primers. Cycle-sequencing was done with 2.5 µl ABI PRISM BigDye Terminator (v 2.0; Applied Biosystems) per 10 µl reaction, following manufacturer's instructions and using approximately 25 ng DNA template as measured with a Hoefer DyNA Quant 200 fluorometer. BigDye cycle-sequencing products were cleaned using the following procedure: the sequencing product was complemented with 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 spun 20 min at 16,000 g, and the supernatant pipetted off. The tube was then rinsed with 190 µl 70% v/v cold ethyl alcohol. Following another centrifugation (16,000 g, 5 min), the supernatant was again pipetted off, and the tube placed for 1 min in a heating block at 90° C.

Sequence data

ABI sequence trace files were checked in Chromas v. 1.45 (Technelysium Pty. Ltd., Australia). Contig assembly was done manually in the Programmer's File Editor v. 0.07.002 (Alan Phillips, Lancaster University, UK).

A single master-file was compiled, containing all new data and imports from the GenBank databases (obtained through BLAST-n: Altschul *et al.* 1997) in FASTA format. Alignment was then done in ClustalX v. 1.64b (Thompson *et al*. 1997), with consecutive manual adjustments on the CLUSTAL-format alignment in a text editor or BioEdit v. 5.0.9 (Hall 1999). Overhangs were trimmed, ambiguously alignable sequences removed. Using the file conversion tool ForCon v. 1.0 (Raes & van de Peer 2002), non-interleaved MEGA2 files (Kumar *et al.* 2001) were created, and indels and parsimony-uninformative sites excluded. In MEGA2, neighbor-joining (NJ: Saitou & Nei 1987; Kimura 2-parameter: Kimura 1980; alignment was checked to have approximately equal nucleotide frequencies) analyses with an interior branch test (Nei & Kumar 2000) or bootstrap (Felsenstein 1985) were run, both with 1,000 pseudoreplicates of character resampling. Likewise, a minimum-evolution (ME: Rzhetsky & Nei 1993) tree was calculated with the Kimura 2-parameter model. The resulting trees were manipulated in TreeVIEW v. 1.6.1. (Page 1996) for importing into graphics and text programs, and converted to NEXUS tree blocks with a text editor.

NEXUS files (Maddison *et al*. 1997) were generated by using the file conversion program SeqVerter v.1.571 (GeneStudio, Inc). PAUP command blocks were manually added. In PAUP* v. 4.0b8 (Swofford 2001) a maximum-parsimony (MP) analysis with heuristic search (TBR swapping, 10 random addition replicates, MAXTREES set at 100 due to lack of computational power, all characters unweighted, corresponding to Fitch 1971) was performed. All resulting MP trees of equal length plus imported MEGA2 NJ and ME trees were evaluated under maximum-likelihood (ML) as optimality criterion, using the Kishino-Hasegawa test (Kishino & Hasegawa 1989) with HKY model selected (Hasegawa *et al.* 1985; no molecular clock, no invariable sites, gamma distribution with estimated shape parameter). The null hypothesis tested was that all trees are NOT significantly different and thus worse in their likelihood, and this test was used to choose one best tree. The NEXUS file was also run in SEPAL v. 1.4 (Salisbury 2001) for the generation of MP decay indices (Bremer 1994). Alignments were posted to the public databases through EMBL's WebInAlign and are available through ftp://ftp.ebi.ac.uk/pub/databases/embl/align/ under accession ALIGN_000377, a TreeBASE accession is pending.

RFLP

Restriction endonucleases *Bfa*I (staggered cut 5/4 bp after ACTGGG; MBI Fermentas) and *Nci*I (CC^SGG; New England Biolabs) were selected using WebCutter (www.firstmarket.com/cutter/cut2.html) to identify potential restriction sites, and ReBASE (http://rebase.neb.com/rebase/rebase.html) to identify suppliers. The enzymes were used to digest ITS 1F/ITS 4B PCR products, according to manufacturer's instructions. Digestion products were electrophoresed alongside a PhiX/*Hae*III marker (Promega G1761).

Specific primers

Primers specific for an intercompatibility group were designed by selecting motifs in the ITS DNA region that differed from other intercompatibility groups but were conserved within. An initial search against GenBank using BLAST-n (Altschul *et al.* 1997) was undertaken to confirm no hits of other microbial/fungal DNA. The following specific primers were prepared: PT-I-UTK (TCGAGTTTTGAAATATGGG, T_m = 57.51◦ C), PT-II-UTK (CCGGGTTGTAACTGG, T_m = 55.27◦ C), PT-III-UTK (CGCTGTGACCGTGA, T_m = 56.67◦ C). We designed ITS-4B-UTK (CAGGAGACTTGTACACGG, T_m = 56.49◦ C) as the return primer with a similar T_m. For PT-I-UTK we obtained a 5' fluorescein-labeled version (PT-I-UTK-F, T_m = 55.27◦ C after manufacturer's measurement). All these primers were synthesized by Sigma-Genosys. PCR products were run on agarose gels without ethidium bromide. Digital photography of flourescein-labeled PCR products required an exposure time of 7 sec as our Kodak DC120 Zoom Digital Camera/Kodak ds1D system (Eastman Kodak Co.) did not have filters for fluorescein. The gel was then stained with ethidium bromide, and another digital photograph taken. We also tried photography with a Kodak MP4 camera, here extended exposure times were also needed. Primer pairs PT-I-UTK-F/ITS-4B-UTK, PT-II-UTK/ITS-4B-UTK, and PT-III-UTK/ITS-4B-UTK were used to screen a variety of culture and specimen DNA extractions, with collections FB9585 (specimen) and FB11536 (dikaryon culture) being of unknown cryptic species identity. Parameters were: initial denaturation 94◦ C/4 min, and 35 cycles of denaturation 94◦ C/1 min - annealing 55◦ C/1 min, extension 72◦C/1 min, plus a final extension of 72◦ C/3 min. Sequencing was also performed with the unlabeled specific primer (only possible on templates initially generated without labeled primer) in order to verify identity of the PCR product. Ideally, the primers should be usable in multiplex PCR applications, as the PCR products would be different in size and by absence/presence of the flourescein label.

RESULTS

Culture morphology

In general, SBIs from Puerto Rico were more vigorous compared to SBIs from other geographical origin, readily forming more aerial hyphae (appearing fluffy) and occasional brown crusts. Continental *P. tricholoma* collections exhibited mostly shallow, translucent hyphal growth. These SBIs tended to lose viability after several rounds of water vial storage. No other culture-morphological differences were observed. Arthroconidia were occasionally formed by cultures.

Self-crosses

In *P. tricholoma*, flats and barrages appeared in both compatible and incompatible SBI pairings, but were not clearly associated with any mating type differences (see Hoffmann 1978, Krüger *et al.* 2002). Figures 1, 2, and 3 show self-crosses of three *P. tricholoma* collections, FB9579, FB4362, and FB10241. The mating system of FB9579 was found to be tetrapolar as was previously reported for this species (Roy & De 1977, David & Rajchenberg 1985). Collections FB4362 and FB10241 lacked a fourth mating type, but it was deduced that the mating system was tetrapolar.

9579		A_1B_1			A_2B_1			A_1B_2			A_2B_2		
		1	7	8	4	5	10	11	6	9	12	2	3
A_1 B_1	1		B-	-	F-	B-	B-	F-	-	F-	+	+	+
	7	B-		-	B-	-	B-	F-	F-	B-	+	+	+
	8	-	-		B-	F-	B-	F-	F-	F-	+	+	+
A_2 B_1	4	F-	B-	B-		B-	-	+	+	+	B-	B-	-
	5	B-	-	F-	B-		B-	+	+	+	B-	B-	B-
	10	B-	B-	B-	-	B-		+	+	+	F-	F-	F-
A_1 B_2	11	F-	F-	F-	+	+	+		-	B-	B-	B-	B-
	6	-	F-	F-	+	+	+	-		B-	B-	B-	-
	9	F-	B-	F-	+	+	+	B-	B-		B-	B-	-
A_2 B_2	12	+	+	+	B-	B-	F-	F-	B-	B-		B-	B-
	2	+	+	+	B-	B-	F-	B-	B-	B-	B-		B-
	3	+	+	+	-	B-	F-	B-	-	-	B-	B-	

Figure 1. Self-cross of *P. tricholoma* FB9579. SBI numbers and assigned mating types indicated on top and left, with tester SBI numbers appearing in shaded cells. + = clamp connections formed. F = flat contact zone morphology. B = barrage contact zone morphology.

4362		A_yB_x		third mating type					A_xB_y		
		7	20	12	9	11	13	19	3	10	18
A_y	7		-	-	(F)-	(F)-	-	-	+	+	+
B_x	20	-		-	-	-	-	-	+	+	+
3^{rd}	12	-	-		F-	-	(F)-	-	F-	F-	B-
	9	(F)-	-	F-		B-	-	(F)-	B-	F-	(F)-
	11	(F)-	-	-	B-		-	-	B-	(B)-	-
	13	-	-	(F)-	-	-		-	-	F-	-
	19	-	-	-	(F)-	-	-		-	F-	-
A_x	3	+	+	F-	B-	B-	-	-		B-	B-
B_y	10	+	+	F-	F-	(B)-	F-	F-	B-		-
	18	+	+	B-	(F)-	-	-	-	B-	-	

Figure 2. Self-cross of *P. tricholoma* FB4362. SBI numbers and assigned mating types indicated on top and left, with tester SBI numbers appearing in shaded cells. + = clamp connections formed. F = flat contact zone morphology. B = barrage contact zone morphology. Brackets indicate a less pronounced contact zone morphology.

10241		A_yB_x				third mating type				A_xB_y	
		5	15	10	7	2	3	4	6	9	13
A_y	5		■	F-	-	BFB-	F-	B-	B-	F-	B+
B_x	15	■		F-	F-	■	■	B-	B-	F+	F+
	10	F-	F-		F-	F-	-	B-	B-	F+	F+
	7	-	F-	F-		F-	F-	F-	B-	F+	F-
	2	BFB-	■	F-	F-		F-	F-	B-	■	F-
3^{rd}	3	F-	■	-	F-	F-		■	■	F-	■
	4	B-	B-	B-	F-	F-	■		F-	BFB-	B+
	6	B-	B-	B-	B-	B-	■	F-		B-	B-
A_x	9	F-	F+	F+	F+	■	F-	BFB-	B-		F-
B_y	13	B+	F+	F+	F-	F-	■	B+	B-	F-	

Figure 3. Self-cross of *P. tricholoma* FB10241. SBI numbers and assigned mating types indicated on top and left, with tester SBI numbers appearing in shaded cells. + = clamp connections formed. F = flat contact zone morphology. B = barrage contact zone morphology. BFB = lips contact zone morphology (barrages with a central flat). ■ = not discernable due to contamination (SBIs 2, 3, 15 tended to become contaminated). Some clamps could only be observed in small pockets after an additional 2 weeks. Knobby-sweaty hyphae often mimicked clamps. Lethal reactions in contact zone common.

Intercollection pairings

The comprehensive intercollection pairing experiment (Fig. 4) revealed the lack of complete intercompatibility among 14 collections of *P. tricholoma*. Instead, there appear to be three compatibility groups or cryptic biological species, tentatively named Group I (FB3870, FB4362, FB8420, FB9909, FB10240), II (FB9286, FB9568, FB9579, FB9591, FB10048, FB10258, FB10261, FB10920), and "III" (FB10241). All three groups exist in sympatry in Costa Rica, where several (FB10258, FB10261, FB10920, FB10240) showed a tendency to not achieve 100% compatibility even within their own group.

Sequence analysis

The ITS region sequence data set contained 30 sequences and 660 sites, of which 25 were variable/parsimony-uninformative, and 75 were variable/parsimony-informative. Sequences of two SBIs of FB10241 (SBIs 2 and 4) and one of FB10258 (SBI 1) were removed for having various, unalignable sequence motifs in the ITS region that were not indels, but completely different.

The unrooted MEGA2 neighbor-joining (NJ) dendrogram depicted as Fig. 5 is of a tree length of 148 steps (counted by PAUP*), CI = 0.791, RI = 0.936, RC = RI X CI = 0.740. The NJ interior branch test tree and the NJ bootstrap tree did not differ. A MEGA2 minimum-evolution (ME) tree with similar topology was described in PAUP* as 150 steps long (CI = 0.780, RI = 0.932).

NJ and ME trees show relationship groups that reflect the two observed cryptic species of *P. tricholoma* (Groups I and II), but fail to differentiate a Group "III". Group I/"III" received high bootstrap and interior branch test support and is separated from Group II by a *P. ciliatus* look-alike (AAU44971) which itself is far from *P. ciliatus* of Europe. Group II is supported with moderately hight bootstrap support, and even higher support in the interior branch test, where, however, the branch of Australian culture DSMZ-H27 collapses into a trichotomy (the position in the interior branch test analysis is marked by a double-line arrow). Closely related to the temperate taxa *P. arcularius*/*brumalis*/*ciliatus* is the Mongolian *Lentinus tigrinus*. *P. arcularius*, *P. brumalis*, and *P. ciliatus* are all supported with high statistical values, and they group together with equally high confidence.

PAUP* found 100 equiprobable MP trees in heuristic search (145 steps, CI = 0.807, RI = 0.943): these and the MEGA2 ME and NJ trees were evaluated by the Kishino-Hasegawa (KH) ML test. The best tree selected by the test was an MP tree with a likelihood of -*ln* L = 1725.88764: the other 102 trees evaluated were not statistically rejected with *P* = 0.0636 to 0.9952 (the MEGA2 trees, not calculated with MP as optimality criterion, scored lowest in *P*). This most-parsimonious tree (Fig. 6) coincided significantly with the topology of Fig. 5 (NJ), except that it appeared upside-down, which is without importance since all trees were unrooted. Decay indices, also based on a 145 step MP tree found in the SEPAL program, gave strong support (decay index = 10) for Group I incl. "III". There also was the inclusive but trichotomous *P. arcularius*/*brumalis*/*ciliatus* relationship group (decay index = 8), with *Lentinus tigrinus* basal to the latter, with a high decay index of 8. The relationship group corresponding to Group II included the Australian culture DSMZ-H27, but received a lower decay support of 2, and was also the relatively weakest group of the NJ tree (Fig. 5).

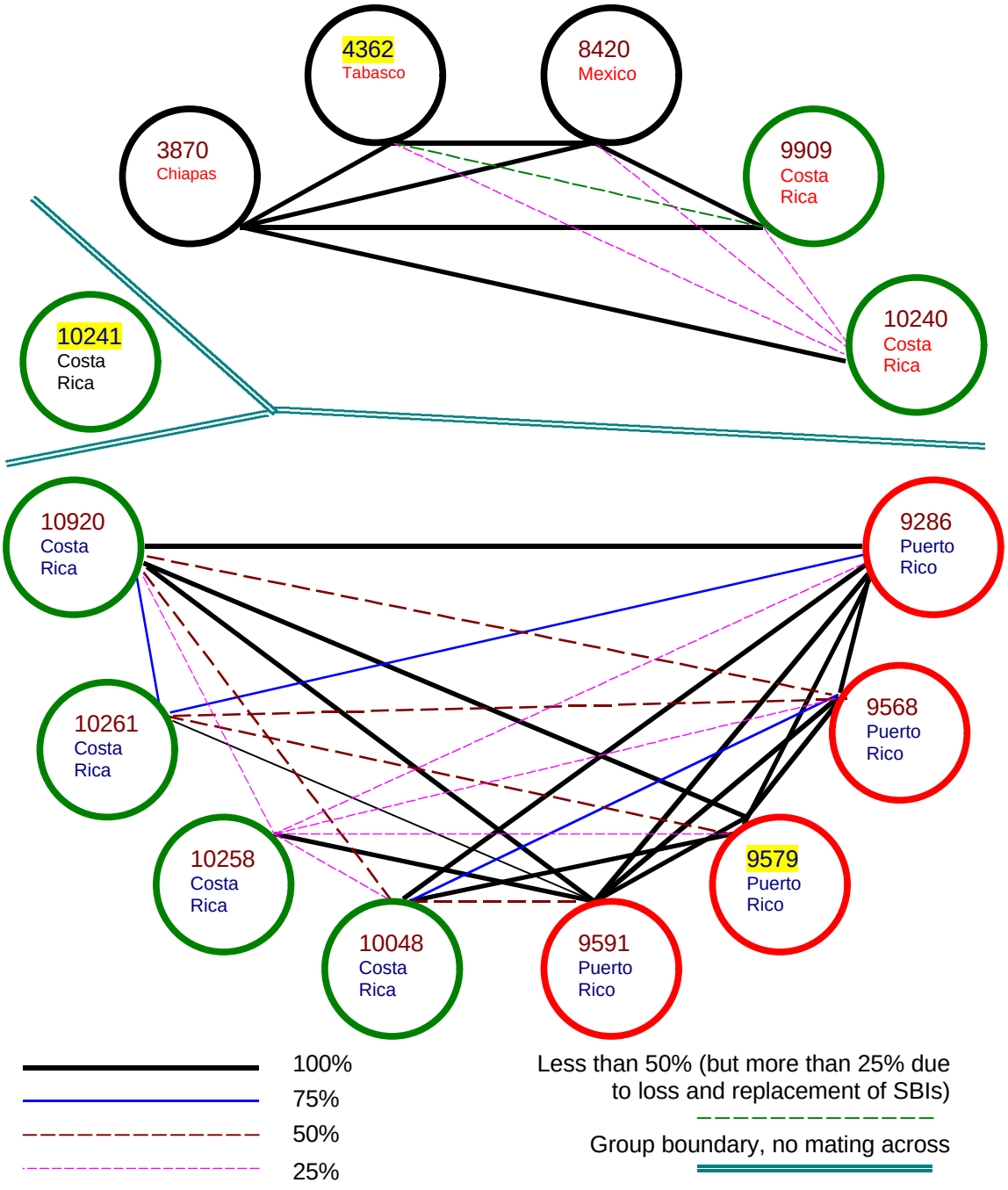

Figure 4. Overview of intercollection mating compatibility as expressed by formation of clamp connections. Upper collections with red label of origin = Group I, lower collections with blue label of origin = Group II, collection 10241 lefthand = "Group III". Black circle = Mexico (with Mexican state specified), red circle = Puerto Rico, green circle = Costa Rica. Exemplar FB numbers highlighted. 0% mating not indicated. FB acronym is omitted.

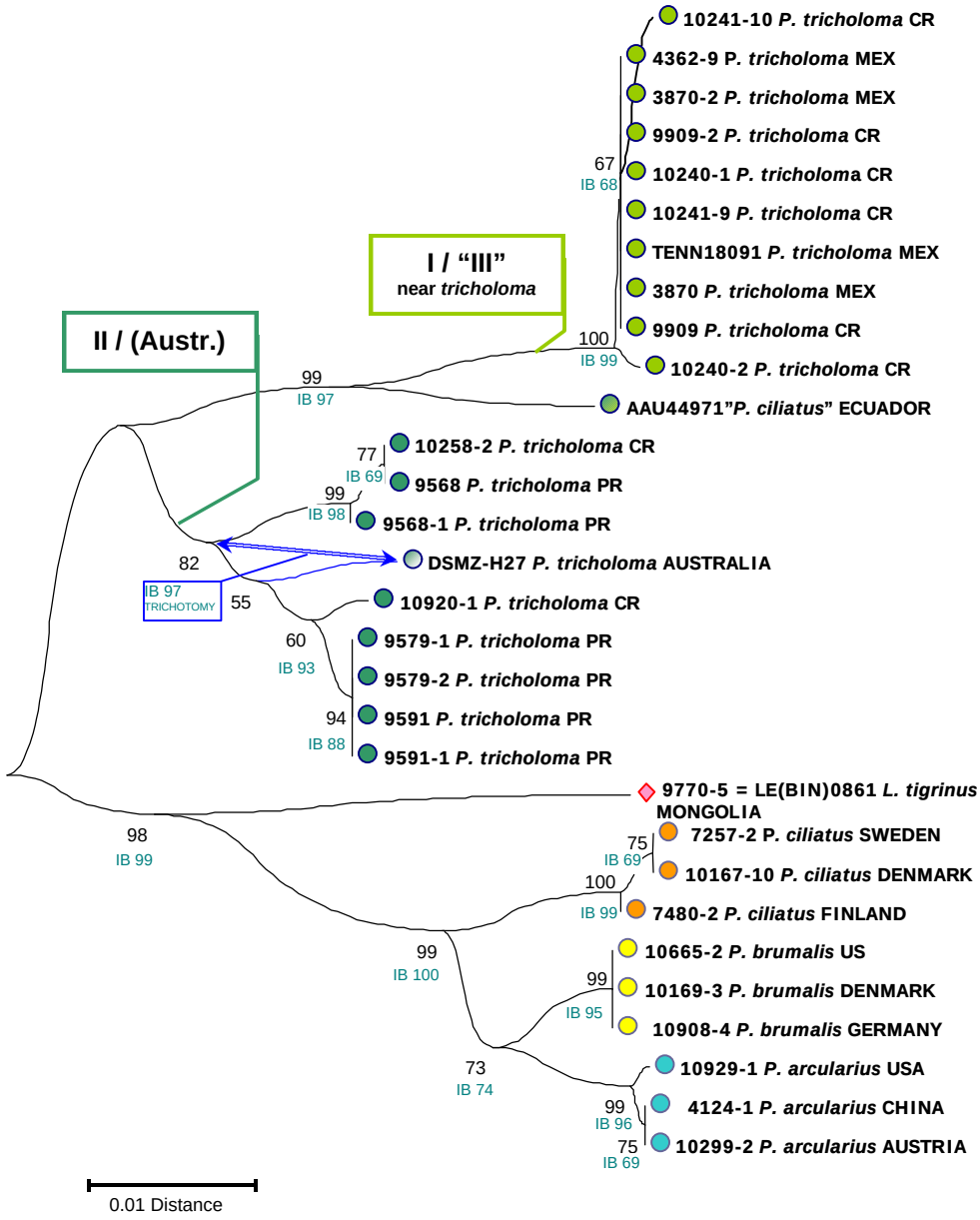

Figure 5. MEGA2 NJ tree based for ITS rDNA sequence data, Kimura2-parameter distance, unrooted, curved MEGA2 tree mode. Number 7480-2 (e.g.) denotes SBI 2 of CulTENN culture of FB7480, whereas the lack of a second number denotes a sequence derived from herbarium specimen or dikaryon culture. Geographic origin next to taxon label (CR = Costa Rica, MEX = Mexico, PR = Puerto Rico, US = continental United States). NJ bootstrap values in black, IB = interior branch test value.

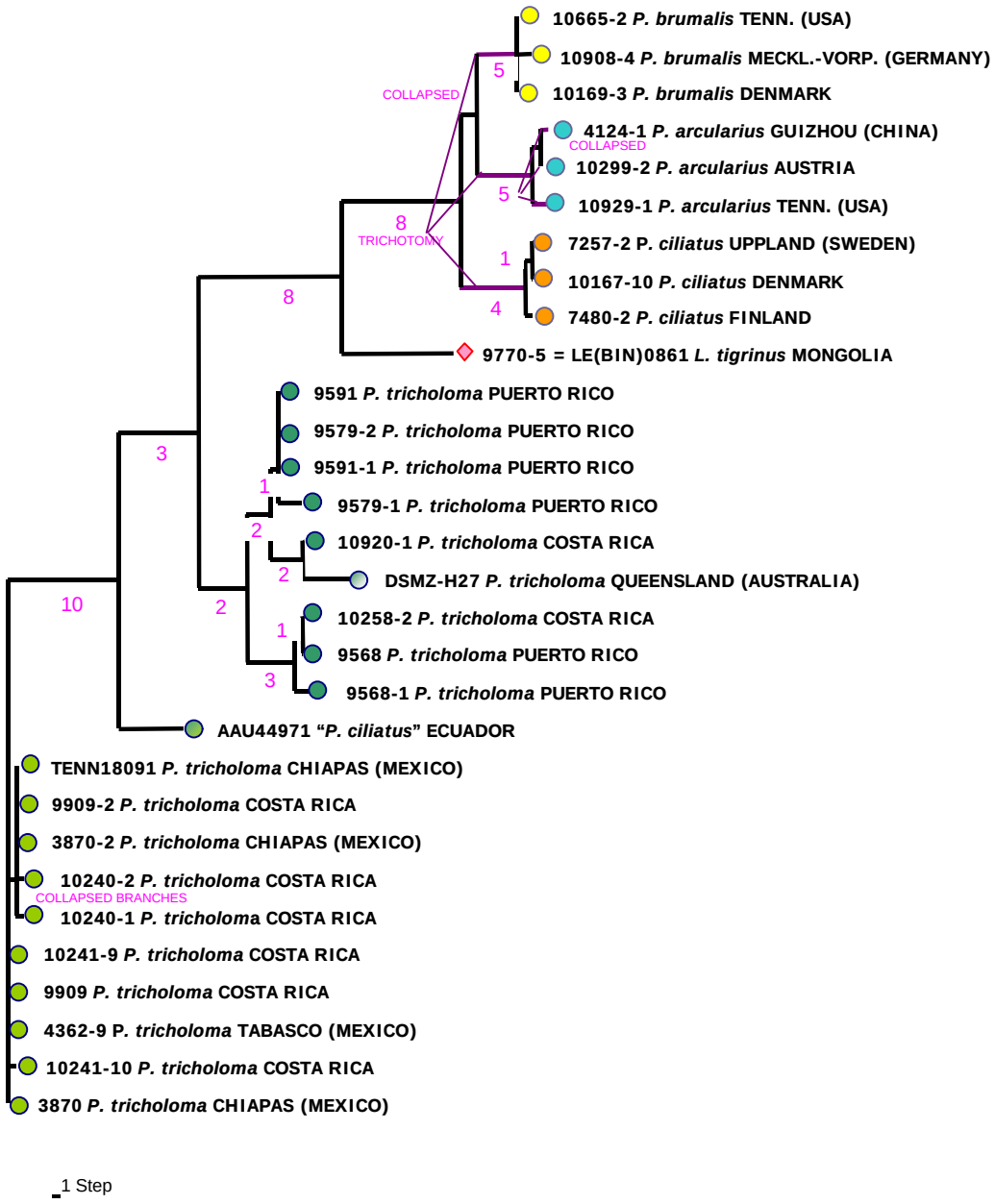

Figure 6. PAUP* MP tree based for ITS rDNA data, unrooted. Number 7480-2 (e.g.) denotes SBI 2 of CulTENN culture of FB7480, whereas the lack of a second number denotes a sequence derived from herbarium specimen or dikaryon culture. Geographic origin next to taxon label. Bremer decay index next to branch.

RFLPs

Figure 7 illustrates the restriction fingerprint found using the enzyme *Nci*I on ITS 1F/4B PCR amplicons. Most Group II amplicons were digested by this enzyme. FB10920 SBI 1 and DSMZ-H27 lacked the restriction site. FB10240 SBI 1 and FB10240 SBI 2, belonging to Group I, revealed incomplete digests. FB10258 SBI 2 also showed a incomplete digest, whereas FB10258 SBI 1 was digested.

With the enzyme *Bfi*I (Fig. 8), Group I and "III" DNA was not digested, whereas it was in most of Group II, incl. DSMZ-H27 (Australia). FB10258 SBI 1 was not digested, but FB10258 SBI 2 showed a incomplete digest. FB9579 SBI 1, FB9591 SBI 1, and FB10920 SBI 1 were cut more than once. Note in Figs. 7 and 8 that small restriction fragments are not visible.

Figure 9 may explain the observed incomplete digests and differences in enzyme specificity. FB10240 SBI 1 at position 134 is heterozygous (C or T), and FB10240 SBI 2 has a C instead of T as in all others depicted, and therefore has gained an *Nci*I restriction site. Heterozygosity was evident in the sequence trace file of FB10240 SBI 1, but not of 10240 SBI 2. DSMZ-H27 does not contain the *Nci*I site at position 166. In collection 10920 SBI 1, a mutation changed the sequence from TCCGGG to TTCGGG, again losing the restriction site. FB10258 SBI 1, which is a longer sequence due to indels, has an *Nci*I site a bit downstream of the regular site, FB10241 SBI 2 contains no *Nci*I site, and FB10241 SBI 4 a site 2 bases upstream of the regular site. Later sequenced FB10241 SBIs 9 and 10 do not contain the *Nci*I site at position 166. In place of a *Bfi*I site there now is a *Nci*I site at position 178 in FB10258 SBI 1. The incomplete digest of FB10258 SBI 2 is not explained by this alignment, however. Also, there is no indication of another *Bfi*I site in the sequences of FB9579 SBI 1, FB9591 SBI 1, and FB10920 SBI 1, however, perhaps undetected sequence heterogeneity may again play a role.

Efficiency of specific primers

An attempt was made to develop primers that would specifically amplify nuclear rDNA sequences of Groups I, II, and "III". Specific primers were designed with only few sequences at hand, and before suspecting sequence heterogeneity as explained in the RFLP results. PT-III-UTK, designed based on FB10241 SBI 2 and 4 sequences and differing in two bases from Group II, and 3 bases from Group I sequences, also yielded amplification with all other *P. tricholoma* DNA extractions. Later sequencing of SBIs 9 and 10 of FB10241 revealed the sequences to be identical to Group I at the primer annealing site. Primer PT-II-UTK also was insufficiently specific, and failed to amplify DNA extractions of FB10258 SBI 1 and FB10920 SBI 1, as explainable in Fig. 9, which highlights matches between primers and sites. The one primer that specifically amplified the intended target group was PT-I-UTK-F (Fig. 10). It expectedly failed to amplify FB10241 SBI 2, and yielded weak amplification of TENN18091. After staining with ethidium bromide, however, very faint amplification product bands from several non-Group I DNA extractions were seen, which again may be due to sequence heterogeneity or insufficient difference among the templates.

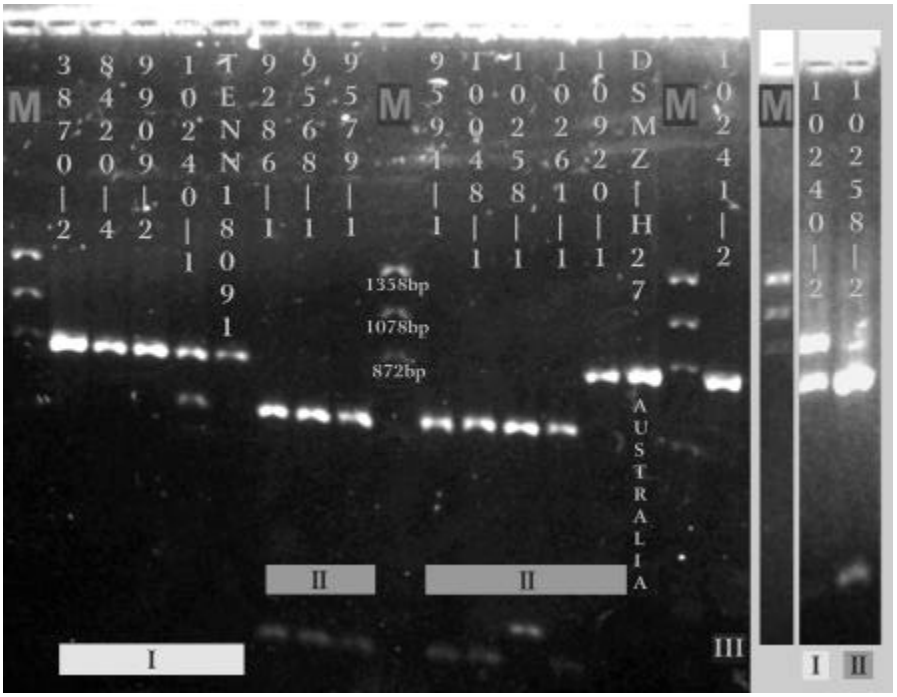

Figure 7. *Nci*I digest of *P. tricholoma*. Mating groups labeled on bottom of image. M = size marker

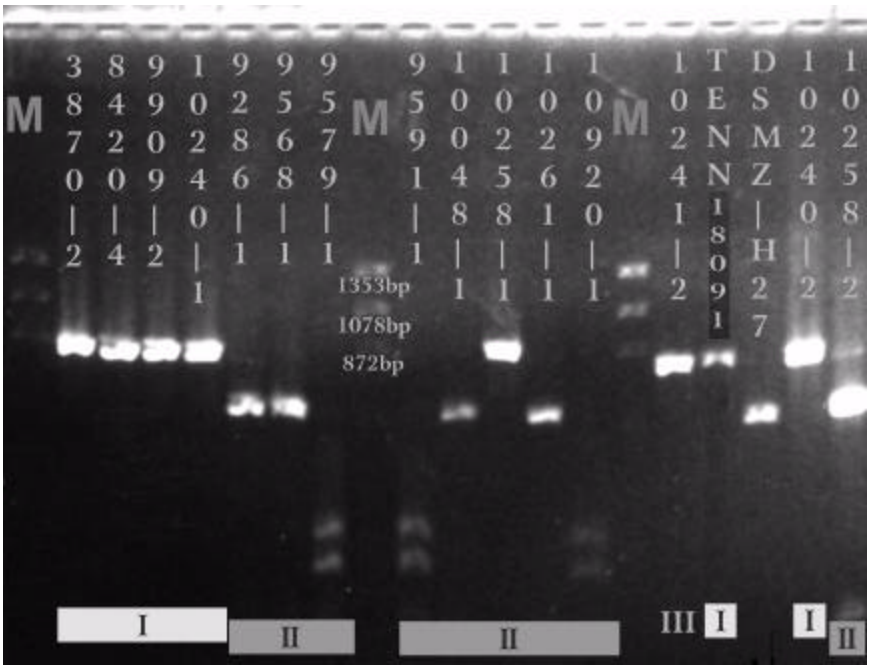

Figure 8. *Bfi*I digest of *P. tricholoma*. Mating groups labeled on bottom of image. M = size marker.

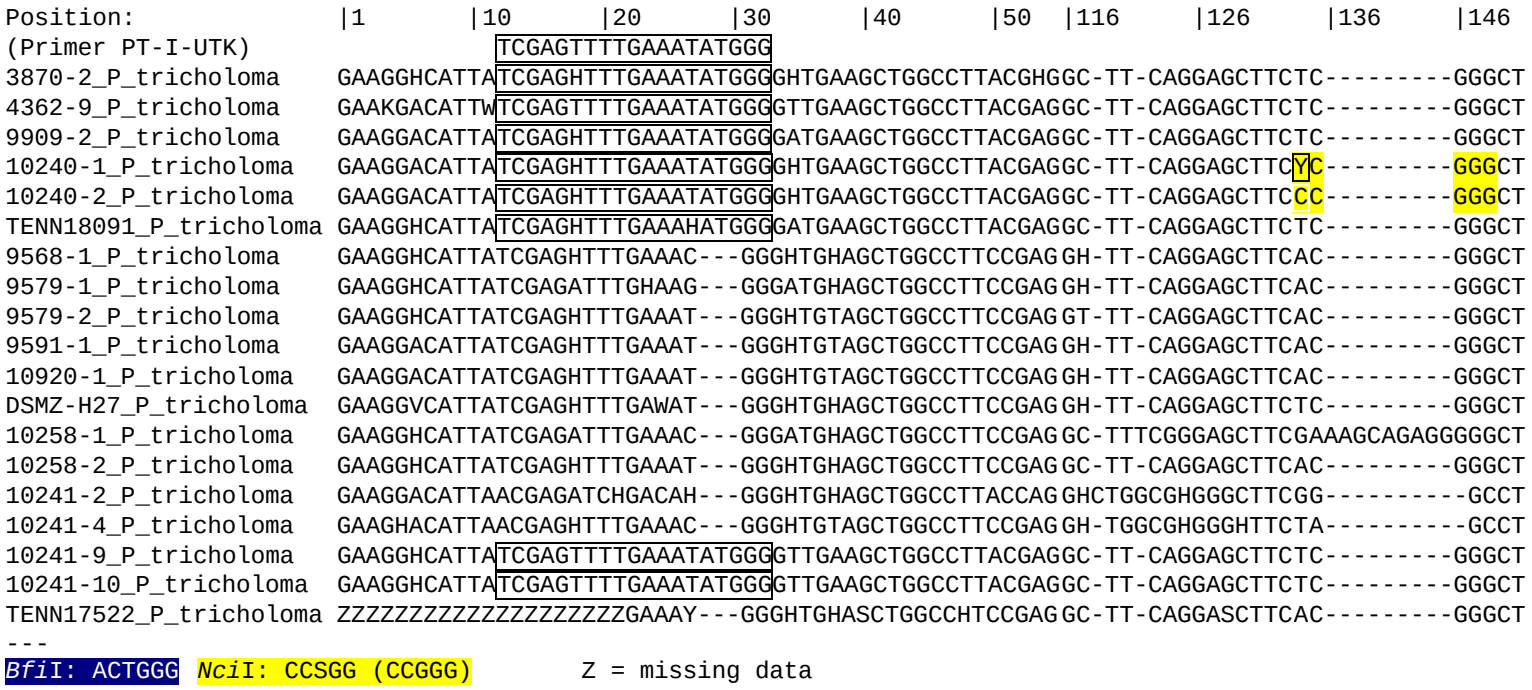

Figure 9. Sequence alignment of *P. tricholoma* ITS sequences with the location of restriction sites and specific primer annealing sites. Position refers to the positions in alignments used for phylogenetic analysis (### = position thus unavailable). FB10241 SBIs 1 and 2, FB10258 SBI 1, and TENN17522 were subsequently excluded from phylogenetic sequence analyses due to depicted sequence ambiguity or missing data. Continued on next two pages.

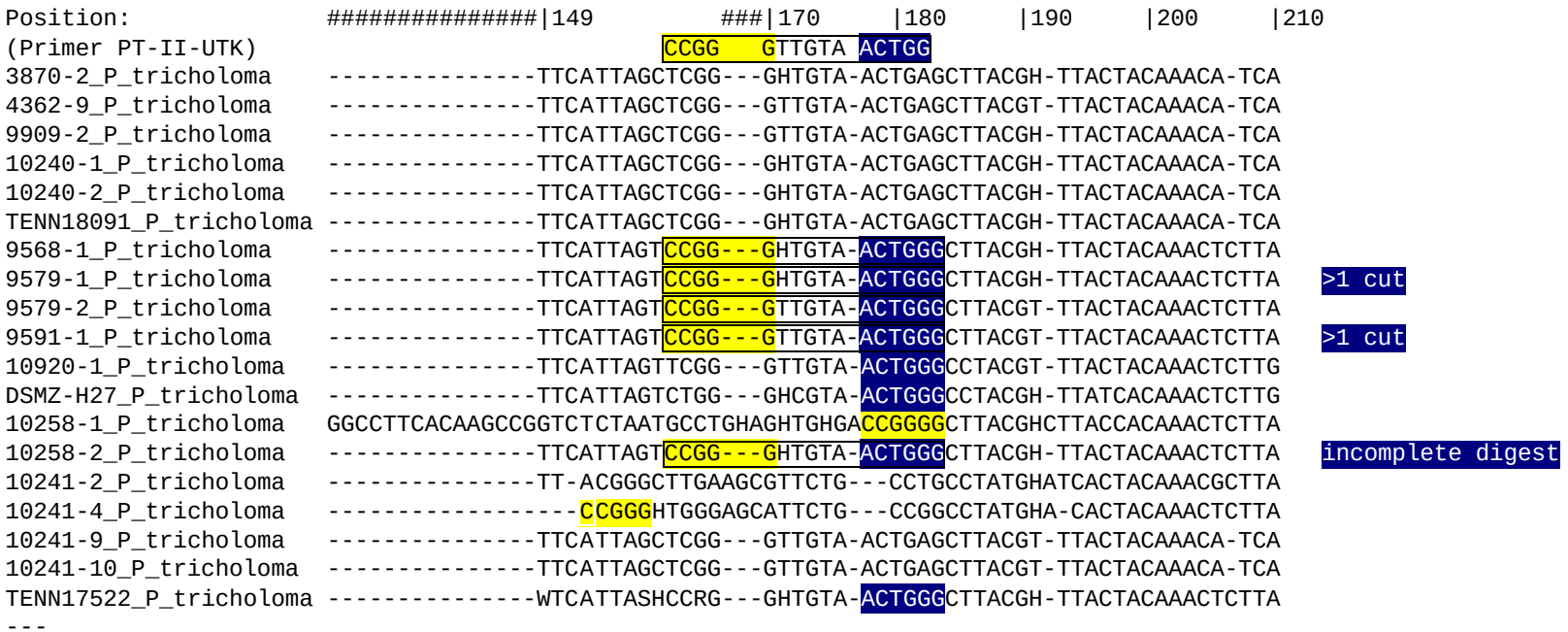

Figure 9. Continued. Sequence alignment of *P. tricholoma* ITS sequences with the location of restriction sites and specific primer annealing sites. Position refers to the positions in alignments used for phylogenetic analysis (### = position thus unavailable). FB10241 SBIs 1 and 2, FB10258 SBI 1, and TENN17522 were subsequently excluded from phylogenetic sequence analyses due to depicted sequence ambiguity or missing data. Continued on next page.

```
Position:              |567|570     |580     |590     |600     |610
(Primer PT-III-UTK)         CGCTGTGACCGT GA
3870-2_P_tricholoma    GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
4362-9_P_tricholoma    GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
9909-2_P_tricholoma    GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
10240-1_P_tricholoma   GTCTACGCCGCGACCGTTGAAGCGTATATAGGCC-AGCTTCTAATCGTCT
10240-2_P_tricholoma   GTCTACGCCGCGACCGTTGAAGCGTHTATAGGCC-AGCTTCTAATCGTCT
TENN18091_P_tricholoma GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
9568-1_P_tricholoma    GTCTACGCCGCGACCGT-GAAGCGTTTATTGACC-AGCTTCTAATCGTCT
9579-1_P_tricholoma    GTCTACGCCGCGACCGT-GAAGCGTTTATTGGCC-AGCTTCTAATCGTCT
9579-2_P_tricholoma    GTCTACGCCGCGACCGT-GAAGCGTTTATTGGCC-AGCTTCTAATCGTCT
9591-1_P_tricholoma    GTCTACGCCGCGACCGT-GAAGCGTTTATTGGCC-AGCTTCTAATCGTCT
10920-1_P_tricholoma   GTCTACGCCGCGACCGT-GAAGCGTTTATTGGCC-AGCTTCTAATCGTCT
DSMZ-H27_P_tricholoma  GTCTACGCCGCGACCGT-GAAGCGTTTATTGGCC-AGCTTCTAATCGTCT
10258-1_P_tricholoma   GTCTACGCCGCGACCGCTGAAGCGTTTAATGGCCCAGCTTCTAATCGTCT
10258-2_P_tricholoma   GTCTACGCCGCGACCGT-GAAGCGTTTATTGACC-AGCTTCTAATCGTCT
10241-2_P_tricholoma   GTCTACGCTGTGACCGT-GAAGCGTTT---GGCG-AGCTTCTAC-CGTCC
10241-4_P_tricholoma   GHCTACGCTGTGACCGT-GAACGTTTT---GGCG-A-CTTCTAC-CGTCC
10241-9_P_tricholoma   GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
10241-10_P_tricholoma  GTCTACGCCGCGACCGTTGAAGCGTTTATAGGCC-AGCTTCTAATCGTCT
TENN17522_P_tricholoma ZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZZ
```

Figure 9. Continued. Sequence alignment of *P. tricholoma* ITS sequences with the location of restriction sites and specific primer annealing sites. Position refers to the positions in alignments used for phylogenetic analysis (### = position thus unavailable). FB10241 SBIs 1 and 2, FB10258 SBI 1, and TENN17522 were subsequently excluded from phylogenetic sequence analyses due to depicted sequence ambiguity or missing data.

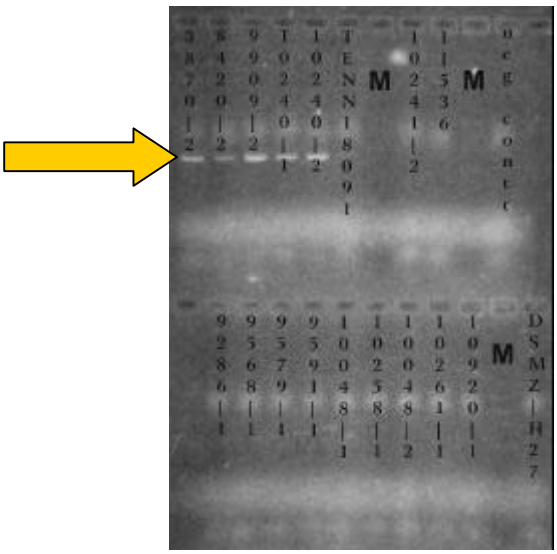

Figure 10. PCR products of fluorescein-labeled primer PT-I-UTK-F without ethidium bromide staining. M = size marker. The arrow marks the PCR product.

Microscopic characters

No difference in macromorphology between collections of the different compatibility groups of collections identified as *P. tricholoma* were detected. Spore sizes and size ranges were calculated. The spore sizes of Group I (66 spores/seven collections measured) were (5.0-) 6.83 (-10.0) µm X (2.0-) 2.86 (-4.0) µm, Q = (1.83-) 2.39 (-4.00). Group II spore measurements (81 spores of 10 collections) were (6.0-) 7.33 (-9.5) µm X (2.0-) 2.56 (-3.0) µm, Q = (2.17-) 2.87 (-4.00). Group "III" spores (11 spores of the one available collection, not included in Group I statistics) measured (6.5-) 6.55 (-8.0) µm X (3.0-) 3.27 (-3.5) µm, Q = (1.86-) 2.00 (-2.33). Group II spores were on average slimmer than those of Groups I and "III". Collection AAU44971 measurements (10 spores) were (5.5-) 6.45 (-7.0) µm X (1.5-) 1.90 (-2.5) µm, Q = (2.80-) 3.39 (-4.33), being more slender than in the *P. tricholoma* compatibility groups above and fitting *P. ciliatus* spores as given by Nuñez & Ryvarden (1995).

DISCUSSION

We (Krüger *et al*. 2002) previously reported morphological, biological, and phylogenetic species of *P. arcularius*, *P. brumalis*, and *P. ciliatus* to be internally cohesive, with the possibility that there may be potential to hybridize. In this study, we detected the existence of interINcompatible groups in Caribbean basin collections of *P. tricholoma*, of which two could be matched with phylogenetic groups with high statistical support. These groups were morphologically indistinguishable. Costa Rica, whence most collections originated, harbors all three Groups. There is DNA sequence evidence that a member, or close relative, of Group II, exists in Australia.

Sequencing, as well as RFLP markers and supposedly specific primers suggested that there is heterogeneity among ITS region sequences of SBIs stemming from the same carpophore, and heterogeneity among rDNA copies.

There was little phylogenetic resolution within the relationship groups representing Groups I and II (Figs. 2, 3). Ambiguity and heterogeneity probably account for the positional instability of the tree tips within those groups. Both Groups I and II are clearly separate from temperate *P. arcularius*, *P. brumalis*, and *P. ciliatus*. The gilled *Lentinus tigrinus*, previously reported as close to *Polyporus* (Hibbett & Vilgalys 1993, Hibbett & Donoghue 1995, PART 2) is a sister to these latter three taxa in the phylogenetic estimates shown.

A look-alike of the temperate *P. ciliatus* (AAU44971), both macromorphologically and in terms of spore sizes, is intermediate between *P. tricholoma* Groups I and II by DNA sequence. This suggests that sampling of more South American *Polyporellus* (Nuñez & Ryvarden 1995, Popoff & Wright 1998) collections is warranted.

Interestingly, Corner (1984) considered *P. tricholoma* to be the aggregate of reduced forms of more than one species, and distinguished five varieties A through E. Of these, the Brazilian "A" formed rather thick-stemmed, large basidiomata, and the short descriptions of the other four Asian and South American varieties which macromophologically appear to agree with our Caribbean collections. None of Corner's variety descriptions agree with our collections in spore sizes. Corner specifically mentioned the close relationship to *P. arcularius* and *P. brumalis*, which our collections did not confirm by DNA data.

Ideally, the specific primers should be usable in multiplex PCR applications, as the PCR products would be different in size and by absence/presence of the flourescein label. Our primer PT-I-UTK-F may be a tool to screen out most of what we called Group I, but there is need to address rDNA copy heterogeneity further in studies of molecular evolution within this species complex in *Polyporellus*.

POLYPORUS TRICHOLOMA TYPE STUDY & TAXONOMIC CONCLUSIONS

We have examined the holotype of *P. tricholoma* (PC), and collection Leprieur 939, authentic material also kept in the Montagne collections at PC (mentioned by Ryvarden 1982). The holotype (labeled as "*Polyporus* (*Mesopus*) *ambiguus P. tricholoma* Ins. Cuba" *s.n.*), *coll*. by Ramon de la Sagra, see Montagne 1837) comprises four stipitate carpophores.

Caps thin, up to 3.6 cm diameter; funnel-shaped, dried in a concentric-wrinkled pattern, but perhaps radially wrinkled before; margin ciliate, orangish to umbrous brown, partly colored darker towards margin as if hygrophanous.

Pores round to angular, decurrent, (3-)6-9(-10)/mm, some radially elongated to ca. 0.5 mm long; dissepiments thin and entire.

Stipe 1.2 to 2.6 cm long, 1-3 mm think, slightly velvety or parallel-fibrillose, grayish brown, olive brown, or ochraceous brown.

Hyphal system dimitic; with clamped generative hyphae of 2-3 µm diam., up to 8 µm in inflated segments; skeleto-ligative hyphae equally sized, variable in lumen width, with inflated stretches up to ca. 8 µm in diam..

Basidioles with clamp connections, 14-16 µm X 5-6 µm.

Basidiospores cylindric, variable in size: (n = 20) (7.0-) 8.35 (-11.0) µm X (2.0-) 2.48 (-3.5) µm, Q = (2.50-) 3.37 (-4.75).

Collection Leprieur 949 is in much worse shape, very flattened and covered with crystalline insecticide. It stems from Guyana, whereas the holotype was from Cuba. Spores are cylindric and variable in size: (n = 20) (5.0-) 6.63 (-9.0) µm X (2.0-) 2.38 (-3.0) µm, Q = (2.17-) 2.79 (-3.60). Among the five carpophores, ciliate margins are less evident, and the fruit bodies are more infundibuliform. One carpophore has an apically much inflated stipe.

The two collections differ somewhat in their morphology, and both may not be of the same species. Likewise, they may belong to any of the compatibility groups, or other, yet undetected ones, which may conform to Corner's varieties. The holotype must be matched with one of our cryptic species pair Group I (incl. "III") and II, without having to attempt sequencing the valuable holotype material. Given the scarce characters, we have chosen our Group II to be the "true" *P. tricholoma*, and therefore designate FB9579/TENN56491 as the EPITYPE (*hic designatum*). This is also justified because the spores appear to be closer in Q to those of Group II. Collection FB9579 does not stem from the original type locality Cuba, but another Caribbean island. The following Group II collections hereby are designated PARA-EPITYPES (FB9286, FB9568, FB9591, FB10048, FB10258, FB10261, FB10920).

Description of epitype

Two carpophores, attached at stipe.
Cap 1-1.5 cm in diameter, less than 1 mm thick, funnel-shaped, Cream-colored, dried in wrinkled manner; margin ciliate.

Pores angular, 6-9/mm; dissepiments thin, entire, cream with whitish shine.

Stipe 1-1.5 mm thick, 2.5 cm long, cream, with grayish tint towards base, parallel-fibrillose to slightly velvety.

Hyphal system dimitic; generative hyphae clamped, granulose in content, 2-4 µm diam.; inflated up to 17 µm, skeleto-ligative hyphae 1-4 µm diam., inflated up to 13 µm, branched, with variable lumen width.

Basidia with clamp connections, 14-16 X 4-6 µm.

Basidiospores: (n = 20) (6.0-) 7.53 (-9.5) µm X (2.0-) 2.28 (-2.5) µm, Q = (3.00-) 3.31 (-4.00). The Q value is almost identical to that of the holotype's spores.

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deposited cultures used in his dissertation and now stored at DSMZ. This study was supported by NSF grants to RHP and KWH (PEET grants DEB9521526/DEB9978011).

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PART 6

Polyporus: mating systems, relationships of gr. *Melanopus*, and transAtlantic compatibility of *P. varius* and *P. tubaeformis*.

This manuscript is intended for publication, with suggested co-authors Karen W. Hughes, Michael Fischer and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research, using some preliminary mating study data done by the co-authors.

Running Title: *Melanopus, Dendropolyporus*: mating system, phylogeny.

Abstract: *Melanopus* is an infrageneric group within *Polyporus.* Basidiomata of species in the *Melanopus* group are characterized by a blackened stipe and small pores, but the pileus is tougher and thinner than in *Polyporus* group *Polyporus sensu stricto* (= *Squamosus* group). Earlier phylogenetic studies showed *Polyporus* to be paraphyletic, with the *Melanopus* and *Squamosus* groups (and other genera) being more closely related to each other (in /core-Polyporus) than to *Polyporus* group *Polyporellus* and *Lentinus* subg. *Lentinus* (/Tigrinus-Polyporellus). In this study we address the systematics of *Polyporus* group *Melanopus* using ITS rDNA sequence data and also elaborate on the mating system and intercontinental mating compatibility of one of its prominent species, *Polyporus varius*. Collections of *P. varius* from both sides of the Atlantic Ocean were found to be intercompatible. This species has a tetrapolar mating system. The small stipitate polypore, *Polyporus tubaeformis*, which was elevated to species rank from *P. varius* subsp. *tubaeformis* less than a decade ago, was studied using mating compatibility tests. A tetrapolar mating system was found. Single-basidiospore isolates (SBIs) representing collections from California and Scotland proved compatible. Molecular analysis of nuclear ribosomal DNA (ITS I – 5.8S – ITS II) confirmed conspecifity of Scottish and Californian collections with a Norwegian collection. Several other herbarium specimens were redetermined as *P. tubaeformis* by morphological characters. This improved on the current knowledge of distribution of the species, tentatively adding Idaho, North Carolina and Germany's Mecklenburg-Western Pomerania. In addition, tetrapolar mating systems are also reported for *Polyporus* (*Dendropolyporus*) *umbellatus*, *P.* (*Melanopus*) *badius*, *P.* (*Melanopus*) *dictyopus*, *P.* (*Melanopus*) *leprieurii, and P.* (*Melanopus*) *melanopus*.

Keywords: *Atroporus*, Aphyllophorales, Basidiomycotina, *Cerioporus*, *Dendropolyporus*, molecular phylogeny, ribosomal DNA, *Polyporus umbellatus*, *Royoporus*, /Tubaeformis-"Phaeopodii".

INTRODUCTION

Melanopus was established as a segregate genus of *Polyporus* by Patouillard (*Melanopus* Pat. 1887, Hym. Eur.: 137), including taxa now contained in *Polyporus* group *Polyporus sensu* Nuñez & Ryvarden (1995) and infrageneric group *Melanopus sensu* Nuñez & Ryvarden (1995). *Melanopus* (group is abbreviated "gr.", referring to *Polyporus* group throughout the remainder of this manuscript) encompassed stipitate polypore species with a blackened stipe. Bourdot & Galzin (1928) distinguished two groups, *Varius* and *Squamosus*, in the genus *Melanopus*. Nuñez (1993) used the name "*Varius* group" in *Polyporus* attributing it to *Polyporus* section *Varii* Bond. & Sing. (1941, Ann. Myc. 39: 58) but superseded it with gr. *Melanopus* in Nuñez & Ryvarden (1995). The "*Polyporus* group" in Nuñez & Ryvarden (1995) was the "*Squamosus* group" of Nuñez (1993), referring to Bourdot & Galzin (1928). Perhaps this is widely congruent with *Polyporus* sect. *Squamosi* Bond. & Singer (1941, Ann. Myc. 39: 58). Nuñez (1993: 40) made no formal taxonomic changes, but apparently changed her opinion about the correct type species for the genus *Polyporus* (Nuñez & Ryvarden 1995). In 1993, it was *P. brumalis*, a member of *Polyporus* section *Genuini* of Bondartsev & Singer (1941: 58). Changing that to *P. tuberaster* (*Squamosi*), resulted in a change in the meaning of group names. The *Genuini* lost the label "*Polyporus* group" and adopted Karsten's

Polyporellus (Karsten 1880, Med. Soc. Fauna Fl. Fenn. 5:37). The *Squamosi* = gr. *Squamosus* took the label gr. *Polyporus*.

One of the gr. *Polyporus*, *P. squamosus*, even was selected as the type species of genus *Melanopus* (Ryvarden 1991), but earlier authors selected *P. melanopus* (Donk 1960). Donk argued that even if unmentioned, Patouillard clearly had *Polyporus melanopus* in mind as a species of his genus. Donk (1960: 240) would want the name *Melanopus* retained for further use if *P. squamosus* were removed from *Melanopus* and served as the type of *Polyporus s. str.* We counter that a tautonym would be created if *P. melanopus* was recombined in a freestanding genus *Melanopus* [but see Ryvarden (1991) for other reasons to reject Donk's typification]. An earlier name available is *Cerioporus* Quélet (1886, Ench. Fung. 1: 167), also with *P. squamosus* as lectotype (Murrill 1903).

Two more names should be considered, one being *Atroporus* Ryvarden (1973, Norw. J. Bot. 20: 2), proposed for species with inflated skeleto-ligative hyphae ("cystidia") around the type *Polyporus diabolicus*. Ryvarden (1991) later suggested that his earlier judgement of basing a genus on this character was wrong. Nuñez & Ryvarden (1995) synonymized *P. diabolicus* under *P. dictyopus*, and both Nuñez (1993) and Nuñez & Ryvarden (1995) furnished examples of inflated skeleto-ligative hyphae resembling setae of *Echinochaete* in several species of *Polyporus*.

The other genus to be discussed is *Royoporus* for species without clamps, with *Royoporus spathulatus* as type [but the basionym *Laschia spathulata* is a synonym of *P.* (*Favolus*) *grammocephalus* according to Nuñez & Ryvarden (1995)], and *Polyporus* (*Melanopus*) *badius* transferred as a second species (De 1996, 1997).

Corner (1984: 72) suggested that *P. badius*, *P. blanchettianus*, *P. dictyopus*, and *P. melanopus* were conspecific, their distinctions being only varietal. *Polyporus blanchettianus*, together with many other names, was referred to synonymy under *P. dictyopus* by Nuñez & Ryvarden (1995). We obtained an Argentine *P. melanopus* collection and a resulting culture also available from M. Nuñez's dissertation work (MN78 = MR10472) which she reported as partly compatible with a Norwegian collection (Nuñez 1993).

LSU rDNA sequence studies showed grs. *Squamosus* and *Melanopus* intermingled in a relationship group named /core-Polyporus (PART 2). We therefore wished to evaluate these groups based on ITS region sequences in this study, with emphasis on gr. *Melanopus*. Since *P. squamosus* (gr. *Polyporus*) and *Datronia mollis* were already shown as closely related to *P. varius* and *P. melanopus* (gr. *Melanopus*) by other rDNA sequences (Hibbett *et al*. 2000, Binder & Hibbett 2002), we also included both species in phylogenetic analyses.

Using mating studies together with molecular data, we also wished to investigate whether Nuñez & Ryvarden's (1995) relegation of *Polyporus elegans* into synonymy with *P. varius* was justified. Earlier reports by Petersen *et al*. (1997) on the mating systems of both *P. elegans* and *P. varius* required reconsideration. *Polyporus elegans* (FB7922) was cautiously reported as bipolar but this would be unusual for *Polyporus* (Petersen *et al*. 1997), and may have been caused by the limited number of monokaryotic cultures (SBIs) used. *Polyporus varius* FB7203 was reported in that publication (Petersen *et al*. 1997) as tetrapolar with one mating type missing.

In 1994, Ryvarden & Gilbertson (1994: 578) elevated *Polyporus tubaeformis* to species status from *P. varius* subsp. *tubaeformis*. Subsequently, it was placed in the infrageneric gr. *Melanopus* (Nuñez & Ryvarden 1995). *Polyporus tubaeformis* had often been confused with *P. melanopus* (Nuñez & Ryvarden 1995), and *P. badius*. *Polyporus tubaeformis* is unique among gr. *Melanopus sensu* Nuñez & Ryvarden (1995): fruit bodies begin with a pale grayish brown,

deeply funnel-shaped cap, and a punctate, pale brown stipe. Only later, the cap develops a dark violaceous-brown or bay brown cuticle, and the stipe cuticle turns blackish as in other gr. *Melanopus spp*.

The range of geographical distribution given by Nuñez & Ryvarden (1995) included northern Europe and Japan, with likely occurrences in Siberia and North America. Dai (1999) listed specimens studied from China and the Russian Far East and included the species in a checklist (Dai 2000). In this publication, we report SBIs from Scottish basidiomata identified as P*. tubaeformis* (*teste* Ryvarden), and putative *P. tubaeformis* from California (Figs 1, 2). These data were corroborated with DNA analysis. In addition, a number of gr. *Melanopus* collections were reidentified as *P. tubaeformis*, and are reported in spite of lack of mating studies and molecular data. Our efforts were undertaken in parallel with phylogenetic studies reported in PART 2 (where no LSU sequence could be generated for *P. tubaeformis*).

We also report the mating system of four other gr. *Melanopus* representatives: *Polyporus badius*, *P. dictyopus*, *P. leprieurii*, *P. melanopus*, and of *Polyporus umbellatus* of gr. *Dendropolyporus*.

MATERIALS & METHODS

All handling, microscopy, and maintenance of cultures and collections followed procedures previously outlined (Krüger *et al*. 2002, PARTS 2, 5). Collections and cultures used in this study are given in Table 1.

In case of collection FB10921, we germinated basidiospores harvested from a herbarium specimen. A piece of hymenophore was placed in sterile water in a 10 ml tube and vortexed for few sec. Ca. 0.5 ml water was pipetted onto the surface of malt extract agar in a Petri dish, then decanted after 30 min allowing the spores to settle. As with all single-basidiospore isolates (SBIs), germinating spores were excised with a sterilized dentist's pick, under observation through a WILD stereoscope.

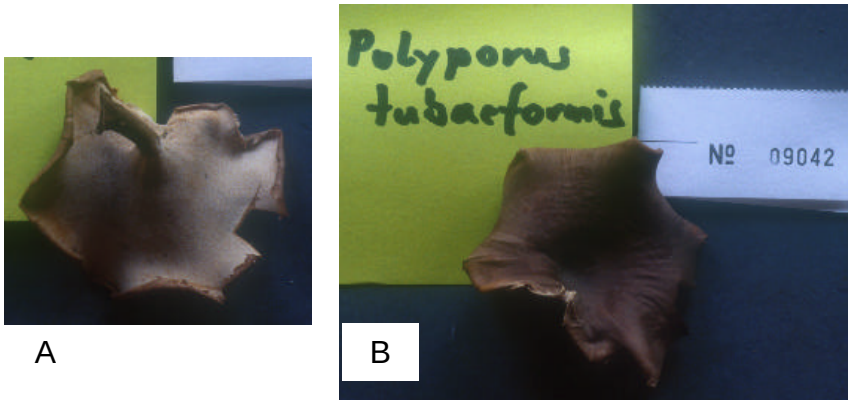

Figure 1. Specimen FB9042 *Polyporus tubaeformis*. **A**: hymenophore, **B**: cap surface. Scale comparison: numbered label small side = 2.3 cm.

Figure 2. Specimen FB8093 *P. tubaeformis*. Scale comparison: square yellow label = 7.6 cm.

Table 1. Fungal specimens and cultures investigated.

Strain numbers and/or herbarium voucher numbers if known	Fungal species and authors	Country of origin	Names of collectors and identifiers	GenBank number
FB10177 SBI 1 (TENN57707)	*Datronia mollis* (Sommerf.: Fr.) Donk	Denmark: Storstrøms Amt	RHP	AF516557
DSH90.36	*P. alveolaris* (DC.: Fr.) Bond. & Sing.		D. Hibbett	AB070828
FB10929 SBI 1 (TENN58412)	*P. arcularius* Batsch: Fr.	USA: Tennessee	H. Voglmayr	AB070867
FB10830 (TENN58379)	*P. badius* (Pers.) Schwein.	USA: Indiana	DK	AF516558
FB7925 SBI 6 (TENN54347)	"	USA: North Carolina	J. Cifuentes	AF516559
FB5085 (TENN59136) hseq	"	USA: Tennessee	RHP	AF511444
FB8856 (TENN55477)	"	USA: Georgia	I. Krisai-Greilhuber	-
X: FB11421 (TENN59126)	"	USA: Kentucky	DK & M. Fischer	-
FB11501 (TENN59385) hseq	*P. dictyopus* Mont.	Belize	E. Lickey	AF516561
FB11241 SBI 1 (TENN 58930)	"	Paraguay: Alto Paraná	RHP & O. Popoff	AF516562
FB11259 (TENN59068)	*P. grammocephalus* Berk.	Paraguay: Alto Paraná	RHP	AF516563

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
FB11288 SBI 1 (TENN59093)	*P. guianensis* Mont.	Argentina: Misiones	E. Albertó	AF516564
FB10064 (TENN57630)	"	Costa Rica: Puntarenas	J. L. Mata, R. Halling & RHP	AF516565
FB10921 SBI 8 = Ryv42383 (TENN58404)	"	Venezuela: Bolivar	T. Iturriaga & L. Ryvarden	AF516566
FB10489 SBI 10 (TENN58597)	*P. leprieurii* Mont.	Costa Rica: Puntarenas	J. L. Mata & RHP	AF516567
CIEFAP148 = MR10701	*P. melanopus* (Schwartz) Fr.	Argentina: Chubut	M. Rajchenberg	AF516569
CIEFAP57 = MN78 = MR10472	"	Argentina: Chubut	M. Rajchenberg	AF516568
X: FB11465 (TENN59326)	"	Austria: Nieder-österreich	H. Voglmayr	-
X: DAOM138015	"	Canada: Ontario	J. Ginns	-
X: Ryv27120 (O)	"	Estonia: Pärnu	L. Ryvarden	-
X: O70124 = Gulden 90/94	"	Norway: Aust-Agder	G. Gulden	-
X: Ryv22005 (O)	"	Norway: Oppland	L. Ryvarden	-
X: L. F. Grand 519 (NCSC)	"	USA: Idaho	L. F. Grand as *P. picipes*, *rev*. L. Ryvarden	-
X: FB9940 (TENN56753)	"	USA: Idaho	KWH	-
FB9562 (TENN56475)	"	USA: North Carolina	RHP	-
MN677 (O) hseq	*P. mikawai* C.G. Lloyd	China: Wuyi	M. Nuñez	AF516570
FB10298 = Thorn567 = Kotiranta6472 (culture)	*P. pseudobetulinus* (Pilát) Thorn, Kotiranta & Niemelä	Finland: Tervola	H. Kotiranta	AF516571
Psurtseva50 SBI 1 (TENN59384)	*P. squamosus* (Huds.) Fr.	Russia: Belgorod Region	N. Psurtseva	AF516572
FB11420 (TENN59125)	"	USA: Michigan	E. Grand	AF516573
FB9579 SBI 1 (TENN56491)	*P. tricholoma* Mont.	USA: Puerto Rico	RHP	AF516553
O63528 hseq	*P. tubaeformis* (Karst.) Gilbn. & Ryv.	Norway: Akershus	R. Anderaa, *det.* L. Ryvarden	AF511443

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
FB6961 SBI 2 (TENN55881)	*P. tubaeformis* (Karst.) Gilbn. & Ryv.	UK: Scotland	RHP as *P. varius*, *rev.* D. Krüger, *teste* L. Ryvarden	AF511439
FB6971 SBI 1 (TENN55889)	"	UK: Scotland	RHP, *det.* A. Henrici as *P. varius*, *rev.* D. Krüger, *teste* L. Ryvarden	AF511437
FB6973 SBI 1 (TENN55891)	"	UK: Scotland	RHP as *P. varius*, *rev.* D. Krüger, *teste* L. Ryvarden	AF511438
FB8093 SBI 1 (TENN55417)	"	USA: California	RHP	AF511442
FB9042 (TENN55689)	"	USA: California	KWH	SBI 1: AF511440 Dikaryon: AF511441
X: Ryv21376 (O)	"	China: Jilin	L. Ryvarden	-
X: DK0546 (TENN58056)	"	Germany: Mecklenburg-Vorp.	H. Ehlert & DK as *P. badius*	-
X: Ryv42621 (O)	"	Norway: Sogn og Fjordane	L. Ryvarden	-
X: L. F. Grand 24 (NCSC)	"	USA: Idaho	L. F. Grand as *P. elegans* or *P. picipes*, *det* DK, *teste* L. Ryvarden	-
X: L. F. Grand 725 (NCSC)	"	USA: North Carolina	L. F. Grand as *P. picipes*, *rev*. DK, *teste* L. Ryvarden.	-
FB6527 (TENN52363)	*P. umbellatus* Fr	USA: North Carolina	RHP	-
Fischer17 = 5079 SBI 8 (TENN59130)	"	USA: Tennessee	M. Fischer	-
SBUG-M1215 (culture only) (GFW-Kreisel)	*P. varius* Fr.	Germany: Mecklenburg-Vorp.	DK	AF516574
FB10513 (TENN57743) (culture)	"	Germany: Sachsen-Anhalt	DK	AF516575

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
FB10807 (TENN58216), fruited on sawdust: TENN58501 (*Fagus*), TENN58502 (*Acer*) TENN58503 (*Betula*)	*P. varius* Fr.	Russia: Leningrad Region	S. Pelchuzhina	AF516576
FB10550 (TENN58172) hseq	"	USA: North Carolina	DK	AF516578
FB10580 (TENN58194)	"	USA: North Carolina	S. Redhead	AF516577
FB7922 (TENN54333)	"	USA: Tennessee	RHP	AF516579
FB10962 SBI 19 (TENN58587)	"	USA: Vermont	DK	AF516580
X: O63529	"	Norway: Telemark	*leg.* C. M. Whist, *det.* M. Nuñez	-
X: L. F. Grand 1070 (NCSC)	"	USA: North Carolina	L. F. Grand	-
X: FB11465 (TENN59326)	"	Austria: Nieder-österreich	H. Voglmayr	-
FB10969 (no specimen)	"	Austria: Wien	H. Voglmayr	-
X: FB10963 (TENN58534)	"	Austria: Wien	T. Barta	-
X: FB10939 (TENN58534)	"	Canada: British Columbia	DK	-
FB10594 = REG97-713B	"	Germany: Bayern	M. Binder, M. Fischer, M. Jarosch & T. Wagner	-
FB10598A = REG97-713C	"	Germany: Bayern	M. Binder, M. Fischer, M. Jarosch & T. Wagner	-
X: DK0526 (TENN58055)	"	Germany: Brandenburg	DK	-
FB8703 (TENN55133)	"	Mexico: Tlaxcala	RHP	-
X: FB8869 (TENN55489)	"	Russia: Krasnodar Region	RHP	-
X: FB3865A (TENN55840)	"	UK: Scotland	RHP	-
X: FB6972 (TENN55890)	"	UK: Scotland	RHP	-

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
FB10340 (TENN57901)	*P. varius* Fr.	USA: North Carolina	KWH & RHP	-
FB10579 (TENN58193)	"	USA: North Carolina	DK	-
FB10948 (TENN58574)	"	USA: North Carolina	E. Grand	-
FB9559 (TENN56472)	"	USA: North Carolina	KWH & RHP	-
X: FB10590 (TENN58204)	"	USA: North Carolina	DK	-
X: FB10591 (TENN58205)	"	USA: North Carolina	DK & A. Sime	-
X: FB10546 (TENN58168)	"	USA: North Carolina	A. Sime	-
X: FB10551 (TENN58173)	"	USA: North Carolina	N. Psurtseva	-
X: FB10552 (TENN58174)	"	USA: North Carolina	DK	-
X: FB10573 (TENN58187)	"	USA: North Carolina	DK	-
X: FB10574 (TENN58188)	"	USA: North Carolina	S. Redhead	-
X: FB10577 (TENN58191)	"	USA: North Carolina	DK	-
X: FB10944 (TENN58571)	"	USA: North Carolina	DK	-
X: FB10945 (TENN58572)	"	USA: North Carolina	A. Euliss	-
X: FB10946 (TENN58573)	"	USA: North Carolina	A. Kovalenko	-
X: FB10949 (TENN58575)	"	USA: North Carolina	DK	-
FB9572 (TENN56485)	"	USA: Puerto Rico	E. Lickey	-
FB10564 (TENN58059)	"	USA: South Carolina	N. Psurtseva	-
FB9158 (TENN55926)	"	USA: Tennessee	RHP	-
X: FB10943 (TENN58538)	"	USA: Tennessee	KWH	-
X: FB10967 (TENN58830)	"	USA: West Virginia	A. Kovalenko	-
FB10955 (TENN58568)	"	USA: Kentucky	E. Lickey & J. Shaw	-

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
FB11219 (TENN58908) (culture)	*P. virgatus* Berk. & Curt.	Argentina: Misiones	E. Albertó	AF516581
FB11406 SBI 1 (TENN59111)	"	Argentina: Misiones	E. Albertó	AF516582
FB11530 (TENN59412)	*P. cf. virgatus* Berk. & Curt.	Belize	E. Lickey	AF516560

DK = Dirk Krüger, KWH = Karen W. Hughes, RHP = Ronald H. Petersen.
FB = TENN herbarium fieldbook number = CulTENN culture number (the acronym FB is omitted in phylogenetic trees). SBI = single-basidiospore isolate. hseq = herbarium specimen sequenced. **X** = identified by morphology alone. All AB accession numbers are from Krüger *et al*. (2002).
Herbarium acronyms follow Index Herbariorum Online (http://www.nybg.org/bsci/ih/ih.html).
If no specification "*leg./det*." the name mentioned in the fourth column is the collector and initial identifier of a collection. All DAOM, FB, NCSC, O collections have been seen by DK and compared with recent taxonomic literature.

Mating studies followed Petersen (1992; for self-crosses) and Krüger *et al*. (2002), using the observation of clamp connections as an indicator of compatibility. Fifteen collections identified as *P. elegans* or *P. varius* were used in intercollection crosses, with one European collection (FB10594) and the United States collection FB7922 (Petersen *et al*. 1997) serving as testers strains. For *Polyporus badius*, DAPI (Bresinsky *et al*. 1987) served as nuclear stain for epifluorescence microscopy (Nikon Optiphot). *Polyporus varius* FB10807 was fruited on sawdust of *Acer*, *Betula*, and *Fagus*, following techniques described by Psurtseva & Mnoukhina (1998). Extraction of DNA and PCR amplification of the ITS region of the nuclear ribosomal rRNA gene cluster (ITS I – 5.8S – ITS II rDNA) was described in Krüger *et al*. (2002) and PART 5.

DNA sequences were assembled, corrected and aligned with GeneDoc v. 2.6.002 (Nicholas *et al.* 1997), BioEdit v. 5.0.9 (Hall 1999), and ClustalX v. 1.64b (Thompson *et al*. 1997). Ambiguously aligned areas were removed from the data analysis, as were invariant regions and indels, leaving only unambiguously aligned variable sites. Results must thus be interpreted with caution as the exclusion of a high number of characters may also diminish autapomorphic characters and decrease branch lengths unduly. This, however, was judged a more conservative approach than leaving characters of questionable homology. Under the optimality criterion minimum-evolution (ME, Rzhetsky & Nei 1993), MEGA2 (Kumar *et al.* 2001) was utilized to infer phylogeny on the basis of the Kimura 2-parameter model of molecular evolution (Kimura 1980; alignment was checked to have approximately equal nucleotide frequencies). As additional support measures for the branches, 1,000 ME bootstrap pseudoreplicates (Felsenstein 1985) and 1,000 ME interior branch test (Nei & Kumar 2000: 168) pseudoreplicates were performed in MEGA2. Minimum-evolution generates a tree topology based on distance alone and seeks a parsimonious explanation in that the tree depicted has the shortest sum of branch lengths (Nei & Kumar 2000: 99), analogous to maximum-parsimony (MP), but otherwise similar to neighbor-joining (NJ). Additionally, congruence of the phylogram topology was compared after running the fastDNAml program (Olsen *et al.* 1994) on the website http://bioweb.pasteur.fr/seqanal/, with default parameters.

Distance-based and fastDNAml analyses were combined into one phylogenetic estimate. Rules followed in this thesis concerning naming of clades and grades were stated in PART 1.

For analyses under the Fitch maximum-parsimony model (Fitch 1971) in PAUP* v.4.0b8 (Swofford 2001), the data set was simplified, i.e. to contain only unique sequences (DAMBE v. 4.0.75: Xia & Xie 2001) as previously explained in PART 2, meaning that identical sequences were allowed to be retained only once, with a combined label. Fitch parsimony, the model that imposes no restrictions on character changes, was deemed as appropriate, lacking knowledge about molecular evolution of the retained nucleotide character positions. MP analyses were undertaken with the following parameters: MAXTREES = 10,000, tree-bisection-reconnection (TBR), starting trees by stepwise addition, 10 replicates, 1 tree held at each step, characters unweighted, steepest descent option not in effect. Trees were condensed by allowing the collapse of branches of zero length.

The Kishino-Hasegawa (Kishino & Hasegawa 1989) test implemented in PAUP* was asked to select one tree of the most-parsimonious trees for presentation in this publication. The same data set was used to calculate Bremer decay indices (Bremer 1994), and jackknife supports (Efron & Gong 1983; 100 MP pseudoreplicates), both in the program SEPAL v. 1.4 (Salisbury 2001). These values were superimposed on the selected MP tree generated in PAUP* to show statistical support for individual branches.

The alignments are available from public sequence databases (ftp://ftp.ebi.ac.uk/pub/databases/embl/align/) under accession numbers ALIGN_000379 (initial alignment), ALIGN_000380 (alignment after exclusion of uninformative sites, indels, and lengths of uncertain homology).

Molecular data were processed differently for another evaluation of *P. tubaeformis* sequences. Sequence analysis here was based on a subset of ALIGN_000379, complemented by one more sequence of *P. tubaeformis*. We retained the entire alignment length (ALIGN_000378) for analysis, and evaluated sequence similarities using BioEdit (Hall 1999) with entropy plots and sequence identity matrix. Analysis was then performed in MEGA2 (Kumar *et al*. 2001) under the minimum-evolution optimality criterion (ME: Rzhetsky & Nei 1993) with the Kimura 2-parameter model of molecular evolution (Kimura 1980), with gaps not participating in the analyses. Branch support was estimated with interior branch support test (Nei & Kumar 2000: 168) as implemented in MEGA2.

RESULTS

Self-crosses

Polyporus varius *FB10594*

When eight SBIs were paired in all possible combinations, a tetrapolar mating system was found (Fig. 3). Flat and barrage contact zone morphologies were not always clearly defined, and were independent of the mating types, so that mating types were assigned arbitrarily. Culture morphology involved patches of brown felty mats mostly starting from the inoculum block, arthroconidia in chains, and dried-up aerial hyphae collapsing onto the agar surface. On some agar plates, the hyphae stuck together in exudates, appearing slimy. Rich production of arthroconidia was also reported for the dikaryotic culture SBUG-M1244, and published by Ingold (1991).

10594		A_2B_1	A_2B_2	A_1B_1		A_1B_2			
		6	3	13	16	15	4	5	14
A_2B_1	6		B- *	F-	F-	+ *	F +	F +	+
A_2B_2	3	B-		+	F +	- slimy	- BFB	F- *	-
A_1	13	F-	+		F-	B-	F-	F-	B- *
B_1	16	F-	F +	F-		B-	- * slimy	-*	-*
	15	+ *	- slimy	B-	B-		- BFB	- * BFB	F-
A_1	4	F +	- BFB	F-	- * slimy	- BFB		B?- *	F-
B_2	5	F +	F- *	F-	-*	- * BFB	B?- *		F- *
	14	+	-	B- *	-*	F-	F-	F- *	

Figure 3. Self-cross of *P. varius* collection FB10594. + = clamp connections, - = no clamp connections, * = aerial hyphae dried up and crumpled, BFB = flat contact zone with raised margin, B = barrage, B? = faint barrage, F = flat contact zone. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

Polyporus badius *FB10830*

Eleven SBIs were paired in all combinations. A tetrapolar mating system was found (Fig. 4). In the self-cross, one mating type was missing. The pairing of seven additional SBIs with SBI 16 did not detect a fourth mating type. Flat and barrage contact zone morphologies appeared independent of the mating types and mating types were assigned arbitrarily. Cultures grew very slowly, formed dark-brown felty patches, and often stained the agar brown as well. Arthroconidia were formed in chains, and intercalary, thick-walled chlamydospores were also occasionally found. Assessing the number of DAPI-stained nuclei per cell was obscured by the occurrence of a green fluorescence of the cell walls which could be overcome by adding 3% KOH. Arthroconidia were found with both one and two nuclei.

Polyporus badius (Fig. 5), as well as many other gr. *Melanopus spp.*, *Polyporus* (*Dendropolyporus*) *umbellatus* and *Polyporus tuberaster* (gr.*Polyporus*) generally have slow growth of cultures. The survival rate of SBIs is low, if spores germinate at all. In gr. *Polyporus* (*P. squamous*), spores often seem to decay on the agar surface, or if germination occurred, the germ tubes were not well attached to the agar, making excision difficult. In gr. *Melanopus*, especially in *P. varius*, *P. badius* (Fig. 6), and *P. dictyopus*, but not *P. tubaeformis*, a “heap germination” was noticed. Such “heap germination” is characterized by multi-branching germ-tubes, forming a puddle of slowly growing, short-celled hyphae on the agar surface. This makes isolation of SBIs extremely difficult, and may result in recovery of mixed monokaryon cultures occasionally mimicking amphithallic mating systems, or lack of certain mating types. After several weeks, longer hyphae emerged from the heap of cells.

10830		A_2B_2		A_1B_1						third		
		17	6	8	1	3	5	9	11	14	16	4
A_2 B_2	17		F? -	+	F+	F+	F+	F+	B+	F-	F-	F? -
	6	F? -		B-	+	+	+	B+	+	B-	F-	B-
A_1 B_1	8	+	B-		B-	F-	F-	B?-	B?-	B?-	-	-
	1	F+	+	B-		B-	F? -	F? -	B-	-	F-	F-
	3	F+	+	F-	B-		F-	B-	B?-	F-	B-	-
	5	F+	+	F-	F? -	F-		-	◙	F-	F-	F-
	9	F+	B+	B?-	F? -	B-	-		F-	◙	-	-
	11	B+	+	B?-	B-	B?-	◙	F-		-	B-	B?-
3rd	14	F-	B-	B?-	-	F-	F-	◙	-		B-	B-
	16	F-	F-	-	F-	B-	F-	-	B-	B-		-
	4	F? -	B-	-	F-	-	F-	-	B?-	B-	-	

Figure 4. Self-cross of *P. badius* collection FB10830. + = dikaryotic, - = monokaryotic, B = barrage, B? = faint barrage, F= flat contact zone, F? = faint flat contact zone, ◙ uncertain nuclear stage (nuclei stretched). All remaining SBIs (18, 20, 21, 22, 24, 25, 26) have been tested against SBI 16 and did not reveal dikaryotic hyphae. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

Figure 5. Specimen FB8856 *P. badius*. Scale comparison: short side of number label = 23 mm.

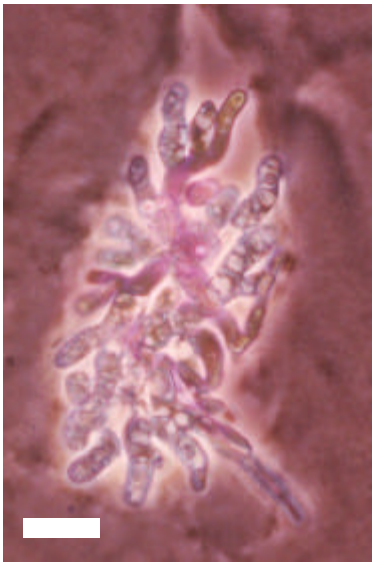

Figure 6. "Heap germination" of a basidiospore of collection FB10830, *P. badius*. Magnification 450X on OLYMPUS BX60, phase contrast, squash mount of agar. Photograph by David Sime. Scale: 10 µm.

Polyporus dictyopus *FB10921*

Six SBIs paired in all possible combinations revealed a tetrapolar mating system (Fig. 7). The pattern of barrages and flats was unrelated to the mating types, so mating types were assigned arbitrarily. Brown hyphal mats and arthroconidia were observed.

Polyporus leprieurii *FB10489*

When 10 SBIs were paired in all combinations, a tetrapolar mating system was revealed (Fig. 8). One mating type was missing in the sample. The pattern of barrages and flats was unrelated to the mating types, which were assigned arbitrarily. SBI combination 7 X 8 produced only sparse clamp connections. Beside flat and barrage or nondescript contact zones, "lip"-like bilateral contact zones of the types "barrages surrounded by flats" and "flat surrounded by barrage" were found among the combinations. The cultures formed patches of brown hyphal mats.

10921		A_1B_2	A_2B_2			A_1B_1	A_2B_1
		7	1	2	4	3	8
A_1B_2	7		F-	-	F-	F-	+
A_2 B_2	1	F-		F-	F-	F+	F-
	2	-	F-		F-	F+	B-
	4	F-	F-	F-		F+	B-
A_1B_1	3	F-	F+	F+	F+		B-
A_2B_1	8	+	F-	B-	B-	B-	

Figure 7. Self-cross of *P. dictyopus* collection FB10921. + = clamp connections, - = no clamp connections, B = barrage, F= flat contact zone. Assigned mating types indicated on the top and to the left. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

10489		A_1B_1		A_2B_2		third mating type					
		7	5	8	9	1	2	3	4	6	10
A_1 B_1	7		F-	F + (few)	F +	F-	F-	B-	F-	F-	F-
	5	B- *		+	B +	-	F-	F-	F-	F-	F-
A_2 B_2	8	F + (few)	+		F-	BFB-	BFB-	B-	F-	F-	FBF-
	9	F +	B +	F-		F-	B-	F-	F-	F-	F-
3^{rd}	1	F-	-	BFB-	F-		F-	B-	F-	F-	B-
	2	F-	F-	BFB-	B-	F-		B-	F-	B-	B-
	3	B-	F-	B-	F-	B-	B-		BFB-	B-	BFB-
	4	F-	F-	F-	F-	F-	F-	BFB-		B-	F-
	6	F-	F-	F-	F-	F-	B-	B-	B-		FBF-
	10	F-	F-	FBF-	F-	B-	B-	BFB-	F-	FBF-	

Figure 8. Self-cross of *P. leprieurii* collection FB10489. + = clamp connections, (few) = few clamp connections, - = no clamp connections, B = barrage, F= flat contact zone, BFB = flat contact zone with raised margin, FBF = barrage surrounded by flat margins. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells

Polyporus tubaeformis *FB6961*

Thirteen monokaryotic single-basidiospore isolates paired in all possible combinations revealed a tetrapolar mating system (Fig. 9). Seven of the cultures belonged to one mating type. Barrage and flat contact zone morphology was unrelated to mating types, and was not stable in that the morphology changed from flat to barrage when the plates were kept for several weeks. Conidiogenesis was not observed, nor did we note a “heap germination” as described previously. Cultures were feathery, white, and grew relatively quickly as compared to *P. varius*, *P. badius*, *P. umbellatus*, or *P. melanopus*. This culture morphology appears to be distinct and of taxonomic relevance in distinguishing *P. tubaeformis* from *P. badius* and *P. melanopus*, the closest known relatives.

Polyporus melanopus *FB9562*

Pairing eleven available SBIs in all possible combinations, a tetrapolar mating system was found, but with one missing mating type (Fig. 10). Barrages and flats were not correlated with the mating types, nor were they stable over time. The cultures typically formed brown mats and often stained the agar brown. Crystalline exudates formed on the hyphae.

Polyporus umbellatus *FB5079*

When 10 SBIs were paired in all combinations, a tetrapolar mating system was detected (Fig. 11). All combinations produced clearly flat reactions with little aerial and submerged hyphal growth in the contact zone. Mating type designation was therefore arbitrary.

Spores germinated slowly and in the "heap fashion" of gr. *Melanopus*. Growth of many cultures was extremely slow: only 1-2 mm in four weeks. These cultures were often brown pigmented, many of which were dikaryotized by close proximity on the polyspore print. Only monokaryotic, faster, feathery white cultures were used for the self-cross. Growth of these was about 10-30 mm in three weeks. Commonly crystalline exudates were found on the agar surface. All cultures featured copious formation of arthroconidia in which the aerial hyphae fragmented into long chains of barrel-shaped conidia of variable length, often with dried-up, constricted segments between them. A few branches of such conidial chains were observed. A very similar conidiogenesis was found in dikaryons of *Polyporus squamosus* (PART 7). Cultures arising from detached conidia and broken-off agar from the inoculum separate from each other by flats with the same appearance as that observed in sexual crosses, so the flat morphology appears independent of sexual compatibility. Any clamp connections formed were only in small pockets without any observable morphological changes at the flat zone and seemed not to proliferate much, making reading of the experiment difficult. This may account for the lack of clamp observation in SBI 12 X SBI 8. Most hyphae, also when not forming conidia, were subject to crumpling and/or excessive exudation of liquid in which hyphae/conidial chains collapsed onto the agar surface.

6961		A_1B_1	A_1B_2							A_2B_1		A_2B_2		
		2	4	5	7	10	12	13	14	15	16	3	6	9
A_1B_1	2		B-	B-	B-	F-	B-	B-	B-	B-	B-	F+	F+	+
A_1 B_2	4	B-		B-	F-	B-	B-	F-	F-	+	+	B-	B-	B-
	5	B-	B-		F-	F-	F-	B-	F-	F+	+	B?-	F-	-
	7	B-	F-	F-		F-	F-	F-	F-	F-	+	B-	B-	F-
	10	F-	B-	F-	F-		F-	B-	F-	+	+	B-	F-	B-
	12	B-	B-	F-	F-	F-		B-	B-	B+	B+L	BFB-	B-	F-
	13	B-	F-	B-	F-	B-	B-		B-	F+	F+	F-	F-	F-
	14	B-	F-	F-	F-	F-	B-	B-		+	+	B-	F-	B-
A_2 B_1	15	B-	+	F+	F-	+	B+	F+	+		F-	F-	B-	B-
	16	F-	+	+	+	+	B+L	F+	+	F-		F-	F-	B-
A_2 B_2	3	F+	B-	B?-	B-	B-	BFB-	F-	B-	F-	F-		F-	B-
	6	F+	B-	F-	B-	F-	B-	F-	F-	B-	F-	F-		F-
	9	+	B-	-	F-	B-	F-	F-	B-	B-	B-	B-	F-	

Figure 9. Self-cross of *P. tubaeformis* collection FB6961. + = clamp connections, - = no clamp connections, BFB = flat contact zone with raised margin, B = barrage, B? = faint barrage, F= flat contact zone, L = lethal reaction in contact zone. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

9562		A_1B_1					3rd	A_2B_2				
		2	4	5	6	9	17	7	8	11	13	14
A_1 B_1	2		B-	-	F-	B-	B-	F+	F+	F+	F+	F+
	4	B-		B-	B-	B-	B-	F+	+	F+	B+	+
	5	-	B-		F-	B-	B-	F+	+	+	F+	F+
	6	F-	B-	F-		F-	B-	B+	F+	F+	B+	F+
	9	B-	B-	B-	F-		F-	F+	B+	F+	F+	+
3rd	17	B-	B-	B-	B-	F-		F-	F-	B-	B-	B-
A_2 B_2	7	F+	F+	F+	B+	F+	F-		(B)-	F-	B-	F-
	8	F+	+	+	F+	B+	F-	(B)-		F-	B-	B-
	11	F+	F+	+	F+	F+	B-	F-	F-		F-	F-
	13	F+	B+	F+	B+	F+	B-	B-	B-	F-		-
	14	F+	+	F+	F+	+	B-	F-	B-	F-	-	

Figure 10. Self-cross of *P. melanopus* collection FB9562. + = clamp connections, - = no clamp connections, BFB = flat contact zone with raised margin, B = barrage, B? = faint barrage, F= flat contact zone, L = lethal reaction in contact zone. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

5079		A_1B_1		A_1B_2		A_2B_1		A_2B_2			
		7	20	8	11	12	17	13	16	18	19
A_1 B_1	7		F-	F-	F-	F-	F-	F+	F+	F+	F+
	20	F-		F-	F-	F+	F-	F+	F+	F+	F+
A_1 B_2	8	F-	F-		F-	F-	F+	F-	F-	F-	F-
	11	F-	F-	F-		F+	F+	F-	F-	F-	F-
A_2 B_1	12	F-	F-	F-	F+		F-	F-	F-	F-	F-
	17	F-	F-	F+	F+	F-		F-	F-	F-	F-
A_2 B_2	13	F +	F+	F-	F-	F-	F-		F-	F-	F-
	16	F+	F+	F-	F-	F-	F-	F-		F-	F-
	18	F+	F+	F-	F-	F-	F-	F-	F-		F-
	19	F+	F+	F-	F-	F-	F-	F-	F-	F-	

Figure 11. Self-cross of *P. umbellatus* collection FB5079. + = clamp connections, - = no clamp connections, B = barrage, F= flat contact zone. Assigned mating types indicated on the top and to the left. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

One of us (RHP) had previously done a self-cross of *P. umbellatus* FB6527. He also observed lips as presumably a form of flat contact zone morphology, and otherwise contact zone morphology was not patterned. The SBIs mainly grew on or beneath the agar surface, with sparse aerial hyphae which were usually converted to masses of conidia making the culture appear granulose. Clamp connection detection was difficult because of knobby protuberances of irregularly torulose hyphae. Lethal reactions in contact zones involved crumpled aerial hyphae and empty submerged hyphae. Also observed were the crumpled, emptied interconidial cells in arthroconidial chains. The self-cross of FB6527 was read as representing tetrapolarity, and also all other characters fully agree with FB5079, except for FB6527 basidiospores germinated within 48 hours and grew quickly. The SBIs of FB6527 subsequently have been lost.

Intercollection pairings & fruiting of P. varius

The results of the intercollection pairings are shown in Fig. 12. Of the 15 strains participating in the pairing experiments, 14 belonged to one intercompatible biological species encompassing collections from Europe, the continental United States, Puerto Rico, and Mexico. Note that not all possible pairings were tested, but only all collections against the examplars (4 SBIs each of FB10594 and FB7922). Collection FB10807, notably, did not form clamp connections in any of the pairings. The dikaryotic culture of FB10807 had been fruited on various substrates, yielding specimens as shown in Figs. 13 and 14. Figure 13 shows that *Polyporus varius* can form almost cap-less stipes. A comparison of fruit bodies fruited on different kinds of wood is given in Fig. 14. The basidiomata appeared somewhat darker and drier after harvesting them in the greenhouse, and were photographed indoors (Fig. 14) as opposed to inside the sunny greenhouse (Fig. 13).

Intercollection pairings involving P. tubaeformis

Intercollection pairing experiments involving *P. tubaeformis* and *P. melanopus* are shown in Fig. 15. The SBIs of the Scottish collections at hand were intercompatible with California SBIs, indicating that they were “conbiospecific” (= belonging to the same biological species as judged by mating ability). *Polyporus tubaeformis* tester SBIs were paired against those of *P. arcularius*, *P. brumalis*, *P. ciliatus*, *P. tricholoma* (gr. *Polyporellus*, PART 2) as young fruit bodies of *P. tubaeformis* share with them a light color, and as *P. tubaeformis* cultures differed from *P. melanopus* and *P. badius*. They were all incompatible. Neither *P. tubaeformis* nor *P. melanopus* were paired with SBIs of *P. badius*, as *P. badius* is clearly different macromorphologically and lacks clamp connections.

Sequence analyses focused on gr. Melanopus

A *P. blanchettianus* sequence could not be generated, but we were able to obtain sequences for *P. badius* and *P. dictyopus*. The sequence alignment (ALIGN_000380) of unambiguously alignable characters contained 107 nucleotide sites. Minimum-evolution (ME)

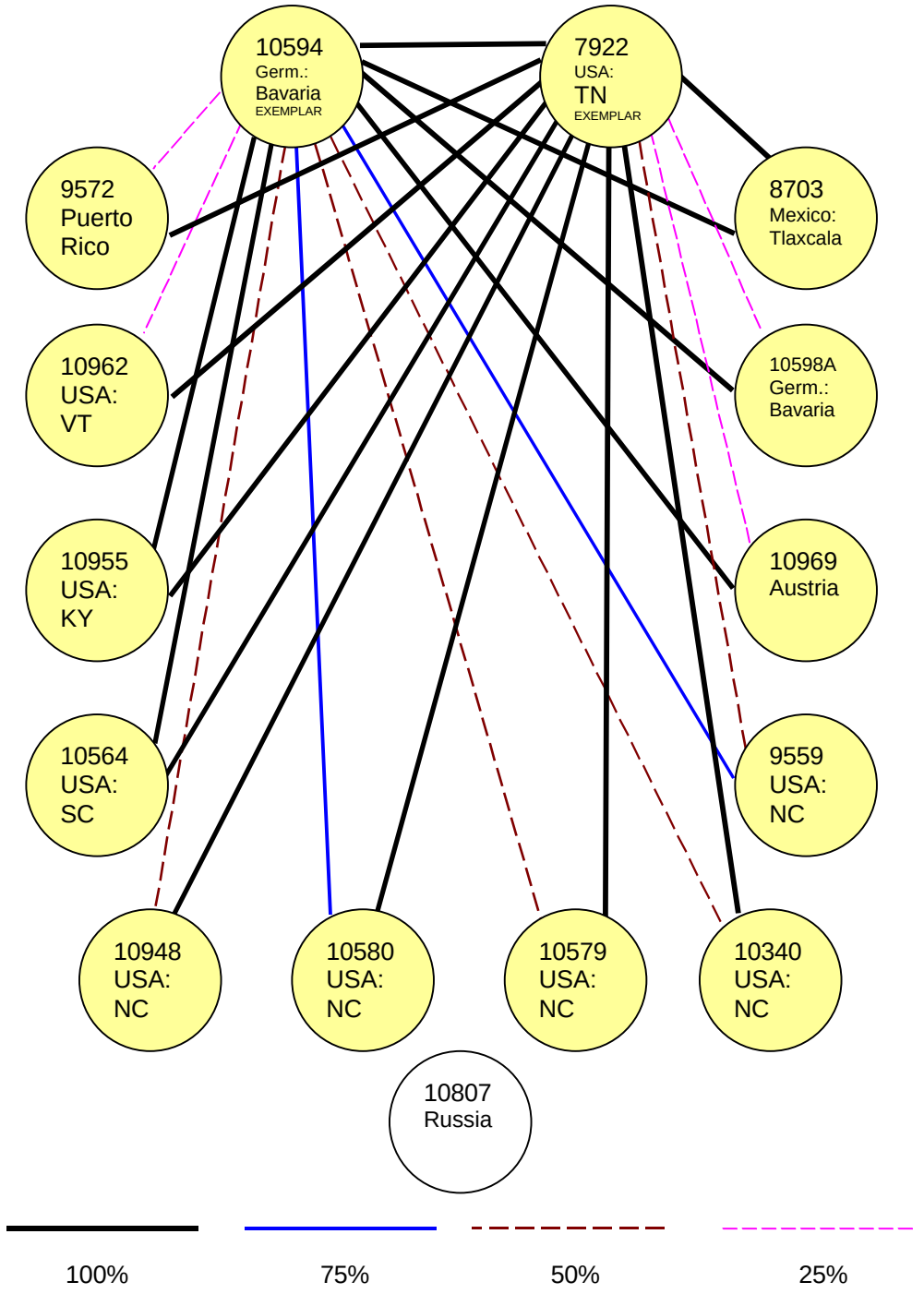

Figure 12. Intercollection pairings tested in the *P. varius*/*P. elegans* complex. Lines representing percentage compatibility as expressed by clamp connections formed (100% = 4/4 SBI pairings, 75% = 3/4, 50% = 2/4, 25% = 1/4, 0% not indicated). Pairing was only against testers (SBIs of FB10594 and FB7922). All numbers are FB numbers.

Figure 13. *Polyporus varius* FB10807 fruited on *Betula* sawdust (TENN58503).

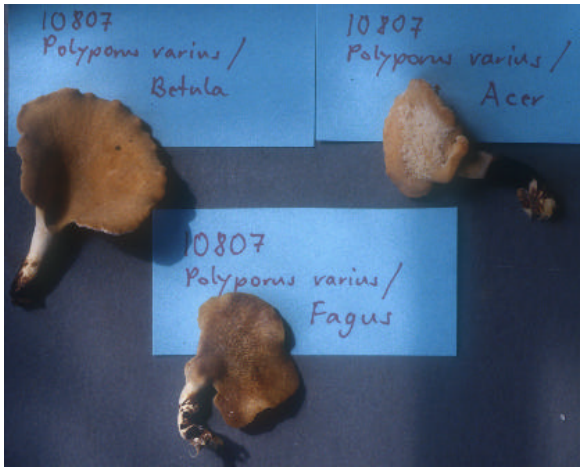

Figure 14. *Polyporus varius* FB10807 specimens from fruiting on sawdust. TENN58501 (*Fagus*), TENN58502 (*Acer*) TENN58503 (*Betula*). Scale comparison: blue label in center = 7.6 cm long.

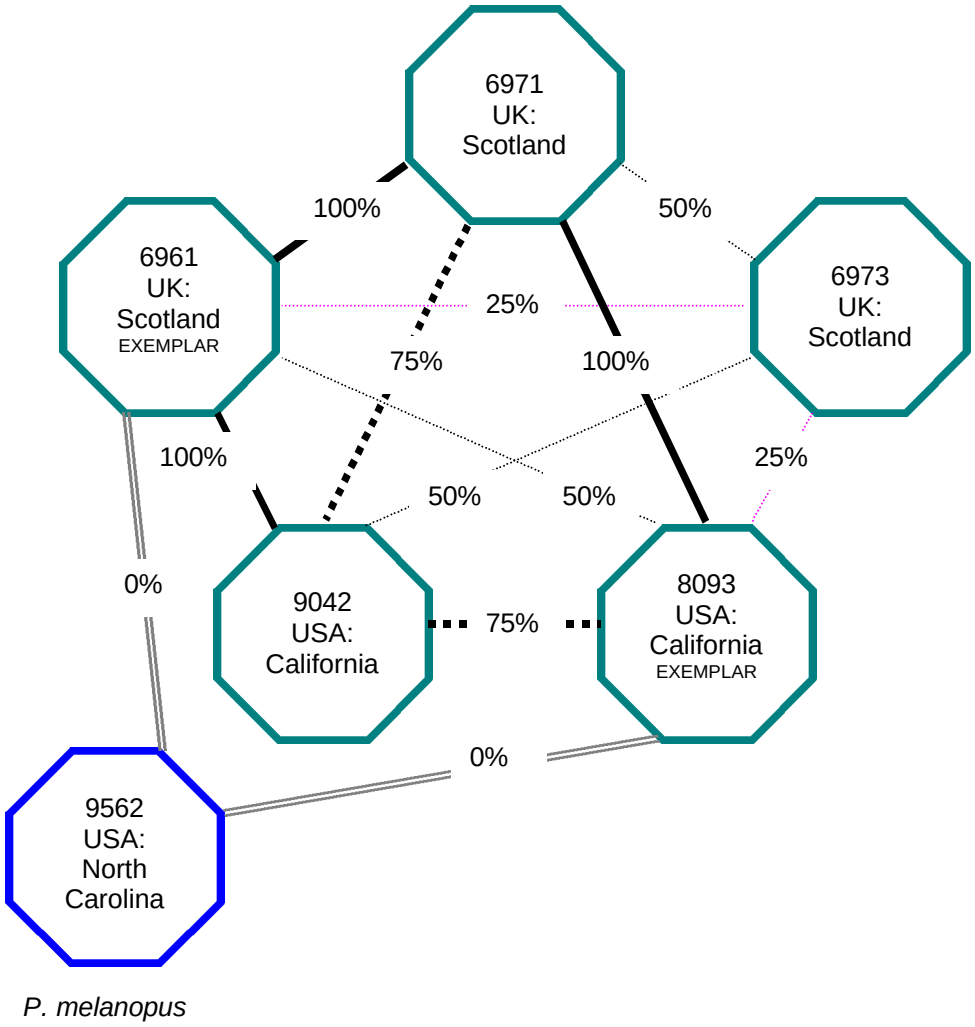

Figure 15. Intercollection pairings tested in *P. tubaeformis*/*P. melanopus*. Percentage compatibility within pairing lines.

analysis in MEGA2 led to the phylogenetic tree depicted as Fig. 16. FastDNAml topology is colored, and ME bootstrap and interior branch test values are shown at nodes. Notably, the backbone in fastDNAml was not recovered in the same branching order as in ME. Support was low for the backbone, and for many of the terminal branches. However, *P. melanopus-P. tubaeformis-P. badius* were highly supported as one inclusive relationship group [bootstrap support: 90, interior branch test support (IB): 97], and each of the three species is highly supported. We refer to this complex as /*Tubaeformis-"Phaeopodii" [hereby defined with Fig. 16 (node-based, internal specifier *Polyporus tubaeformis* (Karst. 1882) Gilbn. & Ryv. 1994)], using the convention of Moncalvo *et al*. 2002, Thomas *et al*. 2002, PART 1). In agreement with rooted MP analyses (see Fig. 17), /*Tubaeformis-"Phaeopodii" can be defined as a monophyletic clade in the strict cladistic sense, called /Tubaeformis-"Phaeopodii". *Polyporus varius* is one inclusive phylogenetic entity (of bootstrap support = 99/IB = 98), with the North American samples forming an inclusive relationship group supported with bootstrap support = 95/IB = 88. *Polyporus virgatus* appears to be non-inclusive and polyphyletic, with two collections from northern Argentina clustering together, and a Belize collection clustering with low support with *P. leprieurii*. *Polyporus guianensis* and *P. dictyopus* seem to be inclusive relationship groups. We hereby name the ladder-like assemblage of *P. varius*, *P. virgatus*, P*. dictyopus*, *P. guianensis,* and *P. leprieurii* the ≠Varius (≠ = referring to ladder or grades as a convention to differentiate from clades). *Polyporus squamosus* and *Datronia mollis* cluster together with high bootstrap support, we refer to this relationship group as the /*Datronia-Squamosus [defined in connection to Fig. 16 (node-based, internal specifier *Polyporus squamosus* Huds. 1778: Fr. 1821; defined as monophyletic clade /Datronia-Squamosus in the strict cladistic sense under consideration of rooted MP phylogenies, Fig. 17). There is a poorly supported assembly of several taxa of grs. *Favolus*, *Admirabilis*, *Polyporellus*, and *Melanopus* (*P. mikawai*). This may be an artifact of the conservative approach of eliminating doubtfully aligned sequence areas in an effort to decrease computing time and improve trust in phylogenetic estimates. As the *P. grammocephalus-P.pseudobetulinus* assemblage is consistent with PART 2, we call it the /Tenuiculus-"Favoliporus".

The PAUP* program considered 102 characters to be parsimony-informative. It found only 18 distinct most-parsimonious trees, of which one is shown in Fig. 17. The other 17 trees differed only in the backbone branching order, leading to polytomies in consensus trees. SEPAL half-deletion jackknife support is given left-hand from nodes, above branches appearing also in SEPAL analysis. Decay indices indicated at branches stem from the program SEPAL, which also generated an MP tree with a length of 258 steps. They are given for the branches that also appeared in SEPAL and corroborate the high support for the same relationship groups as did minimum-evolution analysis (Fig. 16).

The topologies shown in Figs. 16 and 17 are similar in that there is strong support for /Tubaeformis-"Phaeopodii" consisting of *P. badius-P. melanopus-P. tubaeformis*, and for a single *P. varius* species. European *P. varius* (FB10513, FB10807, SBUG-M1215) and American *P. varius* (FB7922, FB10550, FB10580, FB10962) are in close proximity to each other. The non-gr. *Melanopus*/non-gr. *Polyporus* sequences in Fig. 17 are located near *P. arcularius, P. tricholoma*, *P. alveolaris*, *P. grammocephalus*, and *P. pseudobetulinus*. As in Fig. 16, *P.* (*Melanopus*) *mikawai* appears in that part of the tree.

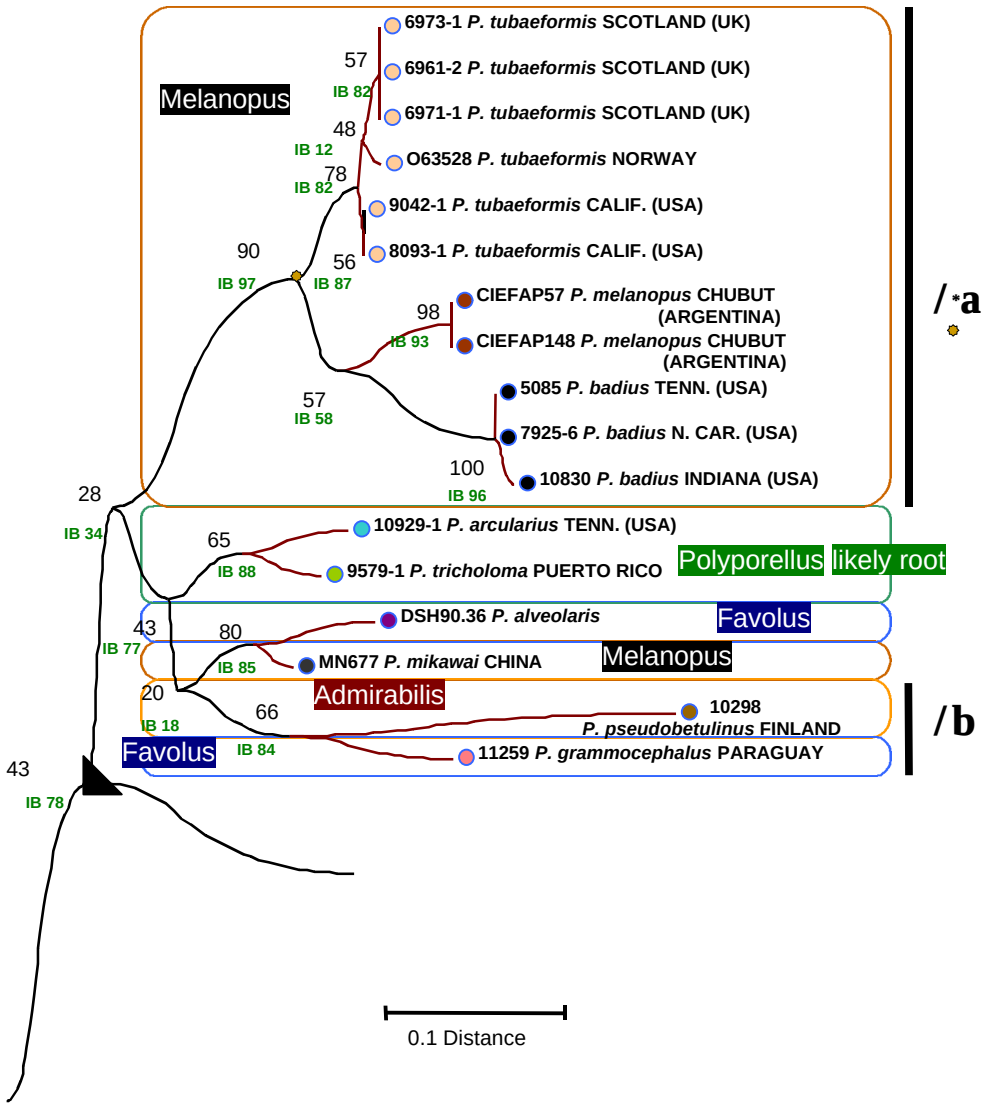

Figure 16. MEGA2 Kimura 2-parameter ME dendrogram (unrooted) for ITS rDNA data, displayed in curved MEGA2 tree mode. The gr. *Polyporellus* is the likely root according to its position in analyses in PART 2. Bootstrap values from MEGA2 (ME, 1,000 pseudoreplicates). IB = MEGA2 interior branch test support (ME, 1,000 pseudoreplicates). Colored branches = topology recovered by fastDNAml. *Polyporus* infrageneric groups indicated in highlighted boxes. Named relationship groups: /*a = /*Tubaeformis-"Phaeopodii" (defined here), /b = /Tenuiculus-"Favoliporus" (unrooted, distance-based: not clades in a strict sense, but provisionally reserved). For new cladonyms defined (node-based) in connection to this dendrogram, a colored node marker is inserted atop the node delimiting the clade, and repeated next to the name. Continued on next page.

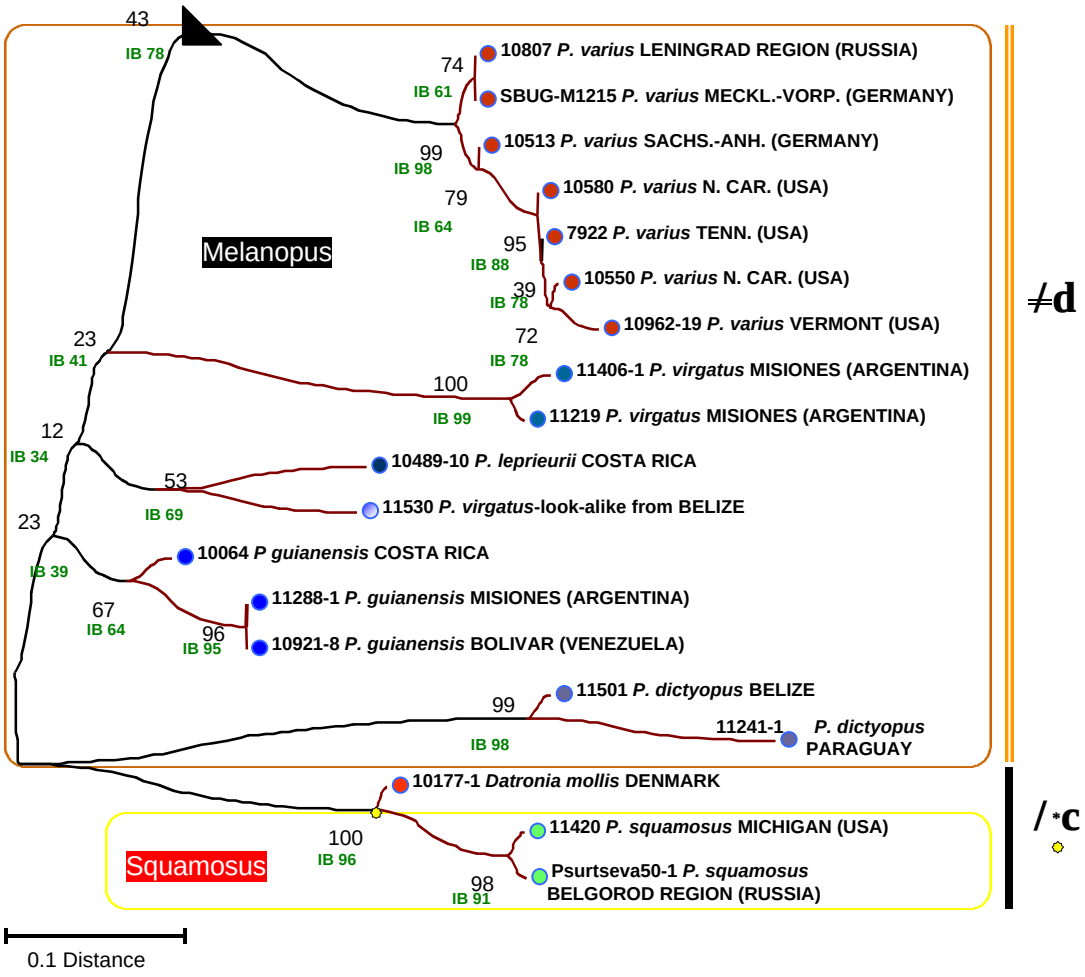

Figure 16. Continued. MEGA2 Kimura 2-parameter ME dendrogram (unrooted) for ITS rDNA data, displayed in curved MEGA2 tree mode. The gr. *Polyporellus* is the likely root according to its position in analyses in PART 2. Bootstrap values from MEGA2 (ME, 1,000 pseudoreplicates). IB = MEGA2 interior branch test support (ME, 1,000 pseudoreplicates). Colored branches = topology recovered by fastDNAml. *Polyporus* infrageneric groups indicated in highlighted boxes. FB acronym are omitted in labels, a number after a slash refers to a SBI number. Named relationship groups: /*c = /*Datronia-Squamosus (defined here, unrooted, distance-based: no clade in a strict sense, but provisionally reserved), ≠d = ≠Varius. For new cladonyms defined (node-based) in connection to this dendrogram, a colored node marker is inserted atop the node delimiting the clade, and repeated next to the name.

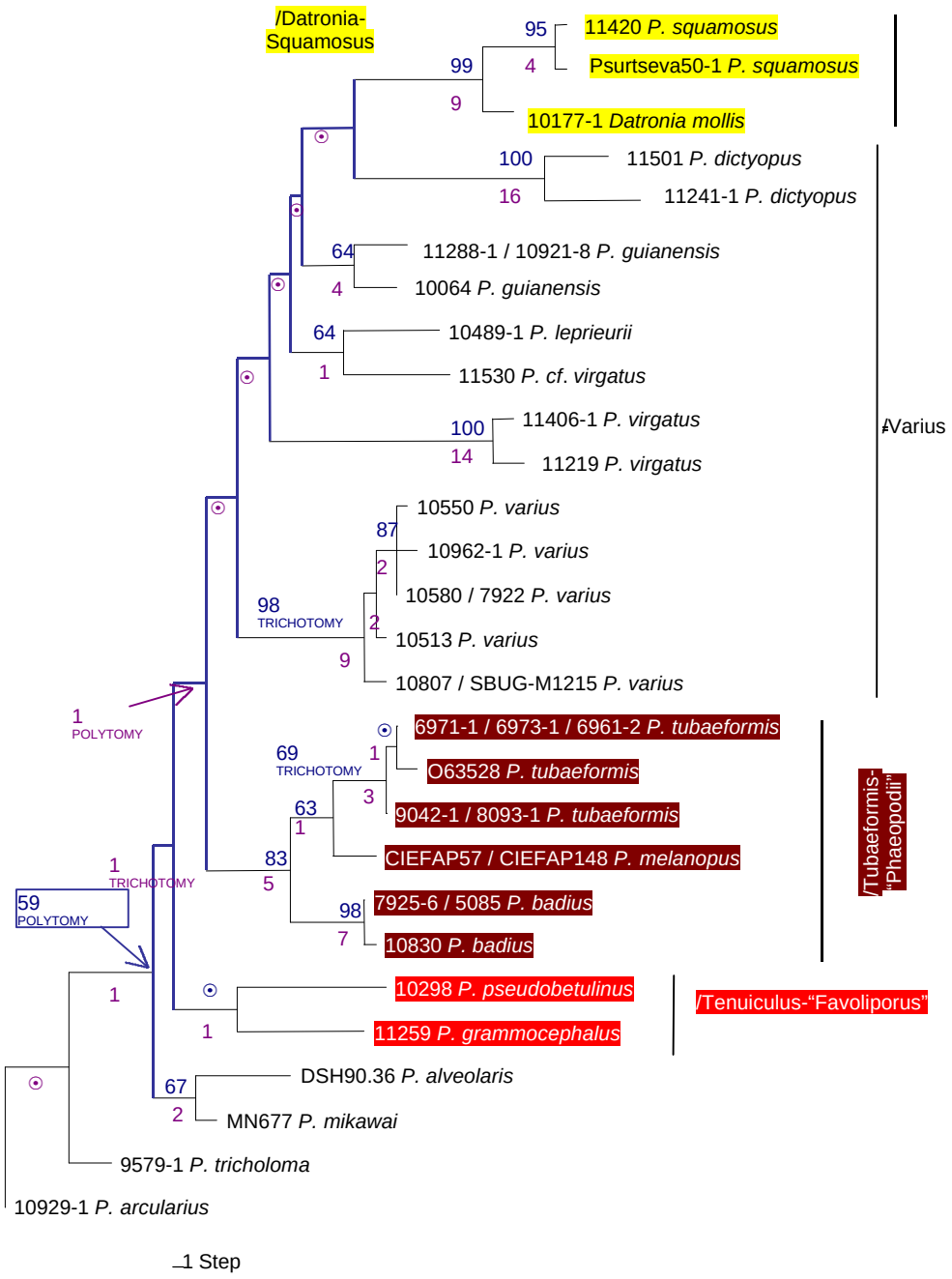

Figure 17. PAUP* MP tree (of 18 found in one tree island) for ITS rDNA data. This dendrogram has been compared to a tree post-analysis rooted (based on position in analyses in PART 2) with 10929-1 *P. arcularius* and 9579-1 *P. tricholoma* (gr. *Polyporellus*), and found identical to the rooted tree. 258 steps, CI = 0.539, RI = 0.703, RC = 0.378. SEPAL MP half-deletion jackknife support in blue (100 pseudoreplicates), SEPAL MP decay indices in lilac. ⊙ = node not recovered or collapsed. FB acronym are omitted in labels, a number after a slash refers to a SBI number.

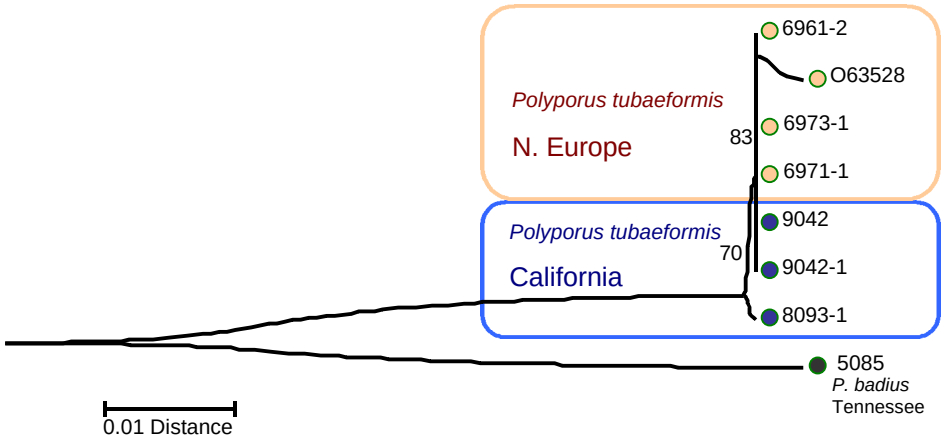

Figure 18. MEGA2 minimum-evolution dendrogram (Kimura 2-parameter) with interior branch support values (1,000 pseudoreplicates). Unrooted, displayed in curved MEGA2 tree mode. Labels represent FB numbers, followed by dash and SBI number if appropriate

Table 2. BioEdit sequence identity matrix for sequences of *P. tubaeformis* and *P. badius* used to generate Fig. 18.

source	6973-1	6961-2	6971-1	O63528	9042	9042-1	8093-1	5085
6973-1	1.000	1.000	1.000	0.973	0.986	0.994	0.993	0.836
6961-2		1.000	1.000	0.973	0.986	0.994	0.993	0.836
6971-1			1.000	0.973	0.986	0.994	0.993	0.836
O63528				1.000	0.966	0.971	0.969	0.814
9042					1.000	0.991	0.989	0.831
9042-1						1.000	0.998	0.838
8093-1							1.000	0.838
5085								1.000

Polyporus tubaeformis infraspecific sequence identity in shaded cells.

Sequence analysis focused on P. tubaeformis

The phylogenetic tree shown as Fig. 18 is based on a part of the data set used to generate the tree shown as Fig. 16, but without exclusion of sites. The analysis was based on 619 aligned nucleotide characters, 61 of which MEGA2 recognized as variable. *Polyporus badius* was used as outgroup to *P. tubaeformis*, with the intention of gaining insight into sequence diversity across the intercompatibility species *P. tubaeformis*.

The sequence identity matrix of the alignment computed by BioEdit is shown in Table 2. The alignment is weak in the entropy plot (Fig. 19) computed by BioEdit. Entropy is described as "lack of predictability for an alignment position", which reflects the "amount of variability" due to either ambiguity or homoplasy. The software author (Hall 1999) implemented the formula

$$H(x) = -\Sigma f(b,x)\ln(f(b,x))$$

where H(x) = uncertainty = entropy at position x (measured in nits), b = a character, and *f*(b,x) = frequency at which character b is found at position x. There is little variability across much of the *P. tubaeformis* sequences, with the non-conserved and ambiguous sites primarily in the non-coding spacers (as recently shown for *Grifola*: Shen *et al*. 2002). Within *P. tubaeformis*, there are only 16 variable sites, and between the dikaryon of FB9042 and SBI 1 of FB9042, the only difference is five ambiguity codes in the dikaryon (all but nucleotide G), which is most likely due to lack of resolution in sequencing. Since this ambiguity includes the possibility of T it did not impact the ME tree topology. The Scottish isolates (FB6961, FB6971, and FB6973) had identical sequences. No maximum-parsimony analysis could be undertaken with this subset of data, as there was no parsimony-informative site available. We did not perform entropy and identity calculations for the badly alignable data set in toto (ALIGN_000379).

DISCUSSION & TAXONOMY

Our analyses had to circumvent error with a high number of unalignable sites. Before exclusion of invariable sites and gaps, sequences were approximately 800 bases (817 in the attempted alignment posted as ALIGN_000379) long, which were reduced to barely 100 informative sites. Therefore, results must be interpreted with caution. As in other phylogenetic analyses in *Polyporus*, there is insufficient information signal to infer geographic origin of taxa. Unlike in gr. *Polyporellus* (PARTS 4, 5), perhaps due to the fact that only one sequence per collection was generated, there is less rearrangement of branches within species clades, and some degree of geographic sorting within species-correlated clades.

The analyses presented mirror previous phylogenetic analyses based on presumably more conservative sequences (Hibbett *et al*. 2000, Binder & Hibbett 2002, PART 2). In the gr. *Melanopus* (as well as the gr. *Polyporus*–PART 7) sequences are very divergent. Gr. *Melanopus* is a non-monophyletic assemblage of dark-footed polypores. A natural grouping is found in species with rather thin, darkening fruit bodies (*P. badius*, *P. melanopus*, *P. tubaeformis* = /Tubaeformis-"Phaeopodii"), making the need for a separate genus *Royoporus* for species without clamp connections (De 1996, 1997) obsolete. These analyses also do not produce relationship groups that correlate with spore size or pore size. Consistent with LSU analyses (PART 2), the *P. pseudobetulinus* sequence (gr. *Admirabilis*) is closest to a gr. *Favolus* sequence (*P. grammocephalus*), representing /Tenuiculus-"Favoliporus". Both taxa

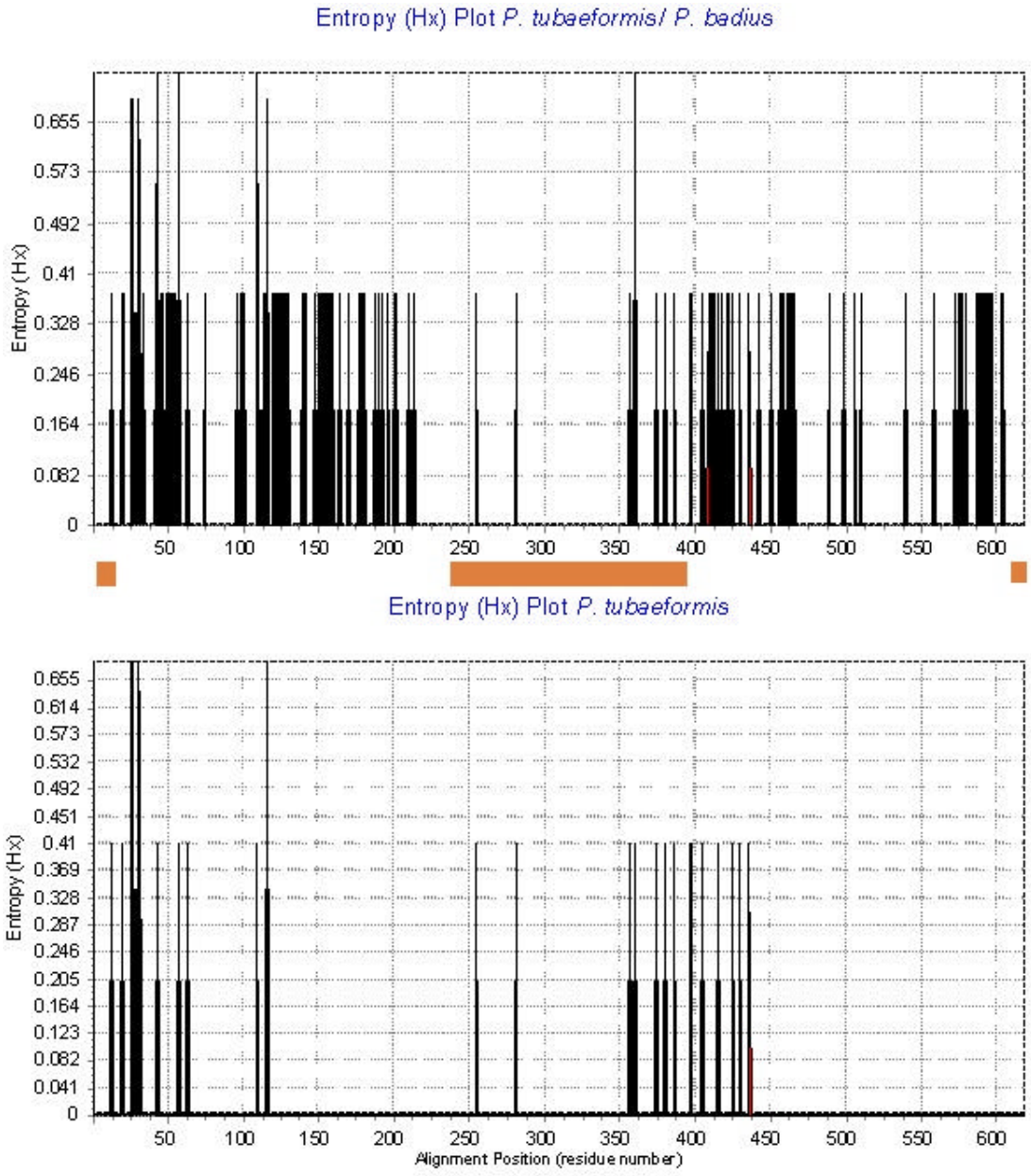

Figure 19. BioEdit plots of entropy (in nits) as a measure of probability of positional homology. Upper plot: all sequences used to produce Fig. 6, lower plot: only *P. tubaeformis* sequences. Bars underneath the upper plot mark coding regions (residue 1 to 12 = 18S rRNA gene, 235 to 394 = 5.8S rRNA gene, 612 to 619 = 28S rRNA gene).

would be members or candidates for *Royoporus*, but are distant from the third, *P. badius*. Corner's (1984) suggestion of *P. dictyopus* and *P. badius* being mere varieties of a variable taxon can also be rejected, as no analysis yields trees where they appear adjacent.

A wrong TENN voucher specimen number for FB7203 was given by Petersen *et al*. (1997), and the collection that really corresponds to the number FB7203 is a *P. badius*, which would have lacked clamps in cultures. Therefore, we offered a new proof for tetrapolarity in *P. varius.*

Many of our North American collections of small gr. *Melanopus* basidiomata with thin, elongated stipes were determined as *P. elegans* using Gilbertson & Ryvarden (1986-1987), but if placed in synonymy, would be determined as *P. varius* according to Nuñez & Ryvarden (1995). Most European collections were also small but without elongated stipes (*P. cf. nummularius*). Collections seen by DK prior to initiation of this study, however, were large-sized and often exhibited radial lines and dark spots on the cap. Kreisel (1963) and Jahn (1969) referred to *P. elegans* and *P. nummularius* as "Zwergformen" (nanisms) of *P. varius*. Kreisel (1983: 38) referred to small *Polyporus* (*Polyporellus*) *ciliatus* and *P. nummularius* as "Hungerformen" (depauperate forms). Perhaps Corner (1984) had similar ideas when suggesting that *P. tricholoma* (gr. *Polyporellus*) was an aggregate of reduced forms of several species. "Hungerformen" and rhizomorphs in grs. *Melanopus* and *Polyporellus* may be correllated with Nuñez's (1993) experimental observation of elongated stipes in non-aerated cultures. We have seen such rhizomorphoid, acapitate, or pseudoclavarioid stipes in monokaryotic cultures of gr. *Polyporellus* [monokaryotic fruiting was also reported by Hoffmann (1978)] and in *Lentinus* subg. *Lentinus* (Ed Grand, pers. comm.) in the laboratory. In nature, such "Hungerformen" and rhizomorphs may be either the result of the microclimate (little aeration, high moisture – especially in the subtropics and tropics), or just the fact that they are often found on tiny twigs with little room and nutrition available. Intercollection mating studies involving tentative *P. varius sensu* Nuñez & Ryvarden (1995) from Europe and North America, and DNA sequencing were used in this study and led us to conclude the conbiospecifity of the *P. varius-P. elegans* complex, which supports the placement of *P. elegans* as synonym under *P. varius*.

Collection FB10807 was not compatible with any other collection against which it was tested, but by sequence and morphology it was *P. varius*. This could be an example of mating barriers predating molecular divergence in the rDNA. We would not conclude that FB10807 belongs to an emerging cryptic species, as this would require completion of more pairing experiments (note that not all possible combinations were tested).

Polyporus varius, based on intercollection matings/molecular data, is a single biological and phylogenetic species with considerable habit variability. Microclimate effects should be investigated in tropical taxa forming rhizomorphs as well.

By intercontinental mating experiments we found *P. tubaeformis* strains from California and Scotland to belong to one intercompatibility group. This does not necessarily mean that populations would be able to mate and produce fertile offspring if they met *in-situ*, as *in-vitro* ability to fuse monokaryotic hyphae and produce clamped dikaryotic mycelium is only a first indicator of being able to intercross.

Polyporus melanopus as we would identify it using Nuñez & Ryvarden (1995) is not compatible with the tester strains of *P. tubaeformis*. By sequence analyses we found *P. tubaeformis* to be distinct from *P. badius* and *P. melanopus*. In phylogenetic analyses the European and California isolates sorted out according to geographic origin. This was not previously found in gr. *Polyporellus* (PARTS 4, 5).

Based on this study alone, we cannot recommend an alternative taxonomy to make gr. *Melanopus* a monophyletic taxon, so that the generic names *Atroporus*, *Melanopus*, and *Cerioporus* are still available if needed. However, if the recommendation to transfer *Polyporellus* to *Lentinus* is followed (PART 2), the name *Polyporus* is retained and may be used for the majority of gr. *Polyporus* and gr. *Melanopus*, reincorporating a number of segregate genera appearing in /core-Polyporus as suggested in the discussion in PART 2. Here we only recommend further fine-tuning of naming phylogenetic entities as clades (/) and grades (≠). The symbol "/*" leads a newly defined relationship group in connection with a non-cladistic or unrooted analysis. The symbol "≠" stands for paraphyletic grade. Further rules followed in this thesis concerning clades and grades were stated in PART 1.

We circumscribe /Tubaeformis-"Phaeopodii" in more detail here, coming short of describing it as a formal taxonomic level such as section. Such act would require agreement on the groups of *Polyporus* having some infrageneric taxonomic status. Also, there is little more than darkening caps and the molecular data to hold these species together.

/Tubaeformis-"Phaeopodii", with three species so far (*P. badius*, *P. melanopus*, and *P. tubaeformis*) is characterized by lignicolous, stipitate fruit bodies with glabrous cap. Caps are shallow to deeply funneled, pale brown (especially in young *P. tubeformis*) to brown or nearly black. Stipes can be whitish in young *P. tubaeformis*, otherwise with a distinct blackish cuticle.

As the three closely related species *P. badius*, *P. melanopus*, and *P. tubaeformis* occur sympatrically [and *P. tubaeformis* was not yet included in Gilbertson & Ryvarden (1986-1987)], and our specimens differ somewhat from the descriptions and key furnished by Nuñez & Ryvarden (1995), we offer a simple key to identify the taxa. This key contains primary taxonomic characters (in bold), but due to overlap of characters, some additional details are included.

1	**Generative hyphae lacking clamp connections**, pores 5-10/mm, carpophores up to 15 cm in diameter, thin. Spores (5 -) 7 – 8 (- 9) X (2 -) 3 – 3.5 (- 4) µm. *P. badius*
1*	**Generative hyphae with clamp connections**, pores usually less than 6/mm; carpophores up to 10 cm in diameter, thin to robust (2 cm). **2**
2	**Pileus deeply funnel-shaped**, young pale grayish brown; stipe punctate, darkening in age; pores 2 - 6 (- 10)/mm; cap context thin; spores (6.5 -) 7 – 9 (- 13) X (2 -) 2.5 – 3.5 (- 4.5) µm. *P. tubaeformis*
2*	**Pileus only shallowly funnel-shaped**, brown even when young; pores 1-5 (-10)/mm; cap context up to 2 cm thick; spores (5 -) 7 – 9 (- 12) X (1.5-) 2 – 3 (- 4) µm. *P. melanopus*

Polyporus tubaeformis hyphae commonly featured branching from clamp connections (cruciform and hooked clamps). Spores were often aberrant from cylindrical shape, with a central constriction, reminiscent of peanuts, which was not mentioned in Nuñez & Ryvarden (1995), where spores were shown more cylindrical.

The known range of *P. tubaeformis* now includes California, North Carolina, Idaho, and Germany's Mecklenburg-Western Pomerania (the latter three only by morphological identification). It was characterized as a boreal species (Nuñez & Ryvarden 1995) and now is known from northern California and the mountains of North Carolina. Likely the distribution is

much wider, and the species chorology reflects affinity to the trees of mountain ranges of Appalachia and the Western US extending south from the nearctic boreal realm. It may be expected that the species is extant across the Canadian boreal forest belt as well as across the Siberian counterpart. More mating studies are highly desirable, and the species should be sought in the low woodlands of Greenland.

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PART 7

Mating system and mating compatibility of *Polyporus tuberaster*, culture morphology and sequence analysis of *Polyporus* sect. *Squamosi*.

This manuscript is intended for publication, with suggested co-authors Karen W. Hughes and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research, using some preliminary mating study data done by the co-authors.

Running Title: *Polyporus* gr. *Polyporus* mating and relationships.

Abstract: One of the infrageneric groups of *Polyporus*, sect. *Squamosi* (= *Polyporus* group *Polyporus*; = *Polyporus* group *Squamosus*) including *P. squamosus* and *P. tuberaster* is central to *Polyporus* because most authors agree on *P. tuberaster* as the type species of *Polyporus*. This group is referred to as gr. *Polyporus* in this publication. We used monokaryotic cultures to revisit mating compatibility of *P. tuberaster*. A tetrapolar mating system was confirmed, as was the biological conspecificity of our *P. tuberaster* cultures. In addition, we addressed the molecular relationships of the group using the nuclear ITS I – 5.8S – ITS II rDNA region. Previous molecular analyses had shown the group to intermix with gr. *Melanopus* and segregate genera. We found ITS sequences to be highly variable, indicating considerable divergence within gr. *Polyporus*. ITS data supported the polyphyletic nature of gr. *Polyporus*. Earlier accounts of cultures of *P. tuberaster* being divergent in their LSU rDNA sequences were not reflected by our mating and ITS sequence data.

Keywords: Aphyllophorales, Basidiomycotina, *Datronia*, molecular systematics, *Pseudofavolus*.

INTRODUCTION

The "*Polyporus* group" of Nuñez & Ryvarden (1995) was the "*Squamosus* group" of Nuñez (1993) and sect. *Squamosi* of Bondartsev & Singer (1941: 58). Nuñez (1993) adopted the name *Squamosus* from *Melanopus* sect. *Squamosus* Bourd. & Galzin. It contained several polypores with large edible fruit bodies of monomitic hyphal structure when young.

There is a long-standing argument about the lectotypification of *Polyporus*. Donk (1960: 262-263) argued against typification of *Polyporus* with a number of available names of stipitate polypores available (now in gr. *Polyporellus*, in *Coltricia*, or *Albatrellus*; "gr." denotes *Polyporus* infrageneric group in this paper), or proposed earlier by a number of authors. Nuñez & Ryvarden (1995) somewhat hesitatingly accepted Donk's (1960: 262) choice of *P. tuberaster* Jacq.: Fr. as the type species of the entire genus *Polyporus*, in spite of prior typifications. Corner (1984: 11) argued against typification with *P. tuberaster* based on confusion and poor knowledge about *P. tuberaster*, suggesting *P. squamosus* as a better lectotype.

Murrill (1903) was the first to typify *Polyporus*, but used the "First Species Rule", now unacceptable as mechanical. He proposed Paulet's *P. ulmi* (= *P. squamosus*). Donk (1960) stated that *P. squamosus* was to be rejected because Fries (1821) had placed it in subg. *Favolus*. Corner (1984) rejected Donk's argument because *Favolus sensu* Corner could be reduced to synonymy under *Polyporus.* Ryvarden (1991: 144) and Nuñez & Ryvarden (1995) agreed with Corner concerning *Favolus*. Nuñez & Ryvarden (1995) also countered Donk's rejection of typification with *P. brumalis* (Clements & Shear 1931) since *P. brumalis* was contained in *Polyporus* Adans.: Fr. On grounds of nomenclatural stability, they accepted *P. tuberaster,* as did Kreisel (1960) and Singer (1986: 170).

We cannot support Corner's (1984) argument against typification with *P. tuberaster* on grounds of being a poorly known species. Neither can we see support in any ICBN provision for Kreisel's (1960) justification for *P. tuberaster* being the *Polyporus* type species. Kreisel

(1960) reasoned that as an organism once cultivated for human consumption it is under protection of name changes, which would be achieved by selecting it as type of *Polyporus*.

Thus, with competing proposals and arguments among the protagonists, *P. squamosus* was one of the proposed types for the segregate genus *Melanopus*. In PART 6 we hesitatingly did not accept Donk's (1960: 240) pro-*P. melanopus* arguments nor Murrill's (1903)/Ryvarden's (1991: 182) pro-*P. squamosus* arguments. Unlike Ryvarden we favor *P. varius* Fr. as type of the genus *Melanopus*, based on Teixeira (1983: 112) but decisively not based on the fact that *P. squamosus* serves as type for *Cerioporus* Quél., or *Polyporus*. With the molecular evidence of PART 2, we must regard the agglomerate of gr. *Melanopus* and gr. *Squamosus* (/Polyporus *p.p.*) as congeneric with *Polyporus* .

Donk (1960: 261) also suggested that *P. tuberaster* and *P. squamosus* might be conspecific. This was refuted by Nuñez (1995) with mating studies. Nuñez (1995) also experimentally confirmed reports (Campbell & Munson 1936) of sclerotia in *P. squamosus*, which makes this particular character unreliable to separate *P. tuberaster* and *P. squamosus*. Synonyms of *P. tuberaster* (*P. forquignoni*) have already (Jahn 1969) been called nanisms (Kreisel 1963), or as "Hungerformen" [environmental variants analogous to those of *P. varius* and in gr. *Polyporellus* (Kreisel 1963, 1983: 38, PART 5)]. As Kreisel (pers. comm.) pointed out, the stipe of *P. tuberaster* has a dark brown cuticle rather then black as in *P. squamosus*. This may be a better character, usable in combination with Jahn's (1969) and Nuñez's (1995) pilear scale and stipe surface characteristics. We also agree with Dai's (1999) characters for the two species.

Regardless of the lectotypification quarrel on the generic level, the ongoing taxonomic and nomenclatural arguments about boundaries and infrageneric taxonomy in *Polyporus* make *P. tuberaster, P. squamosus*, *P. radicatus* and similar taxa worth revisiting using mating studies and molecular data analyses. Lack of monokaryotic single-basidiospore isolates (SBIs) did not allow us to repeat self-crosses in *P. squamosus*. However, the tetrapolarity reported for *P. tuberaster* in Stalpers (1978) could be reassessed. Moreover, large subunit sequence analyses could be complemented with nITS region data, including a new evaluation of *P. varius* being close to *P. squamosus* (*c.f.* Binder & Hibbett 2002, see PART 2). Here we report the latter two endeavors, but note that we lack data for tropical and Southern-Hemisphere representatives of gr. *Polyporus.*

MATERIALS & METHODS

All collecting, maintenance, and use of collections for mating studies followed Krüger *et al*. (2002) and Burdsall & Dorworth (1994). Collections are listed in Table 1. Figure 1A, B shows collections of *P. tuberaster*, Fig. 2 collections of *P. squamosus*, and Fig. 3 shows *Datronia mollis*. DNA isolation, PCR amplification, and sequencing of nuclear ribosomal DNA (ITS I – 5.8S – ITS II) was described by Krüger *et al*. (2002) and PART 5. Alignment of sequences followed procedures described in PART 6, but in this study less data required exclusion for being unalignable. Analyses performed included a MEGA2 (Kumar *et al*. 2001) minimum-evolution analysis [ME (Rzhetsky & Nei 1993), with Kimura 2-parameter distance; Kimura 1980; alignment was checked to have near-equal nucleotide frequencies]. Bootstrap (Felsenstein 1985) and interior branch tests (Nei & Kumar 2000: 168) were performed, both with 1,000 pseudoreplicates of character resampling. Maximum-parsimony (MP) analysis was done under exclusion of gaps. The Kishino-Hasegawa (Kishino & Hasegawa 1989) test was used to select one most-parsimonious tree, and this tree was complemented with branch support obtained by half-deletion jackknifing (Efron & Gong 1983) in DAMBE v. 4.0.75 (Xia & Xie 2001), and decay indices (Bremer 1988, 1994) computed by SEPAL v. 1.4 (Salisbury 2001). The use of "/" to refer to clades follows Moncalvo *et al*. (2002) and Thomas *et al*.

Table 1. Fungal specimens and cultures investigated.

Strain numbers and/or herbarium voucher numbers if known	Fungal species and authors	Country of origin	Names of collectors and identifiers	GenBank number
FB10177 SBI 1 (TENN57707)	*Datronia mollis* (Sommerf.: Fr.) Donk	Denmark: Storstrøms Amt	RHP	AF516557
DSH90.36	*P. alveolaris* (DC.: Fr.) Bond. & Sing.		D. Hibbett	AB070828
FB10929 SBI 1 (TENN58412)	*P. arcularius* Batsch: Fr.	USA: Tennessee	H. Voglmayr	AB070867
FB5085 (TENN59136)	*P. badius* (Pers.) Schwein.	USA: Tennessee	RHP	AF511444
FB11259 (TENN59068)	*P. grammocephalus* Berk.	Paraguay: Alto Paraná	RHP	AF516563
FB10298 = Thorn567 = Kotiranta6472 (culture)	*P. pseudobetulinus* (Pilát) Thorn, Kotiranta & Niemelä	Finland: Tervola	H. Kotiranta	AF516571
DAOM146900 (culture)	*P. radicatus* Schwein.	Canada: Ontario	D. Malloch, *det*. J. Ginns	AF516583
DAOM198916 hseq	"	Canada: Ontario	S. Redhead, *det*. J. Ginns as *P. lentus*	AF516584
FB10968 (TENN58831) (specimen)	"	USA: North Carolina	L. Grand & C. Vernia	AF516585
FB10161 SBI 3 (TENN57692)	*P. squamosus* (Huds.) Fr.	Denmark: Vestsjællands Amt	RHP & DK	AF516586
FB10163 (TENN57694) hseq	"	Denmark: Vestsjællands Amt	H. Knudsen & RHP	AF516587
FB10179 SBI 9 (TENN57709)	"	Germany: Mecklenburg-Vorp.	DK	AF516588
FB10196 (TENN57726) hseq	"	Germany: Mecklenburg-Vorp.	H. Kreisel & DK	AF516589
Psurtseva50 SBI 1 (TENN59384)	"	Russia: Belgorod Region	N. Psurtseva	AF516572
FB7245 SBI 16 (TENN53633) *	"	Sweden: Uppland	Swedish Mycological Club	AF516590
FB10831 (TENN58380) hseq	"	USA : Indiana	M. Scholler & DK	AF516591
FB11420 (TENN59125) (culture)	"	USA: Michigan	E. Grand	AF516573
FB9579 SBI 1 (TENN56491)	*P. tricholoma* Mont.	USA: Puerto Rico	RHP	AF516553

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
FB6973 SBI 1 (TENN55891)	*P. tubaeformis* (Karst.) Gilbn. & Ryv.	UK: Scotland	RHP as *P. varius*, *rev*. D. Krüger, *teste* L. Ryvarden	AF511438
FB11336 (TENN59220) hseq	*P. tuberaster* Jacq.: Fr.	France: Rhône-Alpes	Ed Grand as *P. lentus*	AF516592
FB10197 SBI 5 (TENN57727)	"	Germany: Mecklenburg-Vorp.	RHP	AF516596
FB10316 SBI 1 (TENN57964)	"	Germany: Mecklenburg-Vorp.	RHP	AF516597
FB10511 (TENN57741) hseq	"	Germany: Mecklenburg-Vorp.	RHP	AF516595
FB8976 (TENN55636, TENN55636-2 from fruiting by N. Psurtseva on *Liriodendron* sawdust to obtain SBIs)	"	Russia: Krasnodar Region	B. Tuniyev as *P. lentus*	AF516598
FB10935 SBI 2 (TENN58530)	"	Germany: Bayern	I. Krisai-Greilhuber	AF516593
FB10197 (TENN57727) hseq	"	Germany: Mecklenburg-Vorp.	RHP	AF516596
FB9541 SBI 3 (TENN56254)	"	USA: California	NAMA foray party, *det*. O. K. Miller as *P. mcmurphyii*, *rev*. DK as *P. squamosus*, compatible with *P. tuberaster*	AF516599
FB10317 (TENN57965)	"	Germany: Mecklenburg-Vorp.	RHP	-
FB8893 (TENN55513)	"	Russia: Krasnodar Region	RHP	-
FB10513 (TENN57743) (culture)	*P. varius* Fr.	Germany: Sachsen-Anhalt	DK	AF516575
FB10962 SBI 19 (TENN58587)	*P. varius* Fr.	USA: Vermont	DK	AF516580

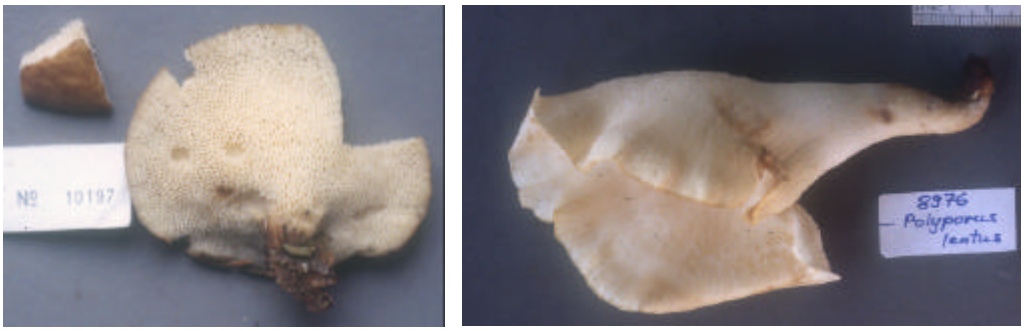
Table 1. Continued.

Strain numbers and/or herbarium voucher numbers if known	Fungal species and authors	Country of origin	Names of collectors and identifiers	GenBank number
FB11221 SBI 1 (TENN58910)	*Pseudofavolus cucculatus* (Mont.) Pat.	Argentina: Misiones	DK & E. Albertó, *teste* L. Ryvarden	AF516600
FB8744 SBI 7 = Ryv *s.n*. (20/7/96) (TENN55173)	"	Mexico: Nayarit	RHP	AF516601

* This collection used to report mating system by Petersen *et al.* (1997)

DK = Dirk Krüger, RHP = Ronald H. Petersen. FB = TENN herbarium fieldbook number = CulTENN culture number (the acronym FB is omitted in phylogenetic trees). SBI = single basidiospore isolate culture. *s.n*. = no number. hseq = herbarium specimen sequenced. All AB accession numbers are from Krüger *et al*. (2002).
Herbarium acronyms from Index Herbariorum Online (http://www.nybg.org/bsci/ih/ih.html).

If no specification "*leg.*/*det*." the name mentioned in the fourth column is the collector and initial identifier of a collection. All available collections (FB, O, DAOM) have been seen by DK and compared with recent taxonomic literature.



A **B**

Figure 1. *Polyporus tuberaster* specimens. **A**: *Polyporus tuberaster* FB10197, pores and upper surface of a section. **B**: *P. tuberaster* FB8976 (TENN55636-2) obtained by fruiting on *Liriodendron* sawdust. Size comparison: small side of numbered label = 23 mm (for A), ruler (for B, mm).

A

B

C

Figure 2. *Polyporus squamosus* specimens. **A**: *Polyporus squamosus* FB10196, upper pileus surface. **B**: *P. squamosus* FB10163 pores and upper surfaces of two fruit bodies. **C**: *P. squamosus* FB10931. Size comparison: small side of numbered label = 23 mm.

Figure 3. Specimen FB10177 *Datronia mollis*. Scale bar = 20 mm.

(2002). The symbol "/*" leads a newly defined relationship group in connection with a non-cladistic or unrooted analysis. The symbol "≠" stands for paraphyletic grade. Further rules followed in this thesis concerning naming of clades and grades were stated in PART 1.

One dikaryotic culture of *P. tuberaster* (FB8976, Fig. 1B) was successfully fruited on pressed *Liriodendron* sawdust in plastic bags in the greenhouse, according to a method devised by Psurtseva & Mnoukhina (1998) with help by Dr. Nadya Psurtseva. Notably, the one basidiome obtained this way was very pale, and the color of the basal stipe cuticle showed only a very faint hint of brown, as depicted for stipes by Michael *et al*. (1983: pl. 38) and according to Kreisel (pers. comm.), rather than black (e.g. Fig. 2B, C with *P. squamosus*). The lack of brown cap squammules in this fruited specimen was unusual when compared to specimens collected in nature. Collection FB8976 was then re-identified as *P. tuberaster* after having it field-identified as *P. lentus*. We had fruited this collection in order to obtain SBIs for intercollection pairing.

RESULTS & DISCUSSION

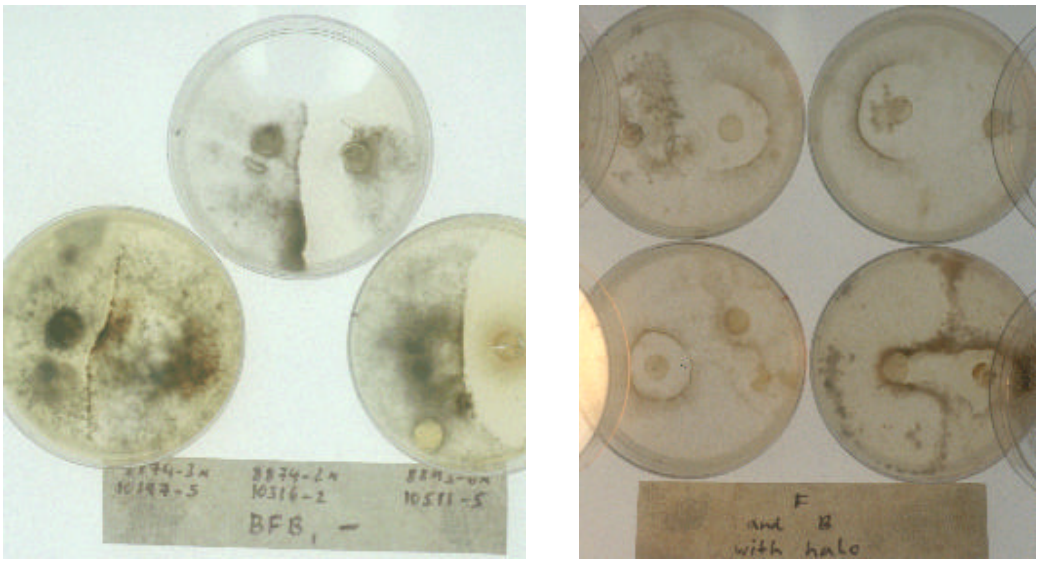
Self-crosses & fruiting of Polyporus tuberaster

When 12 SBIs of FB10316 (Fig. 4) were paired in every combination, a tetrapolar mating system was inferred. Tester SBIs were selected for intercollection pairings (Fig. 5).

Pale brown hyphal mats were formed on MEA plates. The majority of pairings produced a flat contact zone morphology. In some cases, lips (BFB for "barrage-flat-barrage", Fig. 5A) were formed, also reported by Petersen *et al*. (1997) as unilateral barrages for *P. varius*, and also for *P. umbellatus* FB6527 (PART 6). Contact zone morphology was unrelated to mating types. Hyphae were frequently branched, but conidiogenesis was rarely observed. Generally, a sweet smell of almond paste (cyanide) or millipedes was detected. Spore

10316		A_1B_1	A_2B_1						A_1B_2			A_2B_2	
		1	3	8	10	13	14	15	2	11	12	4	16
A_1B_1	1		F-	B-	B-	F-	F-	B?-	F-	F-	F-	+	+
A_2 B_1	3	F-		BFB-	BFB-	F?-	F-	F-	+	+	+	F-	F-
	8	B-	BFB-		F-	F-	F-	F-	+	+	+	F-	B-
	10	B-	BFB-	F-		F-	F-	F-	+	+	+	B-	F-
	13	F-	F?-	F-	F-		F-	F-	+	+	+	F-	F-
	14	F-	F-	F-	F-	F-		F-	+	+	F+	F-	F-
	15	B?-	F-	F-	F-	F-	F-		+	+	+	F-	F-
A_1 B_2	2	F-	+	+	+	+	+	+		-	F-	B?-	B-
	11	F-	+	+	+	+	+	+	-		B-	F-	B-
	12	F-	+	+	+	+	F+	+	F-	B-		B-	BFB-
A_2 B_2	4	+	F-	F-	B-	F-	F-	F-	B?-	F-	B-		F-
	16	+	F-	B-	F-	F-	F-	F-	B-	B-	BFB-	F-	

Figure 4. Self-cross of *P. tuberaster* collection FB10316. + = clamp connections, - = no clamp connections, BFB = flat contact zone with raised margin, B = barrage, B? = faint barrage, F= flat contact zone, F? = faint flat contact zone. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.



A **B**

Figure 5. Contact zone morphology in mating experiments of *P. tuberaster*. **A**: BFB (see Fig. 3 for explanation), **B**: halos.

germination was slow, with germ tubes not quite attached to the agar surface, making SBI isolation difficult. The survival rate of monokaryons was low; recovery from water vial storage was difficult. Dikaryon cultures also grew very slowly.

In the self-cross of FB10316, a halo-like (Fig. 5B) feature around the inoculum block was formed. This was independent of any contact zone morphology or mating type. However, certain SBIs always formed such halos, and if halos were formed by both pairing partners, they seemed to touch in the center of the plate. Barrages and flats were also not stable, but changed, sometimes a barrage line was interrupted by flats. Petersen *et al*. (1997) reported sectoring in *P. alveolaris*, with different growth rates resulting in more raised aerial hyphae or more appressed hyphae. This is similar to “halos”. Next to the inoculum agar piece, hyphae lay appressed on the agar surface, then they form the halo ring, and growth is more vigourous outside that ring. There was no clamp formation seen in relationship with the halo ring. Halos have also been seen in *Polyporus arcularius* and *P. brumalis* (Krüger *et al*. 2002).

Intercollection pairings involving Polyporus tuberaster

Only chance led to the discovery of FB9541 from California being conbiospecific with *P. tuberaster*. Identified as *P. mcmurphyi*, it should have been conspecific with *P. squamosus* (Nuñez & Ryvarden 1995). Since we used all available SBIs of *P. squamosus* in this experiment as well, the compatibility to the Caucasus *P. tuberaster* strains was found. Initially, the German cultures on the bottom of Fig. 6 formed a distinct compatibility group. When later the Austrian collection FB10935 became available, this changed. Now, FB9541 and FB10935

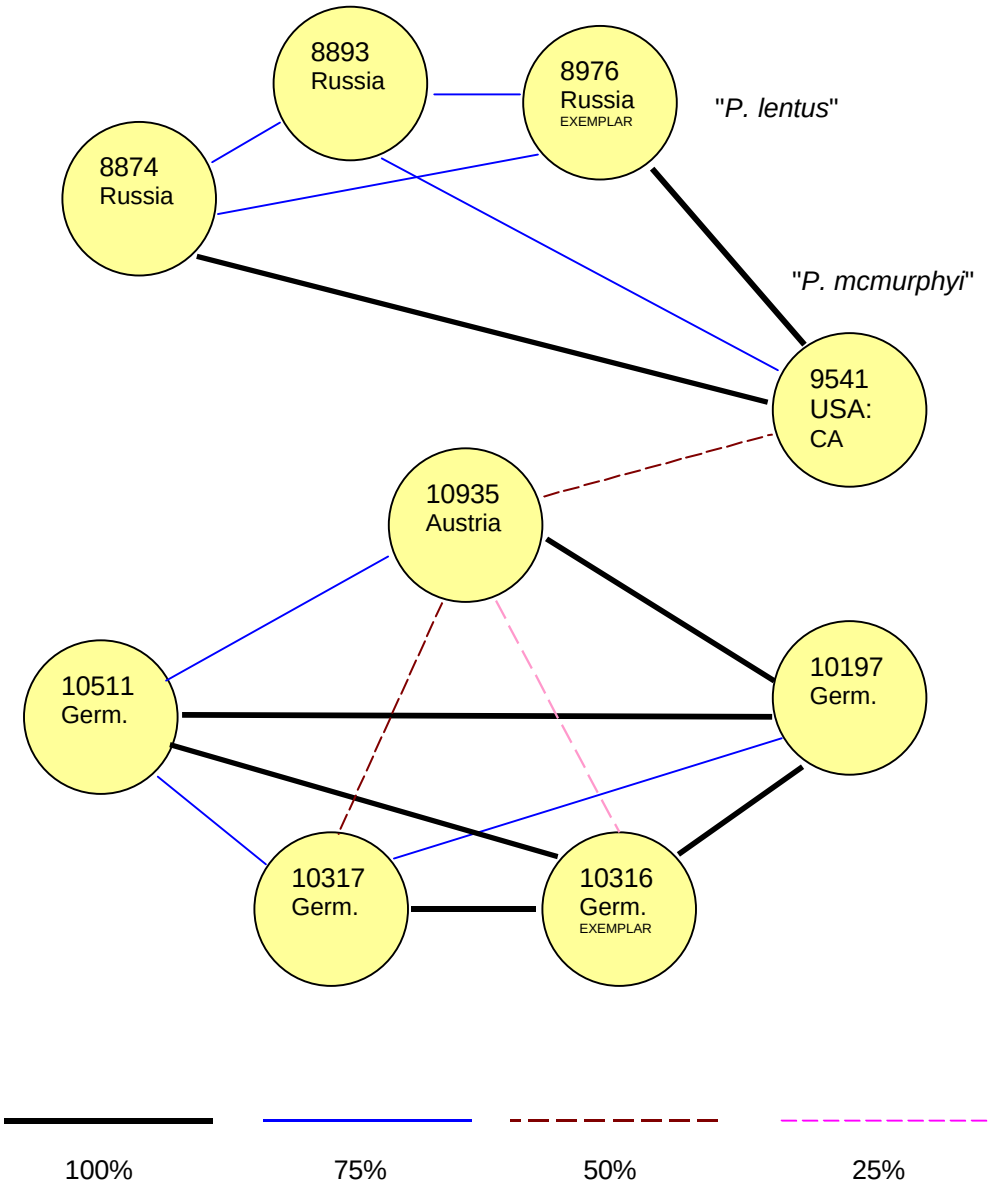


Figure 6. Intercollection compatibility of nine collections of *P. tuberaster*. Deviating original identification noted next to FB8976 and FB9541. All numbers are FB numbers. Lines representing percentage compatibility as expressed by clamp connections formed (100% = 4/4 SBI pairings, 75% = 3/4, 50% = 2/4, 25% = 1/4, 0% not indicated).

appeared to bridge the initially two compatibility groups. Compatibility studies are, of course, always preliminary.

We consider that there is only one compatibility group (= biological species) detected by us, but perhaps there is a speciation process underway that accounts for the gap between Central European and Caucasus collections.

Polyporus tuberaster was paired with the small number of *P. squamosus* SBIs available, including FB7245 from Petersen *et al*. (1997). In these, *P. squamosus* and *P. tuberaster* were found to be incompatible, as Nuñez (1995) reported. Likewise, as we wanted to address the possibility that small specimens of *P. tuberaster* were perhaps *P. arcularius*, we paired SBIs of FB10316 with the FB10929 testers (*P. arcularius*, Krüger *et al*. 2002). These pairings were uniformly incompatible.

Polyporus squamosus *culture morphology*

There was an insufficient number of *Polyporus squamosus* SBIs to perform self-crosses or intercollection matings. Stalpers' (1978) report on *Polyporus squamosus* as tetrapolar was already confirmed by Petersen *et al.* (1997). Basidiospores of *P. squamosus* were slow to germinate, slower than those of *P. tuberaster*, but not as slow as for example *Polyporus badius* or *P. varius* (*Melanopus* group of Nuñez & Ryvarden 1995). However, spore prints sent to us by airmail often completely failed to give any spore germination, and spores collapsed and deteriorated.

Most putative SBIs of *P. squamosus* were not monokaryotic. This may have been caused by harvesting error or it is possible that there is some mechanism promoting amphithallism. Clamp connections easily go undetected. Almost always the entire mass of aerial hyphae is converted to catenulate arthroconidia, between which there are simple septa, and clamped septa, and the latter often are not easy to detect. This copious conidiogenesis was also reported by Petersen *et al*. (1997), but here we show in Fig. 7A some of the peculiarly shaped conidia. Besides conidia, chlamydospores and hyphae rich in vesicles (Fig. 7B) were also seen. Already in 1936, Campbell & Munson reported a dusty appearance of cultures due to rich formation of "oidia". The co-occurrence of clamped and clampless septa, partly thick-walled conidia, and perhaps chlamydospores (as irregular hyphae with protuberances) were noted by Stalpers (1978). We also observed clamp connections combined with branching, as shown in Fig. 7C drawn from a herbarium specimen. Similar cruciform branching was also observed in *P. tubaeformis* (PART 6). Abundant formation of arthroconidia chains was also observed in *P. umbellatus* (PART 6), in *P. varius* (PART 6), and *P. tenuiculus* (Petersen *et al*. 1997), but not to the extent seen here, where the entire aerial hyphae were converted into chains of both shiny dry arthrocondia and duller chlamydiospores.

Slow growth of dikaryotic cultures has also been reported by DAOM (in litt.). *Polyporus squamosus* DAOM172154 could not be sent because it failed, and *P. radicatus* (as *P. lentus*) DAOM198916 took months to revive.

Sequence analyses

The data set generated consisted of 31 sequences; the alignment initially containing 817 sites (available from ftp://ftp.ebi.ac.uk/pub/databases/embl/align/ under accession number ALIGN_000381). After culling positions that were ambiguous in the alignment, 562 characters were left, encompassing 221 variable sites (155 parsimony-informative). This alignment was

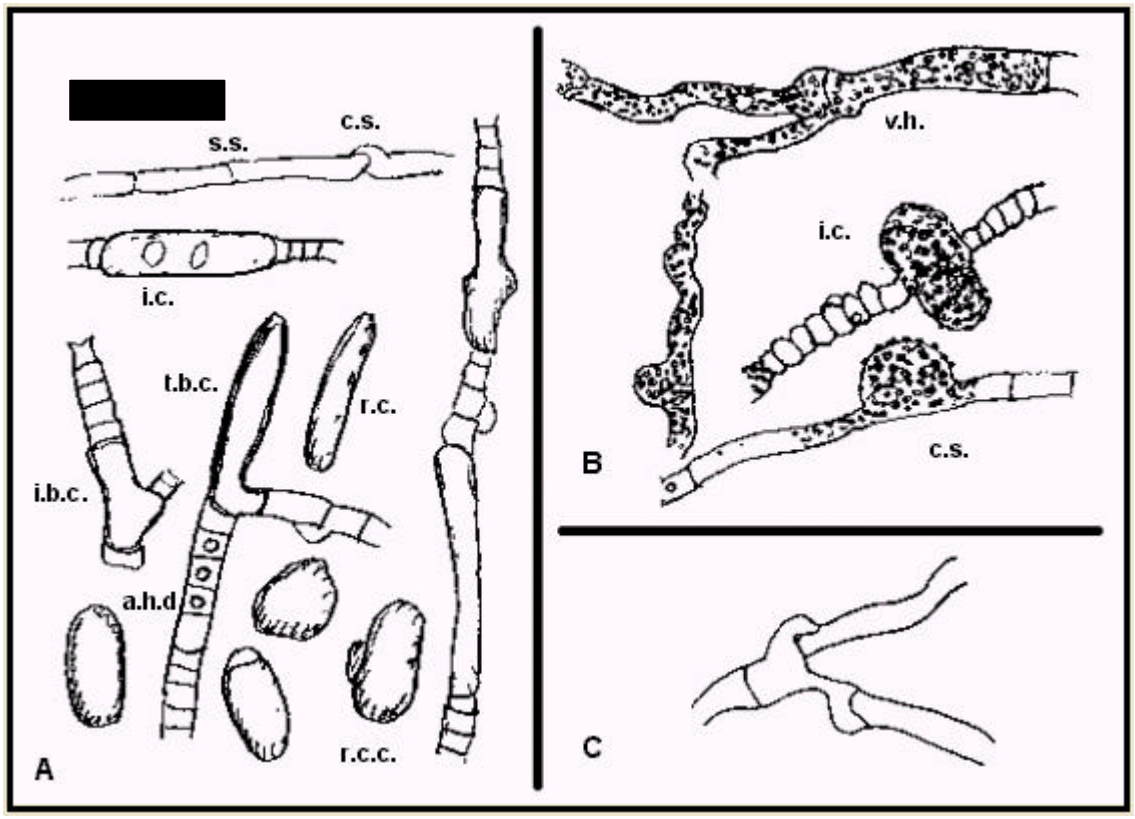


Figure 7. *Polyporus squamosus* microscopic characters. **A**: Arthrocondiogenesis in *P. squamosus* FB10196-10 (for SBI10, but apparent dikaryon). s.s. = simple septum and c.s. = clamped septum in one hypha, i.c. = intercalary conidium, i.b.c. = intercalary branched conidium, t.b.c. = terminal branched conidium, r.c. = released conidium, r.c.c. = released clamp-carying conidium, a.h.d. = annelid-like hyphal deformation. Conidia were refracting light under the microscope. **B**: Chlamydospores in in *P. squamosus* FB10196 SBI 10. v.h. = vesicle-rich, encrusted generative hypha with clamps, i.ch. = intercalary chlamydospore, shown in a chain of annelid-like constrictions with and without clamps, c.s. = clamped septum in hypha rich in vesicles. **C**: *P. squamosus*, Psurtseva50 herbarium specimen (TENN59384): cruciform clamp (a clamp site with outgrowth of branches). Scale bar = 20 µm.

deposited under accession number ALIGN_000382. Figure 8 compares the diversity of aligned sequences expressed as entropy plots (BioEdit, Hall 1999). According to the software author, entropy is a measure of "lack of predictability for an alignment position" (see PART 6). Entropy will thus both be high for a position if sequences are ambiguous or erroneous in an alignment, or if there is information content in homologous positions. Figure 8 shows a baseline entropy level of ca. 0.15 nits over long lengths of the alignment (ALIGN_000381) deemed ambiguous, which is due to long indels with unknown homology. These were thus removed. After this, all sequences were still unique (i.e. differed in at least one position) which allowed analyses in the DAMBE phylogenetic software package without combining identical sequences (Xia & Xie 2001).

The data set partly overlaps with data of PART 6, which dealt with taxa intermingling in LSU analyses (PART 2), but with more emphasis on Nuñez & Ryvarden's (1995) gr. *Melanopus*. The unrooted ME dendrogram (Fig. 9) shows weakness in the deeper branching order or backbone, but assembles sequences from collections in relationship groups corresponding to morphotaxa, and, as far as tested, biological species entities. Support for a single entity *Polyporus squamosus* is very high, with marginal sequence aberrance between European and North American collections.

As discussed in PART 2, *Datronia mollis* was included based on earlier LSU sequence analyses. This grayish-brown, wide-pored, resupinate fungus again (bootstrap support 100), as in PART 6, is basal to *P. squamosus*. The /Datronia-Squamosus (PART 6) is thus confirmed by a variety of sequences and analyses. There is no obvious morphological resemblance to *P. squamosus*. The mating system of *D. mollis* was described as tetrapolar (David 1967), and it shares the resupinate growth with *Dichomitus p. p*. Stalpers (1978) mentioned the lack of arthroconidia (different from *P. squamosus*), but existence of dark lines, in culture, perhaps corresponding to those described *in-vitro* in *P. squamosus* (Campbell & Munson 1936).

So far we have been unable to sequence the ITS rDNA region of *Mycobonia* (near *Pseudofavolus* in PART 2), but *Pseudofavolus cucullatus* is the nearest relative of /Datronia-Squamosus, according to this analysis. This is in agreement with LSU sequence data of PART 2. We call the expanded monophyletic assemblage *P. squamosus-D. mollis-Ps. cucullatus* the /*Squamosus-"Megabasidiophora", after the massive basidia (at least 30 µm long) carrying ellipsoid spores of at least 10 µm length (node-based, internal specifier *Polyporus squamosus* Huds. 1778: Fr. 1821). Notably, both *Ps. cucullatus* and *D. mollis* are reported to have dendrohyphidia (Gilbertson & Ryvarden1986-1987), which we did not observe.

LSU data found *Polyporus radicatus* and *P. varius* as adjacent relationship groups. This result is repeated with the current ITS sequence data, although with weak statistical support. Based on sequence data, the collection DAOM198916 must be redetermined from *P. lentus* to *P. radicatus*. Notably, morphologically both taxa are very similar, and both may perhaps arise from underground sclerotia, as suggested by Nuñez (1995). There is no known correlation with morphology that would point to a particularily close relationship of *P. varius* and *P. radicatus*.

The adjacent, weakly supported relationship groups are /Tubaeformis-"Phaeopodii" (PART 6), and inclusive relationship group limited to *P. tuberaster*. This *P. tuberaster* relationship group comprises collections from Eurasia and North America, as does the *P. squamosus* relationship group, and does not separate *P. tuberaster* FB 10197 from FB10935, as did LSU data of PART 2. The sequence analysis confirms the mating studies, by which the collections FB9541 (California) and FB8976 (Russia) were redetermined to *P. tuberaster*. *Polyporus* (*Favolus*) *grammocephalus*, and *P.* (*Admirabilis*) *pseudobetulinus* are in proximity, but here not directly adjacent, so they do not confirm a monophyletic /Tenuiculus-"Favoliporus".

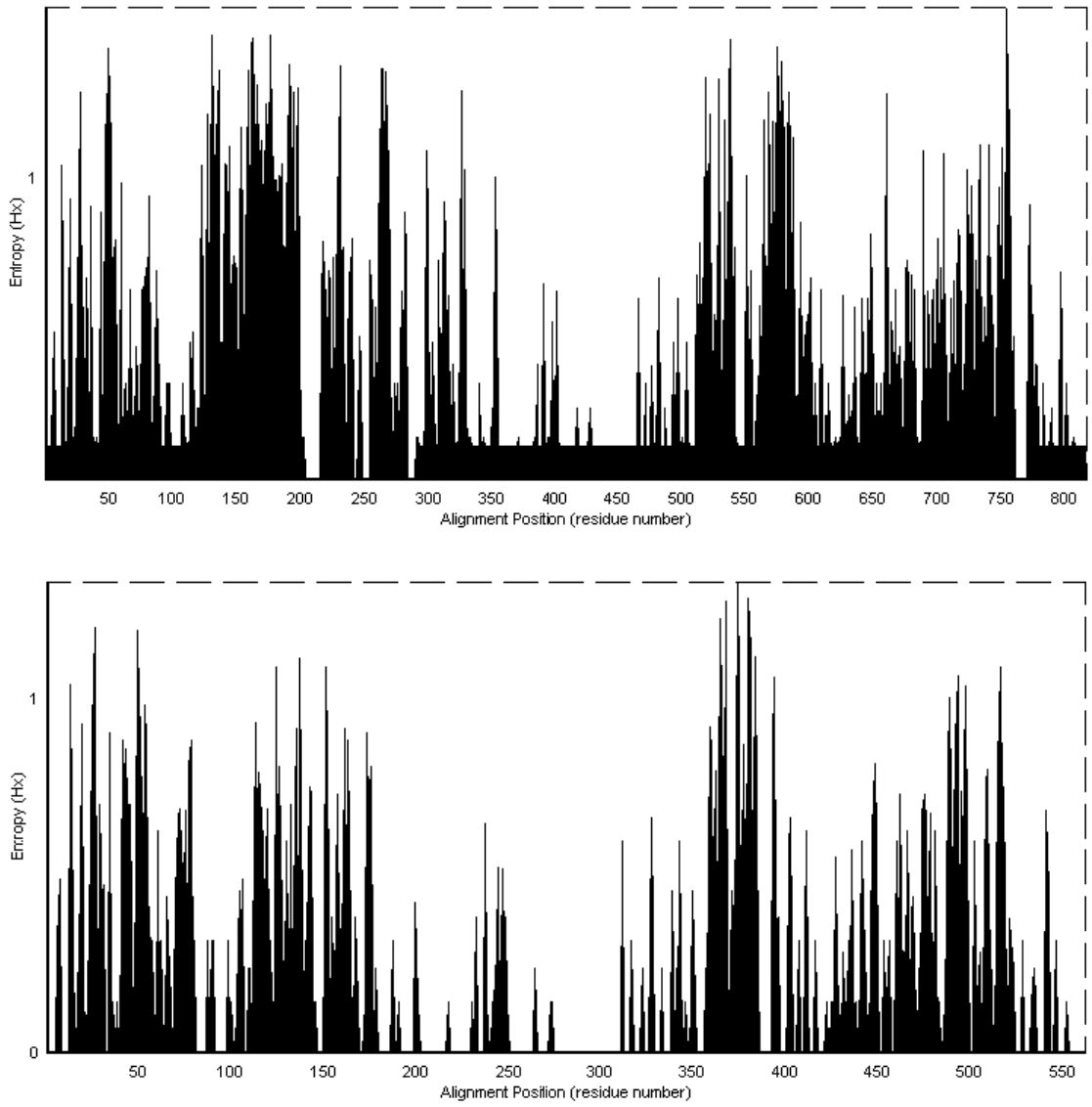
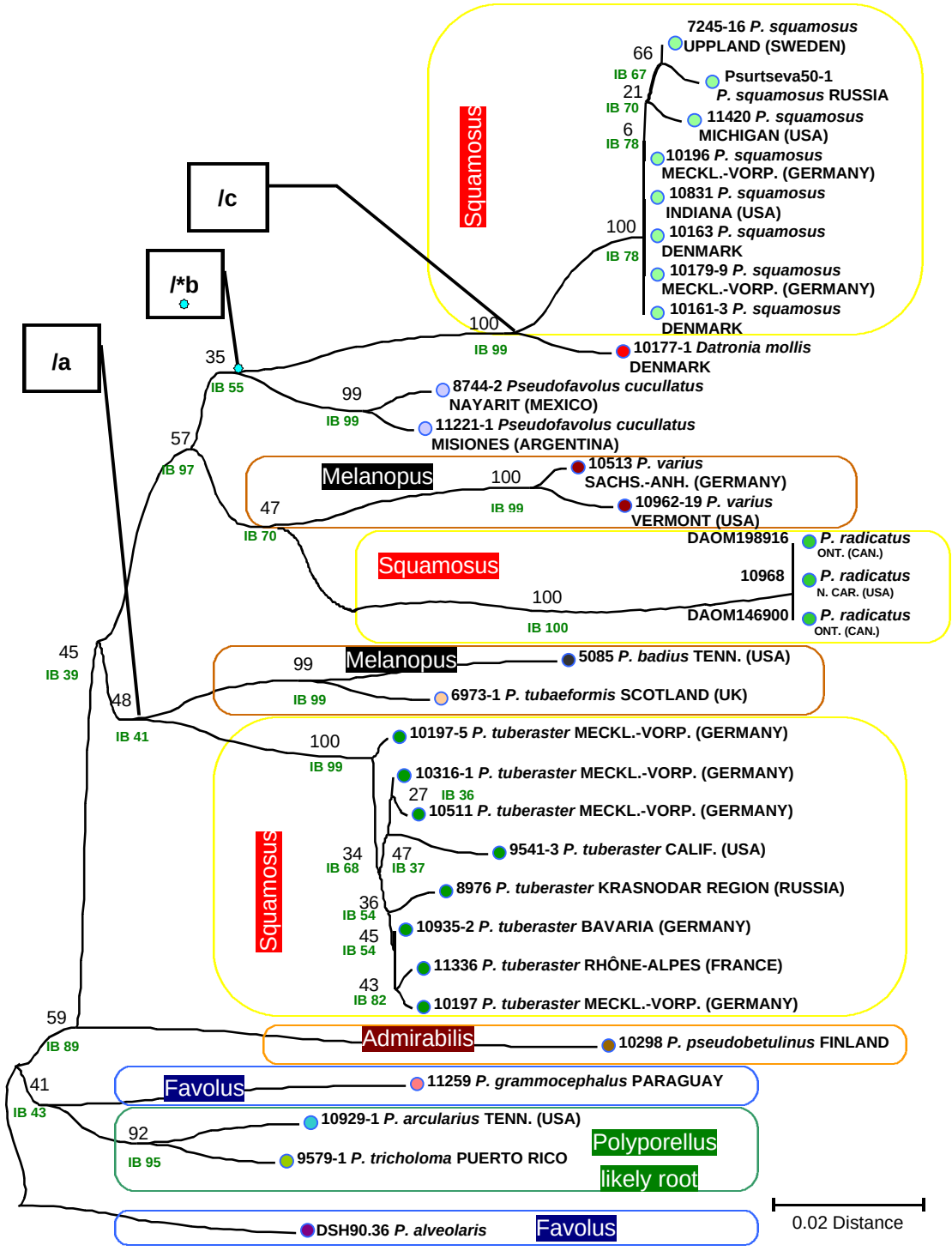


Figure 8. BioEdit plots of entropy (in nits) as measure of probability of positional homology. Upper plot: sequences in attempted alignment including ambiguous sites, lower plot: after culling ambiguous sites.

Figure 9. (next page) MEGA2 Kimura 2-parameter ME dendrogram on ITS sequences (unrooted), displayed in curved MEGA2 tree mode. The gr. *Polyporellus* is the likely root according to its position in analyses in PART 2. Bootstrap values from MEGA2 (ME, 1,000 pseudoreplicates). IB = MEGA2 interior branch test support (ME, 1,000 pseudoreplicates). *Polyporus* infrageneric groups indicated in highlighted boxes. Named relationship groups: /a = /Tubaeformis-"Phaeopodii", /*b = Squamosus-"Megabasidiophora" (defined here, unrooted, distance-based: not a clade in a strict sense, but provisionally reserved), /c = /Datronia-Squamosus. For the new cladonym (see /*b) defined (node-based) in connection to this dendrogram, a colored node marker is inserted atop the node, and repeated next to label "/b". FB abbreviation is omitted, numbers followed by dash and a second number are SBI numbers.


/c
/*b
/a
Squamosus
7245-16 P. squamosus UPPLAND (SWEDEN)
66
IB 67
Psurtseva50-1 P. squamosus RUSSIA
21
IB 70
11420 P. squamosus MICHIGAN (USA)
6
IB 78
10196 P. squamosus MECKL.-VORP. (GERMANY)
10831 P. squamosus INDIANA (USA)
100
IB 78
10163 P. squamosus DENMARK
10179-9 P. squamosus MECKL.-VORP. (GERMANY)
10161-3 P. squamosus DENMARK
100
IB 99
10177-1 Datronia mollis DENMARK
35
IB 55
99
IB 99
8744-2 Pseudofavolus cucullatus NAYARIT (MEXICO)
11221-1 Pseudofavolus cucullatus MISIONES (ARGENTINA)
57
IB 97
Melanopus
100
IB 99
10513 P. varius SACHS.-ANH. (GERMANY)
10962-19 P. varius VERMONT (USA)
47
IB 70
Squamosus
DAOM198916
P. radicatus ONT. (CAN.)
10968
P. radicatus N. CAR. (USA)
100
IB 100
DAOM146900
P. radicatus ONT. (CAN.)
45
IB 39
99
IB 99
Melanopus
5085 P. badius TENN. (USA)
6973-1 P. tubaeformis SCOTLAND (UK)
48
IB 41
100
IB 99
10197-5 P. tuberaster MECKL.-VORP. (GERMANY)
10316-1 P. tuberaster MECKL.-VORP. (GERMANY)
27
IB 36
10511 P. tuberaster MECKL.-VORP. (GERMANY)
Squamosus
34
IB 68
47
IB 37
9541-3 P. tuberaster CALIF. (USA)
8976 P. tuberaster KRASNODAR REGION (RUSSIA)
36
IB 54
10935-2 P. tuberaster BAVARIA (GERMANY)
45
IB 54
11336 P. tuberaster RHÔNE-ALPES (FRANCE)
43
IB 82
10197 P. tuberaster MECKL.-VORP. (GERMANY)
59
IB 89
Admirabilis
10298 P. pseudobetulinus FINLAND
41
IB 43
Favolus
11259 P. grammocephalus PARAGUAY
10929-1 P. arcularius TENN. (USA)
92
IB 95
9579-1 P. tricholoma PUERTO RICO
Polyporellus
likely root
DSH90.36 P. alveolaris
Favolus
0.02 Distance

Two gr. *Polyporellus* taxa (PART 5), and *P. alveolaris* are found there as well. *Polyporus alveolaris* will be discussed further (PART 8).

The MP dendrogram (Fig. 10) shows some differences from Fig. 8. It is one of over 1,000 equiparsimonious trees found in one tree island, all with the following characteristics: 439 steps, CI = 0.645, RI = 0.832. SEPAL's shortest MP tree in the decay index calculation, however, was 453 steps long, and the decay indices are only superimposed on branches consistent between PAUP* and SEPAL analyses. Again, /Datronia-Squamosus is a robust monophyletic entity. The sister-relationship of *P. varius* and *P. radicatus* is not recovered, but replaced by a *Pseudofavolus cucullatus* - *P. radicatus* sister-relationship. This topology, however, is very weakly supported and collapses in consensus trees that are only 2 steps longer. Decay index support is high, and jackknife support low, for the assemblage containing *P. squamosus*, *Datronia*, *Pseudofavolus*, and *P. radicatus*. *Polyporus pseudobetulinus* is found intercollated between *P. varius* and the aforementioned 4-taxon assemblage. Further down to the bottom of the dendrogram species are positioned in a ladder with extremely low support. Several of these are not resolved in decay and jackknife (indicated in the illustration when replaced by polytomies). The MP analyses may suffer from homoplasy, as homoplastic characters are not evaluated and remaining characters may be insufficient to resolve the relationships.

In conclusion, scaly cap surface is a homoplastic morphological character. Regardless of the phylogenetic accuracy of our analyses, such can be found in a variety of white- and brown-rotting polypores and "agarics" with remarkable resemblance [*P. squamosus*, *P. tuberaster*, *P. radicatus*, *P. udus*, *Neolentiporus* (see Rajchenberg 1995), *Neolentinus lepideus* (see Pegler 1983: 185)]. It appears that careful reevaluation of other characters in the future is appropriate. For example, sclerotia/pseudosclerotia have been described in several species of gr. *Polyporus*, as well as segregate genera *Laccocephalum* and *Dendropolyporus* (and *Grifola frondosa* was mentioned by Campbell & Munson 1936), generally with young edible basidiomate. It is possible that the small-pored *P. varius*, for example, forms sclerotia in nature. Perhaps there is homology in pigment chemistry involved in the formation of black lines and stipes and other sclerotized structures. The yellowish-brown cap color found in many representatives of the grs. *Melanopus*, *Polyporus* as well as *Pseudofavolus* and *Mycobonia* may also be a good character if there is some support from pigment chemistry. This color may only be hidden in the darker coloration of basidiomata of the /Tubaeformis-"Phaeopodii" and of genus *Echinochaete*.

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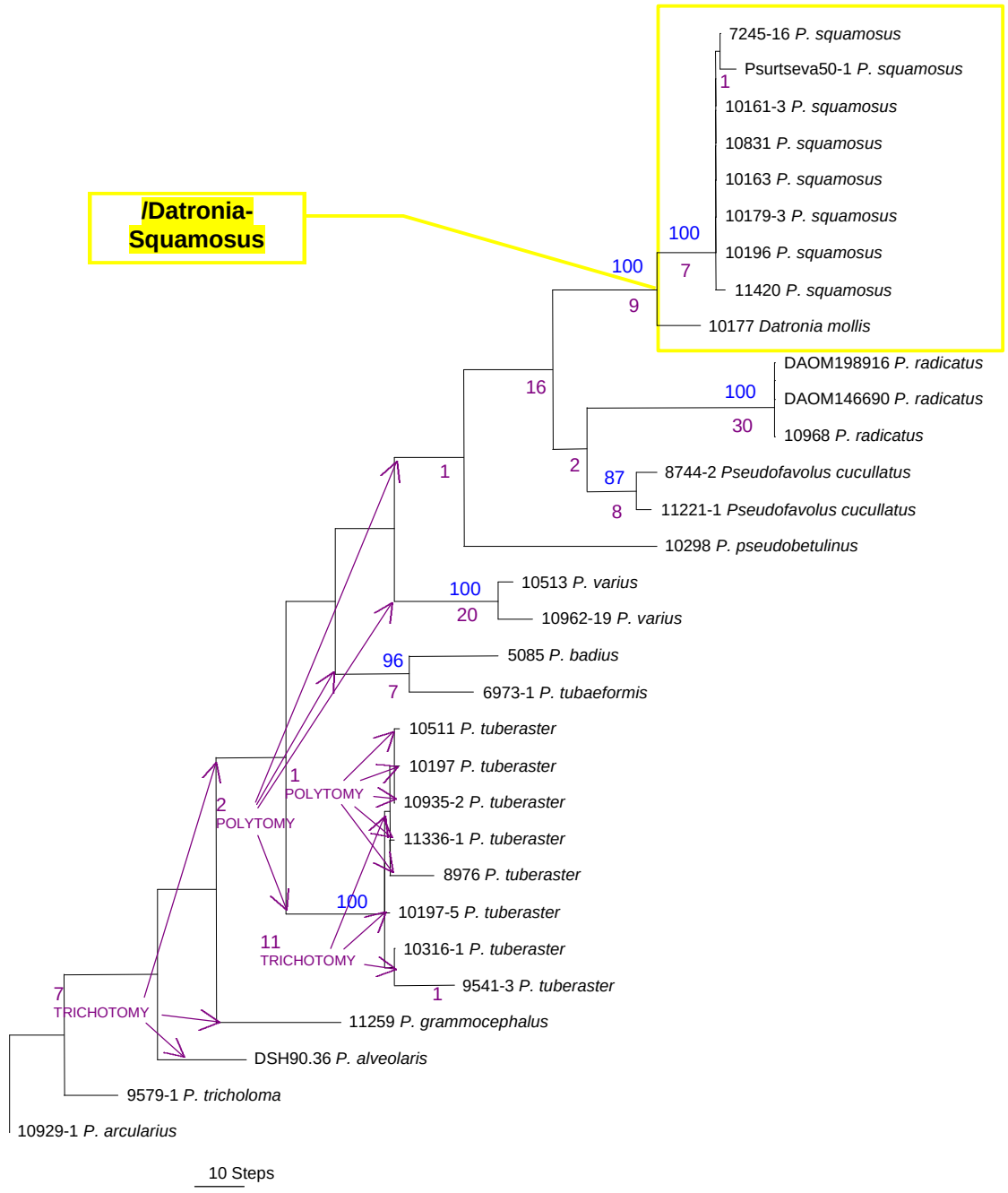

Figure 10. PAUP* best MP tree of ITS sequences selected by Kishino-Hasegawa MP test. This dendrogram has been compared to a tree post-analysis rooted (based on position in analyses in PART 2) with 10929-1 *P. arcularius* and 9579-1 *P. tricholoma* (gr. *Polyporellus*), and found identical to the rooted tree. Only /Datronia-Squamosus is recovered in MP analysis. 439 steps, CI = 0.645, RI = 0.832, RC = 0.536. SEPAL MP half-deletion jackknife support in blue (100 pseudoreplicates), SEPAL MP decay indices in lilac. FB abbreviation is omitted, numbers followed by dash and a second number are SBI numbers.

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PART 8

Polyporus alveolaris: mating studies and an argument to exclude it from proposed subgenus *Favoliporus subgen. nov*.

This manuscript is intended for publication, with suggested co-authors Karen W. Hughes and Ronald H. Petersen. Dirk Krüger is the primary author and has conducted the majority of original research, using some preliminary mating study data done by the co-authors.

Running Title: *Favoliporus* and *Polyporus alveolaris.*

Abstract: *Polyporus alveolaris* (= *Hexagonia mori*), an aberrant member of the *Favolus* group in *Polyporus*, is confirmed to have a tetrapolar mating system. Intercollection mate recognition experiments as an indicator of biological conspecificity implied that cryptic species are contained under this species epithet. No consistent morphological differences were found. Based on earlier studies, we remove the *P. alveolaris* complex from the infrageneric group *Favolus*, and propose subgenus *Favoliporus subgen. nov*. for the *Admirabilis* and *Favolus* groups.

Keywords: Aphyllophorales, Basidiomycotina, *Hexagonia*, mating compatibility, *Royoporus*.

INTRODUCTION

The flabelliform polypore *Polyporus alveolaris* (DC.: Fr.) Bond. & Sing. (*ut* "*alveolarius*"; = *Hexagonia mori* Poll.) was originally described as a *Cantharellus*. Bondartsev & Singer (1941: 58) in their recombination in *Polyporus*, placed the species in their section *Squamosi*. Donk (1960: 214) and Kreisel (1963: 136) thought of *P. alveolaris* as closely related to *P. arcularius* Batsch: Fr. (gr. *Polyporellus* – gr. is used for *Polyporus* infrageneric group *sensu* Nuñez & Ryvarden 1995 throughout). Only Kreisel specifically mentioned the widened pores and brittle trama in both *P. arcularius* and *P. mori* (= *P. alveolaris*) as common characters. The most recent monographic treatment of *Polyporus* (Nuñez & Ryvarden 1995) placed the species in the infrageneric group *Favolus*.

The species can be seen as aberrant in *Favolus* because the basidiospores of *P. alveolaris* are larger (10-14 µm) than those of the tropical *Favoli* (4-12 µm). Basidiomata are more robust and thicker than those of tropical *Favoli* (*P. grammocephalus*, *P. tenuiculus*, *P. philippinensis*) and *P. alveolaris* is not a tropical species, but rather warm-temperate (Nuñez & Ryvarden 1995). There is similarity to the tropical *Favolus spp*. in the diamond-shaped, hexagonal pores and tessellate pattern on the cap, but also similarity to *P. squamosus* and *P. tuberaster* in the squamules on the cap. It is therefore still ambiguous whether *P. alveolaris* should be treated in a subgeneric entity together with what Nuñez & Ryvarden (1995) called *Polyporus* gr. *Melanopus* and *Polyporus* (= *Squamosi* Bond. & Sing.) or with gr. *Polyporellus*, with its affinity to *Lentinus* subg. *Lentinus*.

Previous nLSU rDNA sequence analyses (PART 2) failed to place *P. alveolaris* in topological proximity with gr. *Favolus*. *Polyporus alveolaris* also failed to appear near gr. *Polyporellus*, even though its ITS rDNA region was sufficiently alignable to be included as an outgroup by Krüger *et al*. (2002). Instead, *P. alveolaris* appeared in an assemblage containing grs*. Melanopus* and *Polyporus*.

Imazeki (1943: 47) selected *Favolus alveolaris sensu* Fr. as type species of *Favolus* Fr. 1828. This typification was rejected by Donk (1960: 214) because he saw *P. alveolaris* as related to *P. arcularius*. This was perhaps not warranted, as such taxonomic judgement cannot overrule a valid nomenclatural act; but Donk also suspected that Imazeki used a mechanical "First Species Rule". Donk (1960) also rejected an even earlier proposal for *Favolus europaeus* Fr. [= *P. alveolaris* according to Nuñez & Ryvarden (1995)] by Clements &

Shear (1931: 347), without specifying a reason for rejection (perhaps because *F. europaeus* = *F. alveolaris*). Donk (1960: 212) and Ryvarden (1991: 144) designated *Merulius daedaleus* Link (= *Polyporus dermoporus* Pers.) as type species of *Favolus* Fr. 1828, which is also in synonymy (Nuñez & Ryvarden 1995) under *Daedalea brasiliensis* Fr. [= *Favolus brasiliensis* (Fr.) Fr.; = *Polyporus tenuiculus* (Pal. de Beauv.) Fr.].

Here, we elaborate on mating system and biological species concept of American and European collections identified as *P. alveolaris* and retest the tetrapolarity reported by Nobles *et al*. (1957) and Petersen *et al*. (1997).

MATERIALS & METHODS

All work with specimens and cultures followed the procedures described by Krüger *et al*. (2002), and the formation of clamp connections was used as an early indicator of the biological species concept of Esser & Hoffmann (1977). Mating experiments followed Gordon & Petersen (1991) and Petersen (1992). Collections were identified with keys and descriptions by Gilbertson & Ryvarden (1986-1987) and Nuñez & Ryvarden (1995). Several specimens were examinded by Dr. Leif Ryvarden. All are listed below. A collection constisting of a number of basidiomata of *P. alveolaris* (FB10131) is shown in Fig. 1. This collection generated the SBIs used for the self-cross. The use of "/" to refer to a phylogenetic clade follows Thomas *et al*. (2002) and Larsson (2002).

DK = Dirk Krüger, RHP = Ronald H. Petersen. FB = TENN herbarium fieldbook number = CulTENN culture number.

Austria: Wien: 24 Apr. 1999, W. Till FB10294 (TENN58369); **Mexico:** Chiapas: Nueva Alleman, 21 Oct. 1997, RHP FB3897 (TENN55870); Tlaxcala: Tlaxco, 15 July 1996, A. Kong-Luz FB8701 (TENN55131); Tlaxcala: El Rosario, 14 July 1996, M. Rodriguez FB8477 (TENN55306); **USA:** Minnesota: Bimidji, Beltrami Co., 26 Aug. 1995, NAMA foray FB7979 (TENN54334); Mississippi: Bonita Lake, Meridian, Lauderdale Co., 20 May 1997, RHP FB9072 (TENN54872); North Carolina: Old Fort, McDowell Co., 18 Apr. 1992, RHP FB4436 (TENN50934); Puerto Rico: Luquillo National Forest, Palmer, El Verde Research Station, Rio Grande Co., 09 June 1998, Ed Lickey FB9285 (TENN56363); Tennessee: South Knoxville, Knox Co., 07 Feb. 1999, DK FB10131 (TENN57459).

RESULTS

Self-cross

When twelve monokaryotic SBIs of FB10131 were paired in all possible combinations, heterothallism with a tetrapolar system was confirmed (Fig. 2). Tester SBIs were selected for subsequent intercollection pairings. Similar results were reported by Petersen *et al*. (1997) for collection FB7979.

The majority of contact zones exhibited flat morphology, but the pattern of flats and barrages was unrelated to the mating factors as also reported in Petersen *et al*. (1997) and for other *Polyporus* species (Hoffmann 1978, Krüger *et al*. 2002, PARTS 5, 6, 7). False clamps were observed in one clamp-forming combination and SBI pairings 9 X 12 and 10 X 11 showed clamps without holes, laying directly attached on the hyphal wall. Most cultures, including

Figure 1. Specimen FB10131*P. alveolaris*. **A**: comparison of upper/lower cap surfaces of four basidiomata. **B**: entire collection. Note the pinkish-orangish shades. Scale comparison: short side of number label = 2.3 cm.

10131		A_1B_1				A_2B_1						A_1B_2	A_2B_2
		1	3	8	9	4	5	6	7	2	10	11	12
A_1 B_1	1		F-	B-	F-	B-	F-	F-	F-	B-	B-	F-	+
	3	F-		F-	F-	F-	F-	F-	F-	B-	B-	F-	+
	8	B-	F-		F-	F-	F-	F-	F-	B-	F-	F-	◊
	9	F-	F-	F-		F-	B-	F-	B-	F-	F-	F-	+
A_2 B_1	4	B-	F-	F-	F-		B-	F-	B-	F-	F-	+	F-
	5	F-	F-	F-	B-	B-		B-	F-	F-	F-	+	F-
	6	F-	F-	F-	F-	F-	B-		F-	B-	F-	+	F-
	7	F-	F-	F-	B-	B-	F-	F-		F-	F-	+	F-
	2	B-	B-	B-	F-	F-	F-	B-	F-		B-	+	F-
	10	B-	B-	F-	F-	F-	F-	F-	F-	B-		+	F-
A_1B_2	11	F-	F-	F-	F-	+	+	+	+	+	+		F-
A_2B_2	12	+	+	◊	+	F-	F-	F-	F-	F-	F-	F-	

Figure 2. Self-cross of *P. alveolaris* collection FB10131. + = clamp connections, - = no clamp connections, ◊ = unclosed false-clamps in addition to normal clamps, B = barrage, F= flat contact zone. Assigned mating types indicated on the top and to the left. Assigned mating types and SBI numbers indicated on the top and to the left, with tester SBI numbers in shaded cells.

degrees as were those of FB7979 (Petersen *et al*. 1997). No conidiogenesis was observed. Nobles *et al*. (1957) observed "illegitimate pairings" (rare clamp connections in certain pairings), and Petersen *et al*. (1997) mentioned also few clamps and/or false clamps in certain combinations.

Intercollection pairings

Polyporus arcularius FB7979 has been previously mated with *Polyporus* (*Favolus*) *tenuiculus* and found incompatible (Petersen & McCleneghan 1997). Figure 3 shows the results of new intercollection pairings. One cross between FB7979 and FB10131 was 100% compatible, but the only other compatibility was between FB9072 and FB10294 (50%, collections from Europe and North America). Clamp connections were not produced in any other pairing combination. The experiment was repeated, and DAPI (Bresinsky *et al*. 1987) was used to observe nuclear number. No indication of dikaryotic hyphae was seen.

No consistent morphological differences that could be correlated with the results of pairings experiments were found. FB10131, FB8701, and FB8477 featured a monomitic hyphal layer on the cap surface belonging to the squamules, unreported by Nuñez & Ryvarden (1995). Geographic origin also seemed to be irrelevant to mating compatibility.

DISCUSSION & TAXONOMY

Our mating studies suggested that *P. alveolaris* is perhaps an assemblage of cryptic species still awaiting confirmation by molecular data, as was recently done in the *P. tricholoma* complex in gr. *Polyporellus* (PART 5). The high number of cryptic species, however, is very unusual. It remains to be investigated whether something intrinsic to the experiment prevented successful crossing.

Cultural characters/mating systems were in agreement with those reported for the self-cross of FB7979 by Petersen *et al*. (1997), except that here we recovered all mating types, although unbalanced. There was a clear contrast to culture morphologies in *P.* (*Favolus*) *tenuiculus*, which produced slimy exudates and copious conidiogenesis by branched aerial hyphae (Petersen *et al*. 1997). Imbalance of mating types may be an artifact of germination on agar or sample size as opposed to genetic mechanisms shifting mating type frequencies. Petersen *et al*. (1997) suggested this inbalance to perhaps indicate a relationship to the gilled genus *Pleurotus*, where such imbalance appeared to be very common. This appears not to be the case as a BLAST-n (Altschul *et al*. 1997) search of GenBank did not accept *Pleurotus* as anywhere close. Hibbett & Thorn (2001) found *Pleurotus* in /Euagarics, and Binder & Hibbett (2002: Fig. 4) showed *Pleurotus* as basal to the /core-Euagarics (*ut* core euagarics clade).

Singer (1986: 170) called for a new name to be proposed for *Polyporus* infrageneric group *Favolus* [*nec Favolus* (Pal. de Beauv.: Fr.) Fr.1821, *non Favolus* Fr. 1828, see Donk 1960: 210-214). *Polyporus* subgen. *Favolus* (Pal. de Beauv.: Fr.) Fr. is defined by its monotype *Favolus hirtus* Pal. de Beauv. (Murrill 1903: 90, Donk 1960: 210, Ryvarden 1991: 144, 221). The latter does not pertain to what is understood as *Favolus* now, but is *Scenidium hirtum* (Pal. de Beauv.) O. Kuntze [*Scenidium* (Klotzsch) O. Kuntze = *Hexagonia* Fr*., non Hexagonia* Poll*.* = *Polyporus* Fr.] (Murrill 1903: 90, Donk 1960: 210, Singer 1986: 170, Ryvarden 1991: 144).

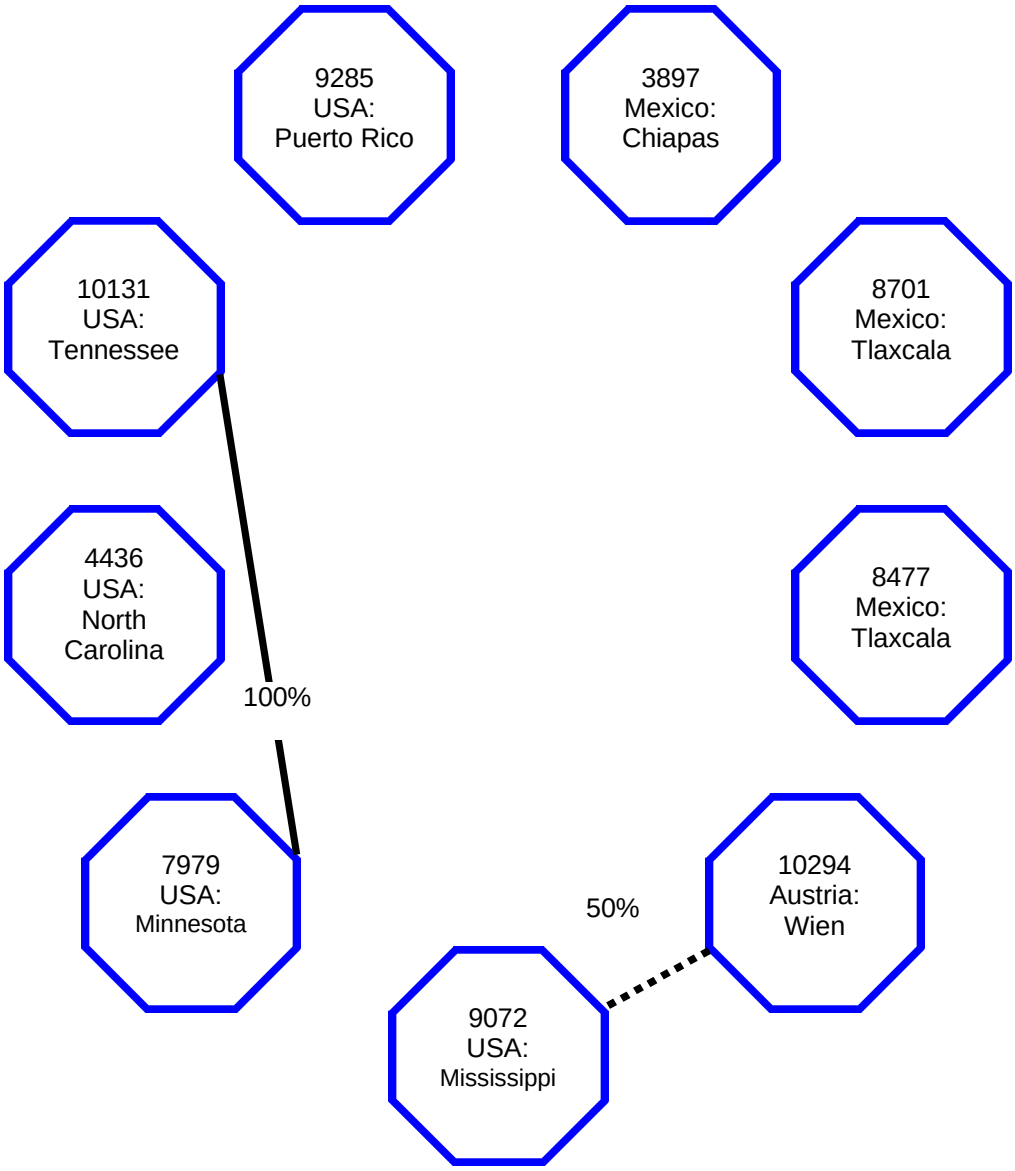

Figure 3. Intercollection pairings tested in *P. alveolaris*. Lines representing percentage compatibility as expressed by clamp connections formed (100% = 4/4 SBI pairings, 75% = 3/4, 50% = 2/4, 25% = 1/4, 0% not indicated).

Primarily based on LSU phylogenies (PART 2) we agree with Singer (1986: 170) who recommended a new subgenus to replace *Favolus* in *Polyporus*, but our data also support inclusion of gr. *Admirabilis sensu* Nuñez & Ryvarden (1995). In our opinion, there is doubt that tesselate caps and widely elongated pores are consistent enough characters to include *P. alveolaris* in one genus or group with the tropical *Favoli*. Also, as we discussed in PART 2, the tendency toward lack of clamp connections in gr. *Favolus* and gr. *Admirabilis* is not evident in *P. alveolaris*. It appears that when *P. alveolaris* is excluded, grs. *Favolus* and *Admirabilis* are natural groups. In this manuscript we propose a new subgenus in *Polyporus* to combine infrageneric grs. *Favolus* and *Admirabilis sensu* Nuñez & Ryvarden (1995), and we exclude *P. alveolaris* from this new taxon. This is deemed necessary for the following reasons: i) as explained above, *Polyporus* subgen. *Favolus* Fr. has a type species that is not a *Favolus* or *Polyporus*, ii) LSU sequence data (PART 2) and ITS region sequence data (PART 6) failed to support the concept of *Royoporus* (De 1996, 1997), with clampless generative hyphae, but showed members of grs. *Admirabilis* and *Favolus sensu* Nuñez & Ryvarden (1995) in a relationship group we have called /Tenuiculus-"Favoliporus".

Polyporus subgenus *Favoliporus* Krüger *apud* Sing., subgen. nov.
Subgenero generis Polyporus. Carpophora lignicola, sessilis, flabelliformis, substipitatus rarus.
Etymology: *favoli* = referring to its historical basis, *Favolus*; *porus* = pore, referring to *Polyporus*
Type: *Polyporus grammocephalus* Berk. = *Laschia spathulata* Jungh.
= "*Admirabilis* group" *sensu* Nuñez & Ryvarden (1995)
= "*Favolus* group" *sensu* Nuñez & Ryvarden (1995)
= *Favolus* Fr. 1828, *non Favolus* (Pal. de Beauv.: Fr.) Fr.1821
/Tenuiculus-"Favoliporus" can thus be considered as a syn-cladonym to /Favoliporus.

Section *Favolipori* (autonym)
Pileus flabelliformis.
Type: *Polyporus grammocephalus* Berk. (= *Laschia spathulata* Jungh.)
Additional species:
Polyporus moluccensis (Mont.) Ryv. (if not synonymous with *P. grammocephalus* Berk.)
Polyporus philippinensis Berk.
Polyporus tenuiculus (Pal. de Beauv.) Fr. [= *P. brasiliensis* (Fr.) Fr.]

Section *Admirabili* Krüger, sect. nov.
Pileus sessilis, substipitatus.
Type: *Polyporus admirabilis* Peck
Additional species:
Polyporus choseniae (Vassilkov) Parmasto [*ut* "*chozeniae*" (see Thorn 2000)]
Polyporus fraxineus (Bond. & Ljub.) Y.-C. Dai 1999 [= later homonym of *P. fraxineus* (Bull.: Fr.) Fr. 1821 1: 374; conspecific with *P. admirabilis* according to Thorn (2000)]
Polyporus gayanus Lév.
Polyporus pseudobetulinus (Pilát) Thorn, Kotiranta & Niemelä

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VITA

Dirk Krüger was born (June 13, 1969) and raised in Neustrelitz, Mecklenburg, in the former German Democratic Republic. After completing the mandatory 10 class polytechnical school in 1986 he went on to learn the profession of a skilled worker in automation technique at electronics and pharmaceutical companies. He moved on to work as a high-voltage electrician with the Deutsche Reichsbahn electrical railway system. At the same time he pursued a university-qualifying high school diploma, the Abitur, in evening school in the outskirts of Berlin at Oranienburg during the stormy times of the collapse of the East Block.

Subsequently he became a university student in biology and languages at Georg-August-Universität Göttingen in 1991, skipping military service. In 1994/1995 he spent a year as visiting graduate student at Memorial University of Newfoundland, St. John's NF, learning molecular techniques to complement his interest in mycology. Upon returning to eastern Germany, he enrolled at Ernst-Moritz-Arndt-Universität Greifswald and ultimately worked on a thesis on gasteromycetes systematics, accompanied by a stint in molecular research at the Universität Regensburg. In 1998, Krüger enrolled in a PhD program in Botany at the University of Tennessee, with emphasis on fungal systematics. After completion in 2002, Dirk hopes to continue research in mycology as a postdoctoral appointee at the University of Wisconsin – Madison.